The Histone Acetyltransferase Dmel\TIP60 Is Essential for Multicellular Development in *Drosophila*

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To my beloved parents and all my teachers.

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Abstract

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Combinatorial histone modifications control chromatin packaging which in turn, contributes to the precise patterning of gene expression during development. Histone acetyltransferases (HATs) are a key class of chromatin regulatory proteins that mediate such developmental chromatin control; however their specific roles during multicellular development remain unclear. Here, we report the first isolation and developmental characterization of a *Drosophila* HAT gene (Dmel\TIP60) that is the homolog of the human HAT gene TIP60. We show that Dmel\TIP60 is differentially expressed during *Drosophila* development, with transcript levels significantly peaking during embryogenesis. We further demonstrate that reducing endogenous Dmel\TIP60 expression in *Drosophila* embryonic cells by RNAi results in cellular defects and lethality. Finally, we use our *Drosophila* GAL4 inducible Dmel\TIP60 knockdown/overexpression system to explore the role of Dmel\TIP60 in a wide variety of specific tissues during *Drosophila* development. We show that ubiquitous and cell/tissue specific reduction of Dmel\TIP60 expression results in lethality and/or cell/tissue specific phenotypes during fly development. Loss of Dmel\TIP60 in the wing leads to a range of wing abnormalities, including the formation of wing blisters

in the most severe cases. Wing surface area and cell count/hair density assays reveal that although the number of cells that compose the wing remain unaffected, their size is significantly smaller than normal and there are defects in wing cell planer polarity. Additionally, we find that loss of Dmel\TIP60 in the CNS leads to lethality and a substantial loss of differentiated neurons in the larval brain, while cyclin E levels and apoptosis remain unaffected. Finally, we show that loss of Dmel\TIP60 in the mesoderm leads to lethality, and malformation or absence of the muscle fibers in the developing embryo. Overexpression of Dmel\TIP60 in each of these tissues has no affect on their development. Taken together, our results support an essential role for Dmel\TIP60 in the differentiation and formation of a variety of specific cell and tissue types. Significantly, our inducible and targeted HAT knockdown system in *Drosophila* now provides a powerful tool to effectively study the roles of these chromatin mediators in specific tissues and cell types during development.

Chapter 1: Background

Chromatin packaging and epigenetic regulation

Metazoans consist of different cell-types that carry out specialized functions essential for proper development. The differentiation of such specialized cell-lineages is achieved through the establishment and maintenance of tightly regulated gene expression profiles distinct for each cell type (Orphanides and Reinberg 2002). In eukaryotes, the differential packaging of genes into chromatin significantly contributes to this regulation.

Chromatin, the genetic material in eukaryotic cells, is constructed by nucleosomes. Each nucleosome consists of 146 bp of DNA wrapped around a histone octamer core, containing two copies each of histone proteins H2A, H2B, H3, and H4 (Luger 2006). There are two different types of eukaryotic chromatin: euchromatin and heterochromatin. Euchomatin has a diffuse appearance during interphase, and undergoes condense and decondense in the cell cycle. Euchromatin is found outside centromere and telomere regions. It contains most of the single-copy genes and lacks meiotic recombination (Dimitri et al. 2005). Acetylation of histone H3 and H4 tails and methylation of lysine 4 residue on H3 (H3K4) are characteristic for euchromatin (Strahl and Allis 2000). In contrast to euchromatin, heterochromatin is condensed throughout the cell cycle. About 30% of D. melanogaster genome and 20% of human

genome are packaged into heterochromatin (Dimitri et al. 2005). With very few meiotic recombinations, heterochromatin replicates late in S phase, and is associated with repressive transcription (Westphal and Reuter 2002). Heterochromatin always resides in centromere and telomere. It has high volume of repetitive DNA copy number but very low single-gene copy number (Dimitri et al. 2005) (Weiler and Wakimoto 1995). For example, only approximately 300 genes in *D. melanogaster* are predicted inside heterochromatin (Lu et al. 2000a; Schulze et al. 2005). The heterochromatin structure is required for the proper expression of heterochromatic genes, because these genes in heterochromatin are found to be repressed when placed into euchromatin by chromosomal rearrangement (Wakimoto and Hearn 1990; Eberl et al. 1993). Taken together, these studies support the premise that chromatin packaging plays an essential role in the regulation of gene expression.

As stated above, epigenetic regulation, i.e., packaging of DNA into chromatin, directly controls gene expression (Felsenfeld and Groudine 2003; Dillon 2006). Epigenetic regulation is achieved by controlling DNA accessibility for cellular processes such as replication, transcription and DNA repair (Woodcock 2006). In general, there are three types of biochemical mechanisms in epigenetic regulation, which are DNA methylation, binding of non-histone proteins (e.g., HP1, Polycomb (PcG) and trithorax (trxG) group complexes), and histone modifications (Bock and Lengauer 2007).

DNA methylation directly replaces a hydrogen atom of the cytosine base by a

methyl group. Although the modified cytosine can still be transcribed into mRNA, DNA methylation leads to inhibition of transcriptional activation because it promotes a highly condensed chromatin structure so that to prevent the binding of transcriptional factors (Bird 2002; Weber and Schubeler 2007). The details of how DNA methylation involves in gene regulation during development will be discussed later.

Epigenetic regulation via non-histone proteins can be categorized into two mechanisms (Bock and Lengauer 2007). One is relied on ATP-dependent chromatin remodeling complexes, which directly move or displace nucleosomes along the DNA (Gangaraju and Bartholomew 2007). The other mechanism is to recruit proteins such as HP1, PcG and trxG complexes, which recognize the epigenetic marks on the chromatin and further modify these marks (e.g., histone modifications and/or DNA methylation) by recruiting other protein complexes (Ringrose and Paro 2004; Schulze and Wallrath 2007). The detailed discussions on PcG and trxG functions during development will be in the section “Epigenetic Regulation during Development”.

Covalent modification of the core histones is another way to remodel chromatin (Berger 2002; Kouzarides 2007). Such modifications include acetylation (L residue), methylation (K and R), phosphorylation (S and T), ubiquitination (K), sumyolation (K), ADP ribosylation, glycosylation, biotinylation, and carbonylation (Margueron et al. 2005). As known, the histone N-terminal tails is unstructured and protrude out of the nucleosome, which can interact with the other neighboring

nucleosomes. The modifications on histone N-terminal tails can physically modulate the affinity between histones and DNA so that to influence the nucleosomal assembly and chromatin structure. For example, histone acetyltransferases (HATs) can transfer an acetyl group to the histone lysine residues, which facilitate gene expression by promoting chromatin decondensation and allowing the transcriptional factors to bind onto DNA (Brownell and Allis 1996). Histone modification has proved to be as important as the genetic code, and as such is called "histone code" (Jenuwein and Allis 2001; Turner 2007). Furthermore, the combinatorial and coordinated histone modifications can determine the chromatin status at different levels (Fischle et al. 2003) and regulate many biological processes including development (Margueron et al. 2005).

In conclusion, epigenetic regulation, which precisely controls chromatin packaging, plays an essential role in regulating gene expression for normal growth and development of multicellular organisms.

Epigenetic regulation during development

The development of multicellular organisms requires the specialization and maintenance of cell lineages, which is achieved by precise regulation of differential gene expression profiles at different developmental stages. As stated above, epigenetic regulation plays an essential role in controlling gene expression. Below are

the detailed mechanisms of epigenetic regulation during development.

Dosage compensation

In both mammals and *Drosophila*, females have two X chromosomes (XX), whereas males have only one (XY), so that X-linked genes have a twofold difference between females and males. Dosage compensation is the precise regulation that equalizes the amount of X chromosomal gene expression between two sexes. However, the mechanisms of dosage compensation in these two species are different. Dosage compensation is achieved by X chromosome inactivation (XCI) in mammalian females, whereas it is achieved by specifically activating transcriptions in *Drosophila* males.

X inactivation in mammalian systems

During mammalian development in the mouse model system, the preimplantation mouse embryonic cells initially undergo imprinted inactivation of the paternal X chromosome (Xp). In the late blastocyst, Xp in the inner cell mass cells is activated, except that in the extraembryonic cells (trophoectoderm, primitive endoderm). Afterwards, the embryo carries out random X inactivation (Allegrucci et al. 2005).

Random X inactivation is initiated by the transient interchromosomal interactions between X inactivation center (Xic) loci on the X chromosomes, which allows for an estimation of the number of X chromosomes and initiates which X chromosome will be inactivated in female cells. Within Xic loci, there are three genes that encode distinctive noncoding RNAs (ncRNAs): Xist, Tsix, and Xite. Accumulation of Xist is required for inactivation of one of the X chromosomes. Tsix is antisense to Xist which leads to Xist silencing. Xite can positively regulate Tsix transcription. On the inactivated X chromosome (Xi), transcription of Tsix is down-regulated, while that of Xist is up-regulated and accumulates to recruit additional histone modifiers. On the active X chromosome (Xa), Tsix represses Xist by introducing H3K4me2 (Sado et al. 2005; Sun et al. 2006). Epigenetic regulations are widely involved in promoting heterochromatization of Xi. In support of this phenomenon, Xi was found to be located within hypermethylated CpG islands (Kratzer et al. 1983). Additionally, the Eed-Ezh2 Polycomb group (PcG) complex associates with Xi and contributes to H3K27me3 (Plath et al. 2003). Furthermore, the histone modifications of H3K9me2 and H3K4me2 are also found in Xi (Valley et al. 2006). Compared to Xa and autosomes that contain the activating histone variants, such as H2ABbd and H2A.Z, Xi contains the most extreme repressive histone variant macroH2A (Chadwick and Willard 2001; Chadwick and Willard 2003).

Dosage compensation in *Drosophila*

In *Drosophila*, a dosage compensation complex (DCC) is the regulating center for activating gene transcription on the X chromosome in males. This complex consists of several protein factors and RNAs. The protein factors are male specific lethal (MSL), the RNA-DNA helicase maleless (MLE), male specific lethal1, 2 and 3 (MSL1, 2 and 3), the acetyltransferase males absent on first (MOF), the histone H3 kinase JIL-1. Two ncRNAs are RNA on the X 1 and 2 (roX1 and roX2).

Within the DCC complex, MSL2 can limit and guide the formation of the functional DCC complex only in males, since all of the MSL proteins, except MSL2, were found to be expressed in females. (Kelley et al. 1995; Zhou et al. 1995). MSL2 further recruits MSL1 and MSL3 and other members. As described below, an epigenetic regulation is essential for DCC mediated dosage compensation when dissecting the functions of each member in this complex. MOF is most likely to be involved in activation of the male X chromosome by acetylating histone H4 on lysine 16 (H4Ac16), a modification associated with transcriptional activation (Akhtar and Becker 2000; Smith et al. 2000) and usually found in the male X chromosome (Turner et al. 1992; Bone et al. 1994). This elevation of gene expression is likely due to the regulation of the rate of elongation, rather than promoter activation as the DCC complex and H4Ac16 are more enriched in the coding regions of compensated genes than on their promoters (Smith et al. 2001; Alekseyenko et al. 2006; Gilfillan et al.

2006). Another histone modification in the male X chromosome is H3 phosphorylated on serine 10 (H3pS10), which is attributed by the JIL-1 (Wang et al. 2001). JIL-1 may antagonize the spread of heterochromatin and promote active euchromatin, most likely because H3pS10 can somewhat block H3K9me2 and thus prohibits HP1 binding on the chromosome region (Zhang et al. 2006). Additional factors that play a role in X activation in males are the noncoding RNAs, roX1 and roX2. These RNAs are found to coat the X chromosome in males and be regulated by one or more members of the MSL complex (Bai et al. 2004; Lee et al. 2004a; Rattner and Meller 2004). For example, MSL3 and MOF have RNA binding activity in vitro. As demonstrated by experiments, MLE and MOF are removed from the X chromosome by RNase A digestion (Richter et al. 1996; Akhtar et al. 2000; Buscaino et al. 2003). Additionally, the MSL proteins can be co-immunoprecipitated with the roX RNAs and JIL-1 (Copps et al. 1998; Jin et al. 2000; Smith et al. 2000). Furthermore, knockout both roX1 and roX2 will significantly reduce the survival rate in males (Meller and Rattner 2002). Thus, the ncRNAs (roX1 and roX2) must play a central role in the interactions among the members within the DCC complex.

Interestingly, the proteins in *Drosophila* DCC also have human homologs; however, their human counterparts may not have the same functions as described. For example, human MOF (hMOF), which also has histone acetyltransferase activity, has nothing to do with dosage compensation in human but is required for normal function of human ATM and DNA repair (Gupta et al. 2005).

Position effect variegation (PEV)

Position effect variegation is defined as differential gene expression in individual cells when such gene is positioned within heterochromatin. This phenomenon was first described when H Muller explored the effect of X ray radiation on *Drosophila* development in 1920s and 1930s (Schulze and Wallrath 2007). The mutated flies had mosaic red and white eyes due to the position of the *white* gene into the heterochromatin region. Multiple screens have been performed to identify the genes that either enhance or suppress PEV (Reuter and Wolff 1981; Sinclair et al. 1989; Wustmann et al. 1989; Dorn et al. 1993). As a result, a series of genes have been identified, ranging from 50 to 150 thus far (Weiler and Wakimoto 1995). Some of them show dosage-dependent effects on variegation, which proves genetically that the amount of protein factors is important for the formation of heterochromatin (Schotta et al. 2003). Such genes fall into 2 categories: enhancer of position variegation effect *E(var)* and suppressor of position variegation effect *Su(var)*. The majority of these genes encode the proteins associated with chromosome structure. In general, Su(var) represses transcription by forming heterochromatin, whereas E(var) enhances gene expression by opening chromatin structure, (Schulze and Wallrath 2007). For example, *Su(var)3-9* encodes a protein containing histone methyltransferase activity that can methylate H3K9 (Rea et al. 2000; Lachner et al.

2001). Methylated H3K9 in the centric heterochromatin regions is always observed to be bound with heterochromatin protein 1 (HP1) encoded by the *Su(var)2-5* gene (James and Elgin 1986; Eissenberg et al. 1990). *Su(var)3-1* encodes JIL-1 as mentioned in dosage compensation, which phosphorylates serine 10 of histone H3 (H3S10) and controls the heterochromatin spreading upstream of the control of HP1 (Ebert et al. 2004; Schulze and Wallrath 2007). In contrast, *E(var)62/trithorax-like* (*trl*) encodes the GAGA factor which can bind the (GA)n motif and open the chromatin structure for transcriptional activation (Granok et al. 1995).

Genomic impriting

Imprinting is the process of monoallelic expression of certain genes from either paternal or maternal allele. In mammals, the epigenetic regulation of imprinting is somewhat similar to XCI (Reik and Lewis 2005). More than one hundred of imprinted genes have been found, most of which function in regulation of late placental and fetal growth (Kiefer 2007). Disruption of genomic imprinting always leads to many human developmental disorders and cancers (Feinberg et al. 2002).

Imprinted genes are differentially expressed from one parental chromatin due to the epigenetic marks (e.g. DNA methylation) established on that particular chromatin (Wood and Oakey 2006; Edwards and Ferguson-Smith 2007). Such genes are usually present in cluster of 3-11 genes and are controlled by an imprint control

region (ICR) located within the cluster. Before oogenesis and spermatogenesis, pre-existing DNA methylation at the ICR is erased in the primordial germ cells by unknown mechanisms (Delaval and Feil 2004). During oogenesis and spermatogenesis, DNA in the ICR is differentially methylated by DNA methyltransferase Dnmt3a and Dnmt3l between two homologous chromosomes in germ-line cells. After a genome-wide reprogramming that erases DNA methylation immediately from the paternal pronucleus and progressively from maternal chromosomes following fertilization (Edwards and Ferguson-Smith 2007), the *de novo* allelic DNA methylation is established and maintained in the somatic cells throughout the development (Wood and Oakey 2006; Edwards and Ferguson-Smith 2007).

Genomic imprinting is achieved by several epigenetic mechanisms such as DNA methylation and histone modifications, serving as a good example of epigenetic gene regulation during development (Delaval and Feil 2004; Kiefer 2007). For instance, the insulin-like growth factor 2 receptor (*Igf2r*) cluster that encodes a fetal growth regulator and potassium voltage-gated channel, subfamily Q, member 1 (*Kcnq1*) cluster that encodes cardiac potassium channel are both expressed on the maternal chromosome and their expression is regulated by the ncRNA antisense *Igf2r* RNA (*Air*) and Kcnq1 overlapping transcript 1 (*Kcnq1ot1*), respectively (Delaval and Feil 2004). On the maternal chromosome, DNA methylation inhibits the ncRNA transcription of *Air* and *Kcnq1ot1*, so that the *Igf2r* and *Kcnq1* clusters can be

expressed (Stoger et al. 1993; Engemann et al. 2000). Although the mechanisms underlying how *Air* and *Kcnq1ot1* can silence *Igf2r* and *Kcnq1* on the paternal chromosome are not clear to date, one recent study found that *Kcnq1ot1* induced silence is associated with H3K9 and H3K27 methylation and concordant with the recruitment of the Eed-Ezh2 PcG complex on the paternal chromosome (Umlauf et al. 2004). The imprinting mechanisms are diverse among different clusters and may be even different within the same cluster. For instance, insulin-like growth factor 2 (*Igf2*) and *H19* are in the same cluster, however, *Igf2* is only expressed on paternal chromosome and *H19* is expressed on maternal chromosome. On the maternal chromosome, the ICR in this cluster is unmethylated, thus blocks *Igf2* expression by recruitment of an insulator CCCTC binding factor (CTCF) and blocking interaction between the promoter and enhancer of *Igf2*. Conversely, the unmethylated ICR directly allows the maternal expression of *H19*. On the paternal chromosome, the opposite events happen allowing *Igf2* expression and blocking *H19* expression (Hark et al. 2000; Engel et al. 2004).

Polycomb group / Trithorax group

Embryonic patterning in *Drosophila* is established by the transient presence of maternal and zygotic transcription factors, which are regulated by the *Polycomb* group (*PcG*) and *trithorax* group (*trxG*) genes. To date, there are 18 *PcG* genes and

17 *trxG* genes (Ringrose and Paro 2004). PcG maintains the spatial pattern of homeobox-containing (*Hox*) genes, a group of conserved genes that lead to anterior/posterior patterning. The name Polycomb comes from the finding in the early 1940s that a fly mutant for the gene that encodes for PcG exhibits sex combs on all the legs of males instead of the normal pattern where sex combs are present only on the first leg (Lewis 1978), which (known as homeosis) is caused by the loss of repression of a *Hox* gene through PcG. (Ringrose and Paro 2004). Mutation of *Trithorax* (*trx*) was initially found to be able to transform body segment into anterior ones (Ingham 1985). It was not until the late 1980s that several other suppressors of *PcG* mutant phenotypes were identified that belonged to the trxG. genes (Kennison and Tamkun 1988). PcG and trxG proteins are well conserved between *Drosophila* and vertebrates. Although *Drosophila* shares a number of vertebrate homologs such as enhancer of Zeste [E(Z)], extra sex combs (ESC), and suppressor of Zeste 12 [SU(Z)12], some *Drosophila* proteins are missing in vertebrates, which include three sequence-specific DNA-binding proteins GAF, Pipsqueak (PSQ), Zeste (Ringrose and Paro 2004).

PcG represses *Hox* genes in regions where they are not normally expressed by marking those regions with the silencing epigenetic marks, H3K27me3 and H3K9me2. Flies and mice mutant for PcG genes exhibit ectopic *Hox* expression, sometimes eliciting homeotic transformations (Jürgens 1985; van der Lugt et al. 1994). The PcG gene *Bmi1* mutation mice have been shown to have numerous defects

in the self-renewal of hematopoietic and neural stem cells, and in the proliferation of primary fibroblasts (Jacobs et al. 1999) (Lessard and Sauvageau 2003; Molofsky et al. 2003; Park et al. 2003). As mentioned previously, trimethylation of H3K27 by PcG also promotes silencing on Xi and imprinted genes. PcG binds to Polycomb group response elements (PREs) in regulatory regions of target genes. In *Drosophila*, PREs are comprised of different combinations of specific binding sites. PcG targets several developmental genes, including the transcription factors Fox, Sox, and Pax and the signaling molecules Wnts, Shh, and BMPs (Bracken et al. 2006; Negre et al. 2006). Additionally, PcG target profiles differ in undifferentiated and differentiated cells. Taken together, these studies support the premise that the PcG complex comprises an important group of epigenetic regulators that are involved in various developmental processes.

There are two PcG complexes derived from the *Drosophila* embryo, Polycomb repressive complex (PRC) 1 and 2 (Kiefer 2007). Another complex, named chromatin associated silencing complex for homeotics (CHRASCH) was purified from *Drosophila* Schneider cells (Huang et al. 2002; Huang and Chang 2004). *Drosophila* PRC1 is composed of Polycomb (PC), Polyhomeotic (PH), Posterior sex combs (PSC), RING, Sex combs on midleg (SCM), Zeste, and some other transcriptional factors (Saurin et al. 2001). The core complex of PC, PH, PSC and RING has no preference for DNA sequences that contain PRE, but will bind to Zeste sites when reconstituted with Zeste protein (Mulholland et al. 2003). GAF and PHO

are also found inside the PRC1 complex as demonstrated by coimmunoprecipitation (Poux et al. 2001). PRC1 can repress transcription by inhibiting chromatin remodeling by SWI/SNF (Francis et al. 2001) and/or preventing transcription initiation by RNA polymerase (Dellino et al. 2004). Recently, it was demonstrated that the mammalian PRC1 complex promotes *Hox* silencing through ubiquitination of H2A (Cao et al. 2005). *Drosophila* PRC2 contains the core proteins extra sex combs (ESC), suppressor of Zeste 12 [SU(Z)12], enhancer of Zeste [E(Z)] and some additional proteins (Ringrose and Paro 2004). PRC2 complex can methylate lysines 9 and 27 on histone H3 , the mark for gene silencing that serves as a binding site for chromodomain of PC (Czermin et al. 2002). It is important to note that PcG regulatory regions do not seem to overlap with those of HP1, suggesting that PcG function during development is different from the general HP1-related heterochromatic silencing during early embryogenesis (Schulze and Wallrath 2007). Although it is related to PRC1 complex, CHRASCH has a unique protein PSQ, which ensures the complex binding to PREs that specifically contain the (GA)n motif. In addition, the complex contains HADC1 that is associated with repression of gene expression (Huang and Chang 2004).

Similar to PcG, trxG functions are also carried out in distinct trxG complexes. There are four trxG complexes that have been found in *Drosophila* so far: BRM complex, small or absent, small or homeotic discs 1 (ASH1) complex, ASH2 complex, and trithorax acetylation complex 1 (TAC1) complex. The BRM complex is

composed of the trxG proteins Brahma (BRM), Moira (MOR), and OSA, as well as additional accessory proteins (Papoulas et al. 1998; Collins et al. 1999). In the BRM complex (the homolog in yeast is the SWI2/SNF2 complex), BRM acts as ATPase for the ATPase-dependent chromatin remodeling (Papoulas et al. 1998). The ASH1 and ASH2 complexes are composed of the trxG proteins ASH1 and ASH2, respetively (Papoulas et al. 1998). The TAC1 complex contains the trxG protein TRX, the histone acetyltransferase CBP and the antiphosphatase sbf1 (Petruk et al. 2001). Of note, TAC1 complex can also repress *Hox* gene *Ultrabithorax* (*Ubx*) by facilitating transcriptional elongation of *bxd* ncRNA transcripts (Petruk et al. 2006). trxG binds the same chromosomal element as PcG. However, this element is called trithorax response element (TRE) and not PRE when trxG binds to it.

The histone modification H3K4me3, which is a well characterized epigenetic mark related to transcriptionally active genes (Strahl and Allis 2000; Cheung and Lau 2005), plays a key role in the trxG-mediated gene activation (Sims and Reinberg 2006; Schuettengruber et al. 2007). For example, both TRX and ASH1 have SET domain that is a catalytic domain responsible for methylating histones, and thus function as histone methyltransferases that specifically methylate lysine 4 on histone H3 (H3K4) (Beisel et al. 2002). Noncoding TRE transcripts, which are required for activation of PRE/TRE, recruit *Drosophila* ASH1 to make the active euchromatin mark H3K4me3 (Sanchez-Elsner et al. 2006). Furthermore, bromodomain PHD finger transcription factor (BPTF), a subunit of nucleosome remodeling factor (NURF) complex,

specifically targets the chromatin remodeling trxG. NURF complex to H3K4me3 on the promoter region and promotes active chromatin by repositioning nucleosomes along DNA (Wysocka et al. 2006). These data highly suggest that trxG activates gene expression (e.g. *Hox* genes) by facilitating euchromatin.

Lineage restriction

During embryogenesis, specific gene programs in multipotent cells are activated thus triggering differentiation of these cells into specialized lineages. Equally important, a cell must also silence expression of genes specific to other cell lineages to secure its fate. Because there gene expression profiles must be maintained throughout the life of the animal, epigenetic mechanisms are ideal for mediating such events.

Nervous systerm

During neuron differentiation in vertebrate systems, a vast array of transcriptional activators and repressors are involved in neuronal and glial fate specification (Bertrand et al. 2002; Ross et al. 2003). Epigenetic regulation provides a modulating center for either recruiting or displacing such different transcription factors, thus allowing for gene activation or repression in a temporal and special

manner. In epigenetic repression, the repressor element 1 (RE-1) silencing transcription factor/neuron restrictive silencing factor (REST/NRSF), a zinc finger protein, plays a central role for epigenetic silencing of neuronal genes (Ballas and Mandel 2005). In support of this, mice lacking REST/NRSF die at E11.5 and show the neural-specific gene βIIItubulin expression in some non-neural tissues (Chen et al. 1998). In non-neuronal cells, REST/NRSF binds a 21 to 23 bp motif RE1 site (also known as neuron-restrictive silencing element, NRSE) in the regulatory regions of target genes and represses their transcription. These genes are essential for neurogenesis, and include ion channels, neurotransmitter receptors, axonal guidance molecules, and the neurogenic gene NeuroD (Bruce et al. 2004). REST/NRSF imposes dynamic, non-permanent repression through its association with several cofactors and complexes. The corepressors that interact with REST/NRSF include CTD phosphatase, which inhibits RNA polymerase II (Yeo et al. 2005), N-CoR (Jepsen et al. 2000), mSin3A/B complex (Naruse et al. 1999), and CoREST/HDAC2 complex (Ballas et al. 2001). The corepressor CoREST coordinates stable, epigenetic repression by directly binding the H3K9 HMT G9a (Roopra et al. 2004) and the H3K4 demethylase LSD1 (Shi et al. 2004). CoREST also recruits additional epigenetic silencing factors, such as the methyl DNA binding protein MeCP2, the H3K9 HMT SUV39H1, and HP1 which binds the regions marked with methylated H3K9 (Lunyak et al. 2002).

Upon neuronal differentiation, the repression of REST/NRSF complexes

would be removed from RE1 site. However, this is not sufficient to activate all neural cell type. Thus, distinct derepression mechanisms are required for different neuronal genes and cell types. For example, in the transformation from pluripotent embryonic stem cells to differentiated neurons, REST/NRSF can be removed from RE1 site, which is sufficient to activate the neuronal genes previously repressed by the complex. For some genes (e.g. brain-derived neurotrophic factor, BDGF and Calbindin), the disassociation of REST/NRSF from RE1 is not enough to trigger the activation of the genes. Additional CoREST/MeCP2 complexes must also be removed from the adjacent methylated DNA CpG regions to accomplish this depression process (Ballas et al. 2005). In neuronal progenitors of the adult hippocampus, a small noncoding double-strand RNA (dsRNA) was found to be associated with NRSF and capable of converting REST/NRSF from a repressor to an activator, most likely due to preventing the interaction of REST/NRSF with other factors by changing conformation of REST/NRSF and not by RNAi mechanisms (Kuwabara et al. 2004).

The ATPase Brahma-related gene 1 (Brg1), the catalytic subunit of SWI/SNF has shown to be required for neuronal differentiation through its association with Neurogenin 1 and NeuroD (Seo et al. 2005). Neurogenins and NeuroD are important neural basic helix-loop-helix (bHLH) transcription factors required for neuronal commitment and cell fate determination respectively. Recent research also showed that Geminin can block gene expression and maintain the undifferentiated state of the cell by disrupting the interactions between brg1 and neural bHLH proteins (Seo

et al. 2005).

Epigenetic regulations also control neural plasticity, memory and behavior. For example, upon stimuli in *Aplysia* (sea hare) neurons, CBP and HDAC5 are found to antagonize each other in the acetylation of histones at the promoter of the early gene C/EBP (Guan et al. 2002). Additionally， polyADP-ribose polymerase 1 is activated supporting the notion that transient chromatin remodeling is required for long-term memory (Cohen-Armon et al. 2004). In mouse Rubinstein-Taybi syndrome (RTS) model, the HAT activity of CBP is also essential for neuronal plasticity, and learning. Remarkably, increasing histone acetylation by HDAC inhibitor suberoylanilide hydroxamic acid or Trichostatin A rescues the RTS phenotypes in CBP mutant mice (Alarcon et al. 2004; Korzus et al. 2004). Additional studies show that behaviors influencing the maternal care in rats can be inherited and is influenced by levels of DNA methylation and histone acetylation at the promoter of glucocorticoid receptor (GR) gene (Weaver et al. 2004). Finally, retrotransposition of the human long interspersed nuclear element-1 (LINE-1) is regulated by epigenetic control mechanisms. Such retrotransposition is thought to influence neuronal differentiation but not glial differentiation (Muotri et al. 2005).

Muscle

During muscle development, lineage commitment is achieved by coordinated activation and repression of specific genes so that appropriate myogenic identity is acquired (Pomerantz and Blau 2004). The undifferentiated muscle precursors will then proliferate, enter the differentiation program, and finally turn into terminally differentiated cells. As shown below, epigenetic mechanisms are of great importance in regulating these complicated events during muscle development.

Myogenesis is generally controlled by several transcriptional factors that include the muscle specific basic helix-loop-helix (bHLH) transcription factors (muscle regulatory factors, MRFs), and the myocyte enhancer factor 2 (MEF2). The four members of MRFs are myogenic factor 5 (Myf5), myogenic differentiation antigen (MyoD), myogenin, and MRF4. These factors bind E-box DNA elements in the muscle specific promoters in a temporal manner and initiate the nuclear reprogramming for myogenic lineage commitment. The homeobox protein Msx-1 was found to inhibit the differentiation of skeletal muscle during embryogenesis (Bendall et al. 1999; Bendall and Abate-Shen 2000). Furthermore, there was evidence that Msx-1 binds to the linker histone H1b and guides H1b to the regulatory region of MyoD, which represses the expression of MyoD and inhibits myogenesis (Lee et al. 2004b). In accordance to this finding, H1b expression in undifferentiated cells, including muscle precursors, decreases as differentiation proceeds (Wang et al. 1997).

In proliferating myoblasts, histone hypoacetylation and hypermethylation within chromatin inhibits premature muscle-gene expression and is achieved by several histone modifiers, including histone deacetylases (HDACs), YY1 and Polycomb proteins. Upon induction of differentiation, As to histone acetylation, the association of class I HDACs with MyoD in undifferentiated myoblasts is disrupted thus allowing for acetylation of genes and concomitant expression (Mal et al. 2001); (Puri et al. 2001). The interactions between class I HDACs and MyoD is regulated by the pRb signaling pathway (Puri et al. 2001) described more in the next section. Class II HDACs bind and repress the MEF2 protein (Lu et al. 2000b; Lu et al. 2000c; McKinsey et al. 2000a; McKinsey et al. 2000b; McKinsey et al. 2002; Zhang et al. 2002), and this repression is facilitated by histone methylation. The NAD(+)-dependent histone deacetylase Sir2 also regulates skeletal muscle differentiation most likely by sensing the redox state of the cell in response to exercise, food intake, or starvation (Fulco et al. 2003). Histone methylation also plays a very important role in maintenance of the myogenic lineage. HDAC4, HDAC5 (class II HDACs) and the MEF2 repressor MITR each associate with HP1. Upon induction of differentiation, H3K9 methylation surrounding the MEF2 binding sites of target genes is decreased with concomitant disruption of the HP1-HDAC and HP1-MITR interactions (Zhang et al. 2002). These data suggest that class II HDACs bind to MEF2 target genes, not only creating a hypoacetylation environment but also promoting H3K9 methylation. The histone methyltransferases Ezh belongs to

Polycomb group (PcG), which also regulates muscle specific gene transcription. Ezh1 and 2 can methylate H3K27 via a conserved SET domain (Cao et al. 2002; Cao and Zhang 2004). Recently, it was shown that Ezh2 is down-regulated in the dermomyotome of the somites during embryonic development, coincident with the activation of the myogenic lineage. During myoblasts differentiation in culture, decreased Ezh2 expression correlates with hypomethylation of H3K9 at muscle regulatory regions while increased Ezh expression inhibits muscle differentiation due to hypermethylation of muscle-specific regulatory regions and the recruitment of the corepressor YY1 (Caretti et al. 2004).

Upon induction of differentiation, the HDACs and their associated co-repressors dissociate from the MRFs and MEF2 factors, allowing for the recruitment of chromatin modifying complexes to the chromatin surrounding the regulatory elements of muscle specific genes. These complexes include HATs CBP/p300, PCAF, p/CIP, SRC1 and glucocorticoid receptor interacting protein (GRIP), the co-activator-associated arginine methyltransferase 1 (CARM-1) and the ATP-dependent switching/sucrose non-fermenting (SWI/SNF) chromatin remodelling complexes (Eckner et al. 1996; Yuan et al. 1996; Puri et al. 1997a; Puri et al. 1997b; Sartorelli et al. 1997; Chen et al. 2000; de la Serna et al. 2001; Chen et al. 2002; Wu et al. 2005). Acetylation of the histone tails by p300 and PCAF results in a relaxed chromatin structure permissive for transcription, while acetylation of MyoD by the same acetyltransferases increases the affinity for its recognition site in the DNA (Puri

et al. 1997b; Sartorelli et al. 1999; Polesskaya et al. 2000; Polesskaya and Harel-Bellan 2001; Polesskaya et al. 2001a; Polesskaya et al. 2001b; Dilworth et al. 2004). Interestingly, p300 is shown to acetylate histones, whereas PCAF preferentially acetylate MyoD on its three conserved lysine residues (K99, K102 and K104) (Dilworth et al. 2004). Similarly, p300-mediated acetylation of multiple lysines is essential for MEF2 function (Ma et al. 2005). Subsequently, it was found that the conserved MADS box of MEF2 binds both p300/CBP and GRIP (Chen et al. 2000). CARM1 then forms the ternary complex with MEF2 and GRIP, leading to decondensed chromatin and cooperative activation of MEF2 target genes (Chen et al. 2002). In support of the importance of histone acetylation in muscle differentiation, inactivation of p300 or PCAF is sufficient to block skeletal myogenesis in cultured cells and in mouse embryos (Puri et al. 1997b; Polesskaya et al. 2001b; Roth et al. 2003).

Along with the expression of muscle-specific genes, terminal differentiation requires an exit from the cell cycle, which is achieved by the induction of cyclin dependent kinase (CDKs) inhibitors such as p21/CIP or p27, and the inhibition of pro-mitogenic genes (Kitzmann and Fernandez 2001). MyoD promotes the transcription of both p21/CIP (Guo et al. 1995; Halevy et al. 1995) and the tumor suppressor pRb (Martelli et al. 1994; Magenta et al. 2003). High levels of p21 allows the cells to exit the cell cycle by targeting and inactivating the CDKs, leading to pRb dephosphorylation (Kitzmann and Fernandez 2001). Hypophosphorylated pRb

represses the transcription of E2F dependent genes that are necessary for cell cycle progression. Involved in these pathways are chromatin modifying enzymes such as the catalytic subunits of the SWI/SNF complex (Brg-1 and Brahma (Brm)), class I HDACs, histone methyltransferases (e.g. Suv39h1) and some members of the Polycomb family of transcriptional repressors (Dunaief et al. 1994; Strober et al. 1996; Trouche et al. 1997; Zhang et al. 1999; Strobeck et al. 2000; Nielsen et al. 2001; Ogawa et al. 2002; Ait-Si-Ali et al. 2004). In quiescent and terminal differentiated cells, these protein complexes and their interaction with the pRb-associated repression of certain genes are necessary for cell cycle progression. Acute pRb deletion in either cultured myotubes or myofibers of adult mice does not cause reactivation of DNA synthesis, despite the re-activation of E2F-dependent transcription of genes leading to G1-S phase progression. (Camarda et al. 2004; Huh et al. 2004). Since pRb-interaction with HDACs and lysine methyltransferases is essential for the establishment of the post-mitotic state in myotubes, it is likely that pRb promotes epigenetic modifications at particular loci (e.g., cell cycle genes), such as histone hypoacetylation and methylation, leading to chromatin condensation and formation of heterochromatin, which eventually persist in the absence of pRb.

DNA methylation is also involved in muscle differentiation. DNA methylation is observed in about 70% of the CpGs in the mammalian genome. DNA methylatransferases (Dnmts) add a methyl group into cytosines within the context of CpG dinucleotides. This will inhibit gene expression by recruiting the methyl binding

domain proteins (MBDs) that specifically recognize methylated CpGs (Bird 1992; Hendrich and Bird 1998). A muscle specific Dnmt-1 was found to be only expressed in differentiated myotubes but not myoblasts (Aguirre-Arteta et al. 2000). The association between MBDs, Dnmts and members of the SWI/SNF chromatin remodellers suggests that DNA methylation may induce transcriptional silencing through local ATP-dependent chromatin remodeling (Geiman et al. 2004; Harikrishnan et al. 2005). The treatment with a methyltransferase inhibitor, 5-azacytidine, can convert embryonic fibroblasts to muscle, leading to the cloning of MyoD gene (Taylor and Jones 1982). Additionally, the transfection of fibroblasts with antisense RNA against DNA methylatransferase (Dnmt-1) leads to the same myoblastic conversion (Szyf et al. 1992). CpG islands in general, and the MyoD promoter in particular (Jones et al. 1990), are constitutively free of methylation in all the tissues of the organism (Bird 1986). The distal control element in MyoD enhancer plays a pivotal role in first activating the expression of human and mouse MyoD during embryonic development (Goldhamer et al. 1995; Kablar et al. 1999). The regulatory region in MyoD, the distal control element, is specifically demethylated prior gene activation during somitogenesis in mice (Brunk et al. 1996). However, mutation of the CpGs found to be methylated in vivo did not lead to precocious activation of MyoD regulatory regions in transgenic mice (Brunk et al. 1996). Taken together, these studies support the notion that DNA demethylation is not sufficient for MyoD-dependent gene activation, but may play an important role in controlling

which genes will be activated during myogenic differentiation. In support of this idea, demethylation of Myogenin promoter is found at the onset of C2C12 muscle cells differentiation (Lucarelli et al. 2001). Furthermore, a genome-wide demethylation has been observed during mouse myoblast differentiation (Jost et al. 2001).

Histone acetylation and histone acetyltransferases

Histone acetylation carried out by histone acetyltransferases (HATs) is one of the most well-studied of the histone modifications. HATs enzymatically transfer an acetyl group from acetyl-coenzyme A (acetyl-CoA) to the ε-terminal group of specific and highly conserved lysine residues within the histone N-terminal tail (Sterner and Berger 2000; Roth et al. 2001). Numerous HATs have been found and categorized into super families, as shown in Table 1 (Adapted from (Sterner and Berger 2000; Yang 2004)).

The distribution patterns of histone acetylation within the eukaryotic genome have been shown to be associated with specific gene expression profiles. For example, transcriptional coactivators that display HAT activity, such as general control of amino-acid synthesis 5 (GCN5)/p300/CBP-associated factor (PCAF), p300/CREB-binding protein (CBP), steroid receptor coactivators 1 (SRC1), and TATA box binding protein (TBP)-associated factor, 250kDa (TAFII250), are recruited to specific promoters through their interaction with DNA-bound transcriptional

activators. This interaction provides a mechanism for promoter-specific histone acetylation and subsequent gene activation (Bannister and Kouzarides 1996; Mizzen et al. 1996; Shiama 1997; Brown et al. 2000; Deckert and Struhl 2001; Nagy and Tora 2007).

HAT function during development is strongly supported by studies demonstrating that histone acetylation plays critical roles in chromatin control and gene expression (Roth et al. 2001). To assess HAT function during development, researchers have utilized gene targeting experiments to create null HAT alleles (p300, CBP, PCAF and GCN5) in mice. The results indicate that specific HATs carry out specialized functions required for proper multicellular development (Xu et al. 2000; Roth et al. 2001). To date, extensive developmental analysis of HAT expression profiles is limited. Such studies have reported cell-type specific CBP distribution patterns that co-localize with CBP target gene expression. Additionally, GCN5 and PCAF, which carry out distinct roles during development, are expressed in complementary amounts during development and in adult mouse tissues (Xu et al. 1998; Xu et al. 2000). These studies suggest that HAT function is controlled, at least in part, through the regulation of HAT expression patterns.

Not surprisingly, misregulation of HATs often leads to disorders and diseases. For example, loss of either CBP, p300 or PCAF HAT function has been implicated in the pathogenesis of Huntington's disease and many other polyglutamine neurodegenerative diseases (Steffan et al. 2001). Particularly, TIP60 and its

relationships to certain diseases will be discussed in the next section. Taken together, these studies demonstrate that deciphering the regulation of HAT (e.g., TIP60) function will not only unveil the mystery of epigenetic regulation during development, but also provide some possible ways to cure different human disorders and diseases.

TIP60 and its function

Tat-interactive protein, 60 KDa (TIP60) belongs to the MYST histone acetyltransferase super family (Sterner and Berger 2000). TIP60 was first identified through its interact with the human immunodeficiency virus, type 1-encoded transactivator protein Tat (Kamine et al. 1996). The human TIP60 (hTIP60) gene is located at 11q13.1 and has 14 exons. There are three variants: TIP60 isoform 1, TIP60 isoform 2 (TIP60α) and TIP60 isoform 3 (TIP60β, PLA2 interacting protein, PLIP) each resulting from alternative RNA splicing (Sapountzi et al. 2006). Isoform 1 is a novel protein with translation of intron 1 (Legube and Trouche 2003). Isoform 3 (TIP60β) is generated from the gene translated without the proline-rich exon 5 (Ran and Pereira-Smith 2000) and may be similar to TIP60α (Sheridan et al. 2001). TIP60 isoforms are expressed at relatively low levels in a broad variety of tissues and cells and exhibit cell type specific functions (Hlubek et al., 2001). TIP60α is the best characterized and thus has been referred to as “TIP60” in most of the published data and in the context below. TIP60 has homologs in different organisms and is

evolutionally conserved (McAllister et al. 2002; Zhu et al. 2007).

hTIP60 (TIP60α) encodes a 513 amino acid protein (58 kDa). It contains an N terminal chromodomain and a C-terminal MYST domain as shown in Figure 1. Chromodomains are present in many chromatin regulatory proteins (e.g. PcG, HP1) which are required for interactions between the protein and methylated histone lysines or RNA molecules (Akhtar et al. 2000). The function of the chromodomain in TIP60 still remains unclear, however, it may carry out the protein-protein interaction function as TIP60 consistently found to interact with different protein complexes. Additionally the chromodomain may offer TIP60 a unique characteristic, allowing it to act as repressor for transcription, similar to HP1. Within the MYST domain, there is a conserved catalytic HAT domain (residues 335–404), which has the HAT activity. A Cys-Cys-His-Cys zinc finger is also present in the MYST domain, which is shown to be essential for its HAT activity and is required for protein–protein interactions (Hlubek et al. 2001) (Nordentoft and Jorgensen 2003); (Xiao et al. 2003).

As shown in Table 2, TIP60 has been found in a highly conserved multi-protein complex which will be further discussed below. Although recombinant TIP60 protein is found to directly acetylate free histone substrates H2A (Lys5), H3 (Lys14) and H4 (Lys5, Lys8, Lys12 and Lys16) in vitro via its C-terminal MYST domain (Yamamoto and Horikoshi 1997; Kimura and Horikoshi 1998), the TIP60 complex can acetylate nucleosomal histones H2A and H4 even when linker histones are present (Ikura et al. 2000). Additionally, *Drosophila* Dmel\TIP60 can acetylate the

histone variant phospho-H2Av at Lys5 (Kusch et al. 2004). Cellular TIP60 can also acetylate additional transcription factors, such as the androgen receptor (AR) (Gaughan et al. 2002), upstream binding transcription factor (UBF) (Halkidou et al. 2004), myelocytomatosis oncogene c (c-Myc) (Patel et al. 2004), and the kinase Ataxia Telangiectasia mutated (ATM) (Sun et al. 2005).

HAT catalysis

Structure information of the chatalytic HAT domain has been obtained from yHat1, Gcn5/PCAF, and yeast TIP60 orthologue in *Saccharomyces cerevisiae*, essential Sas family acetyltransferase 1 (yEsa1) (Marmorstein 2001). The central core domain, which includes A-D motif and the loop-β-strand region, mediates acetyl-CoA binding and catalysis. Inside this central core, Glu122 in Tetraymena Gcn5 (tGcn5) (Glu173 in yGcn5) plays the role as a general base for catalysis. Leu126 functions to polarize the carbonyl group of thioestyer prior to nucleophilic attack of the amino group and stabilize the negative charge that develops on the oxygen atom in the tetrahedral transition state. Additionally, there is a water molecule that may shuttle a proton from the reactive Lys of the histone to Glu122 of the enzyme. In the crystal structure, Glu338 in yEsa1 and Glu255 in yHat1 are thought to have the same function. However, little is known how the acetylated lysine product is released from the catalytic core. Several studies indicate how HATs bind to the histone substrate. By

studying tGcn5, it was found that loop-α2 at the N-terminus and loop-α4 at the C-terminus mediate histone substrate binding. For tGcn5, a small G-K-X-P recognition sequence on the histone H3 N-terminal tail is thought to promote HAT–histone interaction. Finally, it is thought that binding of acetyl-CoA by HATs facilitates a HAT-histone interaction. Unfortunately, neither yHat1/acetyl-CoA nor yEsa1/CoA crystal structure has been co-crystallized with an associated histone. So it is unclear if they apply the same histone binding strategy (Marmorstein 2001). Unlike the GNAT family, MYST proteins (e.g. TIP60) may transfer the acetyl group to the substrate Lys via a "ping-pong" mechanism. A Cys residue of the MYST enzyme (Cys369 in *H. sapiens*) forms an intermediate with acetyl-CoA. The deprotonated substrate Lys residue will carry out direct nucleophilic attack of acetyl-CoA to form the acetylated Lys (Yan et al. 2002). As shown in Figure 2, the recent study of yeast piccolo NuA4 (picNuA4) supports the direct-attack (sequential) mechanism in which Glu338 facilitates the nucleophilic attack on acetyl-CoA by deprotonating histone N-ε-lysine residues while the conserved Cys304 is not required for the catalysis as previously proposed (Berndsen et al. 2007).

TIP60 complex

TIP60 forms distinct complexes with different protein partners allowing it to carry out specific functions in a variety of regulatory events (Sapountzi et al. 2006).

As shown in Table 2, TIP60 co-purifies with a number of additional proteins that form a stable nuclear TIP60 HAT complex. Central to this complex is the transformation/transcription domain-associated protein (TRRAP), thought to act as a scaffold protein (Ikura et al. 2000). p400/Domino is an ATPase that is involved in ATPase-dependent chromatin remodeling (Ikura et al. 2000). BAF53 (BRG-1/human BRM-associated factor, 53 KDa) may have histone chaperone activity via an actin-related domain. Yeast BAF53 is responsible for recruiting chromatin modifying complexes to damaged DNA. RuvBL1 and RuvBL2 are putative helicases, which is related to the bacterial DNA repair RuvB protein. Inhibitor of growth 3 (ING3) has a plant homeodomain (PHD), which is also present in other chromatin modifying complexes (Cai et al. 2003; Doyon and Cote 2004). Mortality factor 4 related gene 15 (Mrg15) and mortality factor 4 related gene X (MrgX), as well as glioma amplified sequence 41 (Gas41) are involved in a variety of cellular processes, such as cell proliferation, viability and senescence. The complex also contains additional protein factors: Mrg binding protein (MrgBP) and bromo-protein bromodomain containing protein 8/thyroid receptor coactivator protein 120 kDa (Brd8/TRCp120), DNA methyltransferase associated protein 1 (DMAP1), enhancer of polycomb 1 (EPC1) and EPC1-like, histone variants H2Av and H2B (Doyon and Cote 2004; Kusch et al. 2004).

TIP60 localization

TIP60 resides in both nucleus and cytoplasm where it plays distinct roles in different cellular processes. As a histone modifier, TIP60 has been shown to primarily reside inside the nucleus where it carries out a variety of functions (Yamamoto and Horikoshi 1997; Gavaravarapu and Kamine 2000; Ran and Pereira-Smith 2000; Cao and Sudhof 2001; Sheridan et al. 2001). However, the cellular localization of TIP60 is dynamic. In certain instances, TIP60 can also be found in the cytoplasm. For example, when prostate cancer (CaP) progresses to the hormone resistant state, a shift in TIP60 cellular distribution from a predominantly cytoplasmic to nuclear localization is observed. This occurs together with an elevated TIP60 mRNA and protein expression level, which facilitates its involvement in the transcription of target genes (Halkidou et al. 2003). In other instances, TIP60 is observed in cytoplasm, instead of nucleus. This is found in the interleukin-9 (IL-9) signaling pathway, in which TIP60 interacts with IL-9 receptor (IL-9R) and represses the downstream transcription factor signal transducer and activator of transcription 3 (STAT3) in the cytoplasm by recruiting HDAC7 (Sliva et al. 1999; Xiao et al. 2003). Furthermore, TIP60 and HDAC7 were shown to translocate from the nucleus to the cytoplasm when interacting with the C-terminus of endothelin receptor A (ETA) in response to endothelin 1 (ET-1) (Lee et al. 2001).

The mechanisms of TIP60 translocation remained unclear. The transport of

TIP60 into and out of the nuclear pore complex (NPC) may be mediated by the conserved karyopherin-β family (Pemberton and Paschal 2005). As TIP60 is found to be associated with many different complexes, perhaps certain protein partners in these complexes may also contribute to the shift of its physical distribution inside the cell.

TIP60 cellular function

TIP60 has been reported to play many essential roles in a wide variety of cellular processes based upon the different protein complexes it involves in. With the presence of ATPase and DNA helicase activity through the association with other proteins in different complexes, TIP60 has been shown to be involved in DNA repair and apoptosis (Ikura et al. 2000).

TIP60 can activate gene expressions through several transcriptional activators. For instance, TIP60 was characterized as a nuclear hormone receptor coactivator (Brady et al. 1999), which enhances the transactivation of the androgen receptor (AR) in a ligand-dependent manner and other steroid receptors such as estrogen and progesterone receptors. Moreover, TIP60 was found to up-regulate class I nuclear hormone receptors through the interaction of its LXXLL motif with AR (Gaughan et al. 2001).

TIP60 has also been characterized as a negative regulator of gene expressions. Studies have demonstrated that overexpression of TIP60 completely blocks activation

of cAMP-response element-binding protein (CREB), a transcriptional activator that mediates hormone and growth factor induction of gene expression, by cyclic AMP-dependent protein kinase A (PKA) (Gavaravarapu and Kamine 2000). In-vitro and in-vivo experiments show that TIP60 is associated with CREB through a 55 amino acid C-terminal half of the praline-rich region. Inhibition of CREB activation by TIP60 is found to be independent on its HAT activity. TIP60 also acts as corepressor of the transcriptional repressor zinc finger E box-binding protein (ZEB) and additively inhibits the CD4 enhancer/promoter activity in Jurkat cells (Hlubek et al. 2001). Interestingly, TIP60 function in these examples is cell-specific and the TIP60 HAT domain is not required for carrying out these functions (Hlubek et al. 2001).

TIP60 itself was found to be regulated by Mdm2-mediated ubiquitination and proteasome-dependent degradation (Legube et al. 2002). Moreover, additional studies showed that HAT activity of TIP60 is controlled by phosphorylation of Ser-86 and Ser-90 amino acid residues in vivo, and that cyclin B/Cdc2 is responsible for the phosphorylation on Ser-90 (Lemercier et al. 2003). Consistent with these studies, phosphorylation has also been shown to stimulate HAT activity of the other HATs, such as transcription factor ATF-2 (Kawasaki et al. 2000), and CREB-binding protein (CBP) at the G1/S boundary (Ait-Si-Ali et al. 1998).

It is worthy to note that the yeast TIP60 homolog Esa1 was found to be essential for viability (Clarke et al. 1999), in accordance with my findings in the

Drosophila model setting (Zhu et al. 2007). Below is a summary of TIP60 function in different pathways, as highlighted by its interactions with other molecules and the diseases that it is associated with.

Nuclear hormone receptor and prostate cancer

Prostate cancer (CaP) is a deadly disease, causing one in every four men diagnosed to eventually die. CaP is initially androgen sensitive and responsive to hormone ablation therapy. Despite the high rate of response to hormone treatment, the median duration of response is less than three years. Consequently, nearly all hormone dependent CaPs eventually relapse into fatal hormone independent diseases (hormone refractory, HR).

TIP60 preferentially interacts with and up-regulates the class I nuclear hormone receptor. TIP60 has a single nuclear receptor box at its C terminus and it interacts with the androgen receptor (AR) in a LXXLL motif-dependent manner. (Gaughan et al. 2001). TIP60 can directly acetylate the AR by its factor acetylation (FAT) activity. AR may be regulated by both acetylation via TIP60 and deacetylation via HDAC1, since chromatin immunoprecipitaion (ChIP) demonstrated that AR, TIP60 and HDAC1 are found in a complex on the endogenous AR-responsive PSA promoter (Gaughan et al. 2002). As the disease progresses to hormone resistance, there is an upregulation of TIP60 mRNA and protein expression combined with a

shift in TIP60 cellular distribution from a predominantly cytoplasmic to nuclear localization. This upregulation and redistribution of TIP60 is believed to be involved in the misregulation of downstream target genes (Halkidou et al. 2003).

Amyloid-β precursor protein and Alzheimer's disease

Amyloid-β precursor protein (APP) is a protein ubiquitously expressed on the cell surface. When APP is cleaved by β- and γ-secretase, the amyloid β-peptide (Aβ) is generated which is found to be accumulated in the amyloid plaques in the brains of Alzheimer's disease (AD) patients (Selkoe 2001; Steiner and Haass 2001). Similar to the proteolytic processing of Notch, an intracellular domain of APP (AICD) is generated and is believe to have nuclear signaling function, which also may contribute to the pathogenesis of AD (Suh and Checler 2002). Yeast two-hybrid assays and coimmunoprecipitation experiments demonstrate that a ternary complex of AICD, TIP60 and adaptor protein Fe65 stimulates transcription in a cell culture model (Cao and Sudhof 2001). Stimulation of transcription by this complex is found to require the interaction of Fe65 with both AICD and TIP60 through the following conserved domains and sequences: the WW domain and PTB domains in Fe65 and the NKSY sequence in TIP60 (Cao and Sudhof 2001). AICD may bind to Janus kinase interacting protein-1 (JIP-1) and activate gene expression independent of TIP60 (Scheinfeld et al. 2003). In addition to AICD, TIP60 is also shown to form

complexes with other proteins processed by proteolytic cleavage, such as the lipoprotein receptor related protein (LRP) (Kinoshita et al. 2003) and APP-like proteins (APLP) (Li and Sudhof 2004).

To date, it remains unclear about the molecular mechanisms including APP/TIP60 mediated transcriptional regulation of target genes and their relationships to the pathogenesis of AD. A variety of pathways have been studied to elucidate the role of TIP60 in its association with APP and transcriptional activation. One study demonstrated that Fe65 is only recruited and activated by a membrane-tethered AICD (but not a free AICD). This complex then translocates from the cytoplasm to the nucleus where it binds to TIP60 and other transcriptional factors to turn on gene expression (Cao and Sudhof 2004). Additionally, AICD has been suggested to cause neurotoxicity via misregulation of the downstream transcription events associated with histone acetylation. In support of this premise, introduction of the histone deacetylase (HDAC) inhibitor, sodium butyrate, in neuronal cells enhances the cytotoxicity induced by AICD (Kim et al. 2004). Additional studies support the existence of an AICD/Fe65/TIP60 complex in nucleus. This complex was found to be at the promoter region of the KAI1 gene, a putative target of APP-mediated transcription (Baek et al. 2002). Subsequently, it was shown that activation of transcription of the KAI1 gene requires both the AICD/Fe65/TIP60 complex and the nucleosome assembly protein SET (Telese et al. 2005). Confocal microscopy and co-immunoprecipitaion experiments suggested that the AICD ternary complex

localizes to the nucleus with different morphology depending on the APP adapter proteins (i.e., Fe65 and Jun-interacting protein Jip1γ) and could upregulate several APP-effectors genes, such as APP, BACE, TIP60, GSK3β, and KAI1, but not the Notch-effector gene Hes1 (von Rotz et al. 2004). Alternatively, other studies show that TIP60 associated signal transduction occurs without γ-secretase cleavage of APP and thus full length APP recruits TIP60 via cyclin dependent kinase (CDK)-dependent phosphorylation (Hass and Yankner 2005). In yet another conflicting study using GAL4DB-luciferase reporter system, Fe65 is found to be the key molecule for transcriptional transactivation, whereas TIP60 acted as repressor (Yang et al. 2006). APP/TIP60 mediated transcriptional regulation of the target genes may lead to different effects in the pathogenesis of AD. In support of this, at least in part, TIP60 and its HAT activity are found to be required for AICD-mediated apoptosis (Kinoshita et al. 2002). However, the function of TIP60 in AICD-mediated apoptosis seems to be redundant, since the other ternary complex of AICD, Fe65 and CP2/LSf/LBP1 could upregulate glycogen synthase kinase-3 beta (GSK3β) and also lead to apoptosis in neurons (Kim et al. 2003).

In summary, TIP60 forms the ternary complex with AICD and FE65 and may play an important role in the pathogenesis of AD. However, the targets genes of this complex and their contributions to the pathogenesis of AD need to be further investigated in the future.

DNA damage

ATM

DNA double stand breaks (DSBs) initiate activation of cell cycle checkpoints that arrest the cell cycle to allow additional time for DNA repair (Abraham 2001). The key component in the signaling pathway that responds to DSBs is a 370 KDa protein kinase termed ataxia telangiectasia mutant (ATM) (Bakkenist and Kastan 2003; Bakkenist and Kastan 2004). ATM resides in the cell as an inactive dimmer. Upon autophosphorylation at amino acid residue serine-1981, ATM disassociates to become an active monomer ATM-S1981(P) (Bakkenist and Kastan 2003). ATM-S1981(P) then phosphorylates other proteins, such as nbs1, p53, chk2, SMC1, and histone variant H2AX, to activate cell cycle arrest and DNA repair (Kim et al. 2002; Bakkenist and Kastan 2004).

TIP60 has been shown to be essential for initiating such cellular responses to DSBs, as well as shutting them down by the removal of phosphor-H2Av after DNA damage. Activation of ATM requires both autophosphorylation and acetylation. Sun *et al* found that TIP60 can directly acetylate ATM before its activation in response to DSBs and forms ATM-TIP60 complex through the C-terminal FATC domain of ATM (Sun et al. 2005). The same research group further demonstrated that lysine 3016 (K3016) in the FATC domain of ATM is the acetylation site (Sun et al. 2007).

Additionally, recent studies show that HAT activity of TIP60 is required for ATM/ATR/CHK2 pathway induced by the tumor suppressor $p14^{ARF}$ in G2 cell cycle arrest (Eymin et al. 2006). To end the damage signal, the ATPase subunit p400/Domino of the *Drosophila* Tip60 (Dmel\TIP60) complex catalyzes exchange of the acetylated and phosphorylated histone variant H2Av (*Drosophila* homolog of H2AX) for an unmodified form (Kusch et al. 2004).

Although it remains unclear how TIP60 recognizes and targets the DSBs, the modified histone variants in the DSBs may serve as marks for TIP60 recruitment. One of these marks is phosphorylated histone H2AX. In support of this, the NuA4 complex (yeast homologous TIP60 complex) is recruited to DNA lesions where H2A is phosphorylated by ATM homologue telomere maintenance 1/mitosis entry checkpoint 1 (Tel1/Mec1). The NuA4 complex further recruits other chromatin remodeling complexes INO80 and SWR1 to facilitate the DNA repair (Downs et al. 2004). It is postulated that methylated histones in DSBs may be recognized by TIP60 through its chromodomain (Sun et al. 2005), since 53BP1 (protein 53 binding protein 1) was shown to be recruited to the methylated histones at the DNA damage site (Huyen et al. 2004). Furthermore, the association of TIP60 with DNA damage may be mediated by histone H4 acetylation. For example, the TIP60-TRRAP complex in mammalian cells is required for the DNA damage-induced H4 hyperacetylation (Murr et al. 2006). Similarly, the yeast NuA4 complex is recruited to the vicinity of DNA damage and induces the transient histone H4 hyperacetylation (Tamburini and Tyler

2005).

p53

p53 is the key molecule in controlling DNA damage so that to maintain the genome integrity (Fuster et al. 2007). Without the appropriate DNA damage signaling, murine double minute 2 (MDM2) inactivates p53 by binding directly to its transactivation domain (Momand et al. 1998), as well as ubiquitylation of p53 for proteasome-dependent degradation (Honda et al. 1997). MDM2 itself is a transcriptional target of p53, thus generating a negative feedback to downregulate the increased p53 activity due to the DNA damage signals (Barak et al. 1993; Wu et al. 1993). This feedback regulation is so important that aberrant accumulation of MDM2 is always associated with many human tumors (Momand et al. 1998). Additionally, this feedback loop is regulated by other protein factors. For example, p53 activation by the oncogenic signals is dependent on the induction of $p19^{ARF}$ (acute renal failure, ARF). $p19^{ARF}$ is the production of an alternative transcript of the tumor suppressor gene *INK4A* (inhibitor of cyclin-dependent kinase 4A) which also encodes $p16^{INK4}$. $p19^{ARF}$ blocks the ubiquitin ligase activity of MDM2, thus preventing MDM2-mediated p53 proteolytic degradation (Kamijo et al. 1997; Pomerantz et al. 1998; Zhang et al. 1998).

Numerous evidences have been found that TIP60 is involved in p53-mediated

apoptosis and cell cycle arrest by its association with different proteins inside the pathway. The tumor repressor Yng2 in the yeast NuA4/TIP60 complex is required for p53 function as transcriptional activator (Nourani et al. 2001). By a large RNA interference (RNAi) screening, TIP60 has been identified to be required for $p19^{ARF}$/p53-mediated proliferation arrest and plays a critical role in genotoxic signaling networks (Berns et al. 2004). Furthermore, studies on Tip60+/- mice showed that TIP60 is a haplo-insufficient tumor suppressor, whose expression level is critical for the DNA-damage induced $p19^{ARF}$/p53 pathway (Gorrini et al. 2007).

The mechanisms of TIP60 function in p53 pathway have not been clear until the recent study showed that the lysine 120 (K120) on DNA binding domain of p53 can be acetylated by TIP60 and this posttranscriptional modification is crucial for p53-dependent apoptosis but not cell growth arrest (Sykes et al. 2006; Tang et al. 2006). As Tip60 dependant acetylation does not appear to influence p53 DNA binding affinity in vitro, it is proposed that the K120 acetylation mark serves to recruit an unidentified p53 co-factor required for apoptotic induction (Sykes et al. 2006; Tang et al. 2006). It should be noted that further investigation of the binding affinity of acetylated vs. non-acetylated p53 is necessary, as Tip60 has been shown in some instances in vivo, to enhance p53 binding to some pro-apoptotic target promoters while non-acetylated p53 binds with greater affinity to the cell cycle control p21 gene promoter. Taken together, these finding support a model by which TIP60 plays a central role in p53 mediated cell fate control by first sensing the level of DNA

damage resulting from cellular stress. When DNA damage levels are too high to be repaired, Tip60 acts to acetylate p53 on residue K120. Acetylation of p53 decreases its binding affinity for cell cycle arrest genes and promotes activation of pro-apoptotic genes, thus tipping the balance of p53 target gene activation in favor of apoptosis.

In summary, TIP60 plays an important role in p53-mediated apoptosis and cell cycle arrest, and is suggested as a tumor suppressor (Squatrito et al. 2006). However, some questions on the detailed mechanisms still remain to be answered by further study. For example, MDM2 is responsible for ubiquitination and degradation of both TIP60 and p53 *in vitro* (Legube et al. 2002). However, acetylation of p53 is found to prevent its degradation by MDM2-dependent ubiquitination (Ito et al. 2002).Furthermore, TIP60 HAT activity can antagonize the repressive effect of MDM2 on AR and HDAC1 (Gaughan et al. 2005). It seems that MDM2 and TIP60 antagonize each other, though this may be the different phenomena observed in different model systems (i.e. cell types).

Cell progression and cancer

RB

Retinoblastoma (RB), a nuclear phosphoprotein, can repress genes required for DNA synthesis and arrest cells during the G1 phase of the cycle. RB is inactivated

when it is phosphorylated by cyclin dependent kinases (CDKs) and their cyclin partners. Phosphorylation of RB allows cell progression through G1 to S phase (Sherr and Roberts 1999). RB regulates cell death by inhibiting apoptosis (Tan and Wang 1998; Chau and Wang 2003). RB is also involved in the differentiation of many tissues and cell types, including skeletal myogenesis, neuronal and epithelial differentiation (Lipinski and Jacks 1999). Besides regulation by phosphorylation, RB function can also be controlled by acetylation. It was demonstrated that RB acetylation by p300/CBP and P/CAF is required for the establishment of permanent cell cycle withdrawal and expression of the late myogenic gene (Nguyen et al. 2004).

TIP60 interacts with RB in a different way compared to its interaction with p53. Acetylation of RB by TIP60 results in the degradation of RB. In support of this, the tumor suppressor $p14^{ARF}$ (mouse homolog of $p19^{ARF}$) can prevent RB degradation by inhibiting TIP60-dependent acetylation of RB C-terminus (Leduc et al. 2006). It is not surprising because several proteins are subject to degradation by acetylation (Caron et al. 2005). Additionally, the acetylation of RB is reported to increase MDM2 binding (Nguyen et al. 2004) and MDM2 can promote ubiquitin-dependent RB degradation (Uchida et al. 2005). In summary, TIP60 can acetylate RB, and further initiate the MDM2-dependent ubiquitination of RB, which leads to the degradation of RB.

MYC

The oncoprotein myelomonocytic leukemia (MYC) is a key regulator of numerous genes that affect growth, proliferation, differentiation and apoptosis. In mammals, there are five MYC genes identified so far, including MYC (formerly *c-MYC*), MYCN, MYCL, MYCS, and a poorly-known MYCB encoding only the N terminus protein (Adhikary and Eilers 2005) (Vervoorts et al. 2006). Genetic alteration of three of these MYC genes (i.e., MYC, MYCN, and MYCL) by either translocations or amplifications always leads to many different types of tumors (Nesbit et al. 1999; Boxer and Dang 2001). MYC promoter is targeted by multiple signal transduction cascades. These cascades, such as WNT, RAS/RAF/MAPK, JAK/STAT, transforming growth factor β, and NF-κB pathways, are misregulated in cancers and cause an elevated MYC expression (Clevers 2004; Liu and Levens 2006). MYC protein is within the MYC/MAX/MAD complex of basic region/helix-loop-helix/leucine zipper (bHLHZ) domain transcriptional regulators (Vervoorts et al. 2006). By recruiting and interacting with additional cofactors, MYC can either activate or repress gene expression (Adhikary and Eilers 2005; Oskarsson and Trumpp 2005; Cole and Nikiforov 2006). The amount of MYC proteins is precisely regulated in part by several posttranslational modifications, such as phosphorylation, acetylation, and ubiquitinylation (Vervoorts et al. 2006).

To enhance MYC transactivation efficiency, TIP60 is recruited to

MYC-dependent promoters, as well as directly acetylates MYC. In support of this, TIP60, as well as p300/CBP, and mammalian mGCN5, is recruited by MYC when MYC is targeted to certain promoters (McMahon et al. 2000; Frank et al. 2001; Frank et al. 2003; Adhikary and Eilers 2005). It had been proposed that these HATs regulate MYC targeted gene activation by their ability to acetylate nucleosomal histones within the promoter regions of these genes. Additionally, TIP60 can also acetylate certain lysine residues on MYC (Patel et al. 2004). It is worthy to note that MYC can also be acetylated by p300/CBP (Vervoorts et al. 2003; Faiola et al. 2005; Zhang et al. 2005), and hGCN5/PCAF (Patel et al. 2004). MYC associated protein MAX can also be acetylated by p300/CBP (Faiola et al. 2007). It is postulated that acetylation of MYC may enhance its stability by antagonizing the degredation of MYC by ubiquitination (Vervoorts et al. 2003; Patel et al. 2004; Faiola et al. 2005; Zhang et al. 2005). MYC protein is believed to be stored in two cell compartments: an unstable S1 pool and a stable S2 pool. Acetylation of MYC may switch MYC from the unstable S1 pool to the more stable S2 pool (Patel et al. 2004). Interestingly, TIP60 was found to acetylate MYC in vivo (Patel et al. 2004) but not in vitro (Faiola et al. 2005). This finding is most likely due to the fact that acetylation of MYC by TIP60 requires additional TIP60 associated proteins (Faiola et al. 2005), emphasizing the importance of TIP60 associating proteins in regulating TIP60 HAT function. One possible mediator that links MYC and TIP60 is TIP60 associated transactivation-transformation domain-associated protein (TRRAP; also called

PAF400). TRRAP binds MYC (McMahon et al. 1998; McMahon et al. 2000) and is recruited to MYC-binding sites within chromatin (Bouchard et al. 2001; Frank et al. 2001).

In summary, TIP60 enhances MYC-mediated transactivation by acetylating MYC itself and/or acetylating the histones on the promoter regions of the MYC target genes. However, the detailed mechanisms are still needed to be investigated in the future.

NF-κB

Nuclear factor kappa light chain gene enhancer in B cells (NF-κB) consists of five members of the Rel family, including Rel-A/p65, Rel-B, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). Different NF-κB complexes are composed of their homo- and heterodimers. NF-κB can both enhance and repress the expression of genes that are involved in many distinct pathways, such as immunity, inflammation, proliferation and apoptosis (Perkins 2007). In general, NF-κB complexes are restrained in the cytoplasm by inhibitors of NF-κB (IκBs). After induction by outside signals, IκBs are phosphorylated by the IκB kinase (IKK) complex. The phosphorylation of IκBs leads to their degradation, which releases NF-κB and allows it to translocate to nucleus.

TIP60 is involved in NF-κB signaling pathway and functions as a coactivator

of the NF-κB regulated genes, though the mechanisms are studied in only one gene: the metastasis suppressor gene KAI1. Before stimulation, *KAI1* promoter is repressed by N-CoR (nuclear receptor corepressor)/TAB2 (TAK1 binding protein 2)/HDAC3 complex. In response to IL-1β signaling, phosphorylation of TAB2 results in the exportation of N-CoR/TAB2/HDAC3 complex so that p50-dependent *KAI1* expression is derepressed. In this process, the AICD/Fe65/TIP60 complex binds to the IκB family protein B-cell leukemia/lymphoma 3 (Bcl3) and displaces N-CoR/TAB2/HDAC3 complex, thus enhancing Bcl3/p50-activated transcription of *KAI1* gene (Dechend et al. 1999; Baek et al. 2002). Additionally, overexpression of TIP60, Fe65 and AICD can directly displace N-CoR and activate transcription of *KAI1* without extracellular stimuli (Kim et al. 2005).

E2F

In eukaryotic cells, E2F family transcription factors regulate cell cycle progression (G1/S transition) (Stevaux and Dyson 2002; Trimarchi and Lees 2002; Blais and Dynlacht 2007). E2F is inactive when bound by the RB family of "pocket proteins" (RB, p107, and p130). This inactive complex binds to DNA and represses transcription of E2F target genes during G1. When RB is hyperphosphorylation by CDKs and thus inactivated, E2F is then released from the former complex and activate genes required for DNA synthesis and entry into S. E2F1/3, which generally

act as transactivators, were discovered to bind to activated E2F target gene promoters with the presence of acetylation on histone H3 and H4 (Takahashi et al. 2000). In addition, E2F itself is likely to be acetylated by PCAF/GCN5 and p300/CBP (Ait-Si-Ali et al. 2000) (Martinez-Balbas et al. 2000; Marzio et al. 2000; Lang et al. 2001; Pediconi et al. 2003).

TIP60 is involved in the E2F-associated cell cycle control, though the detailed mechanisms are unclear. The first evidence is that E2F interacts with TRAPP in the TIP60 complex (McMahon et al. 1998; Lang et al. 2001). E2F, binding to the transcriptional factors Sp1 (specific protein 1) and Sp3, can recruit TIP60 to the promoter region of *MYCN* gene (Kramps et al. 2004). Furthermore, E2F is found to recruit TIP60 complex to the target gene regions in late G1 stage despite that the essential role of TIP60 during E2F-dependent transactivation was not successfully addressed by RNAi or DN mutant (Taubert et al. 2004). This is most likely due to the redundant HAT activity associated with PCAF/GCN5 and p300/CBP as stated above. It is worthy to note that Dmel\E2F is necessary for viability. Its mutation caused lethality at the late larval/pupal stage in *Drosohila* (Royzman et al. 1997). Similarly, our data showed that knockout Dmel\TIP60 by RNAi leads to lethality also in the same stages (Zhu et al. 2007). Perhaps this coincidence suggests that TIP60 and E2F are related at least in some degree during *Drosophila* development.

TRRAP

TRRAP has been found in many HAT complexes that have important cellular regulatory functions. These HAT complexes can be categorized into two families: Spt/Ada/Gcn5 acetyltransferase (SAGA)-like HAT complexes and nucleosome acetyltransferase of H4 (NuA4)-like HAT complexes that is associated with TIP60. The TRRAP associated SAGA-like complexes include *S.c.* SAGA complex (Grant et al. 1997; Grant et al. 1998a; Grant et al. 1998b), mammalian SPT3-TAF(II)31-Gcn5L acetyltransferase (STAGA) complex (Martinez et al. 1998; Martinez et al. 2001), PCAF complex (Ogryzko et al. 1998), and TATA-binding protein-free TAF(II) complex (TFTC) complex (Wieczorek et al. 1998; Brand et al. 2001; Cavusoglu et al. 2003). The TRRAP associated NuA4-like complexes include *S.c.* NuA4 complex (Allard et al. 1999; Galarneau et al. 2000; Brown et al. 2001; Nourani et al. 2001; Boudreault et al. 2003) and mammalian TIP60 complex (Ikura et al. 2000; McMahon et al. 2000). Studies have shown that TRRAP is essential for cell viability in both yeast and mouse (Saleh et al. 1998; Herceg et al. 2001). TRRAP regulates gene expression by acetylation of histone H3 or H4 (Herceg et al. 2003), which is correlated with its association with the different HAT complexes: the GCN5 associated SAGA complexes preferentially acetylate histone H3 while the TIP60 associated NuA4 complexes acetylates histone H4 (Carrozza et al. 2003; Robert et al. 2006).

TIP60 is associated with TRAPP and involved in double-strand break (DSB) repair and cell progression control. In support of this, TIP60 and TRRAP are together recruited to the chromatin sites that have DSBs in vivo. The HAT activity of TIP60 opens the chromatin structure to allow repair complex recruitment (Murr et al. 2006). However, this role of TIP60 in DSB repair may be redundant due to the association of TRRAP with MRE11, RAD50, NBS1 complex (MRN complex) in absence of HAT activity (Robert et al. 2006). In addition, the TIP60 complex has been found to colocalize with TRRAP at the Mad1 and Mad2 promoters which corresponds to histone H4 acetylation, which suggests that TIP60 may play an important role in mitotic checkpoint (Li et al. 2004). This idea is supported by the recent finding that the cyclin E-Cdk2 substrate NPAT (nuclear protein, ataxia-telangiectasia locus) recruits TRAPP-TIP60 complex to the promoter regions of histone genes for their transcriptional activation at the G1/S phase transition (Deran et al. 2007). As shown in my unpublished data in Chapter 3, TIP60 is required for the appropriate wing development in *Drosophila*. This function of TIP60 may be associated with TRAPP. In support of this, Nipped-A (*Drosophila* TRRAP) is found to be involved in the wing development by screening the genes that regulate *cut* and Notch pathways, (Rollins et al. 1999). Furthermore, Nipped-A and Domino (another subunit of TIP60) was found to be required for wing development by regulating mastermind and Notch signaling pathway (Gause et al. 2006).

In summary, TIP60 complex with TRAPP is involved in DNA repair and cell

cycle progression. Further investigation of the mechanisms should be carried out to identify the interaction between these two molecules during the wing development in *Drosophila*.

VHL

The 30 KDa VHL (von Hippel-Lindau), which is encoded by the renal cancer gene *VHL*, binds and stabilizes the protein Jade-1. Jade-1 is expressed in kidney and renal proximal tubule cells and related to renal tubular epithelial cell growth, differentiation, apoptosis (Zhou et al. 2002; Zhou et al. 2004). In addition, Jade-1 is involved in anteroposterior axis development during mouse embryogenesis (Tzouanacou et al. 2003). The recent studies support a role for TIP60 in the recruitment by Jade-1 to Jade1 target genes. TIP60 associates with Jade-1 via Jade-1's plant homeodomain (PHD) and acetylates histone H4 lysine residues on the Jade 1 associated target promoters to active gene expression (Panchenko et al. 2004). Although further studies are needed to investigate the detailed mechanisms, this research suggests that TIP60 may be involved in gene regulation in renal caner and/or embryogenesis by its association with Jade 1.

Conclusion

As stated above, TIP60 has been reported to play many essential roles in a wide variety of cellular processes and is a potential specific regulator during multicellular development. To investigate its mechanisms, I have carried out the functional characterization of a *Drosophila* Dmel\TIP60 that is the homolog of the human TIP60. I present evidence that Dmel\TIP60 is differentially expressed throughout *Drosophila* development, with expression levels significantly peaking during embryogenesis. Using RNAi, I show that reducing endogenous Dmel\TIP60 expression in a *Drosophila* embryonic cell line results in cellular defects and lethality. Finally, I confirm this detrimental *in vitro* effect *in vivo* by using an inducible GAL4 targeted RNAi system in *Drosophila*, and demonstrating that early ubiquitous reduction of Dmel\TIP60 expression results in total lethality of the developing flies. Furthermore, the data from cell/tissue specific knockdown of Dmel\TIP60 suggest that Dmel\TIP60 is a specific regulator which is required for the development of certain tissue/cell type, such as wing, central nervous system (CNS), and muscle. The roles Dmel\TIP60 plays in the development of different tissues are diverse. Dmel\TIP60 is required for the wing development through its essential roles in cell growth and differentiation. In CNS, Dmel\TIP60 is required for the maintenance of differentiated neurons, but not for cell differentiation, progression, or apoptosis. Dmel\TIP60 is essential for the appropriate formation of muscle fibers during

embryogenesis. Taken together, TIP60 plays diverse roles in different pathways and is essential for the multicellular development in *Drosophila*.

Chapter2: The cloning and characterization of the histone acetyltransferase human homolog Dmel\TIP60 in *Drosophila melanogaster*: Dmel\TIP60 is essential for multicellular development

Abstract

Chromatin packaging directly influences gene programming as it permits only certain portions of the genome to be activated in any given developmental stage, cell, and tissue-type. Histone acetyltransferases (HATs) are a key class of chromatin regulatory proteins that mediate such developmental chromatin control, however their specific roles during multicellular development remain unclear. Here, we report the first isolation and developmental characterization of a *Drosophila* HAT gene (Dmel\TIP60) that is the homolog of the human HAT gene TIP60. We show that Dmel\TIP60 is differentially expressed during *Drosophila* development, with transcript levels significantly peaking during embryogenesis. We further demonstrate that reducing endogenous Dmel\TIP60 expression in *Drosophila* embryonic cells by RNAi results in cellular defects and lethality. Finally, using a GAL4 targeted RNAi system in *Drosophila*, we show that ubiquitous or mesoderm/muscle specific reduction of Dmel\TIP60 expression results in lethality during fly development. Our results suggest a mechanism for HAT regulation involving developmental control of HAT expression profiles, and show that Dmel\TIP60 is essential for multicellular development. Significantly, our inducible and targeted HAT knockdown system in

Drosophila now provides a powerful tool to effectively study the roles of these chromatin mediators in specific tissues and cell types during development.

Introduction

Metazoans consist of numerous cell types, each carrying out distinct and essential roles that contribute to the growth and survival of an organism (Wolffe and Dimitrov 1993; Vermaak and Wolffe 1998; Orphanides and Reinberg 2002). Differentiation of such specialized cell-lineages is achieved through the establishment and maintenance of tightly controlled gene expression profiles distinct for each cell type (Wolffe and Dimitrov 1993; Orphanides and Reinberg 2002). Such regulation in eukaryotic cells is determined in large part by the differential packaging of genes into chromatin (Wolffe and Dimitrov 1993; Vermaak and Wolffe 1998). The majority of DNA in the eukaryotic nucleus is packaged into nucleosomes, consisting of 146 base pairs of DNA wrapped around a histone octomer core, containing two subunits each of histones H2A, H2B, H3 and H4. Nucleosomes are in turn, further packaged into a highly organized and compact chromatin structure through their association with nucleosomal-linking histone H1 and additional non-histone proteins (Brand and Perrimon 1993; Wolffe and Dimitrov 1993; Fischle et al. 2003). Chromatin compaction generally makes the DNA of genes and their regulatory regions inaccessible to the transcriptional machinery and co-factor protein binding required

for gene activation (Li et al. 2005). As the genome is largely maintained in this repressive chromatin state, chromatin packaging must be disrupted to accommodate protein factor binding and allow for gene activation (Wolffe and Dimitrov 1993; Roth et al. 2001; Orphanides and Reinberg 2002).

Histone modifying enzymes termed histone acetyltransferases (HATs) are directly involved in promoting chromatin decondensation, generally resulting in positive effects on gene activation (Sterner and Berger 2000; Bottomley 2004). HATs enymatically act to catalyze the transfer of an acetyl group from acetyl-CoA to the ε-amino group of specific and conserved positively charged lysine residues within the N-terminal tails of nucleosomal histones. This modification weakens histone–DNA and neighboring nucleosomal contacts to promote chromatin disruption that in turn, facilitates factor binding and transcriptional activation (Sterner and Berger 2000; Roth et al. 2001). A second way that HATs regulate gene activity is through their distinct substrate preference for specific histone, lysine and gene targets, allowing HATs to generate different acetylation patterns within the genome (Strahl and Allis 2000; Berger 2001; Berger 2002; Fischle et al. 2003; Hake et al. 2004). Such distinct HAT-generated histone and lysine acetylation patterns, as well as additional histone modifications, have been postulated by the "histone code hypothesis" to serve as epigenetic marks that control gene expression by providing recognition sites for downstream regulatory factors (Nowak and Corces 2000; Rice and Allis 2001; Fischle et al. 2003; Bottomley 2004). Specific HATs are also capable of generating

specific local or global acetylation patterns (Hebbes et al. 1994; Elefant et al. 2000a; Elefant et al. 2000b; Fernandez et al. 2001; Smith et al. 2001; Ho et al. 2002; Cooke 2004) that influence gene expression profiles. The ability of certain HATs to acetylate non-histone regulatory proteins adds an additional layer of complexity to their many functions (Sterner and Berger 2000). Finally, histone acetylation is a reversible process that is achieved by histone deacetylase enzymes (HDACs), generally resulting in gene silencing (Alland et al. 1997). Thus, histone acetylation directly influences gene programming during development as it permits only certain portions of the genome to be activated in any given developmental stage, cell, or tissue-type (Wolffe and Dimitrov 1993; Patterton and Wolffe 1996). Understanding how these differentially folded chromatin domains are created and maintained in specific cell types is of central importance to the study of biological regulation during development.

Previous reports have shown that *Drosophila* contains a number of human HAT homologs that belong to each of the three major HAT superfamilies: GNAT (Smith et al. 1998), MYST (Grienenberger et al. 2002) and p300/CREB-binding protein (CBP) (Akimaru et al. 1997; Ludlam et al. 2002). Their genetic analysis in *Drosophila* has provided essential information on the role of acetylation in a wide variety of developmental cellular processes. To gain further understanding into the developmental roles of HATs and acetylation during development, we wished to identify and characterize human HAT homologs in *Drosophila* (Dmel\HATs), with the

reasoning that we could use such Dmel\HATs as "tools" to decipher human relevant HAT function in the multicellular *Drosophila* model setting (Chien et al. 2002). We chose to focus our studies on TIP60, as this HAT is representative of the MYST HAT superfamily, and carries out previously described diverse roles essential for cellular function. TIP60 (tat-interactive protein, 60kD) was identified as part of a multimeric protein complex (Allard et al. 1999; Ikura et al. 2000; Doyon and Cote 2004) that regulates its activity in many essential cellular processes including apoptosis (Ludlam et al. 2002; Legube et al. 2004) DNA repair (Ikura et al. 2000; Bird et al. 2002; Morrison and Shen 2005) cell cycle progression (Clarke et al. 1999), developmental cell signaling (Ceol and Horvitz 2004), ribosomal gene transcription (Reid et al. 2000; Halkidou et al. 2004) and histone variant exchange during DNA repair (Kusch et al. 2004). However, despite the importance of TIP60 in many essential cell processes, it has yet to be studied extensively in a multicellular *in vivo* model setting, and thus its developmental, tissue, and cell type specific roles remain to be explored.

Here, we report the first isolation and developmental characterization of a *Drosophila* HAT gene (Dmel\TIP60) that is the homolog of the human HAT gene TIP60. We present evidence that Dmel\TIP60 is differentially expressed throughout *Drosophila* development, with expression levels significantly peaking during embryogenesis. Using RNAi, we show that reducing endogenous Dmel\TIP60 expression in a *Drosophila* embryonic cell line results in cellular defects and lethality. Finally, we confirm this detrimental *in vitro* effect *in vivo* by using an inducible

GAL4 targeted RNAi system in *Drosophila*, and demonstrating that early ubiquitous and mesoderm specific reduction of Dmel\TIP60 expression results in total lethality of the developing flies. Our results suggest a potential mechanism underlying HAT regulation involving developmental control of HAT expression profiles, and demonstrate an essential role for Dmel\TIP60 during multicellular development.

Materials and methods

Identification of D. melanogaster histone acetyltransferases, isolation of cDNA clones and DNA sequencing

BLAST searches were carried out using the BLAST algorithm at both FLYBASE (1999) and NCBI with sequences corresponding to either hTIP60 (NM_182710) or hELP3 (NM_018091). Two *Drosophila* EST clones were identified that displayed high homology to hTIP60 and hELP3. Embryonic EST cDNA clones were identified that matched each of these sequences (clone LD31064 for Dmel\TIP60 and RE35395 for Dmel\ELP3) and these clones were purchased from Invitrogen (Carlsbad, CA). The full ORFs for each Dmel\HAT were amplified by PCR using the following primer sets. For Dmel\TIP60, the forward primer, 5'-CGG C*GA ATT C***GC CAT C**AT GAA AAT TAA CCA CAA ATA TGA G-3' contained a EcoR1 site (italics), a KOZAK sequence (bold), and sequence corresponding to the

first eight codons of Dmel\TIP60. The reverse strand primer, 5'-GGT T*GG ATC C***TC** **ATC** **A**TC ATT TGG AGC GCT TGG ACC AGT C-3' contained a *Bam*HI site (italics), two in frame stop codons (bold), and the last eight codons of Dmel\TIP60. For Dmel\ELP3, the forward primer 5'-GGC T*GA ATT C***GC** **CAT** **C**AT GAA GGC AAA AAA GAA GTT GGG CG-3' contained a *Eco*RI site (italics), a KOZAK sequence (bold), and sequence corresponding to the first twenty-five bp of Dmel\ELP3. The reverse strand primer, 5'-GGC CGG *TCT AGA* **TCA** **TCA** CTA GTT ATT TTC TTC TAT GCT CTT TGA C-3' contained an *Xba*I site (italics), two in frame stop codons (bold), and the last 28 base pairs of Dmel\ELP3. PCR reactions were carried out using Expand™ High Fidelity PCR System (Roche) according to the manufacture's instructions using 400 nM of each forward and reverse primers. The cycling parameters were 30 cycles of 95° for 2 min, 55° for 1 min, and 72° for 3 min, using Mastercycler (Eppendorf). The correct sized PCR amplification products were cloned into the TOPO pCR2.1 vector (Invitrogen) according to the manufacture's instructions. The entire insert DNA sequence for each of these constructs was determined by the University of Pennsylvania DNA Core Sequencing Facility, Philadelphia, PA.

Real-time PCR analysis of staged Drosophila RNA

Total RNA was isolated from staged *Canton S. D. melanogaster* (12-24h embryo, 1st instar larvae, 2nd instar larvae, 3rd instar larvae, pupae, and adult fly) using TRIzol (Invitrogen) and treated twice with DNA-free™ (Ambion) to remove DNA. First strand cDNA was prepared using the SuperScript™ II reverse transcriptase kit (Invitrogen) according to the manufacture's instructions with 1μg total RNA and 15 ng/μL of random hexamer primers (Roche). Primer sets for Dmel\ELP3 (forward primer: 5'-TCC CCA TGC CGC TTG TTA GT-3'; reverse primer: 5'-CCG CCA TTG GCC ACA TAG TC-3') amplified a 190 bp fragment. Primer sets for Dmel\TIP60 (forward: 5'-CAC AGC GCC ACC ATT CCC TA-3'; reverse: 5'-CCA GAT TGT TGC CAT TCA C-3') amplified a 202 bp fragment. All PCR reactions were carried out in triplicate in 20 μl total reaction volumes containing: 0.5 U Taq (Qiagen), 1 μl cDNA (from the RT reaction described above), 250 μM dNTPs (Amersham Pharmacia Biotech), 500 nM for each forward and reverse primer, and 0.25X SYBR® Green I dye (Molecular Probes, Invitrogen). The PCR was carried out in 96 well microtiter plates and the cycling conditions were: 40 cycles at 95° for 45s, 55° for 45s, and 72° for 1 min with plate readings recorded after each cycle. All results were converted to real cDNA quantities by comparison to a standard curve generated with serial dilutions of either Dmel\TIP60 or Dmel\ELP3 cDNA TOPO pCR2.1 clones. All data analysis was performed using Opticon™2 system software,

MJ Research.

RNAi and control Dmel\TIP60 constructs

To create the inverted repeat Dmel\TIP60/RNAi pUAST construct, a 613 bp target RNAi sequence was amplified by PCR using primer sets specific for the Dmel\TIP60 cDNA sequence and the Dmel\TIP60 cDNA TOPO pCR2.1 clone as template. The forward primer 5'-GGA *GAA TTC* GCA CTG GAG TGA CCA CGC CAC AGC GCC-3' contained an *Eco*RI site (italics). The reverse primer 5'-GCA TAA GA*G CGG CCG C***AT CTA** CTG TAC TTC AGG CAG AAC TCG CAG ATG-3' contained a *Not*I site (italics), and a 5 bp polylinker sequence (bold). PCR reactions were performed as described above for Dmel\HAT cloning. The correct size PCR generated fragment was cloned in the sense direction into *Eco*RI/*Not*I sites in the pUAST vector under the control of the UAS promoter. This construct was designated Dmel\TIP60/pUAST.1. The same target fragment described above was next PCR amplified using the Dmel\TIP60 cDNA TOPO pCR2.1 clone as template. The forward primer 5'-GGA *TCT AGA* GCA CTG GAG TGA CCA CGC CAC AGC GCC-3' contained an *Xba*I site (italics) and the reverse primer 5'-GCA TAA GA*G CGG CCG C*CT GTA CTT CAG GCA GAA CTC GCA GAT G-3' contained a *Not*I site (italic). The PCR generated fragment was cloned in an anti-sense orientiation into *Not*I and *Xba*I sites of the Dmel\TIP60/pUAST.1, thereby creating the inverted repeat

Dmel\TIP60/RNAi/pUAST construct. To create the sense-sense Dmel\TIP60/control construct, the same target RNAi sequence was PCR amplified with the following primers: the forward primer 5'-GCA TAA GA*G CGG CCG C*GC ACT GGA GTG ACC ACG CCA CAG CGC C-3' contained a *Not*I site (italics) and the reverse primer 5'-GCA *TCT AGA* CTG TAC TTC AGG CAG AAC TCG CAG ATG-3' contained a *Xba*I site (italics). The PCR generated fragment was cloned in a sense orientiation into the *Not*I and *Xba*I sites of Dmel\TIP60/pUAST.1, creating a sense-sense Dmel\TIP60/control/pUAST construct. The PCR generated polylinker and the common *Not*I restriction site that joined the two target Dmel\TIP60 repeat fragments served as the "hinge" region of the hairpin in both Dmel\TIP60/RNAi/pUAST and Dmel\TIP60/control/pUAST constructs. All cloning was carried out using standard procedures except that SURE 2 competent bacterial cells (Stratagene) were used for all bacterial transformations to prevent recombination from occurring.

Dmel\TIP60/RNAi and control constructs for transient cell transfection were created by digesting the Dmel\TIP60/RNAi/pUAST and Dmel\TIP60/control/pUAST constructs with *Eco*RI and *Xba*I restriction enzymes, gel-purifying (Qiagen) the released fragments, and sub-cloning each fragment into *Eco*RI and *Xba*I restriction sites within the pAc5.1/V5-HisA vector (Invitrogen). These constructs were designated Dmel\TIP60/RNAi/pAc5.1 and Dmel\TIP60/control/pAc5.1.

Cell culture and transfection

D. Mel-2 Cells (Gibco, Invitrogen) were grown in *Drosophila*-SFM media (Invitrogen) supplemented with 90 mL/L of 200 mM L-Glutamine (Gibco, Invitrogen). The cells were grown in a 28°, non-humidified, ambient air-regulated incubator (Torrey Pines Scientific), and subcultured every three to four days to maintain exponential growth. On day three post-subculture, the cells were seeded to 50-60% confluence into 35 mm plates in 2.0 ml *Drosophila*-SFM with L-Glutamine. After an overnight incubation at 28°, the cells were incubated with the transfection mixture containing 2 μg plasmid DNA, 8 μL Cellfectin (Invitrogen) and 500 μl *Drosophila*-SFM without L-Glutamine for 3 hours. After removal of transfection mixture, and addition of 2 mL of *Drosophila*-SFM with L-Glutamine, each plate was incubated at 28° and observed after 24, 48, and 72 hours. As a transfection efficiency control, separate plates of cells were transfected with pAC5.1/V5-His/lacZ (Invitrogen), cells were stained using the β-Gal staining kit (Invitrogen) according to the manufacture's instructions and blue cells were counted to determine the transfection efficiency. All transient transfections were performed in triplicate.

Semi-quantitative RT-PCR

Total RNA from either a plate of transfected cells or three third instar larvae progeny from a homozygous Dmel\TIP60/RNAi or control x GAL4 337 cross was isolated using TRIzol (Invitrogen) and twice treated with DNA-free™ (Ambion) to remove DNA. First strand cDNA was prepared using the SuperScript™ II reverse transcriptase kit (Invitrogen) according to the manufacture's instructions with 1μg total RNA and 15 ng/μL of random hexamer primers (Roche). PCR reactions were performed in a 40 ul total volume containing 1 U Taq (Qiagen), 1 μl cDNA template, 250 μM dNTPs (Amersham Pharmacia Biotech) and 500 nM of each forward and reverse primer. The cycling conditions were 36 cycles of 95° for 45s, 55° for 45s, and 72° for 1 min. The forward primer (5'-TGG TAT TTC TCA CCC TAT CC-3') and the reverse primer (5'-CAA TGA GCA GCT TGC CGT AG-3') amplified a 427 bp fragment that corresponded to position 1407 to 1833 within the cDNA Dmel\TIP60 sequence.

Creation of P-element-transformed fly lines

P-element germ-line transformations with pUAST constructs were performed as previously described (Elefant and Palter 1999), to create fly lines containing Dmel\TIP60/RNAi or Dmel\TIP60/control pUAST constructs. To determine on which

chromosome the P-element inserted, lines heterozygous for the *TM3* and *TM6* balancer were mated to w^{1118} flies, and segregation of the w^{+} marker was scored: if segregation of w^{+} was neither with the third chromosome balancer or a sex chromosome, it was inferred to segregate with the second chromosome. Balancer chromosomes were subsequently crossed away by successive mating to w^{1118}. Multiple, independent fly lines were created for each construct as the level of gene expression is dependent upon the chromosomal location of the P-element, which occurs randomly.

Drosophila stocks and RNAi crosses

Flies used in this study were as follows: *P{pUAST}/P{pUAST}* flies containing either Dmel\TIP60/RNAi or control constructs were created as described above, y^{1} w^{*}*; P{Act5C-GAL4}25FO1/CyO,* (donated by Bloomington stock center, stock# 4414; Y. Hiromi), *w*;P{GawB}how*^{24B} (Brand and Perrimon 1993), and GAL4 line 337 (Elefant and Palter 1999). All crosses were performed using three males and three newly eclosed virgin females in narrow plastic vials (Applied Scientific) with yeasted *Drosophila* media (Jazz-Mix, Fisher Scientific) at 25 º.

Results

Identification and characterization of two Drosophila HAT (Dmel\HAT) genes that are homologous to human HAT genes TIP60 and ELP3

We first wished to identify the human HAT homolog of MYST family member TIP60. Additionally, we also set out to identify the human HAT homolog of GNAT family member ELP3 in *Drosophila* so that we could compare the developmental expression profiles of two different HAT family members. Conserved sequences within the human TIP60 (hTIP60) and ELP3 (hELP3) genes were used to query the *Drosophila* Genome database for genomic DNA encoding homologous sequences. A single genomic clone mapping to band 4A6-B1 on the X chromosome showed significant homology to hTIP60 while a single genomic clone mapping to band 24F2 on the 2L chromosome demonstrated significant homology to hELP3. Sequences corresponding to these regions were used to conduct a BLAST search of the *Drosophila* expressed sequence tag (EST) library at Flybase and cDNA sequences were identified that diplayed high homology to the hTIP60 sequence (listed as CG6121) and hELP3 sequence (listed as CG15433). Embryonic EST cDNA clones were identified for each Dmel\HAT (clone LD31064 for Dmel\TIP60 and RE35395 for Dmel\ELP3) and these clones were purchased and sequenced. The full sequence was determined for the open reading frame (ORF) of each cDNA Dmel\HAT clone, designated Dmel\TIP60 and Dmel\ELP3, and aligned to its respective cDNA

sequence identified in Flybase, confirming a full ORF and correct sequence identity for each Dmel\HAT construct.

Analysis of the conceptual translation products for both Dmel\TIP60 and Dmel\ELP3 provided evidence that these *Drosophila* genes are homologs of human HATs TIP60 and ELP3. First, alignments between each Dmel\HAT and its human HAT counterpart demonstrated significant homology over their entire coding sequences: Dmel\TIP60 is 58% identical/67% similar and Dmel\Elp3 is 82% identical/91% similar (Figure 1 A and B; Figure 2 A and B). Additionally, the Dmel\TIP60 transcript was found to contain an open reading frame of 1,626 bp, encoding a protein of 541 a.a. with a predicted molecular mass of 61.2 kD, in good agreement with the apparent molecular mass of human TIP60 (Ikura et al. 2000). The ELP3 transcript contained an ORF of 1,659 bp, producing a protein of 552 a.a. with a predicted molecular mass of 62.8 kD, shown to be the approximate molecular mass for the human Elp3 protein (Hawkes et al. 2002). Finally, structural protein data obtained using the conserved domain architecture retrieval tool (CDART) at NCBI revealed that the predicted protein domains specific for Dmel\TIP60 and Dmel\Elp3 and their location within each Dmel\HAT protein are highly conserved between human and Dmel\HAT counterparts (Figure 1 A and B; Figure 2 A and B). Both *Drosophila* and human MYST family member TIP60 contain an N-terminal chromodomain and a C-terminal MYST domain, while both *Drosophila* and human GNAT family member Elp3 contain an N-terminal putative histone demethylation

domain and a C-terminal HAT domain. As expected, each of these conserved domains showed significant homology to one another: for dTIP60 the chromodomain is 70% identical/87% similar and the MYST domain is 80% identical/89% similar, and for Dmel\Elp3 the HAT domain is 85% identical/93% similar while the putative histone demethylase domain is 88% identical/94% similar to their human homolog counterparts. Protein sequence analysis of a number of Dmel\TIP60 and Dmel\Elp3 homologs in a variety of different species in addition to humans, including *Mus musculus*, *Danio rerio*, *Caenorhabditis elegans*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae* demonstrated that such HAT conservation for both Dmel\TIP60 and Dmel\Elp3 is evolutionarily well conserved (Figure 2 A and B). The significant sequence and structural similarity between each Dmel\HAT with its human HAT counterpart strongly indicates that these newly isolated *Drosophila* genes are homologs of human TIP60 and ELP3.

Dmel\TIP60 and Dmel\ELP3 are differentially expressed during Drosophila development

The mechanism underlying the regulation of HAT activity remains unclear. Although detailed analysis of HAT expression throughout development is limited, studies analyzing HAT expression profiles suggest that a number of HATs including HBO1, TIP60, CBP, P/CAF and GCN5 are controlled, at least in part, through their differential regulation in certain tissues (Xu et al. 1998; Iizuka and Stillman 1999;

Stromberg et al. 1999; Xu et al. 2000; Lough 2002; McAllister et al. 2002). To determine whether different families of HATs might also be regulated throughout development, we examined the expression profiles of MYST family member Dmel\TIP60 and GNAT family member Dmel\ELP3 genes in all stages of *Drosophila* development using a real-time RT-PCR assay. RNA was isolated from staged *Drosophila melanogaster* (12-24 h staged embryos, first, second and third instar larvae, pupae, adult flies) and DNaseI treated. cDNAs were generated from equal amounts of RNA for each developmental stage by RT priming with random hexamers. The RT products were then amplified in a real-time PCR assay using primer pairs corresponding to a region specific for each Dmel\HAT and expression levels were displayed in absolute values. We found that transcript levels of both HATs significantly peaked in the embryo, sharply decreased to almost undetectable levels by the second instar larvae stage, then gradually increased as development proceeded, reaching a second, albeit lower, peak of expression in the adult fly (Figure 3). Interestingly, although exact levels of Dmel\TIP60 and Dmel\ELP3 expression differed at each *Drosophila* stage tested, the trend of these levels throughout development was similar for both HATs. These data demonstrate that Dmel\TIP60 and Dmel\ELP3 are each differentially expressed throughout *Drosophila* development.

Plasmid mediated Dmel\TIP60 dsRNA production in a Drosophila embryonic cell line reduces cell viability and Dmel\TIP60 mRNA levels

We found that levels of Dmel\TIP60 and Dmel\ELP3 expression dramatically peaked in the *Drosophila* embryo, supporting an important role for these Dmel\HATs during embryogenesis. Therefore, we wished to decipher their function during early development. As no characterized Dmel\TIP60 and Dmel\ELP3 mutant alleles exist to date, we chose to silence specific endogenous HAT expression in a variety of tissues, cell types, and stages of development of choice by using an inducible GAL4 targeted RNAi based system in *Drosophila*. In this RNAi/GAL4 system, expression of an inverted repeat transgene of choice triggers double-stranded RNA mediated postranscriptional gene silencing (Fortier and Belote 2000; Kennerdell and Carthew 2000). This method is used in conjunction with the targeted GAL4/UAS binary system (Brand and Perrimon 1993) to control expression of the inverted repeat transgene in both a developmental and cell type restricted fashion.

We chose to initially focus our studies on TIP60, as this HAT has been previously reported to play wide range of biological roles essential for numerous cellular processes (Clarke et al. 1999; Ikura et al. 2000; Reid et al. 2000; Bird et al. 2002; Ceol and Horvitz 2004; Halkidou et al. 2004; Kusch et al. 2004; Legube et al. 2004). To create the Dmel\TIP60/RNAi construct, we selected a 613 bp RNAi non-conserved target sequence specific for Dmel\TIP60 (Figure 4 A). BLAST searches using this sequence ensured non-redundancy within the genome. The chosen

Dmel\TIP60 cDNA fragment was cloned into the inducible expression vector (pUAST) under the control of GAL4-UAS binding sites in a sense-antisense inverted gene arrangement predicted to form a double-stranded RNA hairpin that would induce an RNAi response. This plasmid was designated the Dmel\TIP60/RNAi construct (Figure 4B). A control construct was created in which the same RNAi target sequences were cloned into a sense-sense orientation so that it would not induce RNAi. This plasmid was designated the Dmel\TIP60/control construct (Figure 4C). Both the sense-antisense and sense-sense sequences in each of the constructs were separated by a short polylinker that served as the "hinge" region of the hairpin arrangement.

To initially test whether our Dmel\TIP60/RNAi construct would potently down-regulate endogenous Dmel\TIP60 expression and result in phenotypic defects, we utilized the *Drosophila* embryonic D.mel-2 cell culture based system (Figure 5 A and B). The Dmel\TIP60/RNAi sense-antisense repeat and Dmel\TIP60/control sense-sense sequences were each subcloned into the pAc5.1/HisA vector under the control of an active actin promoter. Both the Dmel\TIP60/RNAi and control constructs were each transiently transfected into D.mel-2 cells and visualized using phase/contrast optics 24 hours post transfection. We observed morphological defects in cells transfected with the Dmel\TIP60/RNAi construct. These cells were found to grow poorly, suffering approximately 50-70% lethality 24 hours post-transfection (Figure 5D). Additionally, Dmel\TIP60/RNAi induction appeared to disrupt mitotic

cell cycle progression, as those cells that did survive were larger than the wild-type and control cells and appeared to be arrested during cytokinesis. None of these defects were observed in cells transfected with the Dmel\TIP60/control construct (Figure 5C). These results demonstrate that Dmel\TIP60 /RNAi production in a *Drosophila* embryonic cell line results in cellular defects and lethality, supporting an essential role for Dmel\TIP60 in early development.

To determine whether the Dmel\TIP60/RNAi construct was down-regulating endogenous Dmel\TIP60, RNA was isolated from cells transfected with either the Dmel\TIP60/RNAi or Dmel\TIP60/control construct 24 hours post-transfection, and DNaseI treated. Interestingly, RNA isolated from cell plates transfected with the Dmel\TIP60/RNAi construct was found to be consistently and significantly lower in concentration than RNA isolated from cells transfected with the Dmel\TIP60/control construct (data not shown). This result is likely due to cell lethality occurring in the Dmel\TIP60/RNAi test cell lines (Figure 5D). cDNAs were generated from equal amounts of RNA for each transfection sample by RT priming with random hexamers. The RT products were amplified in a semi-quantitative RT-PCR assay using primer pairs specific for each Dmel\TIP60 that did not amplify dsRNA species. The gene for the RP49 ribosomal protein was also amplified from each sample and served as an internal control. Our results revealed that endogenous Dmel\TIP60 is reduced in RNAi samples when compared to control samples, whereas RP49 expression remained unaffected. These observations indicated that our Dmel\TIP60/RNAi

construct is effectively and specifically inhibiting endogenous Dmel\TIP60 RNA production.

Dmel\TIP60 is essential for Drosophila development

To confirm and further explore our finding that Dmel\TIP60 is required for cell viability, we used a GAL4 targeted RNAi knockdown system to induce silencing of endogenous Dmel\TIP60 expression in the *Drosophila* multicellular model setting. Flies were transformed with our Dmel\TIP60/RNAi and control GAL4 inducible pUAST constructs, and three independently derived transgenic fly lines with insertions for each of the constructs were chosen for use. The insertions were homozygous viable, and did not cause any observable mutant phenotypes in the absence of GAL4 induction.

Based on our previous findings that the actin promoter (*Act5C*) induced potent Dmel\TIP60 RNAi knockdown in the *Drosophila* cell culture line, we chose to induce Dmel\TIP60/RNAi and control transgene expression in the fly using the *Act5c-Gal4* driver strain (Bloomington stock 4414), as this actin driver expresses robust levels of GAL4 constitutively and ubiquitously early in embryogenesis (Chavous et al. 2001; Rollins et al. 2004). We found that when the *Act5c-Gal4* driver was used to induce transgene expression at 25^{o}, each of the three Dmel\TIP60/RNAi insertion lines reduced survival to 0% that of all three Dmel\TIP60/control insertion lines (Table 1). In

each case, lethality for the majority of flies occurred during pupal development, which was the latest stage that flies were able to survive. Those flies that did survive until this stage showed essentially wild-type development. As an internal control, *Act5c* flies are hemizygous for the GAL4 driver over a *CyO* balancer chromosome (*P{Act5c-Gal4}y/CyO* y^+) and thus approximately 50% of flies are expected to eclose due to no GAL4 production in half of the progeny in any given cross. Thus, to determine whether a significant percentage of flies died earlier than the pupal stage, the total number of dead, non-eclosed GAL4$^+$ (*y;Cy*$^+$) pupae was compared to the total number of non-RNAi induced GAL4$^-$ (*y*$^+$*;Cy*) flies that eclosed over a ten day period. We found that although no Dmel\TIP60 RNAi induced GAL4$^+$ (*y;Cy*$^+$) flies were found to eclose, the number of dead pupae was significantly lower than the number of viable GAL4$^-$ (*y*$^+$*;Cy)* flies for one of the Dmel\TIP60/RNAi insertion lines tested. An analysis of the number of such "missing" dead pupae to the total number of eclosed GAL4$^-$ (*y*$^+$*;Cy)* flies demonstrated that for Dmel\TIP60/RNAi/A, 24% of the Dmel\TIP60/RNAi induced flies must have died sometime earlier than pupal development (data not shown). The variation in lethality observed between fly lines is likely due to position effects on transgene expression. Our results demonstrate that early and ubiquitous induction of Dmel\TIP60/RNAi in the fly using an actin specific GAL4 driver results in total lethality for each of the three Dmel\TIP60/RNAi insertions tested, supporting an essential role for Dmel\TIP60 in multicellular development and

the feasibility of our inducible GAL4 targeted HAT/RNAi knockdown system in *Drosophila*.

We next wished to determine whether GAL4 induced expression of Dmel\TIP60/RNAi reduced endogenous Dmel\TIP60 transcripts. Because *Act5c* flies are hemizygous for the GAL4 driver, only 50% of the progeny in any given cross will induce the Dmel\TIP60/RNAi transgene, making analysis of endogenous Dmel\TIP60 down-regulation using this GAL4 driver problematic. We therefore chose to induce Dmel\TIP60/RNAi and control transgenes using the ubiquitous homozygous GAL4 driver 337 (Elefant and Palter 1999). Progeny resulting from a cross between three independently derived homozygous Dmel\TIP60/RNAi or Dmel\TIP60/control fly lines and GAL4 line 337 were allowed to develop to the third instar larval stage, before lethality in the pupal stage was shown to occur (data not shown). RNA was isolated from three third instar larvae from each of the above crosses and DNaseI treated. cDNAs were prepared from equal amounts of each RNA sample by RT priming with random hexamers. The RT products were amplified in a semi-quantitative RT-PCR assay using primer pairs specific for Dmel\TIP60 that did not amplify dsRNA species. The gene for the RP49 ribosomal protein was also amplified from each sample to serve as an internal control. Our results revealed that endogenous Dmel\TIP60 transcript levels were significantly reduced in RNAi samples from each of the three independently derived Dmel\TIP60/RNAi fly lines when compared to samples obtained from each of the three independently derived

Dmel\TIP60/control fly lines (Figure 6). These observations demonstrate that GAL4 induced Dmel\TIP60/RNAi expression is robustly inhibiting endogenous Dmel\TIP60 RNA production.

Targeted expression of Dmel\TIP60/RNAi in the mesoderm and muscle cells of Drosophila results in lethal muscle mutant phenotypes

To further test the specificity of our newly developed GAL4 targeted Dmel\TIP60/RNAi knockdown system, we wished to determine whether targeting Dmel\TIP60/TNAi knockdown to specific tissues would result in phenotypes that were distinctive for a given particular tissue type. As our *in situ* analysis of Dmel\TIP60 transcripts demonstrated that Dmel\TIP60 is expressed in the muscle cells during embryogenesis (our unpublished results, data not shown; similar results in BDGP), we chose to induce Dmel\TIP60/RNAi and control transgene expression in the fly using the GAL4 line 24B (*P{GawB}how*^{24B}), as this driver produces high levels of GAL4 specifically in the presumptive mesoderm and muscle cells during early embryogenesis (Brand and Perrimon 1993). Three independent fly lines containing either Dmel\TIP60/RNAi or control transgenes were crossed to the mesoderm/muscle GAL4 line 24B at 25^{o} and the resulting phenotypes were assessed. We found that all three fly lines expressing the Dmel\TIP60 control transgene showed normal development and no observable defective phenotypes when their expression was targeted to the mesoderm/muscle cells, similar to our results for the actin specific

Act-5c and the ubiquitous 337 GAL4 drivers,. However, when expression of the Dmel\TIP60/RNAi transgene was induced in the mesoderm/muscle cells, we observed a reduction in viability to 0, 40 and 29% (for lines Dmel\TIP60/RNAi/ A, B and C, respectively) that of the Dmel\TIP60 control lines (Table 2). Significantly, the lethal phenotypes we observed were different from that of the *Act-5c* and 337 GAL4 driver lines in that depending on the insertion line tested, the flies died at a broad range of developmental stages, beginning from early pupae to directly before fly eclosion. Importantly, the dying flies resembled those of known muscle mutants (Fyrberg et al. 1994) in that the apparent cause of lethality later in development was due to their inability to eclose from their pupal casings (data not shown). The variation in developmental lethality that we observed for different insertion lines is likely caused by position effects on transgene expression, with higher levels of Dmel\TIP60/RNAi transgene expression resulting in lethality earlier in development. Notably, fly insertion line Dmel\TIP60/RNAi/A consistently resulted in the earliest developmental lethality of all three Dmel\TIP60/RNAi insertion lines when tested with the actin *Act-5c,* ubiquitous 337 and mesoderm/muscle 24B GAL4 drivers, indicating that this is the strongest expresser of our three independent Dmel\TIP60/RNAi fly lines (Tables 2). These results demonstrate the feasibility of targeting different levels of Dmel\TIP60 knockdown specifically to certain cells and tissue types, and also suggest that Dmel\TIP60 is essential for proper muscle formation in the developing fly.

Discussion

The importance of histone acetylation in chromatin control and gene regulation supports a critical role for HAT function in promoting the rapidly changing gene expression profiles that drive developmental processes (Roth et al. 2001). However, the specialized roles of certain HATs in a multicellular developmental setting remains to be explored. Thus, we set out to identify human HAT family homologs in *Drosophila* (Dmel\HATs) in order to elucidate their human relevant developmental functions in the multicellular *Drosophila* model setting. Using homology searches of the *Drosophila* genome, we identified human homologs of MYST family member TIP60 (Dmel\TIP60) and GNAT family member ELP3 (Dmel\ELP3). Our isolation and characterization of the cDNA clones encoding these genes demonstrated high conservation to their human counterparts in terms of both their amino acid sequence identity and location of conserved protein domains. Importantly, while this work was in progress, Kusch et al. (Kusch et al. 2004) purified the dTIP60 multiprotein complex from *Drosophila* embryonic S2 cells and demonstrated by mass spectrometer and sequence analysis that this complex is structurally homologous to its human counterpart and that the dTIP60 protein component is encoded by the Dmel\TIP60 gene we report here, supporting our conclusion that Dmel\TIP60 is the *Drosophila* homolog of human TIP60.

Our analysis of Dmel\TIP60 and Dmel\ELP3 expression levels using real-time PCR demonstrated that both Dmel\HATs are differentially expressed throughout *Drosophila* development. These results suggest that in addition to being regulated by specific protein partners (Marmorstein and Roth 2001), HAT activity may also be controlled, at least in part, by their developmental regulation. In support of this idea is the observation that mice heterozygous for null alleles for each of the p300, CBP and GCN5 HATs show less severe developmental defects than do homozygous null alleles, demonstrating that the overall dosage of HATs is critical for developmental processes (Xu et al. 2000; Roth et al. 2001). We also observed that both Dmel\TIP60 and Dmel\ELP3 expression peaked in the embryo, consistent with studies demonstrating the importance of chromatin control in early development (Patterton and Wolffe 1996). Importantly, high levels of embryonic expression is not the case for all HATs as shown by studies demonstrating that GCN5 is expressed at high levels in the mouse embryo whereas expression levels of the HAT P/CAF are virtually undetectable (Xu et al. 1998). This data, in conjunction with the HAT expression data reported here, suggest that only certain HATs may be essential for embryogenesis to proceed.

Although research on HATs in multicellular systems is still limited to date, knockout studies of p300, CBP (Tanaka et al. 1997; Roth et al. 2003) and GCN5 (Xu et al. 2000) in mice and CBP (Akimaru et al. 1997), HBO1 (Grienenberger et al. 2002) and MOF (Smith et al. 2001) in *Drosophila* have revealed essential roles for these

HATs during development. Significantly, the phenotypic defects that arise from such different HAT knockouts are not identical. GCN5 is essential for mouse development and formation of several mesoderm tissues while PCAF is dispensable (Xu et al. 2000) and differential roles for CBP and p300 in heart, lung, small intestine (Shikama et al. 2003) and muscle development (Roth et al. 2003) have been reported. Taken together, these studies indicate that HATs carry out specific functions required for proper multicellular development (Roth et al. 2001). Here, we show that reducing endogenous Dmel\TIP60 expression by RNAi in either all tissues or specifically in the mesoderm/muscles of the developing fly results in lethality. Our results extend prior HAT knockout studies and add Dmel\TIP60 to the growing list of HATs that carry out potentially specialized roles essential for multicellular development.

Prior studies on the yeast TIP60 homolog, ESA1, demonstrated that temperature-sensitive yeast *esa1* mutant cells were found to be arrested during cell division with a G2/M stage DNA content and partially depleted acetylated H4 levels, thereby linking Esa1 HAT function to cell cycle control via potential transcriptional regulatory events (Clarke et al. 1999). Consistent with these results, we observed that Dmel\TIP60 depletion in the *Drosophila* D.Mel-2 cell culture line resulted in a lethal phenotype reminiscent of mitotic cell cycle progression defects. Cells that did survive were larger than wild-type and control cells and appeared unable to complete cytokinesis, supporting a role for Dmel\TIP60 in metazoan embryonic cell division. We also found that either ubiquitous or mesoderm/muscle specific depletion of

Dmel\TIP60 in our GAL4 inducible HAT knockdown system resulted in lethality for all three independent Dmel\TIP60/RNAi insertion fly lines tested, with the majority of flies dying during early pupal development. Thus, as development proceeds, depletion of Dmel\TIP60 may result in the disruption of cell processes shown to require Dmel\TIP60 such as apoptosis (Ikura et al. 2000; Legube et al. 2004), DNA repair (Bird et al. 2002) , and cell cycle progression (Clarke et al. 1999), culminating in lethality caused by an accumulation of cell defects that accrue over time, all possibilities that we are currently exploring.

HATs execute acetylation profiles required for target gene regulation and thus their misregulation is linked to numerous types of cancers and developmental defects (Petrij et al. 1995; Mahlknecht et al. 2000; Steffan et al. 2001; Roelfsema et al. 2005; Close et al. 2006). The importance of TIP60 is underscored by studies demonstrating its involvement in both normal cellular processes, and abnormal ones, resulting in oncogenesis and developmental disorders. For example, overproduction of TIP60 in the nucleus of prostate cells is associated with androgen–resistant prostate cancer (Halkidou et al. 2003; Sapountzi et al. 2006). TIP60 is also associated with numerous disease related proteins including the c-MYC oncoprotein (Frank et al. 2003; Patel et al. 2004) proteins involved in hematological malignancies (Chambers et al. 2003; Nordentoft and Jorgensen 2003) and Alzheimer's associated ameloid precursor protein (APP-CT) (Baek et al. 2002; Kim et al. 2004). Interestingly, overproduction of APP-CT induces an increase in histone acetylation that significantly enhances

neurotoxicity, implicating TIP60 HAT mistargeting in Alzheimer's disease (Kim et al. 2004). Our isolation and characterization of Dmel\TIP60, in conjunction with our newly developed inducible and targeted HAT knockdown system in *Drosophila,* will now allow us to effectively study the roles of these and other chromatin regulators in both multicellular development and epigenetic-based disorders.

Chapter 3: Dmel\TIP60 is required for the differentiation of a variety of specific cell and tissue types during *Drosophila* development

Abstract

Combinatorial histone modifications control chromatin packaging which in turn, contributes to the precise patterning of gene expression during development. The histone acetyltransferase TIP60 is a histone modifying enzyme that plays essential roles in a wide variety of chromatin mediated cellular processes including gene control, DNA repair and cell cycle control, however little is known about its roles during multicellular development. Here, we use our *Drosophila* GAL4 inducible Dmel\TIP60 knockdown/overexpression system to explore the role of Dmel\TIP60 in a wide variety of specific tissues during *Drosophila* development. We show that loss of Dmel\TIP60 in the wing leads to a range of wing abnormalities, including the formation of wing blisters in the most severe cases. Wing surface area and cell count/hair density assays reveal that although the number of cells that compose the wing remain unaffected, their size is significantly smaller than normal and there are defects in wing cell planer polarity. Additionally, we find that loss of Dmel\TIP60 in the CNS leads to lethality and a substantial loss of differentiated neurons in the larval brain, while cyclin E levels and apoptosis remain unaffected. Finally, we show that loss of Dmel\TIP60 in the mesoderm leads to lethality, and malformation or absence of the muscle fibers in the developing embryo. Expression of an additional copy of

Dmel\TIP60 in each of these tissues has no affect on their development. Taken together, our results support an essential role for Dmel\TIP60 in the differentiation and formation of a variety of specific cell and tissue types.

Introduction

Metazoans are comprised of a multitude of different cell-types that each carry out specialized functions essential for proper development (Orphanides and Reinberg 2002). Differentiation of such specialized cell-lineages is achieved through the establishment and maintenance of tightly regulated gene expression profiles distinct for each cell type (Orphanides and Reinberg 2002; Reik 2007) Such regulation in eukaryotic cells is determined in large part by the differential packaging of genes into chromatin (Kiefer 2007; Reik 2007). All DNA in the eukaryotic nucleus is packaged into nucleosomes, consisting of 146 base pairs of DNA wrapped around a histone octomer core, containing two subunits each of histones H2A, H2B, H3 and H4. The majority of these nucleosomes are further packaged into a highly organized and compact chromatin structure through their association with nucleosomal-linking histone H1 and additional non-histone proteins (Luger 2006). Certain enzymes and protein complexes are found to bring about changes in the state of chromatin structure by numerous mechanisms (Felsenfeld and Groudine 2003). Numerous types of covalent modification of histones are one way to remodel chromatin (Berger 2002;

Fischle et al. 2003). This type of epigenetic gene regulation has been termed the "histone code" (Strahl and Allis 2000; Jenuwein and Allis 2001). Histone modifications include acetylation, methylation, phosphorylation, ubiquitination, and sumyolation. The histone code regulates different levels of chromatin packaging and thus contributes to the precise pattern of gene expression during development (Margueron et al. 2005).

Histone acetylation is carried out by a family of enzymes termed histone acetyltransferases (HATs). This particular modification is one of the most well-studied of the histone modifications. HATs enzymatically transfer an acetyl group from acetyl-coenzyme A (acetyl-CoA) to the ε-terminal group of specific and highly conserved lysine residues within the histone N-terminal tail (Sterner and Berger 2000; Roth et al. 2001). The distribution patterns of histone acetylation within the eukaryotic genome have been shown to be associated with specific gene expression profiles. For example, coactivators that display HAT activity, such as the p300/CREB-binding protein (CBP) and steroid receptor coactivator 1 (SRC1), are recruited to specific promoters through their interaction with DNA-bound transcriptional activators, thus providing a mechanism for promoter-specific histone acetylation (Bannister and Kouzarides 1996; Mizzen et al. 1996; Shiama 1997; Brown et al. 2000; Deckert and Struhl 2001; Nagy and Tora 2007). HAT function during development is strongly supported by studies demonstrating that histone acetylation plays critical roles in chromatin control and gene expression, which is

required for proper multicellular development (Roth et al. 2001).

The tat-interactive protein, 60 KDa (TIP60) belongs to MYST histone acetyltransferase super family (Sterner and Berger 2000), which was identified through its interaction with the human immunodeficiency virus, type 1-encoded transactivator protein Tat (Kamine et al. 1996). Recombinant TIP60 protein was found to directly acetylate free histone substrates H2A, H3 and H4 *in vitro* via its C-terminal MYST domain (Yamamoto and Horikoshi 1997; Kimura and Horikoshi 1998). The TIP60 complex can acetylate nucleosomal histones H2A and H4 even when the linker histones are present (Ikura et al. 2000). Recent data has also shown that *Drosophila* Dmel\TIP60 can acetylate the histone variant phospho-H2Av at Lys5 which is essential for DNA repair (Kusch et al. 2004). Cellular TIP60 can also acetylate a wide variety of transcription factors which influences their function. Such factors include the androgen receptor (AR) (Gaughan et al. 2002), upstream binding transcription factor (UBF) (Halkidou et al. 2004), myelocytomatosis oncogene c (MYC) (Patel et al. 2004), and the kinase Ataxia Telangiectasia mutated (ATM) (Sun et al. 2005).

TIP60 has been reported to play many essential roles in a wide variety of cellular processes based upon the different protein complexes it is associated with. For example, TIP60 is generally found to be associated in a TIP60 protein complex that contains at least18 subunits (Sapountzi et al. 2006). Two such TIP60 associated proteins, p400/Domino and RuvB1/2, carry out DNA remodeling ATPase activity and

DNA helicase activity, respectively, which are essential for the function of TIP60 in DNA repair and apoptosis (Ikura et al. 2000; Kusch et al. 2004). Additionally, TIP60 can activate specific genes via its interaction with different transcriptional activators. For example, TIP60 was found to up-regulate class I nuclear hormone receptors through the interaction of its LXXLL motif with the nuclear receptors (Brady et al. 1999; Gaughan et al. 2001). Furthermore, the ternary complex of TIP60, AICD, and Fe65 activates a variety of specific target genes known to play essential roles in processing of APP and Tau proteins in Alzheimer's disease (Cao and Sudhof 2001; Baek et al. 2002; Cao and Sudhof 2004; von Rotz et al. 2004). Finally, TIP60 also plays a role as a negative regulator of gene expression. For example, it was reported that overexpression of TIP60 completely blocks activation of the cAMP-response element-binding protein (CREB)(Gavaravarapu and Kamine 2000). Other studies show that TIP60 acts as corepressor of the transcriptional repressor zinc finger E box-binding protein (ZEB) and inhibits the CD4 enhancer/promoter activity in Jurkat cells (Hlubek et al. 2001).

Despite the important role that TIP60 plays in regulating gene expression profiles, and the finding that it is essential for both yeast viability as well as *Drosophila* multicellular development (Clarke et al. 1999; Zhu et al. 2007), there are few studies that investigate the specific role that TIP60 plays during multicellular development. One such study examines TIP60 expression profiles in chicken heart development and demonstrates that TIP60 may play a critical role in regulating

transcriptional events essential during myocardial development (Lough 2002). In another study, Brody et al. carried out a genome wide screen to identify neuronal precursor genes in *Drosophila*. Intrigugly, one of the genes identified in the screen was Dmel\TIP60, suggesting that TIP60 may play an important role during the neuroblast lineage development (Brody et al. 2002).

In this report, we utilize our *Drosophila* GAL4 inducible Dmel\TIP60 knockdown/overexpression system to further explore the role of Dmel\TIP60 in a wide variety of specific tissues during *Drosophila* development. We show that loss of Dmel\TIP60 in the wing leads to a range of wing abnormalities, including the formation of wing blisters in the most severe cases. Wing surface area and cell count/hair density assays reveal that although the number of cells that compose the wing remain unaffected, their size is significantly smaller than normal and there are defects in wing cell planer polarity. Additionally, we find that loss of Dmel\TIP60 in the CNS leads to lethality and a substantial loss of differentiated neurons in the larval brain, while cyclin E levels and apoptosis remain unaffected. Finally, we show that loss of Dmel\TIP60 in the mesoderm leads to lethality, and malformation or absence of the muscle fibers in the developing embryo. Expression of an additional copy of Dmel\TIP60 in each of these tissues has no affect on their development. Taken together, our results support an essential role for Dmel\TIP60 in the formation and differentiation of a variety of specific cell and tissue types.

Materials and methods

Drosophila stocks

All stocks were maintained under standard conditions at 25 °C. *P{pUAST}/P{pUAST}* fly line containing Dmel\TIP60/OverEx was created as described below. *P{pUAST}/P{pUAST}* flies containing Dmel\TIP60/RNAi and control were created and maintained in our lab (Zhu et al. 2007). *w*;P{GawB}how*^{24B} (Brand and Perrimon 1993) and GAL4 line 337 (Elefant and Palter 1999) were kindly provided by Dr. Karen Palter (Temple University, PA). *en*-GAL4 was kindly provided by Dr. Marenda (University of the Sciences in Philadelphia, PA). Bloomington Stock Center (Bloomington, IN; http://flystocks.bioindiana.edu/) provided all the other GAL4 drivers: *y*^{1} *w***; P{Act5C-GAL4}25FO1/CyO,* (stock# 4414; Y. Hiromi), *P{GawB}60IIA* (stock# 7029), *P{GawB}109-30* (stock# 7023), *P{GawB}109-69* (stock# 7026), *P{GawB}c179* (stock# 6450), *P{GawB}69B* (stock# 1774), *P{GawB}32B* (stock# 1782), *P{GAL4-ninaE.GMR}12* (stock# 1104), *P{sevEP-GAL4.B}7* (stock# 5793), *P{GAL4-elav.L}2* (stock# 8765).

Creation of Dmel\TIP60/OverEX fly lines

To create the overexpression Dmel\TIP60/OverEX pUAST construct, the open reading frame (ORF) of Dmel\TIP60 was amplified by PCR using primer sets specific for the Dmel\TIP60 cDNA sequence with Dmel\TIP60 cDNA TOPO pCR2.1 clone (Zhu et al. 2007) as template. The forward primer 5'-CGG C*GA ATT C***GC CAT C**AT GAA AAT TAA CCA CAA ATA TGA G-3' contained an EcoRI site (italics), a Kozak consensus sequence (bold), and the sequence corresponding to the first eight codons of Dmel\TIP60. The reverse primer 5'-GGT T*GG TAC C***TC ATC A**TC ATT TGG AGC GCT TGG ACC AGT C-3' contained a *KpnI* site (italics), and two stop codons (bold). PCR reactions were carried out using Expand™ High Fidelity PCR System (Roche) according to the manufacture's instructions using 400 nM of each forward and reverse primers. The cycling parameters were 30 cycles of 95 °C for 2 min, 55 °C for 1 min, and 72 °C for 3 min, using Mastercycler (Eppendorf). The correct size PCR generated fragment was then cloned into *Eco*RI/*KpnI* sites in the pUAST vector downstream of the UAS promoter. P-element germ-line transformations with pUAST constructs were performed as previously described (Elefant and Palter 1999; Zhu et al. 2007), to create fly lines containing Dmel\TIP60/OverEX construct. To determine on which chromosome the P-element inserted, lines heterozygous for the *TM3* and *TM6* balancer were mated to w^{1118} flies, and segregation of the w^{+} marker was scored: if segregation of w^{+} was neither with the third chromosome balancer or a sex

chromosome, it was inferred to segregate with the second chromosome. Balancer chromosomes were subsequently crossed away by successive mating to w^{1118}. Several independent fly lines were created as the level of gene expression is dependent upon the random P-element insertion.

Semi-quantitative RT-PCR

As described previously (Zhu et al. 2007), total RNA from three third instar larvae progeny from a homozygous Dmel\TIP60/OverEX or control to GAL4 337 cross was isolated using TRIzol (Invitrogen) and twice treated with DNA-free™ (Ambion) to remove DNA. First strand cDNA was prepared using the SuperScript™ II reverse transcriptase kit (Invitrogen) with 1μg total RNA and 15 ng/μL of random hexamers (Roche). PCR reactions were performed in a 40 μl total volume containing 1 U Taq (Qiagen), 1 μl cDNA template, 250 μM dNTPs (Amersham Pharmacia Biotech) and 500 nM of each forward and reverse primer. The cycling conditions were 36 cycles of 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min. The forward primer (5'-TGG TAT TTC TCA CCC TAT CC-3') and the reverse primer (5'-CAA TGA GCA GCT TGC CGT AG-3') amplified a 427 bp fragment of position 1407 to 1833 within Dmel\TIP60 cDNA sequence (Zhu et al. 2007).

Viability and mutant phenotype analyses

All crosses were performed using three males and three newly eclosed virgin females in narrow plastic vials (Applied Scientific) with yeasted *Drosophila* media (Jazz-Mix, Fisher Scientific) at 25 ºC. Old flies were removed after 10 days and the number of newly eclosed progeny was scored every 48 h for total 10 days to determine the percent viability per genotype.

As described in the previous protocol (Marenda et al. 2003), ImageJ 1.38X was used to determine wing size of 10 wings per genotype, whose pictures were taken under 10X magnification, by measuring the number of pixels corresponding to anterior and posterior of each wing. The relative wing size was calculated by dividing the number of pixels corresponding to posterior of a wing by that of anterior. To count the hair number, the images of anterior and posterior of each of the 10 wings were taken under 40X magnification. Given a 200 X 200 dpi squire which is measured by the software Pixel Ruler, the number of hairs in anterior and posterior of each wing was counted to determine cell number (Meyer et al. 2000). The relative hair number was calculated by dividing the number of hairs corresponding to posterior of a wing by that of anterior.

Immunohistochemical staining of embryos

The antibodies used in immunohistochemical staining of embryos were as follows: mouse anti-ELAV (Developmental Studies Hybridoma Bank, University of Iowa); mouse anti-REPO (Developmental Studies Hybridoma Bank, University of Iowa); mouse 22C10 (Developmental Studies Hybridoma Bank, University of Iowa); rat anti-filamin (Sokol and Cooley 2003) (Dr. Cooley, Yale University; Gift from Dr. DiNardo, University of Pennsylvania); biotin-conjugated anti-mouse secondary antibody (Vectastain ABC Elite kit; Vector Laboratories); biotin-conjugated anti-rat secondary antibody (Vector Laboratories). Embryos collected from grape-agar plates (Flystuff) were dechorionated in 50% Clorox for 5 min and washed thoroughly with water and 0.1% Triton X-100. Embryos were shaken in 500 μl 4% paraformaldehyde and equal amount of heptane for 2 min. The bottom layer of paraformaldehyde was then removed. The remaining heptane layer with embryos was mixed with 700 μl methanol and shaken vigorously for 15 s. The top heptane layer was removed and the devitellinized embryos that settled to the bottom were washed with methanol twice and stored at 4°C until staining.

Antibody staining was performed by first washing the embryos in phosphate-buffered saline (PBS) with 0.1% Tween (PBT) every 30 min for 3 h at room temperature. Embryos were treated with primary antibody (diluted 1:500 in PBT) overnight at 4°C. Embryos were washed with PBT every 30 min for 3 h at room

temperature. Biotinylated anti-mouse secondary antibody from Vectastain ABC Elite kit (Vector Laboratories; diluted 1:400 in PBT) was added to the embryos for overnight incubation at 4°C. Embryos were washed with PBT every 30 min for 3 h at room temperature. Embryos were incubated in biotin–streptavidin–horseradish peroxidase complex (Vectastain ABC Elite kit; Vector Laboratories) at room temperature for 1.5 h. Embryos were then washed eight times in PBT for 2 h. The signal was developed by incubation with 500 μl ImmPACT DAB (Vector Laboratories) in the presence of 1 μl of 10% H_2O_2. The reaction was terminated by washing the embryos with PBT and then with ethanol. The embryos were stored and mounted in methyl salicylate for microscopy with Zeiss Axioplan2 optics.

Immunohistochemical staining of larval tissues

Imaginal discs and brains were fixed and stained according to standard protocols. The following antibodies with specific dilutions were used: mouse anti-ELAV (1:200; Developmental Studies Hybridoma Bank, University of Iowa); mouse anti-REPO (1:100; Developmental Studies Hybridoma Bank, University of Iowa); mouse anti-cyclin E (1:5; Dr. Marenda, University of the Sciences in Philadelphia); mouse anti-integrin αPS1 (1:200; Developmental Studies Hybridoma Bank, University of Iowa); mouse anti-integrin αPS2 (1:200; Developmental Studies Hybridoma Bank, University of Iowa); biotin-conjugated anti-mouse secondary

antibody (1:100; Vectastain ABC Elite kit; Vector Laboratories); rhodamine (TRITC)-conjugated anti-mouse secondary antibody (1:100; Jackson Immunoresearch). The tissues were fixed in the fixation buffer (PBS with 2% paraformaldehyde, 1% lysine, and 0.25% sodium m-periodate). After being washed with PBT (PBS with 0.1% Triton) 15 min each time for 3 times, the tissues were incubated in block buffer (10% horse or goat serum depending on the secondary antibody) for 15 min. Then the tissues were transferred into the appropriate primary antibody and incubated at 4 ºC overnight. After being washed with PBT for 3 times, the tissues were transferred into the appropriate secondary antibody and incubated at 4 ºC overnight. The tissues were washed with PBT for 3 times. If fluorescence secondary antibody were used, the tissues would be directly mounted in Vectashield mount media (Vector Laboratories) for microscopy using Olympus IX81. If ImmPACT DAB (Vector Laboratories) were used, the color development protocol and reagent concentration were the same as previously described in the immunohistochemical staining of embryos. And the tissues were mounted on the slides and visualized by Zeiss Axioplan2 optics.

TUNEL Assay

TUNEL assays were performed by following the protocol of the *in situ* fluorescein cell death detection kit (Roche) with some modifications (Kim et al.

2007). The imaginal discs and brains from the third instar larvae were fixed the same as stated above in immunohistochemistry. The tissues were washed with 5X PBT (PBS with 0.5% Triton) 10 min each time for three times. Then the tissues were transferred into the reaction buffer at 37 ºC for 10 min before adding terminal deoxynucleotide transferase (TdT) and incubating at 37 ºC for 2 h. The tissues were rinsed with 5X PBT three times and mounted by Vectashield mount media (Vector Laboratories) for microscopy using Zeiss Axioplan2 optics.

Results

An additional copy of Dmel\TIP60 rescues Dmel\TIP60/RNAi induced lethality

We had previously generated transgenic flies carrying either Dmel\TIP60/RNAi or control GAL4 inducible pUAST constructs. (Zhu et al. 2007). Using this system, we demonstrated that loss of Dmel\TIP60 using ubiquitous GAL4 drivers *Act5c-GAL4* and *337* resulted in lethality. We also observed that our Dmel\TIP60/RNAi A line with the P-element located on the X chromosome is our strongest RNAi line, likely due to the position effects on transgene expression.

To confirm that Dmel\TIP60/RNAi induced lethality was specifically caused by the reduction of endogenous Dmel\TIP60, experiments were carried out to determine whether additional Dmel\TIP60 would rescue the lethality resulting from

Dmel\TIP60/RNAi induction. To generate transgenic flies carrying a GAL4 inducible Dmel\TIP60 overexpression construct (Figure 1A), we first subcloned the ORF region of Dmel\TIP60 into the pUAST vector. To facilitate the expression of Dmel\TIP60, we introduced the Kozak consensus sequence (Kozak 1987) at the 5' end and two stop codons at the 3' end by PCR. We then generated Dmel\TIP60/OverEx transgenic fly lines by microinjection of embryos with the Dmel\TIP60/OverEx construct. To ensure that the constructs were indeed overexpressing Dmel\TIP60, we first induced their expression using GAL4 drivers *Act5c-GAL4* or *337* at 25 °C (data not shown). Interestingly, no apparent phenotypes in the progeny were observed. We next assessed the expression level of Dmel\TIP60 in 3rd instar larva progeny from the ubiquitous *337* GAL4 driver cross using RT-PCR with specific primers for Dmel\TIP60 (Zhu et al. 2007) and RP49 as an internal control. We observed increased levels of Dmel\TIP60 mRNA in the three different fly lines carrying Dmel\TIP60/OverEX construct we generated (Figure 1B). Of note, Dmel\TIP60 expression level was relatively low when compared to the internal control RP49, which was in accordance with my previous real-time RT-PCR results (Zhu et al. 2007).

We next conducted rescue experiments to determine whether an additional copy of Dmel\TIP60 would rescue the lethal phenotype caused by induction of Dmel\TIP60\RNAi. To perform the rescue crosses, I chose to use the Dmel\TIP60/RNAi A line to provide the RNAi construct on X chromosome as it

displays the strongest RNAi expression level. Three separate fly strains were produced that each carried the P-element transposed GAL4 inducible Dmel\TIP60/RNAi construct on the X chromosome as well as an additional P-element transposed GAL4 inducible Dmel\TIP60 overexpression construct derived from each of my three independent overexpression Dmel\TIP60 lines on the third chromosome balancer (*TM3*). These three rescue fly lines were designated Dmel\TIP60/Rescue/A, B and C. Each of the three fly lines were then crossed to the ubiquitous GAL4 driver 337 and the viability of the progeny was scored. The results showed that when the Dmel\TIP60 overexpression construct was present in flies also expressing the Dmel\TIP60/RNAi construct, a significant number of flies were rescued (an average of 54% flies surviving). Of note, the survival rates were different among the three different rescue fly constructs likely due to the different levels of wild-type Dmel\TIP60 protein produced from each of the three Dmel\TIP60 independent constructs used. Intriguingly, although the majority of such rescued flies displayed a normal phenotype, a number of these flies (an average of 23%) were found to exhibit a wing blister phenotype (Figure 2B) that is typically characteristic of defects in cell adhesion and signaling molecules termed integrins (Li et al. 1998) (discussed further in next section).

Our results demonstrate that Dmel\TIP60 induced lethality is specifically caused by the reduction of endogenous Dmel\TIP60 as it can be partially rescued by increasing the level of wild-type Dmel\TIP60. Additionally, our results suggest that a

critical threshold expression level of Dmel\TIP60 is required for the appropriate *Drosophila* development.

Dmel\TIP60 is required for the cell growth and differentiation during wing development

We found that 23% of the flies that were rescued from Dmel\TIP60/RNAi induced lethality by increasing levels of wild-type Dmel\TIP60 exhibited a distinct wing blister phenotype. This well characterized phenotype reflects the inability of the dorsal and ventral wing epithelial layers to become permanently fused and often results from misexpression of the integrin genes (Brown 1993). As the integrin protein family carries out essential functions in both cell adhesion and cell signaling (Hynes 1992; Li et al. 1998), we wished to further explore the role of Dmel\TIP60 in these cell processes. Thus, Dmel\TIP60/RNAi, overexpression and control transgene expression were each induced specifically in the wing discs of the fly using GAL4 drivers *69B* and *32B*. Three independent fly lines containing either Dmel\TIP60/RNAi or control transgenes were crossed to flies homozygous for either GAL4 *69B* or *32B* driver at 25^{o}C and the resulting phenotypes were assessed. All three fly lines expressing the Dmel\TIP60 overexpression and control transgenes showed normal development and no observable defective phenotypes. However, when expression of the Dmel\TIP60/RNAi transgene was induced in the wing disc, a range of wing defective phenotypes were observed , including wing blisters in the

most severe cases when the *69B* GAL4 driver was used (Figure 3 and Figure 4). Of note, I also observed lethality when Dmel\TIP60/RNAi was induced using these drivers, likely due to expression of GAL4 in tissues other than the wing discs in both of these drivers (our laboratories unpublished results). These results suggested that TIP60 plays a role in integrin-mediated cell adhesion and cell signaling pathways**.**

To further explore the wing blister integrin related phenotype in a more controlled fashion, we targeted expression of Dmel\TIP60/RNAi specifically to the posterior portion of the wing disc using the *engrailed* GAL4 driver (*en-GAL4*), enabling us to use the anterior portion as an internal control for these and subsequent studies. Use of this driver led to wing defects, including blisters, which were specifically confined to the posterior portion of the wing, confirming that reduction of Dmel\TIP60 was indeed responsible for causing such wing defects (Figure 5B). Further study of the wing hairs revealed that the hair orientation was disordered, indicative of defects in the planar cell polarity (PCP; Figure 5C to F). Of note, all of these defects were again confined to the posterior region (Figure 5F), while the anterior was not affected by RNAi (Figure 5D), further indicating that loss of Dmel\TIP60 was responsible in causing such defects.

To explore the mechanisms of DmelTIP60 in regulations of wing development, we collected ten wings from each of the male and female progeny of Dmel\TIP60/RNAi A and Dmel\TIP60/Control C crossed to *en-GAL4*. After measuring the sizes of the posterior and anterior of the wings, we found that the wing

area in the posterior of both male and female wings was significantly reduced ($p<0.0001$) when RNAi was induced in the posterior compartment of the wing when compared to controls (Figure 6A). The reduction on wing area could have resulted from either cell death or a change of cell size (or shape) due to loss of Dmel\Tip60. To distinguish which of these two mechanisms is responsible for the phenotypes we observed, cell count\hair density assays were performed. As each of the cells of the wing contain one hair, we counted the hair number (which represents cell number) in a certain area located in either the posterior or anterior regions to determine whether the number of cells was affected by loss of Dmel\TIP60 in the posterior portion of the wing compared to the anterior portion(Meyer et al. 2000). By examining ten wings from both males and females, we found that the number of cells was not changed in the posterior of the wing in both males and females when Dmel\TIP60/RNAi was expressed in the posterior of the wing (Figure 6B). This result suggested that the reduced size of the posterior wing is caused by a decrease of the size of cells likely due to defects in cell growth and differentiation. We also used the TUNEL assay on wing discs dissected from these animals to determine whether apoptosis levels were affected. No significant differences of apoptosis signals were observed between the posterior and anterior portions of the wing (Supplement Figure 1), further indicating that the reduced size of the posterior wing was caused by a decrease in actual cell size and not cell number caused by apoptotic cell death. As wing blister phenotypes have been shown to be associated with the molecule integrin, we next examined whether

the integrin expression pattern in the wing disc of these animals was altered. Immunohistochemical staining with anti-PS1 and anti-PS2 antibodies revealed no significant changes in stain between the posterior and anterior portions of the wing disc (Supplement Figure 2). These defects in cell size and their planar organization in the wing resulting from Dmel\TIP60 loss suggests that Dmel\TIP60 is required specifically for cell growth and differentiation, and not cell proliferation, in this particular tissue type.

Dmel\TIP60 is required for the development of central nervous system

Our *in-situ* analysis of Dmel\TIP60 transcripts demonstrated that Dmel\TIP60 seems to be expressed in the nervous system during embryogenesis (data not shown). Additionally, our Dmel\TIP60 was identified as an uncharacterized gene in a screen for neural precursor genes in *Drosophila* (Brody et al. 2002). These findings suggested that Dmel\TIP60 may be involved in neural development. To determine whether Dmel\Tip60 is required for the development of the nervous system, we crossed our Dmel\TIP60/RNAi and /Control flies to each of three different nervous system GAL4 driver lines, i.e. *60IIA*, *109-30* and *109-69* at 25 ºC. GAL4 driver *60IIA* drives GAL4 expression in the both central and peripheral nervous system (CNS and PNS; Figure 7A); while both *109-30* and *109-69* drive GAL4 expression in certain subset of PNS tissues. Compared to the Dmel\TIP60/Control lines, we observed

lethality in the progeny when expressing Dmel\TIP60/RNAi in both CNS and PNS via GAL4 driver *60IIA* (Figure 7A), and no apparent phenotypes when expressing Dmel\TIP60/RNAi in the subsets of PNS respectively by either *109-30* or *109-69* (Figure 7B and C). Again, Dmel\TIP60/RNAi A was characterized as the strongest RNAi line with a 100% lethality of progeny. The data led to the hypothesis that Dmel\TIP60 may be involved in the development of nervous system, and especially be essential for the development of the CNS.

To test our hypothesis, we carried out the immunohistochemistry experiments as follows. First, we crossed our strongest RNAi line Dmel\TIP60/RNAi A and control Dmel\TIP60/Control line C to *60IIA*. We next collected the progeny embryos and stained them with the antibodies 22C10, anti-REPO and anti-ELAV respectively. Antibody 22C10 is used to visualize the PNS by detecting a microtubule-associated protein. We also used the antibodies against the well characterized neuronal marker protein ELAV (embryonic lethal abnormal visual system), an RNA binding protein present in all differentiated neurons; and REPO (reserved polarity), a homeodomain protein expressed specifically in glial cells. We detected no significant defects in the embryos that expressed Dmel\TIP60/RNAi by *60IIA* when compared to controls using each of the three antibodies 22C10, anti-REPO and anti-ELAV (Figure 8). As Dmel\TIP60 induced lethality is not observed until the pupal stage (Figure 7A), we thought that the phenotypic defects may have not accumulated significantly enough to be detected in the early embryonic stages. Thus, we decided to investigate the effect

of RNAi knockdown in later developmental stages, i.e. the 3rd instar larval stage. We dissected the brains out of the 3rd instar larvae derived from a cross between Dmel\TIP60/RNAi A to *60IIA* with Dmel\TIP60/Control C as control. We then fixed and stained the dissected brains with the anti-ELAV antibody. The control brains showed a well defined pattern of differentiated neurons particularly within the ganglion (Figure 9A to C). The ventral portion of the control ganglia showed the symmetrical condensed dots on the two edges and the neuronal "web" to link the two lobe portions of the brain (Figure 9A). The dorsal region of the control ganglia showed a middle line and symmetrical stripes on the two edges (Figure 8B). A closer look of the dorsal region showed that the middle line and the stripes are composed of symmetrical dots, indicating differentiated neurons (Figure 8C). In contrast, our results revealed that the wild-type ELAV staining pattern was substantially disrupted in the brains of all Dmel\TIP60/RNAi larvae examined when compared to control larvae (Figure 9 D to F). Staining was substantially reduced in the posterior portion of the ventral brain lobes, particularly within the region connecting the brain lobes with the ventral ganglion, and virtually absent within the thoracic and abdominal regions of the ganglion (Figure 9D to F). These ELAV staining defects indicate the absence of differentiated neurons specifically in these regions of the CNS. We next stained Dmel\TIP60/RNAi and control brains using the anti-REPO antibody and observed no obvious differences (Supplement Figure 3), indicating that the defects were neuron specific. TUNEL staining using whole mount Dmel\TIP60/RNAi and control brains

revealed no obvious apoptotic abnormalities (Supplement Figure 4), indicating that neuronal loss was not due to an increase in cell death. We next wanted to determine whether loss of differentiated neurons was due to defects in cell division. Since cyclin E is shown to be essential for the asymmetrical division of neuroblast in *Drosophila* CNS (Berger et al. 2005), we decided to test whether cyclin E expression was affected by loss of Dmel\TIP60. After staining the brains with the anti-cyclin E antibody, we did not observe any obvious differences between the Dmel\TIP60 RNAi and control brains (Supplement Figure 5). Taken together, our data suggest that Dmel\TIP60 is required for promoting differentiation of the neurons during the CNS development.

Dmel\TIP60 is required for muscle formation in the developing embryo

As reported in our previous paper (Zhu et al. 2007), targeted expression of Dmel\TIP60/RNAi in the mesoderm of *Drosophila* using the GAL4 line *24B* results in a lethal muscle mutant phenotypes at 25 ºC. To extend these studies, we utilized a second muscle specific *C179* GAL4 driver that induces higher levels of GAL4 in the *Drosophila* mesoderm and muscle system. When I crossed Dmel\TIP60/RNAi and Dmel\TIP60/Control fly lines to *C179* at 25 ºC, I observed 0% viability for all three Dmel\TIP60/RNAi lines (Figure 10A) and no apparent defects for the control. Interestingly, I observed that the progeny died shortly after the 1st larvae stage, the stage directly after embryogenesis (data not shown). These data suggested that *C179*

is a stronger muscle-specific GAL4 driver compared to *24B* and would be a valuable tool to study muscle defects caused by Dmel\TIP60 at an early developmental stage in *Drosophila*.

To determine whether the muscle development pathway is disrupted by Dmel\TIP60 knockdown, we crossed Dmel\TIP60/RNAi A or Dmel\TIP60/Control to GAL4 driver *C179*, collecting the resulting progeny embryos and stained them with the anti-filamin antibody. Filamin is an actin associated protein (Sokol and Cooley 2003), and thus shows an immunohistochemical muscle staining pattern where in the control embryos, the muscle fibers were well defined and oriented (Figure 10B). In contrast, I observed that this muscle pattern was significantly altered in embryos expressing Dmel\TIP60/RNAi (Figure 9C). First, I observed defects in the shape of the embryos likely due to the disordered formation of muscle fibers. Second, I observed that the muscle fibers were very thin when compared to that of the controls and that their orientations were disrupted. Taken together, our data suggested that Dmel\TIP60 is an important regulator during muscle development and is required for appropriate muscle pattern formation during embryogenesis.

Discussion

HATs carry out important functions during development by their regulation of chromatin packaging that in turn, controls gene expression profiles both spatially and temporarily (Margueron et al. 2005), however little is known about specific HAT function in the differentiation of distinct cell types. In this paper, we utilize our GAL4 inducible Dmel\TIP60 knockdown/overexpression system to demonstrate that Dmel\TIP60 plays an essential role in the differentiation of a wide variety of cell and tissue types during *Drosophila* development. We show that loss of Dmel\TIP60 in the wing leads to a range of wing abnormalities, including the formation of wing blisters in the most severe cases. Wing surface area and cell count/hair density assays reveal that although the number of cells that compose the wing remain unaffected, their size is significantly smaller than normal and there are defects in wing cell planer polarity. Additionally, we find that loss of Dmel\TIP60 in the CNS leads to lethality and a substantial loss of differentiated neurons in the larval brain, while cyclin E levels and apoptosis remain unaffected. Finally, we show that loss of Dmel\TIP60 in the mesoderm leads to lethality, and malformation or absence of the muscle fibers in the developing embryo. An additional copy of Dmel\TIP60 in each of these tissues has no affect on their development. Taken together, our results support an essential role for Dmel\TIP60 in promoting the differentiation of a wide variety of specific cell and tissue types, thus shedding light on the importance of HATs in the development of amulticellular organisms.

First, our data suggest that endogenous Dmel\TIP60 levels are precisely regulated during development. In support of this concept, the Dmel\TIP60/RNAi A line exhibited more severe phenotypes earlier in development than those of the other two RNAi lines, likely due to the fact that it displays the highest RNAi expression level due to the position effects on transgene expression. Additionally, we do not observe any apparent phenotype of in flies carrying an additional copy of Dmel\TIP60 driven by either ubiquitous or tissue/cell specific GAL4 drivers (data not shown), even though overexpression was confirmed by our RT-PCR results. Based on these observations, we conclude that knockdown or an additional copy of Dmel\TIP60 does not show a distinctive phenotype until the perturbation of Dmel\TIP60 protein level reaches a certain threshold. Similar results have been shown for the mouse homolog of Tip60 in that a critical level of TIP60 is found to be required for its tumor suppressor activity in the mouse model system (Gorrini et al. 2007).

Our results are the first to demonstrate that Dmel\TIP60 is required for the appropriate development and formation of the wing in *Drosophila*. As TIP60 is involved in many different protein complexes and carries out a variety of different functions, the roles Dmel\TIP60 plays during wing development may be related to its association with other subunits within the Dmel\TIP60 multi-protein complex and/or other factors that interact with Dme\TIP60. One of the TIP60 associated molecules is most likely to be involved in wing development is TRRAP. As shown by others,

TRRAP has been found in many HAT complexes that have important cellular regulatory functions (Carrozza et al. 2003). These studies demonstrate that TRRAP in both yeast and mouse is essential for cell viability (Saleh et al. 1998; Herceg et al. 2001). When associated with the NuA4 (TIP60) complex, TRRAP was found to be involved in the regulation of double-strand break (DSB) repair (Murr et al. 2006) and mitotic checkpoint (Li et al. 2004; Deran et al. 2007). It is important to note that Nipped-A (*Drosophila* TRRAP) was found to be involved in the wing development by screening the genes that regulate *cut* and Notch pathways (Rollins et al. 1999). In addition, Nipped-A and Domino (another subunit of TIP60 complex) were both found to be required for wing development by regulating the mastermind and Notch signaling pathway (Gause et al. 2006). Myelomonocytic leukemia (MYC) may be another molecule that is associated with the function of Dmel\Tip60 during wing development. MYC recruits TIP60, and other HATs including p300/CBP and mammalian mGCN5, to the promoters of specific target genes to activate their experssion (McMahon et al. 2000; Frank et al. 2001; Frank et al. 2003; Adhikary and Eilers 2005). It has been shown that such MYC recruited HATs (TIP60 included) regulate MYC target gene expression by their ability to acetylate both nucleosomal histones at the promoter regions as well as the specific lysine residues on MYC (Vervoorts et al. 2003; Patel et al. 2004; Faiola et al. 2005; Zhang et al. 2005). Of note, Dmel\MYC, the single MYC homolog in *Drosophila*, is a key molecule to regulate cell growth and organ size (Secombe et al. 2004). While the Dmel\MYC null

mutation is lethal, the viable hypomorphic mutants show smaller body size due to smaller cells and the ectopic expression of Dmel\MYC leads to larger cells (Johnston et al. 1999; Maines et al. 2004; Pierce et al. 2004). These findings are consistent with our results demonstrating that loss of function of Dmel\TIP60 results in the reduction of wing size due to defects in cell growth and not cell number. Although Dmel\MYC regulation of cell growth is also linked to apoptosis, we did not observe a significant increase in cell death in response to Dmel\TIP60 loss in the wing discs of the fly. However, we can not rule out the fact that we did not detect apoptosis perhaps due to not obtaining the appropriate expression threshold of Dmel\TIP60/RNAi loss as we discussed previously. Interestingly, we also observed that the planar cell polarity (PCP) of the cells that compose the fly wings was disordered due to Dmel\TIP60 loss. Since it is well characterized that PCP is maintained and regulated by Fz/PCP pathway (Seifert and Mlodzik 2007; Zallen 2007), this finding leads us to believe that Dmel\TIP60 may be involved in Frizzeld (Fz)/PCP signaling pathways. Further supporting a role for Dmel\TIP60 in the control of cell size are studies demonstrating that the PCP related gene *Dmel\ft* (*fat*) affects cell growth by modulating the Hippo/Warts pathway (Silva et al. 2006; Willecke et al. 2006). Collectively, we show that Dmel\TIP60 is required for regulating the size and shape of the cells composing the fly wing likely through its association with specific protein factors. Additionally, we observed that driving Dmel\TIP60/RNAi in the wing using GAL4 drivers *69B* and *32B* results in wing defective phenotypes that range severity as well as lethality. The

lethality is likely due to the fact that GAL4 expression using these GAL4 drivers are not limited in the wing tissues but also occurs in other tissues in the embryo (our unpublished results) as lethality was not observed when the expression of Dmel\TIP60/RNAi was confined to the posterior of the wing using GAL4 driver *en-GAL4*. Such easily observable wing defective phenotypes will be offer a powerful tool for therapeutic screening of either enhancers or inhibitors of TIP60.

Epigenetic gene regulation plays an essential and distinct role in neural stem cell fate specification, neural plasticity, learning and memory (Hsieh and Gage 2005; Kiefer 2007). For example, the HAT activity of CBP in mouse Rubinstein-Taybi syndrome (RTS) model was found to be essential for neuronal plasticity and learning (Alarcon et al. 2004; Korzus et al. 2004). TIP60 has been found to be involved in the neural degenerative disease Alzheimer's disease (AD) through its association with the intracellular domain of amyloid precursor protein (AICD). Yeast two-hybrid assays and coimmunoprecipitation experiments demonstrate that a ternary complex of AICD, TIP60 and adaptor protein Fe65 stimulates transcription in a cell culture model (Cao and Sudhof 2001). This complex was found at the promoter region of the KAI1 gene, a putative target of APP-mediated transcription (Baek et al. 2002). Subsequently, it was shown that activation of transcription of the KAI1 gene requires both the AICD/Fe65/TIP60 complex and the nucleosome assembly protein SET (Telese et al. 2005). In addition, the TIP60/AICD/FE65 ternary complex was found to localize in nucleus with different morphology depending on the APP adapter proteins (i.e., Fe65

and Jun-interacting protein Jip1γ) and upregulate several APP-effector genes (von Rotz et al. 2004). AICD has been suggested to cause neurotoxicity via misregulation of the downstream transcription events associated with histone acetylation. In support of this premise, introduction of the histone deacetylase (HDAC) inhibitor, sodium butyrate, in neuronal cells enhances the cytotoxicity induced by AICD (Kim et al. 2004). Furthermore, TIP60 may also play a role in apoptotic cell death in AD (Kinoshita et al. 2002).

We show here that Dmel\TIP60 is required for the development of central nervous system (CNS), establishing yet another example of epigenetic gene regulation during neural development. Consistent with our finding, Dmel\Tip60 was identified as a neural precursor gene in *Drosophila* (Brody et al. 2002), though its function remained uncharacterized at that time. Thus, our work is the first to identify TIP60 as an essential regulator for neural development. We find that Dmel\TIP60 may be specifically required for CNS, as loss of function in subsets of PNS did not result in any significant defects in flies. However, we do not rule out the possibility that these PNS GAL4 drivers may not be strong enough to drive sufficient Dmel\TIP60 knockdown to achieve and observable defect in these cell types. We observed that the loss of differentiated neurons in the brains did not occur until the 3rd instar larvae stage, as we did not detect any abnormalities in embryos. This observation is likely due to the fact that this nervous GAL4 line drives GAL4 expression later during embryonic development. Interestingly, we did not find any

defects in our immunohistological examination of the glial cells in both embryos and third instar larval brains, suggesting that Dmel\TIP60 is a specific regulator for the differentiation of neuronal and not glial cells and that the loss of neurons is not due to the deprivation of nutrients. Additionally, we did not observe cell division defects and apoptotic abnormality as indicated by our anti-cyclin E and TUNEL assays respectively. This finding suggest that Dmel\TIP60 is specifically required promotion and/or maintenance of differentiated neurons which is independent of cell lethality via cell cycle defects and apoptosis. TIP60 has been shown to play an essential role in a variety of cell processes and gene conrol via its interaction with a variety of different cell-specific transcription factors (Sapountzi et al. 2006; Squatrito et al. 2006). Thus, our finding that Dmel\TIP60 is specifically required for promoting the differentiation of neuronal cells extends the well established role of TIP60 in cell progression and apoptosis by adding yet another layer of complexity to the important role that TIP60 plays during the development of multicellular organisms.

Our previous report suggests that Dmel\TIP60 is essential for the appropriate development of muscle tissue (Zhu et al. 2007). To extend these studies, we show here that knockdown of endogenous Dmel\TIP60 results in defects in embryo shape as well as defects in the muscle fibers themselves, as visualized by the actin-associated filamin antibody. Our finding is consistent with the important roles that different HATs and HDACs play in regulating the complicated events that promote muscle differentiation (McKinsey et al. 2001; McKinsey et al. 2002;

Palacios and Puri 2006; Kiefer 2007). In proliferating myoblasts, the premature muscle-gene expression is inhibited by several histone modifiers, such as histone deacetylases (HDACs), YY1 and Polycomb proteins. Class I HDACs is found to be associated with MyoD in undifferentiated myoblasts (Mal et al. 2001); (Puri et al. 2001), while Class II HDACs specifically bind and repress MEF2 protein (Lu et al. 2000b; Lu et al. 2000c; McKinsey et al. 2000a; McKinsey et al. 2000b; McKinsey et al. 2002; Zhang et al. 2002). Upon induction of differentiation, the HDACs and their associated co-repressors dissociate from the MRFs and MEF2 factors, allowing for the recruitment of chromatin modifying complexes to the chromatin surrounding the regulatory elements of muscle specific genes. These complexes include HATs CBP/p300, PCAF, p/CIP, SRC1 and glucocorticoid receptor interacting protein (GRIP), the co-activator-associated arginine methyltransferase 1 (CARM-1) and the ATP-dependent switching/sucrose non-fermenting (SWI/SNF) chromatin remodelling complexes (Eckner et al. 1996; Yuan et al. 1996; Puri et al. 1997a; Puri et al. 1997b; Sartorelli et al. 1997; Chen et al. 2000; de la Serna et al. 2001; Chen et al. 2002; Wu et al. 2005). Acetylation of the histone tails by p300 and PCAF results in a relaxed chromatin structure permissive for transcription, while acetylation of MyoD by the same acetyltransferases increases the affinity for its recognition site in the DNA (Puri et al. 1997b; Sartorelli et al. 1999; Polesskaya et al. 2000; Polesskaya and Harel-Bellan 2001; Polesskaya et al. 2001a; Polesskaya et al. 2001b; Dilworth et al. 2004). Similarly, p300-mediated acetylation of multiple lysines is essential for MEF2

function (Ma et al. 2005). It is worthwhile to note that TIP60 can interact with both class I (HDAC 1) and class II (HDAC 7) HDACs in different pathways associated with androgen receptor (AR), endothelin receptor A (ETA), signal transducer and activator of transcription 3 (STAT3) respectively (Lee et al. 2001; Gaughan et al. 2002; Xiao et al. 2003). Taken together, our data have linked Dmel\TIP60 to the muscle development in *Drosophila*. Future studies can now entail further dissection of the particular pathways which Dmel\TIP60 is involved in and the detailed regulatory mechanisms on how Dmel\TIP60 contributes to each pathways.

In summary, the results presented here support an essential role for Dmel\TIP60 in the differentiation of different cell and tissue types during *Drosophila* development. We show that the roles Dmel\TIP60 plays in the development of different tissues are diverse. These important findings will allow us and the other researchers to further investigate such TIP60 cell type specific roles in particular pathways in more detail. Furthermore, our Dmel\TIP60/RNAi, overexpression and rescue system will offer a powerful and unique tool to study epigenetic modes of gene regulation during development as well as serve as a multicellular drug screening tool to identify therapeutics for TIP60 associated disorders.

Chapter 4: Conclusion and Future Direction

The work eminating from my Ph.D. project has made a significant contribution to deciphering the roles of epigenetic regulation during multicellular multicellular development. I am the first to have cloned and characterized the TIP60 human homolog in *Drosophila*. Using this information, I have created a GAL4-UAS inducible Dmel\TIP60 transgenic fly system. This system is the first to allow for the induction of overexpression and knockdown of Dmel/TIP60 in a variety of specific tissues, cell types and developmental stages of choice, thus enabling me to explore the role of TIP60 in multicellular development (Zhu et al. 2007). Using this system, I am the first to demonstrate that Dmel\TIP60 is required for the differentiation of a variety of tissues that include wing, CNS and muscle during *Drosophila* development (unpublished data, Chapter 3). Thus, my studies on Dmel\TIP60 have shed new light on the important epigenetic regulatory roles that TIP60 plays in the development of a multicellular organism.

As the last is not the least, the first does not always mean the best. Although my Ph.D. work has opened a door to the new world of epigenetic study, further work is still needed to enhance our understanding of epigenetic regulation during development. Thus, I feel that future directions should entail research that reveals the

molecular mechanisms underlying the genetic phenotypes that I observed during my Ph.D. research. I feel that the most important and urgent questions to be answered in the future is identifying the specific target genes that are regulated by Dmel\TIP60 and how the proteins they encode interact with each other. Since our lab has been focusing on the study of relationships between epigenetic regulation and development, I would focus this question to understanding the mechanism(s) by which the HAT activity of Dmel\TIP60 regulates such genes during development. By using the GAL4-UAS inducible Dmel\TIP60 system that I have created, my strategies to address the above questions would be to utilize DNA microarrays and chromatin immunoprecipitation (Chip) assays for the genome-wide screening of Dmel\TIP60 regulated genes, and then choose several specific targets for further study. I will now outline my strategy to carry out these experiments.

First, I will use DNA microarrays to explore how many and which genes are directly and/or indirectly affected at mRNA level by Dmel\TIP60 knockdown Although an additional copy of Dmel\TIP60 did not induce any phenotypes it will serve as a control for the knockdown system. I will extract mRNA from third instar larvae that are ubiquitously expressing Dmel\TIP60/control and Dmel\TIP60/RNAi transgenes using the ubiquitous GAL4 driver 337. I have chosen the 3rd instar larval stage because our previous experiments showed that my transgenes are expressed at the highest expression level in this stage and hence the genes controlled by Dmel\TIP60 should be affected the most at this stage. Then the mRNA will be labeled

with fluorescent dyes and hybridized on DNA microarrays composed of *Drosophila* genes. The fluorescent signals on the DNA arrays will be detected by a confocal scanner. Finally, image and statistical analysis will determine the differences of gene expression profiles between the control and RNAi knockdown. I expect that several clusters of genes will be downregulated or upregulated by Dmel\TIP60/RNAi. Based on my results that Dmel\TIP60 is required for wing, muscle and CNS development, I would expect that the TIP60 genes that I will identify will be involved in developmental pathways critical for the development of these tissues. I would also like to identify specific TIP60 target genes that are exclusively regulated by the HAT activity of Dmel\TIP60. One of the easiest ways to achieve this goal is to use a dominant negative (DN) version of Dmel\TIP60 defective only in its HAT activity. Meredith Toth, a member of our laboratory, has recently generated such a TIP60 DN protein and her unpublished data demonstrates that expressing TIP60/DN protein using the ubiquitous *Act5-GAL4* driver leads to total lethity of the progeny during early stages of their development. Since the DN mutants carry a specific amino acid substituition in the catalytic region of the MYST HAT domain, her results suggest that the HAT activity of TIP60 is required for multicellular development. Thus, the same DNA microarray procedures as described above would be carried out using mRNA extracted from larvae expressing TIP60/DN proteins. Such experiments would enable me to identify Tip60 target genes exclusively affected by the HAT activity of Dmel\TIP60.

Several chromatin immunoprecipitation (ChIP) assays will next be applied to further narrow down the genes that are regulated by Dmel\TIP60. Instead of using the conventional Chip assays, I propose to use the state-of-art Chip-Seq (Sequence) assay, which combines high-throughput DNA sequencing and Chip. The advantage of Chip-Seq is that the data are read in absolute counts so that no normalization is necessary (Bock and Lengauer 2007; Euskirchen et al. 2007). Chip-Seq is also cost-efficient because of the new sequencing-by synthesis methods (e.g. Roche/454 (Margulies et al. 2005) and Illumina/Solexa (Barski et al. 2007)). The general procedure is as follows. Cell samples are treated with formaldehyde to cross-link DNA-bound proteins to DNA. The chromatin extract is then prepared and sheared into fragments (approximately 500 bp in length). The targeted fragments are enriched by incubating them with an antibody of choice. The DNA released from the immunoprecipitated protein-DNA complex is then purified and ready for sequencing and analysis.

As previously demonstrated, TIP60 can acetylate H2A, H3 and H4 (Yamamoto and Horikoshi 1997; Kimura and Horikoshi 1998; Ikura et al. 2000). To investigate the specificity of histone acetylation profiles created by TIP60 during development, I propose to use the antibodies that specifically recognize histone acetylation on H2A, H3 and H4 respectively for Chip-Seq. The chromatin will be extracted from the 3rd instar larvae of DmelTIP60/control and RNAi respectively. Compared to those of the controls, the data of RNAi samples will reveal most of gene

regions where the histone acetylation is reduced due to Dmel\TIP60 knockdown. As histone acetylation is generally related to the transcriptional activation, the genes identified by the Chip-Seq described above should overlap with the genes found in DNA microarrays using dominant negative mutants. However, the data generated by the Chip-Seq assay described above will be more specific because of the usage of antibodies against specific histone acetylation sites. It is worthwhile to note that a rough acetylation map could be relatively easy to obtain by immunochemical staining of the polytene chromatin in the 3rd instar larvae of DmelTIP60/control and RNAi respectively. This would be a backup or pilot experiment because the resolution obtained by such experiments would not be as high as through Chip-Seq.

Another Chip-Seq assay I would propose to carry out is one that would investigate how Dmel\TIP60 directly binds the regulatory regions of certain genes and controls their expressions during the development of specific tissue or cell types. For such experiments, I would use wild type flies (e.g. Canton S) and an antibody that directly recognizes Dmel\TIP60 (Kusch et al. 2004). My unpublished data revealed that Dmel\TIP60 is required for the development of wing, CNS and muscle. To identify the TIP60 regulated target genes that are required for these specific tissues, I will utilize new techniques that enable the isolation of chromatin from specific cell types. These techniches originated from the laboratory of Dr. Michelson at NIH (Estrada et al. 2006). To isolate chromatin from muscles cells, I will utilize the *twi-Gal4* to drive *UAS-GFP* specifically in the mesoderm. These embryos will be

gently disassociated to form a single cell suspension. The GFP expressing mesoderm cells will be separated and isolated by fluorescence activated cell sorting (FACS). I will then isolate chromatin from these mesoderm specific cells and utilize the Chip-Seq assay as described above. I anticipate that by using these methods, I will identify muscle specific target genes that are directly controlled by Dmel\TIP60 in muscle developmental pathway.

After the genome-wide screening, I would next carry out several conventional experiments to validate the data and further explore the function of such Dmel\TIP60 target genes. For example, real-time RT PCR, Western blots will be used to examine the expression levels of the genes. Immunochemistry assays in *Drosophila* embryos using Abs specific for the proteins that the Dmel\TIP60 target genes encode will provide the information on the localization of such proteins in the embryo. Finally, I expect to identify genes with both known and unknown functions. For the genes whose functions have been previously identifies, I would suggest to explore their positions in particular developmental pathways. I would then dissect the pathways genetically and biochemically to determine the detailed interactions between Dmel\TIP60 and the target genes they regulate. I believe that the most exciting findings would be discovering novel genes regulated by Dmel\TIP60, thus leading to new exciting future studies on their function. Thus, the perfect end of science is no end.

I believe that the significance of my studies on Dmel\TIP60 will not be

limited to expanding our knowledge on epigenetic regulation during development. I can foresee that the information obtained from my work could ultimately benefit the entire scientific society. For example, genetic variation in environmental studies is greatly influenced by epigenetic regulation (Fraga et al. 2005; Heijmans et al. 2007). Additionally, epigentics is believed to regulate the orthologous regions of different mammals that arise during evolution (Enard et al. 2004; Bernstein et al. 2005). Thus, there are many potential collaborations we can create to aid in understanding the role of epigenetic regulation in a variety of broad fields that include nutrition, ecology and evolution. Finally, epigenetic disorders have been associated with causing many different cancers, mental disorders, autoimmune diseases and other complex diseases (Bjornsson et al. 2004; Feinberg 2007). Thus, our studies on Dmel\TIP60 should ultimately lead to finding the innovative therapeutics for human diseases caused by misregulation of TIP60.

In summary, although I have completed my Ph.D. research project, I believe the important data generated by it and the ideas inspired by it will never be covered by the dust in history. My hope is that my doctoral research has enhanced the understanding of epigenetic regulation during development which in turn, will lead to the future discover of innovative and novel therapeutics for TIP60 associated human diseases as well as a better understanding of human beings themselves.

Chapter 5: Tables and Figures

Chapter 1

Tables

Table 1. Summary of known and putative HATs

HAT	Organisms known to have the HAT	Known transcription-related functions/effects	HAT activity demonstrated in vitro	Histone Specificty of recombinant enzyme in vitro
GNAT super family				
Hat1	Various (yeast to mammals)	None (histone deposition-related B-type HAT)	Yes	**H4**
Gcn5	Various (yeast to mammals)	Coactivator (adaptor)	Yes	**H3**/H4
P/CAF	Yeast to mammals	Coactivator	Yes	**H3**/H4
Elp3	Yeast to mammalss	Transcription elongation	Yes	ND
Hpa2	Yeast	unknown	Yes	**H3**/H4
MYST family				
Sas2	Yeast	Silencing	ND	
Sas3	Yeast	Silencing	Yes	**H3**/**H4**/H2A
Esa1/TIP60	**Various (yeast to mammals)**	Cell cycle progression	Yes	**H4**/H3/H2A
MOF	*Drosophila*	Dosage compensation	Yes	**H4**/H3/H2A

Table 1 *(Continued)*

MOZ	Mammals	Leukemogenesis, upon chromosomal translocation	ND	
MORF	Mammals	Unknown (strong homology to MOZ)	Yes	**H4/H3/H2A**
HBO1	Humans, *Drosophila* (Chameau)	ORC interaction	Yes	**ND**
P300/CBP	Various multicellular	Global coactivator	Yes	**H2A/H2B/H3/H4**
Nuclear receptor coactivators		Nuclear receptor coactivators		
SRC-1	Mammals	(transcriptional	Yes	**H3**/H4
ACTR	Mammals	response to hormone	Yes	**H3/H4**
TIF2	Mammals	signals)	ND	
TAFII250	Various (yeast to Mammals)		Yes	**H3**/H4
TFIIIC				
TFIIIC220	Mammals		Yes	ND
TFIIIC110	Mammals		Yes	ND
TFIIIC90	Mammals		Yes	**H3**
CIITA	Mammals		Yes	H4
ATF2	Mammals		Yes	H4/H2B
CDY				
CDY	Humans		Yes	H4
CDYL	Mammals	Histone-to-protamine transition during spermatogenesis	Yes	H4

Table 2. TIP60 complexes that show homology between yeast, human and *Drosophila* Adapted from (Doyon et al. 2004) (Kusch et al. 2004)

Yeast NuA4 Subunit	Human	*Drosophila*
Act1	Actin	Act87E
Arp4	BAF53a (BRG1-associated protein)	BAP55
Eaf1/Swr1	hDomino? (p400)	Domino
Eaf2	DMAP1 (DNMT1-associated protein)	dDMA
Eaf3	Mortality factor related genes (MORF4, MRG15/X)	dMrg15
Eaf5	?	
Eaf6	hEaf6 (FLJ11730)	dEaf6
Esa1	TIP60/TIP60b	Dmel\TIP60
Epl1	Enhancer of Polycomb (EPC1, EPC-like)	E(Pc)
Tra1	TRRAP	dTra1
Yaf9	YEATS family (AF9, ENL, GAS41)	dGas41
Yng2	Inhibitor of growth gene family (ING 1 to 5)	dIng3
Rvb1	Pontin	dPontin
Rvb2	Peptin	dPeptin
Eaf7	MrgBP	dMrgBP
Bdf1	Brd8/TRCp12	dBrd8
Vps72	YL-1	dYL-1
H2A/H2A.Z	H2A.X/H2A.Z	H2A.V
H2B	H2B	H2B

Figures

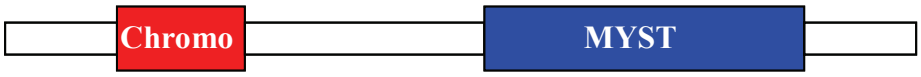


Figure 1. Schematic diagram of the conserved domains and their locations in TIP60. TIP60 is a ~60 KDa protein, containing (from left to right) an N-terminal chromodomain and a C-terminal MYST functional domain.

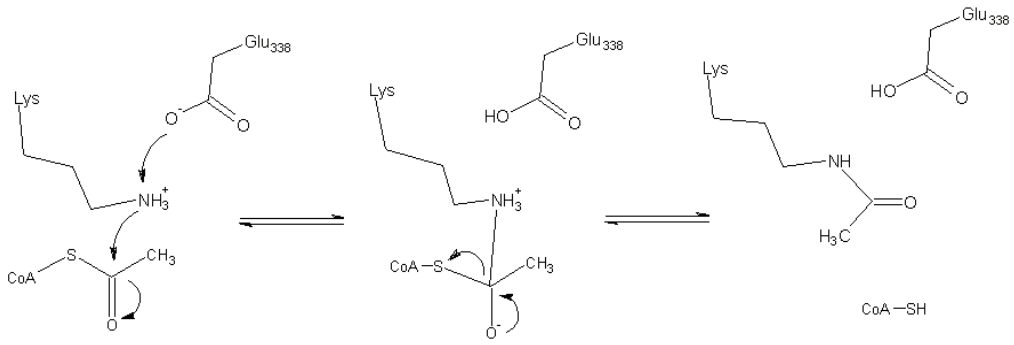


Figure 2. Sequential mechanism of ESA1 enzyme kinetics. Glu338 in ESA1 deprotonates the N-ε-lysine in the histone tails when the ternary complex of acetyl-CoA, histone substrate and ESA1 is formed. Then lysine attacks the carbonyl carbon on acetyl-CoA to form a tetrahedral intermediate. CoA-SH and acetylated histone tail are released.

Chapter 2

Tables

Table 1. Ubiquitous expression of Dmel\TIP60/RNAi in three independent fly lines results in total lethality of developing flies.

Fly lines[a]	GAL4^{+}(*y; Cy*$^{+}$)[b]	GAL4^{-}(*y*$^{+}$*; Cy*)[b]
Dmel\TIP60/RNAi/A	0±0	49±11
Dmel\TIP60/RNAi/B	0±0	53±12
Dmel\TIP60/RNAi/C	0±0	57±14
Dmel\TIP60/control/A	67±16	63±7
Dmel\TIP60/control/B	57±0	59±8
Dmel\TIP60/control/C	69±3	67±12

[a] Three flies homozygous for either Dmel\TIP60/RNAi or Dmel\TIP60/control P-element insertions were mated to three flies homozygous for the actin GAL4 driver line *Act5c-GAL4*: (Dmel\TIP60/RNAi or control x P{*Act5c*-GAL4}/*CyO,y*$^{+}$*).* For Dmel\TIP60/RNAi lines, the P element insertion is located on the X chromosome for line A and on the second chromosome for lines B and C. For Dmel\TIP60/control lines, the P-element insertion is located on the second chromosome for line A and on the X chromosome for lines B and C. [b] Adult progeny were counted over a ten day period and scored for either GAL4^{+}(*y;Cy*$^{+}$*)* or GAL4^{-}(*y*$^{+}$*;Cy*) phenotypes. All three Dmel\TIP60/RNAi lines strongly reduced viability to 0% that of the Dmel\TIP60/control lines. Lethality for the majority of flies occurred during pupal development. The results are reported as Mean ± SD (n=3).

Table 2. Mesoderm/muscle specific expression of Dmel\TIP60/RNAi in three independent fly lines results in a range of lethal effects during fly development.

Fly lines[a]	Adult[b]	Dead Pupae[b]
Dmel\TIP60/RNAi/A	0±0	113±26
Dmel\TIP60/RNAi/B	63±15	74±13
Dmel\TIP60/RNAi/C	46±5	101±10
Dmel\TIP60/control/A	120±19	1±1
Dmel\TIP60/control/B	179±40	1±1
Dmel\TIP60/control/C	173±14	2±1

[a] Three flies homozygous for either Dmel\TIP60/RNAi or Dmel\TIP60/control P-element insertions (for P-element chromosomal locations, see Table 1) were mated to three flies homozygous for the mesoderm/muscle GAL4 driver line 24B. [b] Progeny were counted over a ten day period and scored for either viable adults or dead pupae. To calculate the effect of RNAi on viability, viable progeny for each of the Dmel\TIP60/RNAi independent lines was divided by the total combined number of viable progeny for the three Dmel\TIP60/control lines. Independent insertions Dmel\TIP60/RNAi A, B and C reduced viability to 0, 40 and 29%, respectively that of the Dmel\TIP60/control lines. Lethality for the flies occurred during a broad range of developmental stages, beginning from early pupae to directly before fly eclosion. The results are reported as Mean ± SD (n=3).

Figures

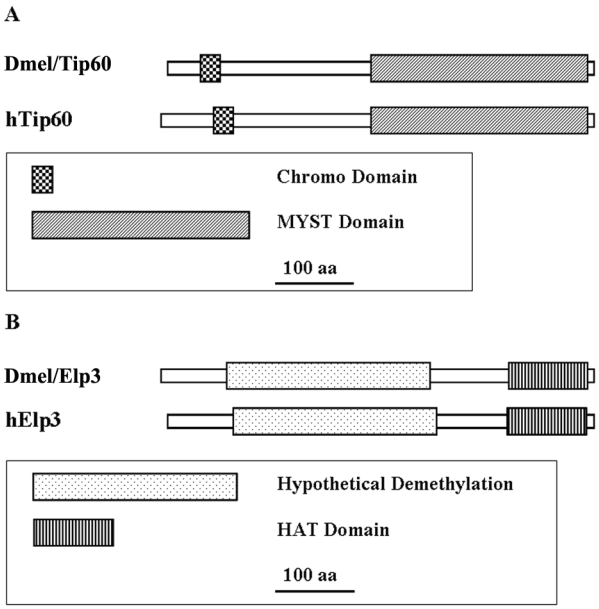


Figure 1. MYST family member Dmel\Tip60 and GNAT family member Dmel\Elp3 proteins are highly conserved with their human homolog counterparts. (A) Shown is a schematic representation (drawn to scale) of the conserved domains and their location within the Dmel\Tip60 and hTip60 proteins. Both proteins contain (from left to right): an N-terminal chromodomain and a C-terminal MYST functional domain. For Dmel\Tip60, the chromodomain is 70% identical/87% similar and the MYST domain is 80% identical/89% similar to hTip60. (B) Schematic representation (drawn to scale) of the conserved domains and their location within Dmel\Elp3 and hElp3 proteins. Both proteins contain an N-terminal putative histone demethylation domain and a C terminal HAT domain. For Dmel\Elp3, the putative histone demethylation domain is 88%identical/94%similar and the HAT domain is 85% identical/93% similar to hElp3. (Structural domains were obtained by CDART, NCBI).

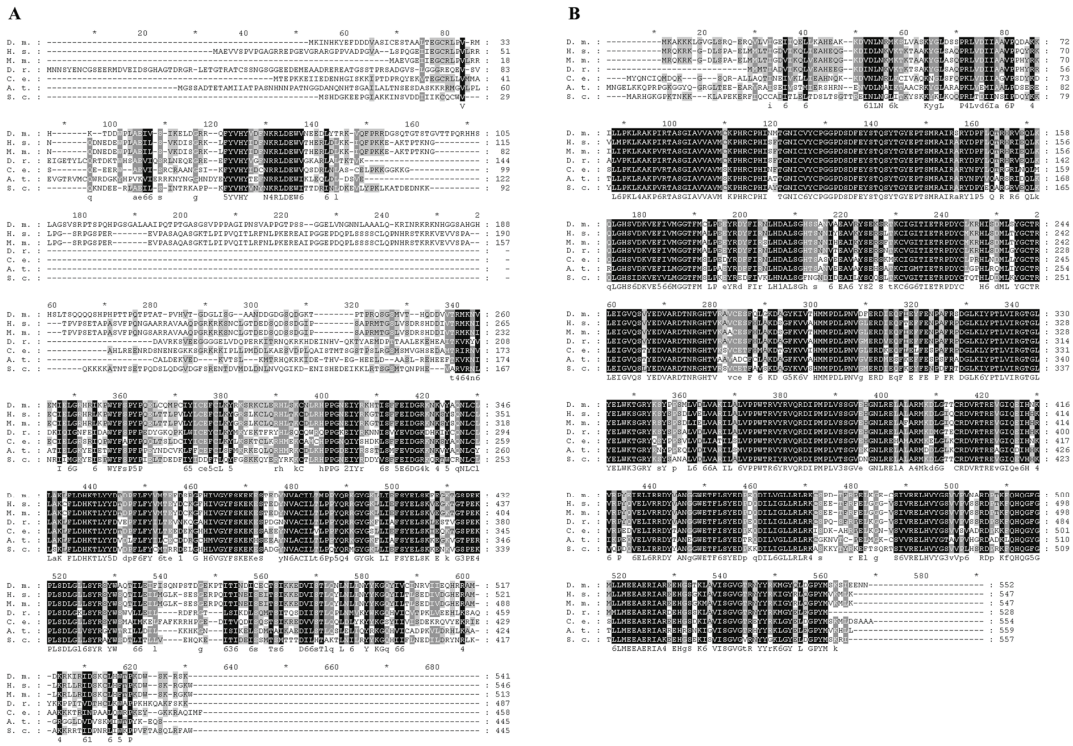


Figure 2. Dmel\Tip60 and Dmel\Elp3 are evolutionarily conserved among different species. Shown is the predicted amino acid sequences for the proteins encoded by (A) Dmel\Tip60 and (B) Dmel\Elp3 and their alignment with sequences encoded by ORFs from H.s., *Homo sapiens*; M.m., *Mus musculus*; D.r., *Danio rerio*; C.e., *Caenorhabditis elegans*; A.t., *Arabidopsis thaliana*; S.c., *Saccharomyces cerevisiae.* Interspecies homology ranges from 29%-56% (D.r. to M.m.) identity/41%-68% (D.r. to M.m.) similarity for Dmel\Tip60 and 70%-82% identity (A.t. to H.s.)/82%-92% (A.t. to H.s.) similarity for Dmel\Elp3 over their entire coding region. Black boxes and grey boxes represent identical and similar amino acids, respectively. Alignment was carried out by Genedoc.

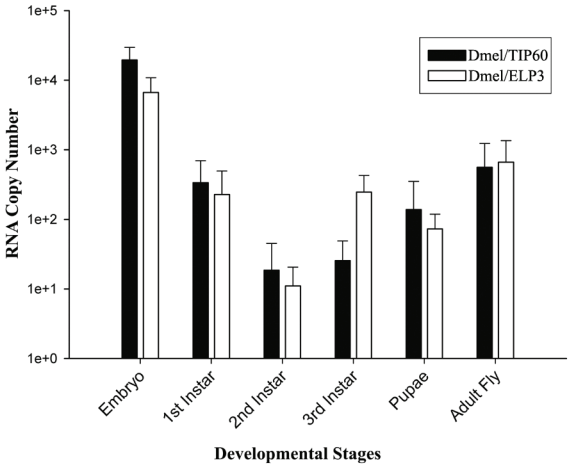


Figure 3. Dmel\TIP60 and Dmel\ELP3 are each differentially expressed during *Drosophila* development. Real time PCR analysis of Dmel\TIP60 and Dmel\ELP3 transcript levels using stage specific *Drosophila melanogaster* cDNAs (12-24 h staged embryos, first, second and third instar larvae, pupae, adult flies) prepared by RT priming of DNase treated RNA with random hexamers and PCR primer sets amplifying 200bp regions specific for each dHAT. Histogram depicts RNA copy number (Mean + SD) in logarithmic scale of at least three independent experiments for both Dmel\TIP60 and Dmel\ELP3 in each stage of development. SYBR-Green kit and Opticon^{TM}2 system (MJ Research) were used for real-time detection and data analysis. All data shown is corrected for –RT background.

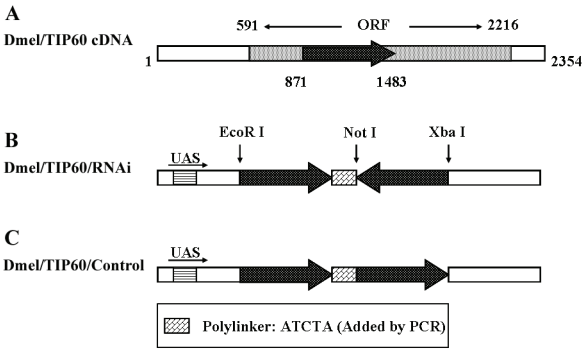


Figure 4. Structure of the pUAST Dmel\TIP60/RNAi and control constructs. (A) Schematic representation of the Dmel\TIP60 ORF. Black arrow represents the location of the 613 bp RNAi non-conserved target sequence chosen for use in creating the following constructs. (B) Schematic diagram of the Dmel\TIP60/RNAi construct. The 613 bp RNAi target cDNA sequence was amplified by PCR using the cDNA Dmel\TIP60 clone reported here as template, and cloned into a sense-antisense inverted gene arrangement into the inducible expression vector (pUAST) under the control of GAL4-UAS binding sites. A PCR generated polylinker and the common restriction site that joins the inverted cDNA fragments separates the cloned repeats and serves as the "hinge" region of the hairpin. (C) Schematic diagram of the Dmel\TIP60/control construct. The same RNAi cDNA target sequence was cloned into a sense- sense orientation and separated by the same short polylinker as described above.

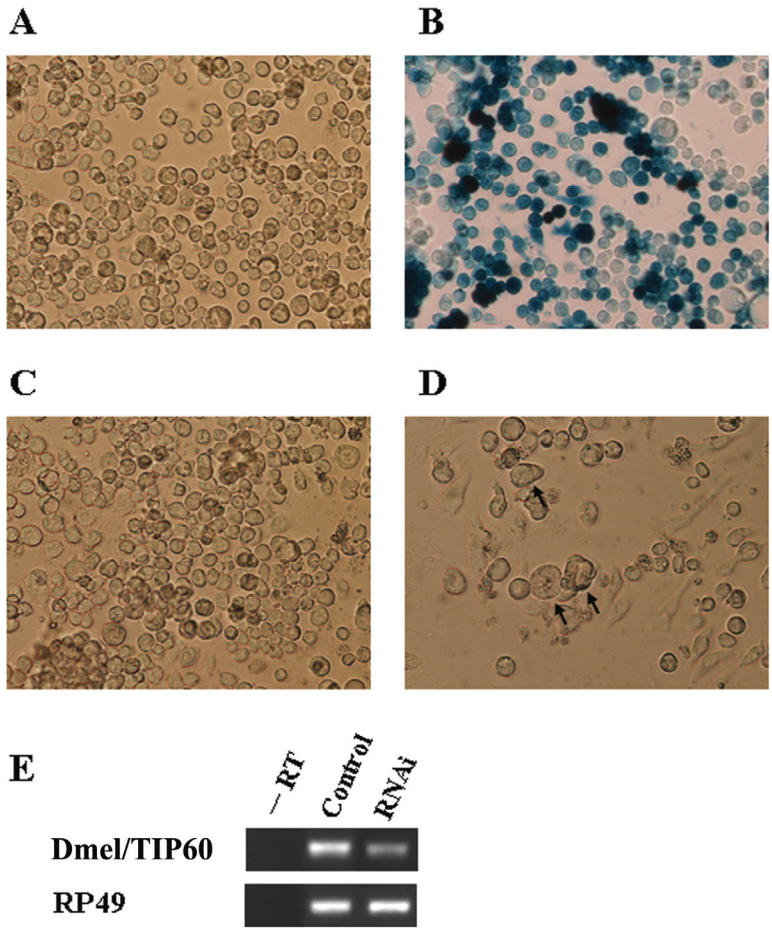


Figure 5. The transient transfection of D. Mel-2 cells with the Dmel\TIP60/RNAi construct results in deleterious effects on cell growth and reduction of endogenous Dmel\TIP60 transcript levels. Shown in panels A-D are D. Mel-2 cells visualized at 200X magnification using phase/contrast optics. (A) Cells transiently transfected with pAc5.1/V-5-His/LacZ (unstained). (B) Same cells as in A stained with X-Gal showing transfection efficiency at 77%. (C) Cells transiently transfected with Dmel\TIP60/control construct, shown 24 hours post-transfection. (D) Cells transiently transfected with Dmel\TIP60/RNAi construct, shown 24 hours post-transfection. Arrows point to morphologically defective cells. (E) Semi-quantitative RT-PCR analysis of Dmel\TIP60 and RP49 transcript levels. RNA was isolated from cells (shown above) 24 hours post-transfection. Equal amounts of RNA for each sample was subjected to cDNA preparation using RT priming with random hexamers and PCR using primer sets specific for Dmel\TIP60 that did not amplify RNAi target sequences and primer sets specific for RP49 internal control. All experiments shown were repeated at least 3 independent times with consistent results.

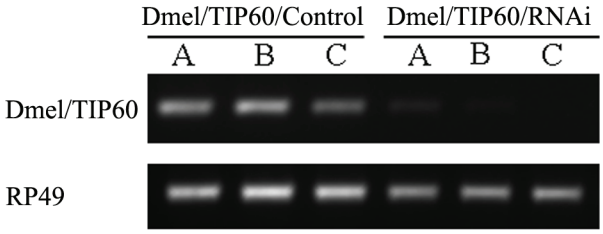


Figure 6. Expression of Dmel\TIP60/RNAi in three independent fly lines reduces endogenous Dmel\TIP60 levels. (A) Progeny resulting from a cross between homozygous Dmel\TIP60/RNAi (independent lines Dmel\TIP60/RNAi/A,B and C) or Dmel\TIP60/control (independent lines Dmel\TIP60/control/A,B and C) and ubiquitous GAL4 line 337 were allowed to develop to the third instar larval stage. RNA was isolated from three third instar larvae progeny and subjected to semi-quantitative RT-PCR analysis. cDNAs were obtained from equal amounts of RNA for each sample using RT priming with random hexamers. PCR primer sets were specific for either Dmel\TIP60 that did not amplify Dmel\TIP60 RNAi target sequences or RP49. All RT-PCR experiments included negative (-RT) controls for both Dmel\TIP60 and RP49 which showed no background in all samples tested (data not shown). All experiments were repeated at least twice with consistent results. This figure shows RT-PCR analysis of one representative experiment.

Chapter 3

Tables

Table 1. Ubiquitous expression[a] of Dmel\TIP60/OverEx in three independent fly lines results in the rescue of lethal effects caused by Dmel\TIP60/RNAi during fly development.

	Female[b]		Male[b]	
Fly lines	Tm3	Tm6	Tm3	Tm6
Rescue /A	20±5[c]	0±0	26±5[c]	32±9
Rescue /B	9±5[d]	0±0	29±8[d]	31±9
Rescue /C	16±11[e]	0±0	29±12[e]	29±13

a. The expression was induced by *337* ubiquitous GAL4 drvier.

b. Progeny were counted over a ten day period and scored for viable adults (n=3). The genotypes of female Tm3 and Tm6 are *337-GAL4>Tip60/RNAi/+; Tm3, Tip60/OverEX/+* and *337-GAL4> Tip60/RNAi/X; Tm6/+* respectively. The genotypes of male Tm3 and Tm6 are *337-GAL4> Tm3, Tip60/OverEX/+* and *337-GAL4>Tm6/+* respectively.

c. The full rescure was observed statistically as there is no signicant difference of the viability ($p > 0.05$).

d. The rescue was partial showing a statistical difference of the viability ($p < 0.05$).

e. The full rescure was observed statistically as there is no signicant difference of the viability ($p > 0.05$).

Figures

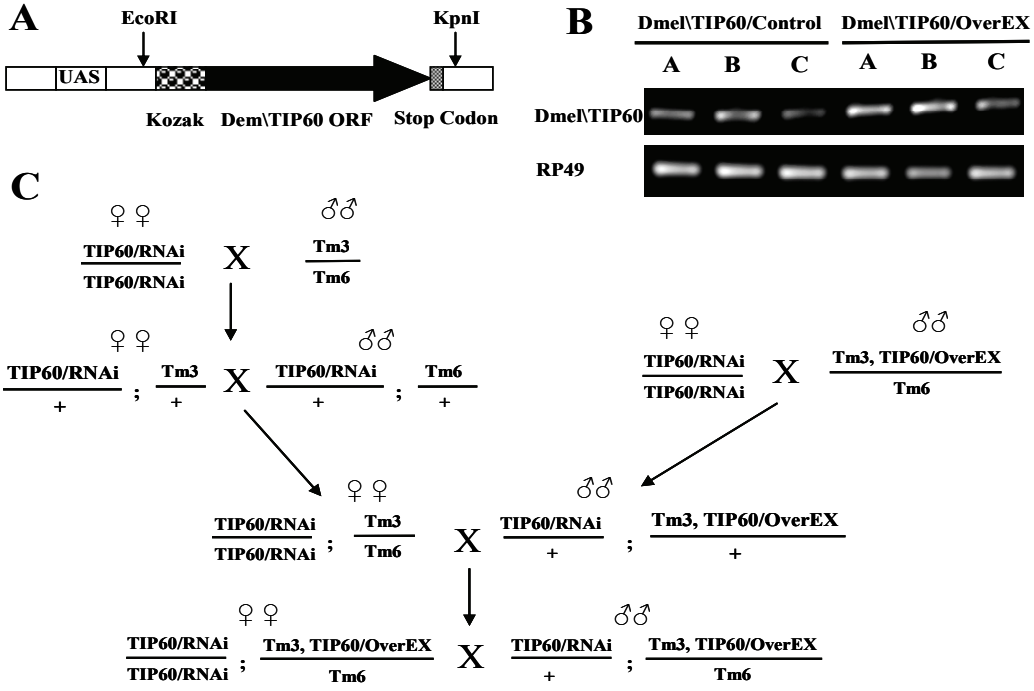


Figure 1. Determination of the specificity of Dmel\TIP60/RNAi knockdown system by rescue experiment. (A) Schematic diagram of the Dmel\TIP60/OverEx construct. This overexpression construct was created by subcloning Dmel\TIP60 ORF into pUAST vector between EcoRI and KpnI sites. Kozak consensus sequence was added at the 5' end and two stop codons were added at the 3' end by PCR using specific primers. (B) Expression of Dmel\TIP60/OverEx in three independent fly lines increases endogenous Dmel\TIP60 expression levels. Progeny resulting from a cross between homozygous Dmel\TIP60/OverEx (independent lines Dmel\TIP60/OverEx/A, B and C) or Dmel\TIP60/control (independent lines Dmel\TIP60/control/A, B and C) and ubiquitous GAL4 line 337 were allowed to develop to the third instar larval stage. RNA was isolated from three third instar larvae and subjected to semi-quantitative RT-PCR analysis. PCR primer sets were specific for either Dmel\TIP60 that did not amplify Dmel\TIP60 RNAi target sequences or RP49. All RT-PCR experiments included negative (-RT) controls for both Dmel\TIP60 and RP49 which showed no background in all samples tested (data not shown). This figure shows RT-PCR analysis of one representative experiment from at least twice repeats of the consistent results. (C) Scheme to make

Dmel\TIP60/Resue lines. The ebony homozygous Dmel\TIP60/RNAi fly line (*Tip60/RNAi / Tip60/RNAi; Tm3/Tm6*) was generated by crossing Dmel\TIP60/RNAi A to the fly line Tm3/Tm6. Meanwhile, Dmel\TIP60/RNAi A was crossed to three independent fly lines with Dmel\TIP60/OverEx on Tm3 so that to obtain three independent fly lines (males with *Tip60/RNAi / +; Tm3, Tip60/OverEX /* +) which is screened by the stubble phenotype (Tm3). The fly line *Tip60/RNAi* / *Tip60/RNAi*; Tm3/Tm6 females was crossed to three independent *Tip60/RNAi / +; Tm3, Tip60/OverEX /* + males to create three independent rescue fly lines with Dmel\TIP60/RNAi construct on X chromosome and Dmel\TIP60/OverEx construct on the 3rd chromosome (Tm3) which is screened by the dosage-dependent eye color due to *w*+ gene.

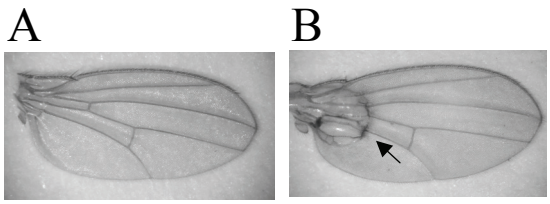


Figure 2. The survivors (23±9%) from the rescue experiment carry the blister phenotype. (A) and (B) are the wings of the Tm3 female progeny from the cross of *337* to the rescue C line. Thus their genotypes are both *337-GAL4>Tip60/RNAi / +; Tm3, Tip60/OverEX /+*. (A) appears to be normal, while (B) carries the blister as the arrow points out.

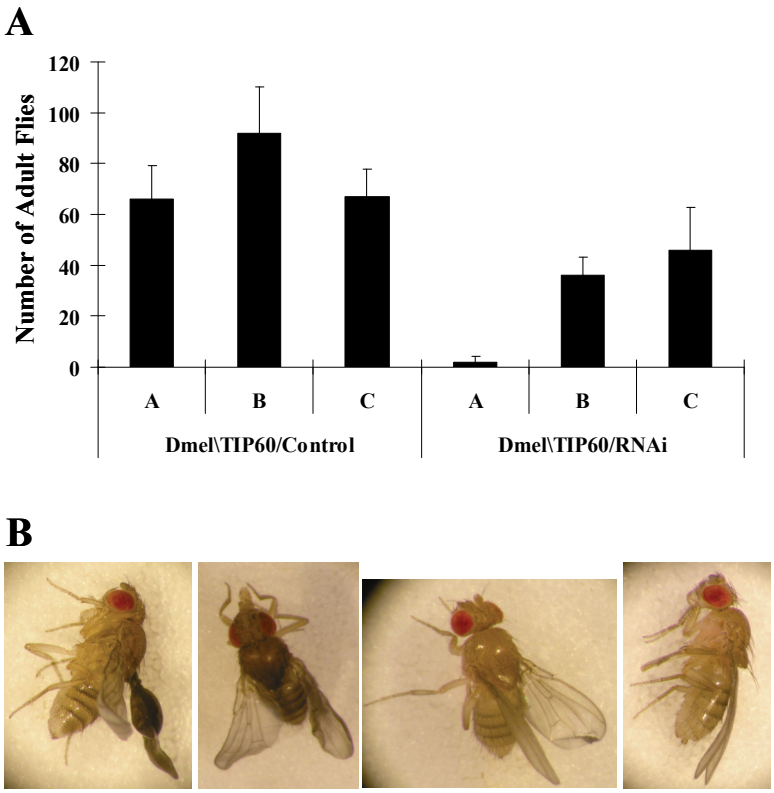


Figure 3. Induction of Dmel\TIP60/RNAi expression by GAL4 driver *69B* at 25 ºC results in lethality and a variety of wing phenotypes. (A) Three flies homozygous for either Dmel\TIP60/RNAi or Dmel\TIP60/control P-element insertions (for P-element chromosomal locations, see (Zhu et al. 2007)) were mated to three of GAL4 driver line *69B*. Progeny were counted over a ten day period and scored for either viable adults or dead pupae. To calculate the effect of RNAi on viability, viable progeny for each of the Dmel\TIP60/RNAi independent lines was divided by the total combined number of viable progeny for the three Dmel\TIP60/control lines. Independent insertions Dmel\TIP60/RNAi A, B and C reduced viability to 0, 48 and 61%, respectively that of the Dmel\TIP60/control lines. (B) shows the wing phenotypes in a descendent order of the severity of the phenotype. From the left, the first two flies are females *69B>Dmel\TIP60/RNAi A*; the third is female *69B>Dmel\TIP60/RNAi B*; The fourth is female from *69B>Dmel\TIP60/RNAi C*.

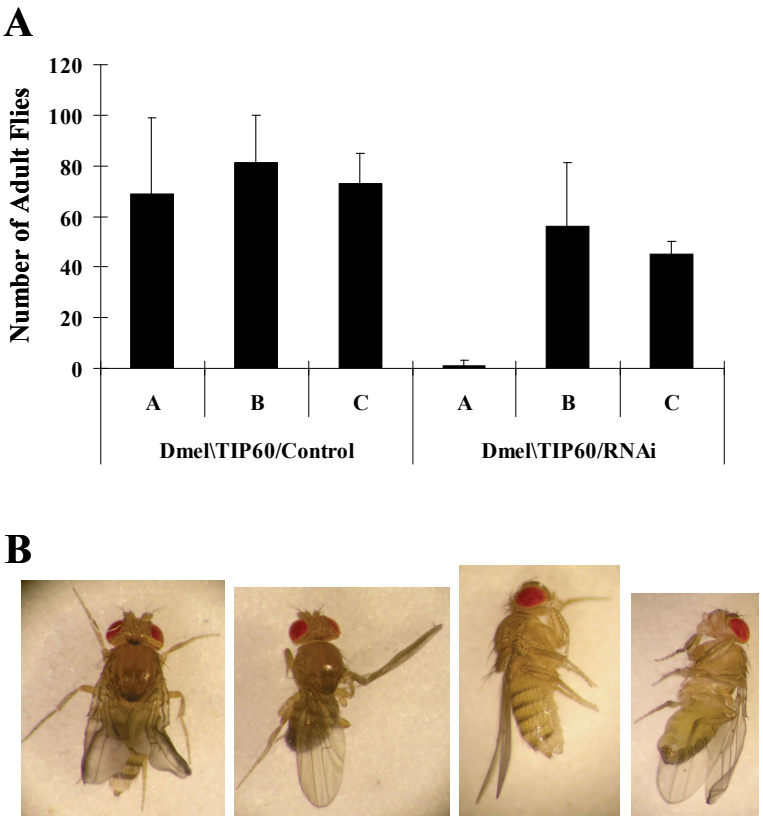


Figure 4. Induction of Dmel\TIP60/RNAi expression by GAL4 driver *32B* at 25 ºC results in lethality and a variety of wing phenotypes. (A) Three flies homozygous for either Dmel\TIP60/RNAi or Dmel\TIP60/control P-element insertions were mated to three of GAL4 driver line *32B*. Progeny were counted over a ten day period and scored for either viable adults or dead pupae. The viability was calculated as described in Figure 2. Independent insertions Dmel\TIP60/RNAi A, B and C reduced viability to 0, 76 and 61%, respectively that of the Dmel\TIP60/control lines. (B) shows the wing phenotypes in a descendent order of the severity of the phenotype. From the left, the first fly is male and the second is female, both from *32B>Dmel\TIP60/RNAi A*; the third is female *32B>Dmel\TIP60/RNAi B*; the fourth is male *32B>Dmel\TIP60/RNAi C*.

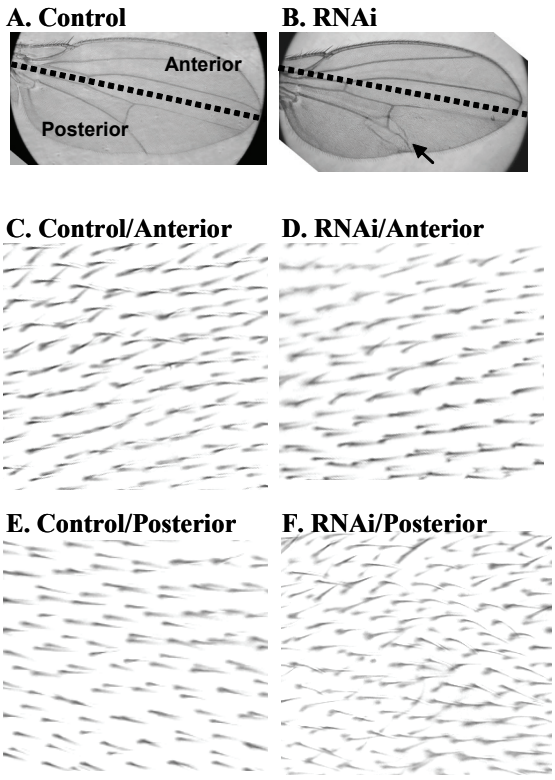


Figure 5. Wing phenotypes in the posterior compartment of the wing result from induction of Dmel\TIP60/RNAi in the posterior of the wing by GAL4 driver *en-GAL4*. The wings are from the progeny of crosses at 25 ºC of UAS P-element lines Dmel\TIP60/RNAi A or Dmel\TIP60/control C to GAL4 driver *en-GAL4*. Compared to Control (A), the blister phenotype (B, as the arrow points out) was only observed in the posterior of the wing in 49±20% of the *en-GAL4>Dmel\TIP60/RNAi A*. The planar cell polarity (PCP) was not affected in the anterior of the wing in both control and RNAi (C and D). But PCP was changed in the posterior of the wing in RNAi (F) compared to that of the control.

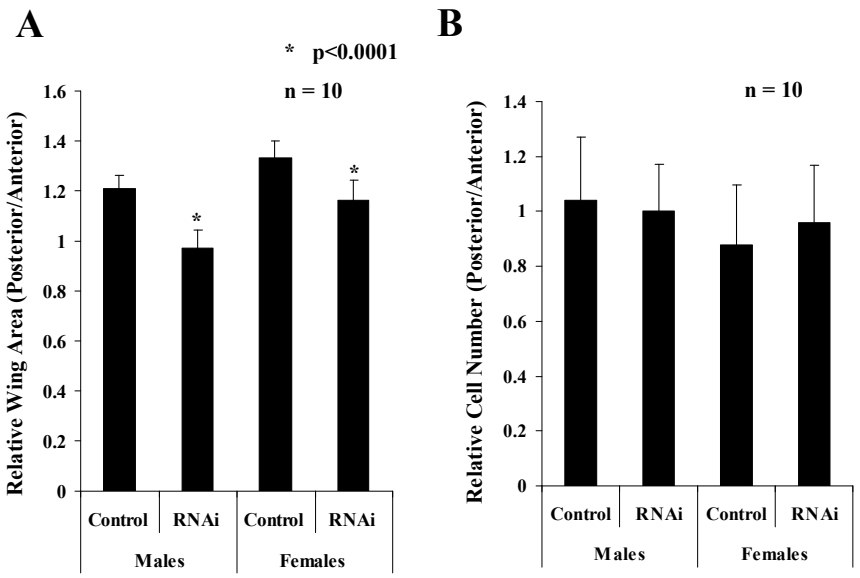


Figure 6. Induction of Dmel\TIP60/RNAi in the posterior of the wing by GAL4 driver *en-GAL4* reduces the wing area due to the decrease of the cell size. (A) The wing area in the posterior of both male and female wings was significantly reduced when Dmel\TIP60/RNAi A was induced in the posterior compartment of the wing by using *en-GAL4* compared to the control Dmel\TIP60/Control C. The pixels of anterior and posterior in each of 10 wings per genotype were measured by ImageJ 1.38X. The relative wing size was calculated by dividing the number of pixels corresponding to posterior of a wing by that of anterior. (B) The cell number in the posterior of both male and female wings remained unchanged when Dmel\TIP60/RNAi A was induced in the posterior compartment of the wing by using *en-GAL4* compared to the control Dmel\TIP60/Control C., suggesting that the reduction on wing area in RNAi is resulted from the reduction of cell size. The hair number of a 200 X 200 dpi squire (measured by Pixel Ruler) was counted in both anterior and posterior, since one cell of the wing has one hair (Meyer et al. 2000). Using 10 wings from each control and RNAi, the relative hair number was calculated by dividing the number of hairs corresponding to posterior of a wing by that of anterior.

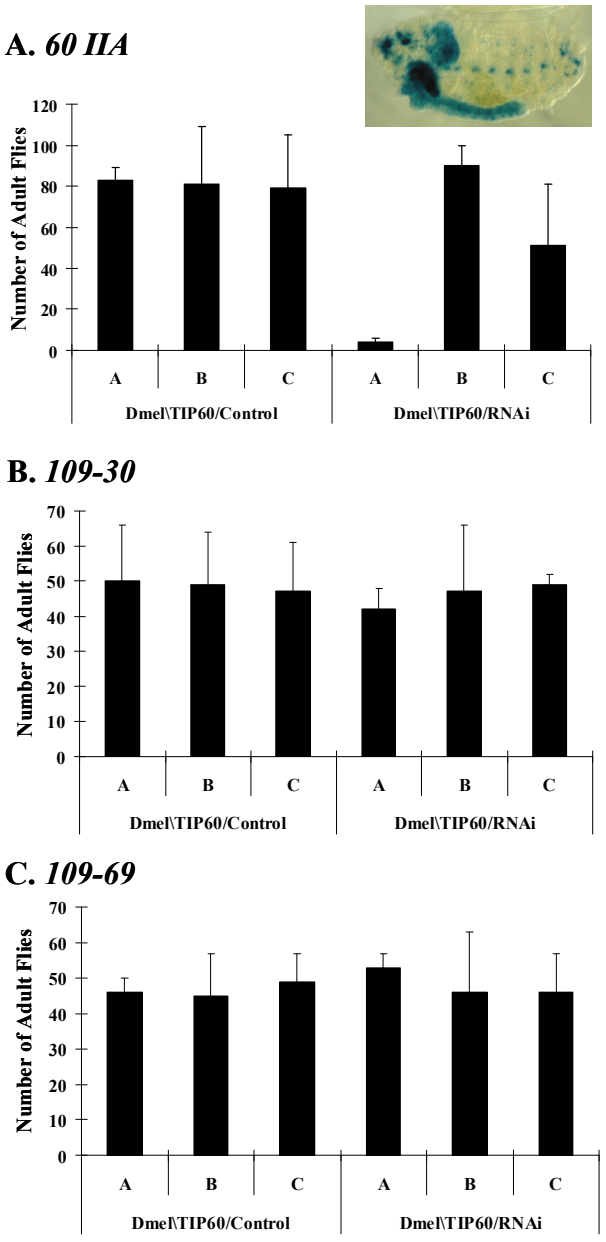


Figure 7. Expression of Dmel\TIP60/RNAi in central nervous system (CNS) at 25 ºC results in lethality. Three flies homozygous for either Dmel\TIP60/RNAi or Dmel\TIP60/control P-element insertions were mated to three of GAL4 driver *32B*. Progeny were counted over a ten day period and scored for either viable adults or dead pupae. The viability was calculated as described in Figure 2. (A) Induction of Dmel\TIP60/RNAi in CNS by GAL4 driver *60IIA* causes lethality. The top panel shows GAL4 expression in the nervous system driven by *60IIA* using X-Gal staining. Independent insertions Dmel\TIP60/RNAi A, B and C reduced viability to 0, 111 and

63%, respectively that of the Dmel\TIP60/control lines. (B) and (C) Viability was not affected by Dmel\TIP60/RNAi compared to the controls, when Dmel\TIP60/RNAi was induced by GAL4 drivers *109-30* and *109-69* respectively, which drive GAL4 expression in the subsets of peripheral nervous system.

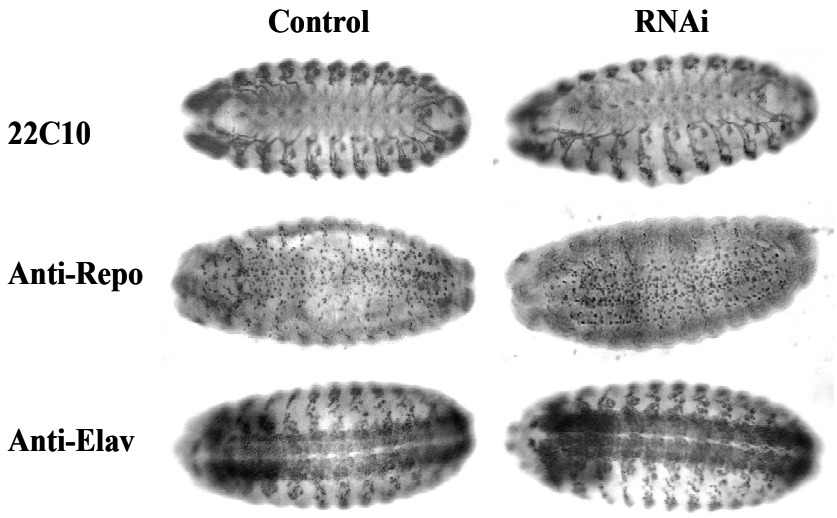


Figure 8. Expression of Dmel\TIP60/RNAi in the brains at the 15th embryonic stage by GAL4 driver *60IIA* at 25 ºC shows no apparent defect phenotypes. The embryos were collected from *60IIA>Dmel\TIP60/RNAi A* and *60IIA>Dmel\TIP60/Control C* respectively and then stained by using the primary antibody mouse 22C10, anti-Repo, and anti-Elav respectively.

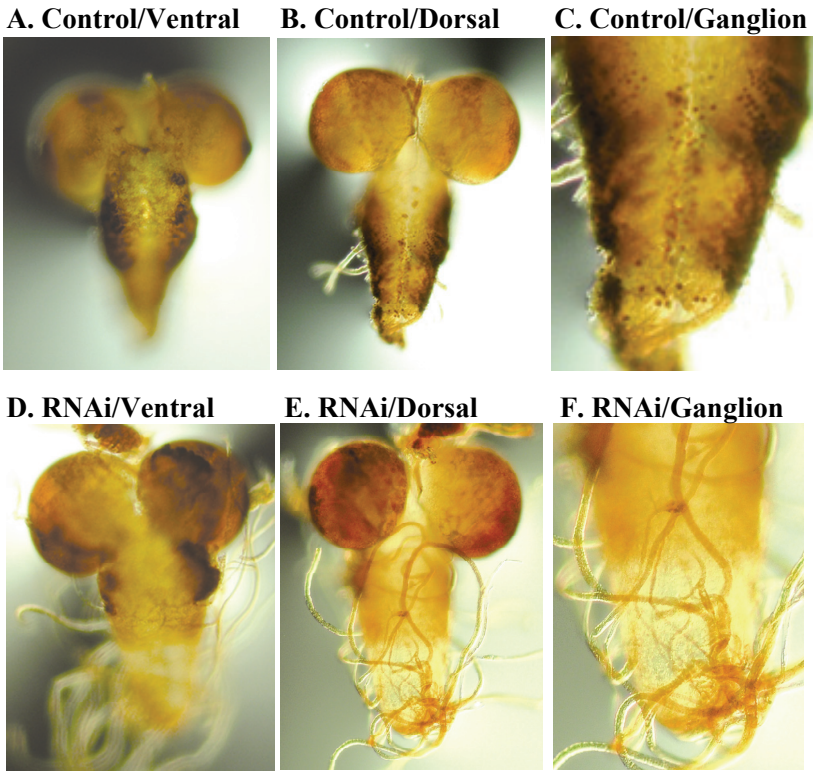


Figure 9. Expression of Dmel\TIP60/RNAi in the brains at the 3rd instar larva stage by GAL4 driver *60IIA* at 25 ºC causes loss of differentiated neurons in the ganglion. The 3rd instar larvae brains were collected from *60IIA>Dmel\TIP60/RNAi A* and *60IIA>Dmel\TIP60/Control C* respectively and then stained by using the primary antibody mouse anti-Elav. (A) Ventral view of *60IIA>Dmel\TIP60/Control C* brain, which showed the symmetrical signals and linkage between two sides. (B) Dorsal view of *60IIA>Dmel\TIP60/Control C* brain, which showed the symmetrical signals made of stripes of dots representative of differentiated neurons in the ganglion (magnified in C). (D) and (E) Ventral and Dorsal view of *60IIA>Dmel\TIP60/RNAi A* brain respectively, showing the absence of the signals compared to the control (A and B), which is magnified in (F).

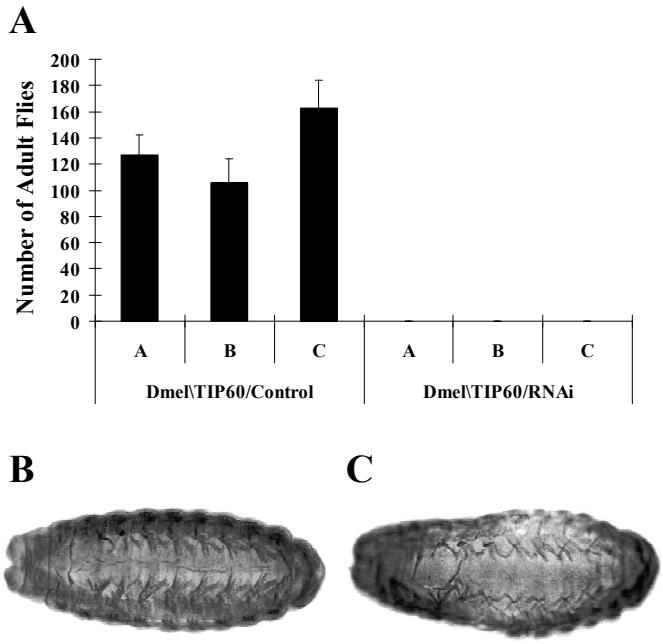
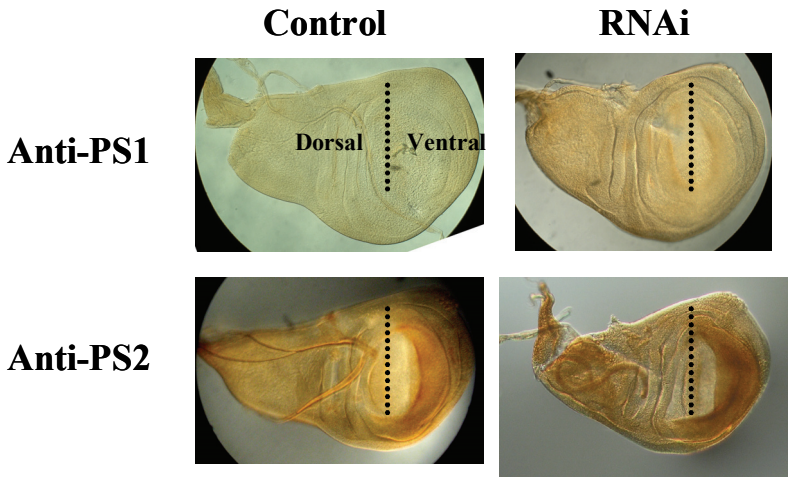
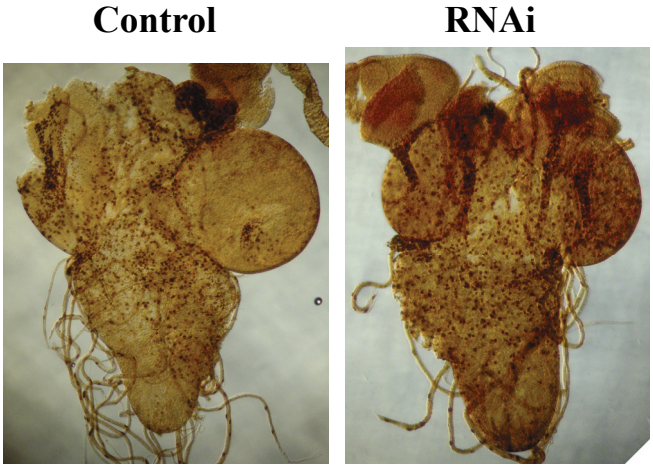


Figure 10. Induction of Dmel\TIP60/RNAi expression by muscle GAL4 driver *c179* at 25 ºC results in lethality and muscle disorder. Three flies homozygous for either Dmel\TIP60/RNAi or Dmel\TIP60/control P-element insertions were mated to three of GAL4 driver line *c179* at 25 ºC. Progeny were counted over a ten day period and scored for either viable adults or dead pupae. The viability was calculated as described in Figure 2. (A) 0% viability was observed in all three independent Dmel\TIP60/RNAi lines. Compared to that of *C179>Dmel\TIP60/Control C* as shown in (B), the embryo shape of *C179>Dmel\TIP60/RNAi A* was affected and the muscle fibers in the embryo of *C179>Dmel\TIP60/RNAi A* became thinner as shown in (C).

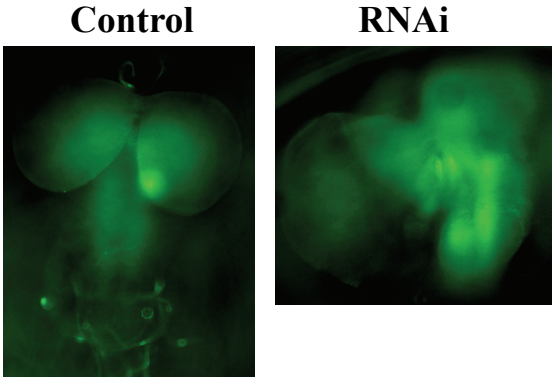
Supplement Figure 1. Induction of Dmel\TIP60/RNAi in the posterior of the wing by GAL4 driver *en*-GAL4 does not affect apoptosis. The 3rd instar larvae wing discs were dissected from the progeny of crosses at 25 ºC of UAS P-element lines Dmel\TIP60/RNAi A or Dmel\TIP60/control C to GAL4 driver *en-GAL4*. The TUNEL assay was applied to the wing discs after fixation.



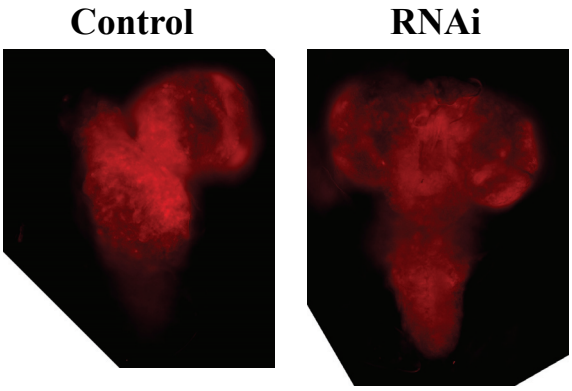
Supplement Figure 2. Induction of Dmel\TIP60/RNAi in the posterior of the wing by GAL4 driver *en*-GAL4 does not affect the expression pattern of integrin PS1 and PS2. The 3rd instar larvae wing discs were dissected from the progeny of crosses at 25 ºC of UAS P-element lines Dmel\TIP60/RNAi A or Dmel\TIP60/control C to GAL4 driver *en-GAL4*. Then the wing discs were stained with primary antibody mouse anti-PS1 and anti-PS2 respectively.



Supplement Figure 3. Induction of Dmel\TIP60/RNAi in the brains at the 3rd instar larval stage does not affect the glial cells. The 3rd instar larvae brains were collected from *60IIA>Dmel\TIP60/RNAi A* and *60IIA>Dmel\TIP60/Control C* respectively and then stained by using the primary antibody mouse anti-Repo.



Supplement Figure 4. Induction of Dmel\TIP60/RNAi in the brains at the 3rd instar larval stage does not affect apoptosis. The 3rd instar larvae brains were dissected from the progeny of crosses at 25 ºC of UAS P-element lines Dmel\TIP60/RNAi A or Dmel\TIP60/control C to GAL4 driver *60IIA*. The TUNEL assay was applied to the brains after fixation.



Supplement Figure 5. Induction of Dmel\TIP60/RNAi in the brains at the 3rd instar larval stage does not affect the expression pattern of cyclin E. The 3rd instar larvae brains were collected from *60IIA>Dmel\TIP60/RNAi A* and *60IIA>Dmel\TIP60/Control C* respectively and then stained by using the primary antibody mouse anti-cyclin E.

List of References

1999. The FlyBase database of the *Drosophila* Genome Projects and community literature. The FlyBase Consortium. *Nucleic Acids Res* 27(1): 85-88.

Abraham, R.T. 2001. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 15(17): 2177-2196.

Adhikary, S. and Eilers, M. 2005. Transcriptional regulation and transformation by Myc proteins. *Nature reviews* 6(8): 635-645.

Aguirre-Arteta, A.M., Grunewald, I., Cardoso, M.C., and Leonhardt, H. 2000. Expression of an alternative Dnmt1 isoform during muscle differentiation. *Cell Growth Differ* 11(10): 551-559.

Ait-Si-Ali, S., Guasconi, V., Fritsch, L., Yahi, H., Sekhri, R., Naguibneva, I., Robin, P., Cabon, F., Polesskaya, A., and Harel-Bellan, A. 2004. A Suv39h-dependent mechanism for silencing S-phase genes in differentiating but not in cycling cells. *The EMBO journal* 23(3): 605-615.

Ait-Si-Ali, S., Polesskaya, A., Filleur, S., Ferreira, R., Duquet, A., Robin, P., Vervish, A., Trouche, D., Cabon, F., and Harel-Bellan, A. 2000. CBP/p300 histone acetyl-transferase activity is important for the G1/S transition. *Oncogene* 19(20): 2430-2437.

Ait-Si-Ali, S., Ramirez, S., Barre, F.X., Dkhissi, F., Magnaghi-Jaulin, L., Girault, J.A., Robin, P., Knibiehler, M., Pritchard, L.L., Ducommun, B., Trouche, D., and Harel-Bellan, A. 1998. Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature* 396(6707): 184-186.

Akhtar, A. and Becker, P.B. 2000. Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Mol Cell* 5(2): 367-375.

Akhtar, A., Zink, D., and Becker, P.B. 2000. Chromodomains are protein-RNA interaction modules. *Nature* 407(6802): 405-409.

Akimaru, H., Chen, Y., Dai, P., Hou, D.X., Nonaka, M., Smolik, S.M., Armstrong, S., Goodman, R.H., and Ishii, S. 1997. *Drosophila* CBP is a co-activator of cubitus interruptus in hedgehog signalling. *Nature* 386(6626): 735-738.

Alarcon, J.M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E.R., and Barco, A. 2004. Chromatin acetylation, memory, and LTP are impaired in CBP+/- mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. *Neuron* 42(6): 947-959.

Alekseyenko, A.A., Larschan, E., Lai, W.R., Park, P.J., and Kuroda, M.I. 2006. High-resolution ChIP-chip analysis reveals that the *Drosophila* MSL complex selectively identifies active genes on the male X chromosome. *Genes Dev* 20(7): 848-857.

Alland, L., Muhle, R., Hou, H., Jr., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R.A. 1997. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. *Nature* 387(6628): 49-55.

Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L., Workman, J.L., and Cote, J. 1999. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *The EMBO journal* 18(18): 5108-5119.

Allegrucci, C., Thurston, A., Lucas, E., and Young, L. 2005. Epigenetics and the germline. *Reproduction* 129(2): 137-149.

Baek, S.H., Ohgi, K.A., Rose, D.W., Koo, E.H., Glass, C.K., and Rosenfeld, M.G. 2002. Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kappaB and beta-amyloid precursor protein. *Cell* 110(1): 55-67.

Bai, X., Alekseyenko, A.A., and Kuroda, M.I. 2004. Sequence-specific targeting of MSL complex regulates transcription of the roX RNA genes. *The EMBO journal* 23(14): 2853-2861.

Bakkenist, C.J. and Kastan, M.B. 2003. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 421(6922): 499-506.

-. 2004. Initiating cellular stress responses. *Cell* 118(1): 9-17.

Ballas, N., Battaglioli, E., Atouf, F., Andres, M.E., Chenoweth, J., Anderson, M.E., Burger, C., Moniwa, M., Davie, J.R., Bowers, W.J., Federoff, H.J., Rose, D.W., Rosenfeld, M.G., Brehm, P., and Mandel, G. 2001. Regulation of neuronal traits by a novel transcriptional complex. *Neuron* 31(3): 353-365.

Ballas, N., Grunseich, C., Lu, D.D., Speh, J.C., and Mandel, G. 2005. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 121(4): 645-657.

Ballas, N. and Mandel, G. 2005. The many faces of REST oversee epigenetic programming of neuronal genes. *Curr Opin Neurobiol* 15(5): 500-506.

Bannister, A.J. and Kouzarides, T. 1996. The CBP co-activator is a histone acetyltransferase. *Nature* 384(6610): 641-643.

Barak, Y., Juven, T., Haffner, R., and Oren, M. 1993. mdm2 expression is induced by wild type p53 activity. *The EMBO journal* 12(2): 461-468.

Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* 129(4): 823-837.

Beisel, C., Imhof, A., Greene, J., Kremmer, E., and Sauer, F. 2002. Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. *Nature* 419(6909): 857-862.

Bendall, A.J. and Abate-Shen, C. 2000. Roles for Msx and Dlx homeoproteins in vertebrate development. *Gene* 247(1-2): 17-31.

Bendall, A.J., Ding, J., Hu, G., Shen, M.M., and Abate-Shen, C. 1999. Msx1 antagonizes the myogenic activity of Pax3 in migrating limb muscle precursors. *Development (Cambridge, England)* 126(22): 4965-4976.

Berger, C., Pallavi, S.K., Prasad, M., Shashidhara, L.S., and Technau, G.M. 2005. A critical role for cyclin E in cell fate determination in the central nervous system of *Drosophila* melanogaster. *Nat Cell Biol* 7(1): 56-62.

Berger, S.L. 2001. An embarrassment of niches: the many covalent modifications of histones in transcriptional regulation. *Oncogene* 20(24): 3007-3013.

-. 2002. Histone modifications in transcriptional regulation. *Curr Opin Genet Dev* 12(2): 142-148.

Berndsen, C.E., Albaugh, B.N., Tan, S., and Denu, J.M. 2007. Catalytic mechanism of a MYST family histone acetyltransferase. *Biochemistry* 46(3): 623-629.

Berns, K., Hijmans, E.M., Mullenders, J., Brummelkamp, T.R., Velds, A., Heimerikx, M., Kerkhoven, R.M., Madiredjo, M., Nijkamp, W., Weigelt, B., Agami, R., Ge, W., Cavet, G., Linsley, P.S., Beijersbergen, R.L., and Bernards, R. 2004. A large-scale RNAi screen in human cells identifies new components of the p53 pathway. *Nature* 428(6981): 431-437.

Bernstein, B.E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D.K., Huebert, D.J., McMahon, S., Karlsson, E.K., Kulbokas, E.J., 3rd, Gingeras, T.R., Schreiber, S.L., and Lander, E.S. 2005. Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* 120(2): 169-181.

Bertrand, N., Castro, D.S., and Guillemot, F. 2002. Proneural genes and the specification of neural cell types. *Nat Rev Neurosci* 3(7): 517-530.

Bird, A. 1992. The essentials of DNA methylation. *Cell* 70(1): 5-8.

-. 2002. DNA methylation patterns and epigenetic memory. *Genes Dev* 16(1): 6-21.

Bird, A.P. 1986. CpG-rich islands and the function of DNA methylation. *Nature* 321(6067): 209-213.

Bird, A.W., Yu, D.Y., Pray-Grant, M.G., Qiu, Q., Harmon, K.E., Megee, P.C., Grant, P.A., Smith, M.M., and Christman, M.F. 2002. Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* 419(6905): 411-415.

Bjornsson, H.T., Fallin, M.D., and Feinberg, A.P. 2004. An integrated epigenetic and genetic approach to common human disease. *Trends Genet* 20(8): 350-358.

Blais, A. and Dynlacht, B.D. 2007. E2F-associated chromatin modifiers and cell cycle control. *Curr Opin Cell Biol* 19(6): 658-662.

Bock, C. and Lengauer, T. 2007. Computational Epigenetics. *Bioinformatics*.

Bone, J.R., Lavender, J., Richman, R., Palmer, M.J., Turner, B.M., and Kuroda, M.I. 1994. Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes Dev* 8(1): 96-104.

Bottomley, M.J. 2004. Structures of protein domains that create or recognize histone modifications. *EMBO Rep* 5(5): 464-469.

Bouchard, C., Dittrich, O., Kiermaier, A., Dohmann, K., Menkel, A., Eilers, M., and Luscher, B. 2001. Regulation of cyclin D2 gene expression by the Myc/Max/Mad network: Myc-dependent TRRAP recruitment and histone acetylation at the cyclin D2 promoter. *Genes Dev* 15(16): 2042-2047.

Boudreault, A.A., Cronier, D., Selleck, W., Lacoste, N., Utley, R.T., Allard, S., Savard, J., Lane, W.S., Tan, S., and Cote, J. 2003. Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev* 17(11): 1415-1428.

Boxer, L.M. and Dang, C.V. 2001. Translocations involving c-myc and c-myc function. *Oncogene* 20(40): 5595-5610.

Bracken, A.P., Dietrich, N., Pasini, D., Hansen, K.H., and Helin, K. 2006. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev* 20(9): 1123-1136.

Brady, M.E., Ozanne, D.M., Gaughan, L., Waite, I., Cook, S., Neal, D.E., and Robson, C.N. 1999. Tip60 is a nuclear hormone receptor coactivator. *J Biol Chem* 274(25): 17599-17604.

Brand, A.H. and Perrimon, N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development (Cambridge, England)* 118(2): 401-415.

Brand, M., Moggs, J.G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F.J., Stevenin, J., Almouzni, G., and Tora, L. 2001. UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation. *The EMBO journal* 20(12): 3187-3196.

Brody, T., Stivers, C., Nagle, J., and Odenwald, W.F. 2002. Identification of novel *Drosophila* neural precursor genes using a differential embryonic head cDNA screen. *Mechanisms of development* 113(1): 41-59.

Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S., and Workman, J.L. 2001. Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science (New York, NY* 292(5525): 2333-2337.

Brown, C.E., Lechner, T., Howe, L., and Workman, J.L. 2000. The many HATs of transcription coactivators. *Trends Biochem Sci* 25(1): 15-19.

Brown, N.H. 1993. Integrins hold *Drosophila* together. *Bioessays* 15(6): 383-390.

Brownell, J.E. and Allis, C.D. 1996. Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr Opin Genet Dev* 6(2): 176-184.

Bruce, A.W., Donaldson, I.J., Wood, I.C., Yerbury, S.A., Sadowski, M.I., Chapman, M., Gottgens, B., and Buckley, N.J. 2004. Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes. *Proceedings of the National Academy of Sciences of the United States of America* 101(28): 10458-10463.

Brunk, B.P., Goldhamer, D.J., and Emerson, C.P., Jr. 1996. Regulated demethylation of the myoD distal enhancer during skeletal myogenesis. *Developmental biology* 177(2): 490-503.

Buscaino, A., Kocher, T., Kind, J.H., Holz, H., Taipale, M., Wagner, K., Wilm, M., and Akhtar, A. 2003. MOF-regulated acetylation of MSL-3 in the *Drosophila* dosage compensation complex. *Mol Cell* 11(5): 1265-1277.

Cai, Y., Jin, J., Tomomori-Sato, C., Sato, S., Sorokina, I., Parmely, T.J., Conaway, R.C., and Conaway, J.W. 2003. Identification of new subunits of the multiprotein mammalian TRRAP/TIP60-containing histone acetyltransferase complex. *J Biol Chem* 278(44): 42733-42736.

Camarda, G., Siepi, F., Pajalunga, D., Bernardini, C., Rossi, R., Montecucco, A., Meccia, E., and Crescenzi, M. 2004. A pRb-independent mechanism preserves the postmitotic state in terminally differentiated skeletal muscle cells. *J Cell Biol* 167(3): 417-423.

Cao, R., Tsukada, Y., and Zhang, Y. 2005. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. *Mol Cell* 20(6): 845-854.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S., and Zhang, Y. 2002. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science (New York, NY* 298(5595): 1039-1043.

Cao, R. and Zhang, Y. 2004. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. *Curr Opin Genet Dev* 14(2): 155-164.

Cao, X. and Sudhof, T.C. 2001. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science (New York, NY* 293(5527): 115-120.

-. 2004. Dissection of amyloid-beta precursor protein-dependent transcriptional transactivation. *J Biol Chem* 279(23): 24601-24611.

Caretti, G., Di Padova, M., Micales, B., Lyons, G.E., and Sartorelli, V. 2004. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes Dev* 18(21): 2627-2638.

Caron, C., Boyault, C., and Khochbin, S. 2005. Regulatory cross-talk between lysine acetylation and ubiquitination: role in the control of protein stability. *Bioessays* 27(4): 408-415.

Carrozza, M.J., Utley, R.T., Workman, J.L., and Cote, J. 2003. The diverse functions of histone acetyltransferase complexes. *Trends Genet* 19(6): 321-329.

Cavusoglu, N., Brand, M., Tora, L., and Van Dorsselaer, A. 2003. Novel subunits of the TATA binding protein free TAFII-containing transcription complex identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry following one-dimensional gel electrophoresis. *Proteomics* 3(2): 217-223.

Ceol, C.J. and Horvitz, H.R. 2004. A new class of C. elegans synMuv genes implicates a Tip60/NuA4-like HAT complex as a negative regulator of Ras signaling. *Dev Cell* 6(4): 563-576.

Chadwick, B.P. and Willard, H.F. 2001. A novel chromatin protein, distantly related to histone H2A, is largely excluded from the inactive X chromosome. *J Cell Biol* 152(2): 375-384.

-. 2003. Chromatin of the Barr body: histone and non-histone proteins associated with or excluded from the inactive X chromosome. *Hum Mol Genet* 12(17): 2167-2178.

Chambers, A.E., Banerjee, S., Chaplin, T., Dunne, J., Debernardi, S., Joel, S.P., and Young, B.D. 2003. Histone acetylation-mediated regulation of genes in leukaemic cells. *Eur J Cancer* 39(8): 1165-1175.

Chau, B.N. and Wang, J.Y. 2003. Coordinated regulation of life and death by RB. *Nat Rev Cancer* 3(2): 130-138.

Chavous, D.A., Jackson, F.R., and O'Connor, C.M. 2001. Extension of the *Drosophila* lifespan by overexpression of a protein repair methyltransferase. *Proceedings of the National Academy of Sciences of the United States of America* 98(26): 14814-14818.

Chen, S.L., Dowhan, D.H., Hosking, B.M., and Muscat, G.E. 2000. The steroid receptor coactivator, GRIP-1, is necessary for MEF-2C-dependent gene expression and skeletal muscle differentiation. *Genes Dev* 14(10): 1209-1228.

Chen, S.L., Loffler, K.A., Chen, D., Stallcup, M.R., and Muscat, G.E. 2002. The coactivator-associated arginine methyltransferase is necessary for muscle differentiation: CARM1 coactivates myocyte enhancer factor-2. *J Biol Chem* 277(6): 4324-4333.

Chen, Z.F., Paquette, A.J., and Anderson, D.J. 1998. NRSF/REST is required in vivo for repression of multiple neuronal target genes during embryogenesis. *Nature genetics* 20(2): 136-142.

Cheung, P. and Lau, P. 2005. Epigenetic regulation by histone methylation and histone variants. *Mol Endocrinol* 19(3): 563-573.

Chien, S., Reiter, L.T., Bier, E., and Gribskov, M. 2002. Homophila: human disease gene cognates in *Drosophila*. *Nucleic Acids Res* 30(1): 149-151.

Clarke, A.S., Lowell, J.E., Jacobson, S.J., and Pillus, L. 1999. Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Molecular and cellular biology* 19(4): 2515-2526.

Clevers, H. 2004. Wnt breakers in colon cancer. *Cancer Cell* 5(1): 5-6.

Close, P., Hawkes, N., Cornez, I., Creppe, C., Lambert, C.A., Rogister, B., Siebenlist, U., Merville, M.P., Slaugenhaupt, S.A., Bours, V., Svejstrup, J.Q., and Chariot, A. 2006. Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. *Mol Cell* 22(4): 521-531.

Cohen-Armon, M., Visochek, L., Katzoff, A., Levitan, D., Susswein, A.J., Klein, R., Valbrun, M., and Schwartz, J.H. 2004. Long-term memory requires polyADP-ribosylation. *Science (New York, NY* 304(5678): 1820-1822.

Cole, M.D. and Nikiforov, M.A. 2006. Transcriptional activation by the Myc oncoprotein. *Curr Top Microbiol Immunol* 302: 33-50.

Collins, R.T., Furukawa, T., Tanese, N., and Treisman, J.E. 1999. Osa associates with the Brahma chromatin remodeling complex and promotes the activation of some target genes. *The EMBO journal* 18(24): 7029-7040.

Cooke, N., Ho, Y., Shewchuk, B., Kimura, A., Elefant, F., Cajiao, I., and Liebhaber, S. 2004. Distinct epigenetic pathways activate the human growth hormone genes in pituitary and placenta. *12th International Congress of Endocrinology, Medimond International Proceedings*: 79-85.

Copps, K., Richman, R., Lyman, L.M., Chang, K.A., Rampersad-Ammons, J., and Kuroda, M.I. 1998. Complex formation by the *Drosophila* MSL proteins: role of the MSL2 RING finger in protein complex assembly. *The EMBO journal* 17(18): 5409-5417.

Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A., and Pirrotta, V. 2002. *Drosophila* enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* 111(2): 185-196.

de la Serna, I.L., Carlson, K.A., and Imbalzano, A.N. 2001. Mammalian SWI/SNF complexes promote MyoD-mediated muscle differentiation. *Nature genetics* 27(2): 187-190.

Dechend, R., Hirano, F., Lehmann, K., Heissmeyer, V., Ansieau, S., Wulczyn, F.G., Scheidereit, C., and Leutz, A. 1999. The Bcl-3 oncoprotein acts as a bridging factor between NF-kappaB/Rel and nuclear co-regulators. *Oncogene* 18(22): 3316-3323.

Deckert, J. and Struhl, K. 2001. Histone acetylation at promoters is differentially affected by specific activators and repressors. *Molecular and cellular biology* 21(8): 2726-2735.

Delaval, K. and Feil, R. 2004. Epigenetic regulation of mammalian genomic imprinting. *Curr Opin Genet Dev* 14(2): 188-195.

Dellino, G.I., Schwartz, Y.B., Farkas, G., McCabe, D., Elgin, S.C., and Pirrotta, V. 2004. Polycomb silencing blocks transcription initiation. *Mol Cell* 13(6): 887-893.

Deran, M., Pulvino, M., Greene, E., Su, C., and Zhao, J. 2007. Transcriptional activation of histone genes requires NPAT-dependent recruitment of TRRAP-Tip60 complex to histone promoters during the G1/S phase transition. *Molecular and cellular biology*.

Dillon, N. 2006. Gene regulation and large-scale chromatin organization in the nucleus. *Chromosome Res* 14(1): 117-126.

Dilworth, F.J., Seaver, K.J., Fishburn, A.L., Htet, S.L., and Tapscott, S.J. 2004. In vitro transcription system delineates the distinct roles of the coactivators pCAF and p300 during MyoD/E47-dependent transactivation. *Proceedings of the National Academy of Sciences of the United States of America* 101(32): 11593-11598.

Dimitri, P., Corradini, N., Rossi, F., and Verni, F. 2005. The paradox of functional heterochromatin. *Bioessays* 27(1): 29-41.

Dorn, R., Szidonya, J., Korge, G., Sehnert, M., Taubert, H., Archoukieh, E., Tschiersch, B., Morawietz, H., Wustmann, G., Hoffmann, G., and et al. 1993. P transposon-induced dominant enhancer mutations of position-effect variegation in *Drosophila* melanogaster. *Genetics* 133(2): 279-290.

Downs, J.A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., Kron, S.J., Jackson, S.P., and Cote, J. 2004. Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol Cell* 16(6): 979-990.

Doyon, Y. and Cote, J. 2004. The highly conserved and multifunctional NuA4 HAT complex. *Curr Opin Genet Dev* 14(2): 147-154.

Doyon, Y., Selleck, W., Lane, W.S., Tan, S., and Cote, J. 2004. Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. *Molecular and cellular biology* 24(5): 1884-1896.

Dunaief, J.L., Strober, B.E., Guha, S., Khavari, P.A., Alin, K., Luban, J., Begemann, M., Crabtree, G.R., and Goff, S.P. 1994. The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* 79(1): 119-130.

Eberl, D.F., Duyf, B.J., and Hilliker, A.J. 1993. The role of heterochromatin in the expression of a heterochromatic gene, the rolled locus of *Drosophila* melanogaster. *Genetics* 134(1): 277-292.

Ebert, A., Schotta, G., Lein, S., Kubicek, S., Krauss, V., Jenuwein, T., and Reuter, G. 2004. Su(var) genes regulate the balance between euchromatin and heterochromatin in *Drosophila*. *Genes Dev* 18(23): 2973-2983.

Eckner, R., Yao, T.P., Oldread, E., and Livingston, D.M. 1996. Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. *Genes Dev* 10(19): 2478-2490.

Edwards, C.A. and Ferguson-Smith, A.C. 2007. Mechanisms regulating imprinted genes in clusters. *Curr Opin Cell Biol* 19(3): 281-289.

Eissenberg, J.C., James, T.C., Foster-Hartnett, D.M., Hartnett, T., Ngan, V., and Elgin, S.C. 1990. Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila* melanogaster. *Proceedings of the National Academy of Sciences of the United States of America* 87(24): 9923-9927.

Elefant, F., Cooke, N.E., and Liebhaber, S.A. 2000a. Targeted recruitment of histone acetyltransferase activity to a locus control region. *J Biol Chem* 275(18): 13827-13834.

Elefant, F. and Palter, K.B. 1999. Tissue-specific expression of dominant negative mutant *Drosophila* HSC70 causes developmental defects and lethality. *Mol Biol Cell* 10(7): 2101-2117.

Elefant, F., Su, Y., Liebhaber, S.A., and Cooke, N.E. 2000b. Patterns of histone acetylation suggest dual pathways for gene activation by a bifunctional locus control region. *The EMBO journal* 19(24): 6814-6822.

Enard, W., Fassbender, A., Model, F., Adorjan, P., Paabo, S., and Olek, A. 2004. Differences in DNA methylation patterns between humans and chimpanzees. *Curr Biol* 14(4): R148-149.

Engel, N., West, A.G., Felsenfeld, G., and Bartolomei, M.S. 2004. Antagonism between DNA hypermethylation and enhancer-blocking activity at the H19 DMD is uncovered by CpG mutations. *Nature genetics* 36(8): 883-888.

Engemann, S., Strodicke, M., Paulsen, M., Franck, O., Reinhardt, R., Lane, N., Reik, W., and Walter, J. 2000. Sequence and functional comparison in the Beckwith-Wiedemann region: implications for a novel imprinting centre and extended imprinting. *Hum Mol Genet* 9(18): 2691-2706.

Estrada, B., Choe, S.E., Gisselbrecht, S.S., Michaud, S., Raj, L., Busser, B.W., Halfon, M.S., Church, G.M., and Michelson, A.M. 2006. An integrated strategy for analyzing the unique developmental programs of different myoblast subtypes. *PLoS Genet* 2(2): e16.

Euskirchen, G.M., Rozowsky, J.S., Wei, C.L., Lee, W.H., Zhang, Z.D., Hartman, S., Emanuelsson, O., Stolc, V., Weissman, S., Gerstein, M.B., Ruan, Y., and Snyder, M. 2007. Mapping of transcription factor binding regions in mammalian cells by ChIP: comparison of array- and sequencing-based technologies. *Genome Res* 17(6): 898-909.

Eymin, B., Claverie, P., Salon, C., Leduc, C., Col, E., Brambilla, E., Khochbin, S., and Gazzeri, S. 2006. p14ARF activates a Tip60-dependent and p53-independent ATM/ATR/CHK pathway in response to genotoxic stress. *Molecular and cellular biology* 26(11): 4339-4350.

Faiola, F., Liu, X., Lo, S., Pan, S., Zhang, K., Lymar, E., Farina, A., and Martinez, E. 2005. Dual regulation of c-Myc by p300 via acetylation-dependent control of Myc protein turnover and coactivation of Myc-induced transcription. *Molecular and cellular biology* 25(23): 10220-10234.

Faiola, F., Wu, Y.T., Pan, S., Zhang, K., Farina, A., and Martinez, E. 2007. Max is acetylated by p300 at several nuclear localization residues. *Biochem J* 403(3): 397-407.

Feinberg, A.P. 2007. Phenotypic plasticity and the epigenetics of human disease. *Nature* 447(7143): 433-440.

Feinberg, A.P., Cui, H., and Ohlsson, R. 2002. DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms. *Seminars in cancer biology* 12(5): 389-398.

Felsenfeld, G. and Groudine, M. 2003. Controlling the double helix. *Nature* 421(6921): 448-453.

Fernandez, L.A., Winkler, M., and Grosschedl, R. 2001. Matrix attachment region-dependent function of the immunoglobulin mu enhancer involves histone acetylation at a distance without changes in enhancer occupancy. *Molecular and cellular biology* 21(1): 196-208.

Fischle, W., Wang, Y., and Allis, C.D. 2003. Histone and chromatin cross-talk. *Curr Opin Cell Biol* 15(2): 172-183.

Fortier, E. and Belote, J.M. 2000. Temperature-dependent gene silencing by an expressed inverted repeat in *Drosophila*. *Genesis* 26(4): 240-244.

Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C., and Esteller, M. 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences of the United States of America* 102(30): 10604-10609.

Francis, N.J., Saurin, A.J., Shao, Z., and Kingston, R.E. 2001. Reconstitution of a functional core polycomb repressive complex. *Mol Cell* 8(3): 545-556.

Frank, S.R., Parisi, T., Taubert, S., Fernandez, P., Fuchs, M., Chan, H.M., Livingston, D.M., and Amati, B. 2003. MYC recruits the TIP60 histone acetyltransferase complex to chromatin. *EMBO Rep* 4(6): 575-580.

Frank, S.R., Schroeder, M., Fernandez, P., Taubert, S., and Amati, B. 2001. Binding of c-Myc to chromatin mediates mitogen-induced acetylation of histone H4 and gene activation. *Genes Dev* 15(16): 2069-2082.

Fulco, M., Schiltz, R.L., Iezzi, S., King, M.T., Zhao, P., Kashiwaya, Y., Hoffman, E., Veech, R.L., and Sartorelli, V. 2003. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. *Mol Cell* 12(1): 51-62.

Fuster, J.J., Sanz-Gonzalez, S.M., Moll, U.M., and Andres, V. 2007. Classic and novel roles of p53: prospects for anticancer therapy. *Trends in molecular medicine* 13(5): 192-199.

Fyrberg, E.A., Bernstein, S.I., and VijayRaghavan, K. 1994. Basic methods for *Drosophila* muscle biology. *Methods Cell Biol* 44: 237-258.

Galarneau, L., Nourani, A., Boudreault, A.A., Zhang, Y., Heliot, L., Allard, S., Savard, J., Lane, W.S., Stillman, D.J., and Cote, J. 2000. Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Mol Cell* 5(6): 927-937.

Gangaraju, V.K. and Bartholomew, B. 2007. Mechanisms of ATP dependent chromatin remodeling. *Mutat Res* 618(1-2): 3-17.

Gaughan, L., Brady, M.E., Cook, S., Neal, D.E., and Robson, C.N. 2001. Tip60 is a co-activator specific for class I nuclear hormone receptors. *J Biol Chem* 276(50): 46841-46848.

Gaughan, L., Logan, I.R., Cook, S., Neal, D.E., and Robson, C.N. 2002. Tip60 and histone deacetylase 1 regulate androgen receptor activity through changes to the acetylation status of the receptor. *J Biol Chem* 277(29): 25904-25913.

Gaughan, L., Logan, I.R., Neal, D.E., and Robson, C.N. 2005. Regulation of androgen receptor and histone deacetylase 1 by Mdm2-mediated ubiquitylation. *Nucleic Acids Res* 33(1): 13-26.

Gause, M., Eissenberg, J.C., Macrae, A.F., Dorsett, M., Misulovin, Z., and Dorsett, D. 2006. Nipped-A, the Tra1/TRRAP subunit of the *Drosophila* SAGA and Tip60 complexes, has multiple roles in Notch signaling during wing development. *Molecular and cellular biology* 26(6): 2347-2359.

Gavaravarapu, S. and Kamine, J. 2000. Tip60 inhibits activation of CREB protein by protein kinase A. *Biochemical and biophysical research communications* 269(3): 758-766.

Geiman, T.M., Sankpal, U.T., Robertson, A.K., Zhao, Y., Zhao, Y., and Robertson, K.D. 2004. DNMT3B interacts with hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system. *Biochemical and biophysical research communications* 318(2): 544-555.

Gilfillan, G.D., Straub, T., de Wit, E., Greil, F., Lamm, R., van Steensel, B., and Becker, P.B. 2006. Chromosome-wide gene-specific targeting of the *Drosophila* dosage compensation complex. *Genes Dev* 20(7): 858-870.

Goldhamer, D.J., Brunk, B.P., Faerman, A., King, A., Shani, M., and Emerson, C.P., Jr. 1995. Embryonic activation of the myoD gene is regulated by a highly conserved distal control element. *Development (Cambridge, England)* 121(3): 637-649.

Gorrini, C., Squatrito, M., Luise, C., Syed, N., Perna, D., Wark, L., Martinato, F., Sardella, D., Verrecchia, A., Bennett, S., Confalonieri, S., Cesaroni, M., Marchesi, F., Gasco, M., Scanziani, E., Capra, M., Mai, S., Nuciforo, P., Crook, T., Lough, J., and Amati, B. 2007. Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* 448(7157): 1063-1067.

Granok, H., Leibovitch, B.A., Shaffer, C.D., and Elgin, S.C. 1995. Chromatin. Ga-ga over GAGA factor. *Curr Biol* 5(3): 238-241.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L., and Workman, J.L. 1997. Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev* 11(13): 1640-1650.

Grant, P.A., Schieltz, D., Pray-Grant, M.G., Steger, D.J., Reese, J.C., Yates, J.R., 3rd, and Workman, J.L. 1998a. A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* 94(1): 45-53.

Grant, P.A., Sterner, D.E., Duggan, L.J., Workman, J.L., and Berger, S.L. 1998b. The SAGA unfolds: convergence of transcription regulators in chromatin-modifying complexes. *Trends in cell biology* 8(5): 193-197.

Grienenberger, A., Miotto, B., Sagnier, T., Cavalli, G., Schramke, V., Geli, V., Mariol, M.C., Berenger, H., Graba, Y., and Pradel, J. 2002. The MYST domain acetyltransferase Chameau functions in epigenetic mechanisms of transcriptional repression. *Curr Biol* 12(9): 762-766.

Guan, Z., Giustetto, M., Lomvardas, S., Kim, J.H., Miniaci, M.C., Schwartz, J.H., Thanos, D., and Kandel, E.R. 2002. Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. *Cell* 111(4): 483-493.

Guo, K., Wang, J., Andres, V., Smith, R.C., and Walsh, K. 1995. MyoD-induced expression of p21 inhibits cyclin-dependent kinase activity upon myocyte terminal differentiation. *Molecular and cellular biology* 15(7): 3823-3829.

Gupta, A., Sharma, G.G., Young, C.S., Agarwal, M., Smith, E.R., Paull, T.T., Lucchesi, J.C., Khanna, K.K., Ludwig, T., and Pandita, T.K. 2005. Involvement of human MOF in ATM function. *Molecular and cellular biology* 25(12): 5292-5305.

Hake, S.B., Xiao, A., and Allis, C.D. 2004. Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br J Cancer* 90(4): 761-769.

Halevy, O., Novitch, B.G., Spicer, D.B., Skapek, S.X., Rhee, J., Hannon, G.J., Beach, D., and Lassar, A.B. 1995. Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD. *Science (New York, NY* 267(5200): 1018-1021.

Halkidou, K., Gnanapragasam, V.J., Mehta, P.B., Logan, I.R., Brady, M.E., Cook, S., Leung, H.Y., Neal, D.E., and Robson, C.N. 2003. Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene* 22(16): 2466-2477.

Halkidou, K., Logan, I.R., Cook, S., Neal, D.E., and Robson, C.N. 2004. Putative involvement of the histone acetyltransferase Tip60 in ribosomal gene transcription. *Nucleic Acids Res* 32(5): 1654-1665.

Harikrishnan, K.N., Chow, M.Z., Baker, E.K., Pal, S., Bassal, S., Brasacchio, D., Wang, L., Craig, J.M., Jones, P.L., Sif, S., and El-Osta, A. 2005. Brahma links the SWI/SNF chromatin-remodeling complex with MeCP2-dependent transcriptional silencing. *Nature genetics* 37(3): 254-264.

Hark, A.T., Schoenherr, C.J., Katz, D.J., Ingram, R.S., Levorse, J.M., and Tilghman, S.M. 2000. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature* 405(6785): 486-489.

Hass, M.R. and Yankner, B.A. 2005. A {gamma}-secretase-independent mechanism of signal transduction by the amyloid precursor protein. *J Biol Chem* 280(44): 36895-36904.

Hawkes, N.A., Otero, G., Winkler, G.S., Marshall, N., Dahmus, M.E., Krappmann, D., Scheidereit, C., Thomas, C.L., Schiavo, G., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. 2002. Purification and characterization of the human elongator complex. *J Biol Chem* 277(4): 3047-3052.

Hebbes, T.R., Clayton, A.L., Thorne, A.W., and Crane-Robinson, C. 1994. Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. *The EMBO journal* 13(8): 1823-1830.

Heijmans, B.T., Kremer, D., Tobi, E.W., Boomsma, D.I., and Slagboom, P.E. 2007. Heritable rather than age-related environmental and stochastic factors dominate variation in DNA methylation of the human IGF2/H19 locus. *Hum Mol Genet* 16(5): 547-554.

Hendrich, B. and Bird, A. 1998. Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Molecular and cellular biology* 18(11): 6538-6547.

Herceg, Z., Hulla, W., Gell, D., Cuenin, C., Lleonart, M., Jackson, S., and Wang, Z.Q. 2001. Disruption of Trrap causes early embryonic lethality and defects in cell cycle progression. *Nature genetics* 29(2): 206-211.

Herceg, Z., Li, H., Cuenin, C., Shukla, V., Radolf, M., Steinlein, P., and Wang, Z.Q. 2003. Genome-wide analysis of gene expression regulated by the HAT cofactor Trrap in conditional knockout cells. *Nucleic Acids Res* 31(23): 7011-7023.

Hlubek, F., Lohberg, C., Meiler, J., Jung, A., Kirchner, T., and Brabletz, T. 2001. Tip60 is a cell-type-specific transcriptional regulator. *Journal of biochemistry* 129(4): 635-641.

Ho, Y., Elefant, F., Cooke, N., and Liebhaber, S. 2002. A defined locus control region determinant links chromatin domain acetylation with long-range gene activation. *Mol Cell* 9(2): 291-302.

Honda, R., Tanaka, H., and Yasuda, H. 1997. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett* 420(1): 25-27.

Hsieh, J. and Gage, F.H. 2005. Chromatin remodeling in neural development and plasticity. *Curr Opin Cell Biol* 17(6): 664-671.

Huang, D.H. and Chang, Y.L. 2004. Isolation and characterization of CHRASCH, a polycomb-containing silencing complex. *Methods Enzymol* 377: 267-282.

Huang, D.H., Chang, Y.L., Yang, C.C., Pan, I.C., and King, B. 2002. pipsqueak encodes a factor essential for sequence-specific targeting of a polycomb group protein complex. *Molecular and cellular biology* 22(17): 6261-6271.

Huh, M.S., Parker, M.H., Scime, A., Parks, R., and Rudnicki, M.A. 2004. Rb is required for progression through myogenic differentiation but not maintenance of terminal differentiation. *J Cell Biol* 166(6): 865-876.

Huyen, Y., Zgheib, O., Ditullio, R.A., Jr., Gorgoulis, V.G., Zacharatos, P., Petty, T.J., Sheston, E.A., Mellert, H.S., Stavridi, E.S., and Halazonetis, T.D. 2004. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 432(7015): 406-411.

Hynes, R.O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69(1): 11-25.

Iizuka, M. and Stillman, B. 1999. Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. *J Biol Chem* 274(33): 23027-23034.

Ikura, T., Ogryzko, V.V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. 2000. Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* 102(4): 463-473.

Ingham, P.W. 1985. A clonal analysis of the requirement for the trithorax gene in the diversification of segments in *Drosophila*. *J Embryol Exp Morphol* 89: 349-365.

Ito, A., Kawaguchi, Y., Lai, C.H., Kovacs, J.J., Higashimoto, Y., Appella, E., and Yao, T.P. 2002. MDM2-HDAC1-mediated deacetylation of p53 is required for its degradation. *The EMBO journal* 21(22): 6236-6245.

Jacobs, J.J., Kieboom, K., Marino, S., DePinho, R.A., and van Lohuizen, M. 1999. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 397(6715): 164-168.

James, T.C. and Elgin, S.C. 1986. Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila* melanogaster and its gene. *Molecular and cellular biology* 6(11): 3862-3872.

Jenuwein, T. and Allis, C.D. 2001. Translating the histone code. *Science (New York, NY* 293(5532): 1074-1080.

Jepsen, K., Hermanson, O., Onami, T.M., Gleiberman, A.S., Lunyak, V., McEvilly, R.J., Kurokawa, R., Kumar, V., Liu, F., Seto, E., Hedrick, S.M., Mandel, G., Glass, C.K., Rose, D.W., and Rosenfeld, M.G. 2000. Combinatorial roles of the nuclear receptor corepressor in transcription and development. *Cell* 102(6): 753-763.

Jin, Y., Wang, Y., Johansen, J., and Johansen, K.M. 2000. JIL-1, a chromosomal kinase implicated in regulation of chromatin structure, associates with the male specific lethal (MSL) dosage compensation complex. *J Cell Biol* 149(5): 1005-1010.

Johnston, L.A., Prober, D.A., Edgar, B.A., Eisenman, R.N., and Gallant, P. 1999. *Drosophila* myc regulates cellular growth during development. *Cell* 98(6): 779-790.

Jones, P.A., Wolkowicz, M.J., Harrington, M.A., and Gonzales, F. 1990. Methylation and expression of the Myo D1 determination gene. *Philos Trans R Soc Lond B Biol Sci* 326(1235): 277-284.

Jost, J.P., Oakeley, E.J., Zhu, B., Benjamin, D., Thiry, S., Siegmann, M., and Jost, Y.C. 2001. 5-Methylcytosine DNA glycosylase participates in the genome-wide loss of DNA methylation occurring during mouse myoblast differentiation. *Nucleic Acids Res* 29(21): 4452-4461.

Jürgens, G. 1985. A group of genes controlling the spatial expression of the bithorax complex in *Drosophila*. *Nature* 316: 153-155.

Kablar, B., Krastel, K., Ying, C., Tapscott, S.J., Goldhamer, D.J., and Rudnicki, M.A. 1999. Myogenic determination occurs independently in somites and limb buds. *Developmental biology* 206(2): 219-231.

Kamijo, T., Zindy, F., Roussel, M.F., Quelle, D.E., Downing, J.R., Ashmun, R.A., Grosveld, G., and Sherr, C.J. 1997. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* 91(5): 649-659.

Kamine, J., Elangovan, B., Subramanian, T., Coleman, D., and Chinnadurai, G. 1996. Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. *Virology* 216(2): 357-366.

Kawasaki, H., Schiltz, L., Chiu, R., Itakura, K., Taira, K., Nakatani, Y., and Yokoyama, K.K. 2000. ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. *Nature* 405(6783): 195-200.

Kelley, R.L., Solovyeva, I., Lyman, L.M., Richman, R., Solovyev, V., and Kuroda, M.I. 1995. Expression of msl-2 causes assembly of dosage compensation regulators on the X chromosomes and female lethality in *Drosophila*. *Cell* 81(6): 867-877.

Kennerdell, J.R. and Carthew, R.W. 2000. Heritable gene silencing in *Drosophila* using double-stranded RNA. *Nat Biotechnol* 18(8): 896-898.

Kennison, J.A. and Tamkun, J.W. 1988. Dosage-dependent modifiers of polycomb and antennapedia mutations in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* 85(21): 8136-8140.

Kiefer, J.C. 2007. Epigenetics in development. *Dev Dyn* 236(4): 1144-1156.

Kim, H.J., Kim, S.H., Shim, S.O., Park, E., Kim, C., Kim, K., Tanouye, M.A., and Yim, J. 2007. *Drosophila* homolog of APP-BP1 (dAPP-BP1) interacts antagonistically with APPL during *Drosophila* development. *Cell death and differentiation* 14(1): 103-115.

Kim, H.S., Kim, E.M., Kim, N.J., Chang, K.A., Choi, Y., Ahn, K.W., Lee, J.H., Kim, S., Park, C.H., and Suh, Y.H. 2004. Inhibition of histone deacetylation enhances the neurotoxicity induced by the C-terminal fragments of amyloid precursor protein. *Journal of neuroscience research* 75(1): 117-124.

Kim, H.S., Kim, E.M., Lee, J.P., Park, C.H., Kim, S., Seo, J.H., Chang, K.A., Yu, E., Jeong, S.J., Chong, Y.H., and Suh, Y.H. 2003. C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression. *Faseb J* 17(13): 1951-1953.

Kim, J.H., Kim, B., Cai, L., Choi, H.J., Ohgi, K.A., Tran, C., Chen, C., Chung, C.H., Huber, O., Rose, D.W., Sawyers, C.L., Rosenfeld, M.G., and Baek, S.H. 2005. Transcriptional regulation of a metastasis suppressor gene by Tip60 and beta-catenin complexes. *Nature* 434(7035): 921-926.

Kim, S.T., Xu, B., and Kastan, M.B. 2002. Involvement of the cohesin protein, Smc1, in Atm-dependent and independent responses to DNA damage. *Genes Dev* 16(5): 560-570.

Kimura, A. and Horikoshi, M. 1998. Tip60 acetylates six lysines of a specific class in core histones in vitro. *Genes Cells* 3(12): 789-800.

Kinoshita, A., Shah, T., Tangredi, M.M., Strickland, D.K., and Hyman, B.T. 2003. The intracellular domain of the low density lipoprotein receptor-related protein modulates transactivation mediated by amyloid precursor protein and Fe65. *J Biol Chem* 278(42): 41182-41188.

Kinoshita, A., Whelan, C.M., Berezovska, O., and Hyman, B.T. 2002. The gamma secretase-generated carboxyl-terminal domain of the amyloid precursor protein induces apoptosis via Tip60 in H4 cells. *J Biol Chem* 277(32): 28530-28536.

Kitzmann, M. and Fernandez, A. 2001. Crosstalk between cell cycle regulators and the myogenic factor MyoD in skeletal myoblasts. *Cell Mol Life Sci* 58(4): 571-579.

Korzus, E., Rosenfeld, M.G., and Mayford, M. 2004. CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron* 42(6): 961-972.

Kouzarides, T. 2007. Chromatin modifications and their function. *Cell* 128(4): 693-705.

Kozak, M. 1987. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res* 15(20): 8125-8148.

Kramps, C., Strieder, V., Sapetschnig, A., Suske, G., and Lutz, W. 2004. E2F and Sp1/Sp3 Synergize but are not sufficient to activate the MYCN gene in neuroblastomas. *J Biol Chem* 279(7): 5110-5117.

Kratzer, P.G., Chapman, V.M., Lambert, H., Evans, R.E., and Liskay, R.M. 1983. Differences in the DNA of the inactive X chromosomes of fetal and extraembryonic tissues of mice. *Cell* 33(1): 37-42.

Kusch, T., Florens, L., Macdonald, W.H., Swanson, S.K., Glaser, R.L., Yates, J.R., 3rd, Abmayr, S.M., Washburn, M.P., and Workman, J.L. 2004. Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science (New York, NY* 306(5704): 2084-2087.

Kuwabara, T., Hsieh, J., Nakashima, K., Taira, K., and Gage, F.H. 2004. A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell* 116(6): 779-793.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410(6824): 116-120.

Lang, S.E., McMahon, S.B., Cole, M.D., and Hearing, P. 2001. E2F transcriptional activation requires TRRAP and GCN5 cofactors. *J Biol Chem* 276(35): 32627-32634.

Leduc, C., Claverie, P., Eymin, B., Col, E., Khochbin, S., Brambilla, E., and Gazzeri, S. 2006. p14ARF promotes RB accumulation through inhibition of its Tip60-dependent acetylation. *Oncogene* 25(30): 4147-4154.

Lee, C.G., Reichman, T.W., Baik, T., and Mathews, M.B. 2004a. MLE functions as a transcriptional regulator of the roX2 gene. *J Biol Chem* 279(46): 47740-47745.

Lee, H., Habas, R., and Abate-Shen, C. 2004b. MSX1 cooperates with histone H1b for inhibition of transcription and myogenesis. *Science (New York, NY* 304(5677): 1675-1678.

Lee, H.J., Chun, M., and Kandror, K.V. 2001. Tip60 and HDAC7 interact with the endothelin receptor a and may be involved in downstream signaling. *J Biol Chem* 276(20): 16597-16600.

Legube, G., Linares, L.K., Lemercier, C., Scheffner, M., Khochbin, S., and Trouche, D. 2002. Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation. *The EMBO journal* 21(7): 1704-1712.

Legube, G., Linares, L.K., Tyteca, S., Caron, C., Scheffner, M., Chevillard-Briet, M., and Trouche, D. 2004. Role of the histone acetyl transferase Tip60 in the p53 pathway. *J Biol Chem* 279(43): 44825-44833.

Legube, G. and Trouche, D. 2003. Identification of a larger form of the histone acetyl transferase Tip60. *Gene* 310: 161-168.

Lemercier, C., Legube, G., Caron, C., Louwagie, M., Garin, J., Trouche, D., and Khochbin, S. 2003. Tip60 acetyltransferase activity is controlled by phosphorylation. *J Biol Chem* 278(7): 4713-4718.

Lessard, J. and Sauvageau, G. 2003. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* 423(6937): 255-260.

Lewis, E.B. 1978. A gene complex controlling segmentation in *Drosophila*. *Nature* 276(5688): 565-570.

Li, B., Ruan, C., and Workman, J.L. 2005. Histones: should I stay or should I go? *Genome Biol* 6(2): 306.

Li, H., Cuenin, C., Murr, R., Wang, Z.Q., and Herceg, Z. 2004. HAT cofactor Trrap regulates the mitotic checkpoint by modulation of Mad1 and Mad2 expression. *The EMBO journal* 23(24): 4824-4834.

Li, Q. and Sudhof, T.C. 2004. Cleavage of amyloid-beta precursor protein and amyloid-beta precursor-like protein by BACE 1. *J Biol Chem* 279(11): 10542-10550.

Li, X., Graner, M.W., Williams, E.L., Roote, C.E., Bunch, T.A., and Zusman, S. 1998. Requirements for the cytoplasmic domain of the alphaPS1, alphaPS2 and betaPS integrin subunits during *Drosophila* development. *Development (Cambridge, England)* 125(4): 701-711.

Lipinski, M.M. and Jacks, T. 1999. The retinoblastoma gene family in differentiation and development. *Oncogene* 18(55): 7873-7882.

Liu, J. and Levens, D. 2006. Making myc. *Curr Top Microbiol Immunol* 302: 1-32.

Lough, J.W. 2002. Transient expression of TIP60 protein during early chick heart development. *Dev Dyn* 223(3): 419-425.

Lu, B.Y., Emtage, P.C., Duyf, B.J., Hilliker, A.J., and Eissenberg, J.C. 2000a. Heterochromatin protein 1 is required for the normal expression of two heterochromatin genes in *Drosophila*. *Genetics* 155(2): 699-708.

Lu, J., McKinsey, T.A., Nicol, R.L., and Olson, E.N. 2000b. Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proceedings of the National Academy of Sciences of the United States of America* 97(8): 4070-4075.

Lu, J., McKinsey, T.A., Zhang, C.L., and Olson, E.N. 2000c. Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol Cell* 6(2): 233-244.

Lucarelli, M., Fuso, A., Strom, R., and Scarpa, S. 2001. The dynamics of myogenin site-specific demethylation is strongly correlated with its expression and with muscle differentiation. *J Biol Chem* 276(10): 7500-7506.

Ludlam, W.H., Taylor, M.H., Tanner, K.G., Denu, J.M., Goodman, R.H., and Smolik, S.M. 2002. The acetyltransferase activity of CBP is required for wingless activation and H4 acetylation in *Drosophila* melanogaster. *Molecular and cellular biology* 22(11): 3832-3841.

Luger, K. 2006. Dynamic nucleosomes. *Chromosome Res* 14(1): 5-16.

Lunyak, V.V., Burgess, R., Prefontaine, G.G., Nelson, C., Sze, S.H., Chenoweth, J., Schwartz, P., Pevzner, P.A., Glass, C., Mandel, G., and Rosenfeld, M.G. 2002. Corepressor-dependent silencing of chromosomal regions encoding neuronal genes. *Science (New York, NY* 298(5599): 1747-1752.

Ma, K., Chan, J.K., Zhu, G., and Wu, Z. 2005. Myocyte enhancer factor 2 acetylation by p300 enhances its DNA binding activity, transcriptional activity, and myogenic differentiation. *Molecular and cellular biology* 25(9): 3575-3582.

Magenta, A., Cenciarelli, C., De Santa, F., Fuschi, P., Martelli, F., Caruso, M., and Felsani, A. 2003. MyoD stimulates RB promoter activity via the CREB/p300 nuclear transduction pathway. *Molecular and cellular biology* 23(8): 2893-2906.

Mahlknecht, U., Ottmann, O.G., and Hoelzer, D. 2000. When the band begins to play: histone acetylation caught in the crossfire of gene control. *Mol Carcinog* 27(4): 268-271.

Maines, J.Z., Stevens, L.M., Tong, X., and Stein, D. 2004. *Drosophila* dMyc is required for ovary cell growth and endoreplication. *Development (Cambridge, England)* 131(4): 775-786.

Mal, A., Sturniolo, M., Schiltz, R.L., Ghosh, M.K., and Harter, M.L. 2001. A role for histone deacetylase HDAC1 in modulating the transcriptional activity of MyoD: inhibition of the myogenic program. *The EMBO journal* 20(7): 1739-1753.

Marenda, D.R., Zraly, C.B., Feng, Y., Egan, S., and Dingwall, A.K. 2003. The *Drosophila* SNR1 (SNF5/INI1) subunit directs essential developmental functions of the Brahma chromatin remodeling complex. *Molecular and cellular biology* 23(1): 289-305.

Margueron, R., Trojer, P., and Reinberg, D. 2005. The key to development: interpreting the histone code? *Curr Opin Genet Dev* 15(2): 163-176.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.J., Chen, Z., Dewell, S.B., Du, L., Fierro, J.M., Gomes, X.V., Godwin, B.C., He, W., Helgesen, S., Ho, C.H., Irzyk, G.P., Jando, S.C., Alenquer, M.L., Jarvie, T.P., Jirage, K.B., Kim, J.B., Knight, J.R., Lanza, J.R., Leamon, J.H., Lefkowitz, S.M., Lei, M., Li, J., Lohman, K.L., Lu, H., Makhijani, V.B., McDade, K.E., McKenna, M.P., Myers, E.W., Nickerson, E., Nobile, J.R., Plant, R., Puc, B.P., Ronan, M.T., Roth, G.T., Sarkis, G.J., Simons, J.F., Simpson, J.W., Srinivasan, M., Tartaro, K.R., Tomasz, A., Vogt, K.A., Volkmer, G.A., Wang, S.H., Wang, Y., Weiner, M.P., Yu, P., Begley, R.F., and Rothberg, J.M. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437(7057): 376-380.

Marmorstein, R. 2001. Structure of histone acetyltransferases. *J Mol Biol* 311(3): 433-444.

Marmorstein, R. and Roth, S.Y. 2001. Histone acetyltransferases: function, structure, and catalysis. *Curr Opin Genet Dev* 11(2): 155-161.

Martelli, F., Cenciarelli, C., Santarelli, G., Polikar, B., Felsani, A., and Caruso, M. 1994. MyoD induces retinoblastoma gene expression during myogenic differentiation. *Oncogene* 9(12): 3579-3590.

Martinez-Balbas, M.A., Bauer, U.M., Nielsen, S.J., Brehm, A., and Kouzarides, T. 2000. Regulation of E2F1 activity by acetylation. *The EMBO journal* 19(4): 662-671.

Martinez, E., Kundu, T.K., Fu, J., and Roeder, R.G. 1998. A human SPT3-TAFII31-GCN5-L acetylase complex distinct from transcription factor IID. *J Biol Chem* 273(37): 23781-23785.

Martinez, E., Palhan, V.B., Tjernberg, A., Lymar, E.S., Gamper, A.M., Kundu, T.K., Chait, B.T., and Roeder, R.G. 2001. Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. *Molecular and cellular biology* 21(20): 6782-6795.

Marzio, G., Wagener, C., Gutierrez, M.I., Cartwright, P., Helin, K., and Giacca, M. 2000. E2F family members are differentially regulated by reversible acetylation. *J Biol Chem* 275(15): 10887-10892.

McAllister, D., Merlo, X., and Lough, J. 2002. Characterization and expression of the mouse tat interactive protein 60 kD (TIP60) gene. *Gene* 289(1-2): 169-176.

McKinsey, T.A., Zhang, C.L., Lu, J., and Olson, E.N. 2000a. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* 408(6808): 106-111.

McKinsey, T.A., Zhang, C.L., and Olson, E.N. 2000b. Activation of the myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. *Proceedings of the National Academy of Sciences of the United States of America* 97(26): 14400-14405.

-. 2001. Control of muscle development by dueling HATs and HDACs. *Curr Opin Genet Dev* 11(5): 497-504.

-. 2002. Signaling chromatin to make muscle. *Curr Opin Cell Biol* 14(6): 763-772.

McMahon, S.B., Van Buskirk, H.A., Dugan, K.A., Copeland, T.D., and Cole, M.D. 1998. The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell* 94(3): 363-374.

McMahon, S.B., Wood, M.A., and Cole, M.D. 2000. The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. *Molecular and cellular biology* 20(2): 556-562.

Meller, V.H. and Rattner, B.P. 2002. The roX genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex. *The EMBO journal* 21(5): 1084-1091.

Meyer, C.A., Jacobs, H.W., Datar, S.A., Du, W., Edgar, B.A., and Lehner, C.F. 2000. *Drosophila* Cdk4 is required for normal growth and is dispensable for cell cycle progression. *The EMBO journal* 19(17): 4533-4542.

Mizzen, C.A., Yang, X.J., Kokubo, T., Brownell, J.E., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., Nakatani, Y., and Allis, C.D. 1996. The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. *Cell* 87(7): 1261-1270.

Molofsky, A.V., Pardal, R., Iwashita, T., Park, I.K., Clarke, M.F., and Morrison, S.J. 2003. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* 425(6961): 962-967.

Momand, J., Jung, D., Wilczynski, S., and Niland, J. 1998. The MDM2 gene amplification database. *Nucleic Acids Res* 26(15): 3453-3459.

Morrison, A.J. and Shen, X. 2005. DNA repair in the context of chromatin. *Cell Cycle* 4(4): 568-571.

Mulholland, N.M., King, I.F., and Kingston, R.E. 2003. Regulation of Polycomb group complexes by the sequence-specific DNA binding proteins Zeste and GAGA. *Genes Dev* 17(22): 2741-2746.

Muotri, A.R., Chu, V.T., Marchetto, M.C., Deng, W., Moran, J.V., and Gage, F.H. 2005. Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. *Nature* 435(7044): 903-910.

Murr, R., Loizou, J.I., Yang, Y.G., Cuenin, C., Li, H., Wang, Z.Q., and Herceg, Z. 2006. Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. *Nat Cell Biol* 8(1): 91-99.

Nagy, Z. and Tora, L. 2007. Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene* 26(37): 5341-5357.

Naruse, Y., Aoki, T., Kojima, T., and Mori, N. 1999. Neural restrictive silencer factor recruits mSin3 and histone deacetylase complex to repress neuron-specific target genes. *Proceedings of the National Academy of Sciences of the United States of America* 96(24): 13691-13696.

Negre, N., Hennetin, J., Sun, L.V., Lavrov, S., Bellis, M., White, K.P., and Cavalli, G. 2006. Chromosomal distribution of PcG proteins during *Drosophila* development. *PLoS Biol* 4(6): e170.

Nesbit, C.E., Tersak, J.M., and Prochownik, E.V. 1999. MYC oncogenes and human neoplastic disease. *Oncogene* 18(19): 3004-3016.

Nguyen, D.X., Baglia, L.A., Huang, S.M., Baker, C.M., and McCance, D.J. 2004. Acetylation regulates the differentiation-specific functions of the retinoblastoma protein. *The EMBO journal* 23(7): 1609-1618.

Nielsen, S.J., Schneider, R., Bauer, U.M., Bannister, A.J., Morrison, A., O'Carroll, D., Firestein, R., Cleary, M., Jenuwein, T., Herrera, R.E., and Kouzarides, T. 2001. Rb targets histone H3 methylation and HP1 to promoters. *Nature* 412(6846): 561-565.

Nordentoft, I. and Jorgensen, P. 2003. The acetyltransferase 60 kDa trans-acting regulatory protein of HIV type 1-interacting protein (Tip60) interacts with the translocation E26 transforming-specific leukaemia gene (TEL) and functions as a transcriptional co-repressor. *Biochem J* 374(Pt 1): 165-173.

Nourani, A., Doyon, Y., Utley, R.T., Allard, S., Lane, W.S., and Cote, J. 2001. Role of an ING1 growth regulator in transcriptional activation and targeted histone acetylation by the NuA4 complex. *Molecular and cellular biology* 21(22): 7629-7640.

Nowak, S.J. and Corces, V.G. 2000. Phosphorylation of histone H3 correlates with transcriptionally active loci. *Genes Dev* 14(23): 3003-3013.

Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D.M., and Nakatani, Y. 2002. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science (New York, NY* 296(5570): 1132-1136.

Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.J., Howard, B.H., Qin, J., and Nakatani, Y. 1998. Histone-like TAFs within the PCAF histone acetylase complex. *Cell* 94(1): 35-44.

Orphanides, G. and Reinberg, D. 2002. A unified theory of gene expression. *Cell* 108(4): 439-451.

Oskarsson, T. and Trumpp, A. 2005. The Myc trilogy: lord of RNA polymerases. *Nat Cell Biol* 7(3): 215-217.

Palacios, D. and Puri, P.L. 2006. The epigenetic network regulating muscle development and regeneration. *J Cell Physiol* 207(1): 1-11.

Panchenko, M.V., Zhou, M.I., and Cohen, H.T. 2004. von Hippel-Lindau partner Jade-1 is a transcriptional co-activator associated with histone acetyltransferase activity. *J Biol Chem* 279(53): 56032-56041.

Papoulas, O., Beek, S.J., Moseley, S.L., McCallum, C.M., Sarte, M., Shearn, A., and Tamkun, J.W. 1998. The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. *Development (Cambridge, England)* 125(20): 3955-3966.

Park, I.K., Qian, D., Kiel, M., Becker, M.W., Pihalja, M., Weissman, I.L., Morrison, S.J., and Clarke, M.F. 2003. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 423(6937): 302-305.

Patel, J.H., Du, Y., Ard, P.G., Phillips, C., Carella, B., Chen, C.J., Rakowski, C., Chatterjee, C., Lieberman, P.M., Lane, W.S., Blobel, G.A., and McMahon, S.B. 2004. The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. *Molecular and cellular biology* 24(24): 10826-10834.

Patterton, D. and Wolffe, A.P. 1996. Developmental roles for chromatin and chromosomal structure. *Developmental biology* 173(1): 2-13.

Pediconi, N., Ianari, A., Costanzo, A., Belloni, L., Gallo, R., Cimino, L., Porcellini, A., Screpanti, I., Balsano, C., Alesse, E., Gulino, A., and Levrero, M. 2003. Differential regulation of E2F1 apoptotic target genes in response to DNA damage. *Nat Cell Biol* 5(6): 552-558.

Pemberton, L.F. and Paschal, B.M. 2005. Mechanisms of receptor-mediated nuclear import and nuclear export. *Traffic (Copenhagen, Denmark)* 6(3): 187-198.

Perkins, N.D. 2007. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nature reviews* 8(1): 49-62.

Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C., Masuno, M., Tommerup, N., van Ommen, G.J., Goodman, R.H., Peters, D.J., and et al. 1995. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 376(6538): 348-351.

Petruk, S., Sedkov, Y., Riley, K.M., Hodgson, J., Schweisguth, F., Hirose, S., Jaynes, J.B., Brock, H.W., and Mazo, A. 2006. Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. *Cell* 127(6): 1209-1221.

Petruk, S., Sedkov, Y., Smith, S., Tillib, S., Kraevski, V., Nakamura, T., Canaani, E., Croce, C.M., and Mazo, A. 2001. Trithorax and dCBP acting in a complex to maintain expression of a homeotic gene. *Science (New York, NY* 294(5545): 1331-1334.

Pierce, S.B., Yost, C., Britton, J.S., Loo, L.W., Flynn, E.M., Edgar, B.A., and Eisenman, R.N. 2004. dMyc is required for larval growth and endoreplication in *Drosophila*. *Development (Cambridge, England)* 131(10): 2317-2327.

Plath, K., Fang, J., Mlynarczyk-Evans, S.K., Cao, R., Worringer, K.A., Wang, H., de la Cruz, C.C., Otte, A.P., Panning, B., and Zhang, Y. 2003. Role of histone H3 lysine 27 methylation in X inactivation. *Science (New York, NY* 300(5616): 131-135.

Polesskaya, A., Duquet, A., Naguibneva, I., Weise, C., Vervisch, A., Bengal, E., Hucho, F., Robin, P., and Harel-Bellan, A. 2000. CREB-binding protein/p300 activates MyoD by acetylation. *J Biol Chem* 275(44): 34359-34364.

Polesskaya, A. and Harel-Bellan, A. 2001. Acetylation of MyoD by p300 requires more than its histone acetyltransferase domain. *J Biol Chem* 276(48): 44502-44503.

Polesskaya, A., Naguibneva, I., Duquet, A., Bengal, E., Robin, P., and Harel-Bellan, A. 2001a. Interaction between acetylated MyoD and the bromodomain of CBP and/or p300. *Molecular and cellular biology* 21(16): 5312-5320.

Polesskaya, A., Naguibneva, I., Fritsch, L., Duquet, A., Ait-Si-Ali, S., Robin, P., Vervisch, A., Pritchard, L.L., Cole, P., and Harel-Bellan, A. 2001b. CBP/p300 and muscle differentiation: no HAT, no muscle. *The EMBO journal* 20(23): 6816-6825.

Pomerantz, J. and Blau, H.M. 2004. Nuclear reprogramming: a key to stem cell function in regenerative medicine. *Nat Cell Biol* 6(9): 810-816.

Pomerantz, J., Schreiber-Agus, N., Liegeois, N.J., Silverman, A., Alland, L., Chin, L., Potes, J., Chen, K., Orlow, I., Lee, H.W., Cordon-Cardo, C., and DePinho, R.A. 1998. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell* 92(6): 713-723.

Poux, S., Melfi, R., and Pirrotta, V. 2001. Establishment of Polycomb silencing requires a transient interaction between PC and ESC. *Genes Dev* 15(19): 2509-2514.

Puri, P.L., Avantaggiati, M.L., Balsano, C., Sang, N., Graessmann, A., Giordano, A., and Levrero, M. 1997a. p300 is required for MyoD-dependent cell cycle arrest and muscle-specific gene transcription. *The EMBO journal* 16(2): 369-383.

Puri, P.L., Iezzi, S., Stiegler, P., Chen, T.T., Schiltz, R.L., Muscat, G.E., Giordano, A., Kedes, L., Wang, J.Y., and Sartorelli, V. 2001. Class I histone deacetylases sequentially interact with MyoD and pRb during skeletal myogenesis. *Mol Cell* 8(4): 885-897.

Puri, P.L., Sartorelli, V., Yang, X.J., Hamamori, Y., Ogryzko, V.V., Howard, B.H., Kedes, L., Wang, J.Y., Graessmann, A., Nakatani, Y., and Levrero, M. 1997b. Differential roles of p300 and PCAF acetyltransferases in muscle differentiation. *Mol Cell* 1(1): 35-45.

Ran, Q. and Pereira-Smith, O.M. 2000. Identification of an alternatively spliced form of the Tat interactive protein (Tip60), Tip60(beta). *Gene* 258(1-2): 141-146.

Rattner, B.P. and Meller, V.H. 2004. *Drosophila* male-specific lethal 2 protein controls sex-specific expression of the roX genes. *Genetics* 166(4): 1825-1832.

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., and Jenuwein, T. 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406(6796): 593-599.

Reid, J.L., Iyer, V.R., Brown, P.O., and Struhl, K. 2000. Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol Cell* 6(6): 1297-1307.

Reik, W. 2007. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 447(7143): 425-432.

Reik, W. and Lewis, A. 2005. Co-evolution of X-chromosome inactivation and imprinting in mammals. *Nat Rev Genet* 6(5): 403-410.

Reuter, G. and Wolff, I. 1981. Isolation of dominant suppressor mutations for position-effect variegation in *Drosophila* melanogaster. *Mol Gen Genet* 182(3): 516-519.

Rice, J.C. and Allis, C.D. 2001. Histone methylation versus histone acetylation: new insights into epigenetic regulation. *Curr Opin Cell Biol* 13(3): 263-273.

Richter, L., Bone, J.R., and Kuroda, M.I. 1996. RNA-dependent association of the *Drosophila* maleless protein with the male X chromosome. *Genes Cells* 1(3): 325-336.

Ringrose, L. and Paro, R. 2004. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu Rev Genet* 38: 413-443.

Robert, F., Hardy, S., Nagy, Z., Baldeyron, C., Murr, R., Dery, U., Masson, J.Y., Papadopoulo, D., Herceg, Z., and Tora, L. 2006. The transcriptional histone acetyltransferase cofactor TRRAP associates with the MRN repair complex and plays a role in DNA double-strand break repair. *Molecular and cellular biology* 26(2): 402-412.

Roelfsema, J.H., White, S.J., Ariyurek, Y., Bartholdi, D., Niedrist, D., Papadia, F., Bacino, C.A., den Dunnen, J.T., van Ommen, G.J., Breuning, M.H., Hennekam, R.C., and Peters, D.J. 2005. Genetic heterogeneity in Rubinstein-Taybi syndrome: mutations in both the CBP and EP300 genes cause disease. *Am J Hum Genet* 76(4): 572-580.

Rollins, R.A., Korom, M., Aulner, N., Martens, A., and Dorsett, D. 2004. *Drosophila* nipped-B protein supports sister chromatid cohesion and opposes the stromalin/Scc3 cohesion factor to facilitate long-range activation of the cut gene. *Molecular and cellular biology* 24(8): 3100-3111.

Rollins, R.A., Morcillo, P., and Dorsett, D. 1999. Nipped-B, a *Drosophila* homologue of chromosomal adherins, participates in activation by remote enhancers in the cut and Ultrabithorax genes. *Genetics* 152(2): 577-593.

Roopra, A., Qazi, R., Schoenike, B., Daley, T.J., and Morrison, J.F. 2004. Localized domains of G9a-mediated histone methylation are required for silencing of neuronal genes. *Mol Cell* 14(6): 727-738.

Ross, S.E., Greenberg, M.E., and Stiles, C.D. 2003. Basic helix-loop-helix factors in cortical development. *Neuron* 39(1): 13-25.

Roth, J.F., Shikama, N., Henzen, C., Desbaillets, I., Lutz, W., Marino, S., Wittwer, J., Schorle, H., Gassmann, M., and Eckner, R. 2003. Differential role of p300 and CBP acetyltransferase during myogenesis: p300 acts upstream of MyoD and Myf5. *The EMBO journal* 22(19): 5186-5196.

Roth, S.Y., Denu, J.M., and Allis, C.D. 2001. Histone acetyltransferases. *Annu Rev Biochem* 70: 81-120.

Royzman, I., Whittaker, A.J., and Orr-Weaver, T.L. 1997. Mutations in *Drosophila* DP and E2F distinguish G1-S progression from an associated transcriptional program. *Genes Dev* 11(15): 1999-2011.

Sado, T., Hoki, Y., and Sasaki, H. 2005. Tsix silences Xist through modification of chromatin structure. *Dev Cell* 9(1): 159-165.

Saleh, A., Schieltz, D., Ting, N., McMahon, S.B., Litchfield, D.W., Yates, J.R., 3rd, Lees-Miller, S.P., Cole, M.D., and Brandl, C.J. 1998. Tra1p is a component of the yeast Ada.Spt transcriptional regulatory complexes. *J Biol Chem* 273(41): 26559-26565.

Sanchez-Elsner, T., Gou, D., Kremmer, E., and Sauer, F. 2006. Noncoding RNAs of trithorax response elements recruit *Drosophila* Ash1 to Ultrabithorax. *Science (New York, NY* 311(5764): 1118-1123.

Sapountzi, V., Logan, I.R., and Robson, C.N. 2006. Cellular functions of TIP60. *Int J Biochem Cell Biol* 38(9): 1496-1509.

Sartorelli, V., Huang, J., Hamamori, Y., and Kedes, L. 1997. Molecular mechanisms of myogenic coactivation by p300: direct interaction with the activation domain of MyoD and with the MADS box of MEF2C. *Molecular and cellular biology* 17(2): 1010-1026.

Sartorelli, V., Puri, P.L., Hamamori, Y., Ogryzko, V., Chung, G., Nakatani, Y., Wang, J.Y., and Kedes, L. 1999. Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. *Mol Cell* 4(5): 725-734.

Saurin, A.J., Shao, Z., Erdjument-Bromage, H., Tempst, P., and Kingston, R.E. 2001. A *Drosophila* Polycomb group complex includes Zeste and dTAFII proteins. *Nature* 412(6847): 655-660.

Scheinfeld, M.H., Matsuda, S., and D'Adamio, L. 2003. JNK-interacting protein-1 promotes transcription of A beta protein precursor but not A beta precursor-like proteins, mechanistically different than Fe65. *Proceedings of the National Academy of Sciences of the United States of America* 100(4): 1729-1734.

Schotta, G., Ebert, A., Dorn, R., and Reuter, G. 2003. Position-effect variegation and the genetic dissection of chromatin regulation in *Drosophila*. *Semin Cell Dev Biol* 14(1): 67-75.

Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B., and Cavalli, G. 2007. Genome regulation by polycomb and trithorax proteins. *Cell* 128(4): 735-745.

Schulze, S.R., Sinclair, D.A., Fitzpatrick, K.A., and Honda, B.M. 2005. A genetic and molecular characterization of two proximal heterochromatic genes on chromosome 3 of *Drosophila* melanogaster. *Genetics* 169(4): 2165-2177.

Schulze, S.R. and Wallrath, L.L. 2007. Gene regulation by chromatin structure: paradigms established in *Drosophila* melanogaster. *Annu Rev Entomol* 52: 171-192.

Secombe, J., Pierce, S.B., and Eisenman, R.N. 2004. Myc: a weapon of mass destruction. *Cell* 117(2): 153-156.

Seifert, J.R. and Mlodzik, M. 2007. Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. *Nat Rev Genet* 8(2): 126-138.

Selkoe, D.J. 2001. Alzheimer's disease: genes, proteins, and therapy. *Physiological reviews* 81(2): 741-766.

Seo, S., Richardson, G.A., and Kroll, K.L. 2005. The SWI/SNF chromatin remodeling protein Brg1 is required for vertebrate neurogenesis and mediates transactivation of Ngn and NeuroD. *Development (Cambridge, England)* 132(1): 105-115.

Sheridan, A.M., Force, T., Yoon, H.J., O'Leary, E., Choukroun, G., Taheri, M.R., and Bonventre, J.V. 2001. PLIP, a novel splice variant of Tip60, interacts with group IV cytosolic phospholipase A(2), induces apoptosis, and potentiates prostaglandin production. *Molecular and cellular biology* 21(14): 4470-4481.

Sherr, C.J. and Roberts, J.M. 1999. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 13(12): 1501-1512.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., Casero, R.A., and Shi, Y. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119(7): 941-953.

Shiama, N. 1997. The p300/CBP family: integrating signals with transcription factors and chromatin. *Trends in cell biology* 7(6): 230-236.

Shikama, N., Lutz, W., Kretzschmar, R., Sauter, N., Roth, J.F., Marino, S., Wittwer, J., Scheidweiler, A., and Eckner, R. 2003. Essential function of p300 acetyltransferase activity in heart, lung and small intestine formation. *The EMBO journal* 22(19): 5175-5185.

Silva, E., Tsatskis, Y., Gardano, L., Tapon, N., and McNeill, H. 2006. The tumor-suppressor gene fat controls tissue growth upstream of expanded in the hippo signaling pathway. *Curr Biol* 16(21): 2081-2089.

Sims, R.J., 3rd and Reinberg, D. 2006. Histone H3 Lys 4 methylation: caught in a bind? *Genes Dev* 20(20): 2779-2786.

Sinclair, D.A., Lloyd, V.K., and Grigliatti, T.A. 1989. Characterization of mutations that enhance position-effect variegation in *Drosophila* melanogaster. *Mol Gen Genet* 216(2-3): 328-333.

Sliva, D., Zhu, Y.X., Tsai, S., Kamine, J., and Yang, Y.C. 1999. Tip60 interacts with human interleukin-9 receptor alpha-chain. *Biochemical and biophysical research communications* 263(1): 149-155.

Smith, E.R., Allis, C.D., and Lucchesi, J.C. 2001. Linking global histone acetylation to the transcription enhancement of X-chromosomal genes in *Drosophila* males. *J Biol Chem* 276(34): 31483-31486.

Smith, E.R., Belote, J.M., Schiltz, R.L., Yang, X.J., Moore, P.A., Berger, S.L., Nakatani, Y., and Allis, C.D. 1998. Cloning of *Drosophila* GCN5: conserved features among metazoan GCN5 family members. *Nucleic Acids Res* 26(12): 2948-2954.

Smith, E.R., Pannuti, A., Gu, W., Steurnagel, A., Cook, R.G., Allis, C.D., and Lucchesi, J.C. 2000. The *Drosophila* MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. *Molecular and cellular biology* 20(1): 312-318.

Sokol, N.S. and Cooley, L. 2003. *Drosophila* filamin is required for follicle cell motility during oogenesis. *Developmental biology* 260(1): 260-272.

Squatrito, M., Gorrini, C., and Amati, B. 2006. Tip60 in DNA damage response and growth control: many tricks in one HAT. *Trends in cell biology* 16(9): 433-442.

Steffan, J.S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B.L., Kazantsev, A., Schmidt, E., Zhu, Y.Z., Greenwald, M., Kurokawa, R., Housman, D.E., Jackson, G.R., Marsh, J.L., and Thompson, L.M. 2001. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. *Nature* 413(6857): 739-743.

Steiner, H. and Haass, C. 2001. Nuclear signaling: a common function of presenilin substrates? *J Mol Neurosci* 17(2): 193-198.

Sterner, D.E. and Berger, S.L. 2000. Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* 64(2): 435-459.

Stevaux, O. and Dyson, N.J. 2002. A revised picture of the E2F transcriptional network and RB function. *Curr Opin Cell Biol* 14(6): 684-691.

Stoger, R., Kubicka, P., Liu, C.G., Kafri, T., Razin, A., Cedar, H., and Barlow, D.P. 1993. Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. *Cell* 73(1): 61-71.

Strahl, B.D. and Allis, C.D. 2000. The language of covalent histone modifications. *Nature* 403(6765): 41-45.

Strobeck, M.W., Knudsen, K.E., Fribourg, A.F., DeCristofaro, M.F., Weissman, B.E., Imbalzano, A.N., and Knudsen, E.S. 2000. BRG-1 is required for RB-mediated cell cycle arrest. *Proceedings of the National Academy of Sciences of the United States of America* 97(14): 7748-7753.

Strober, B.E., Dunaief, J.L., Guha, and Goff, S.P. 1996. Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. *Molecular and cellular biology* 16(4): 1576-1583.

Stromberg, H., Svensson, S.P., and Hermanson, O. 1999. Distribution of CREB-binding protein immunoreactivity in the adult rat brain. *Brain Res* 818(2): 510-514.

Suh, Y.H. and Checler, F. 2002. Amyloid precursor protein, presenilins, and alpha-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. *Pharmacological reviews* 54(3): 469-525.

Sun, B.K., Deaton, A.M., and Lee, J.T. 2006. A transient heterochromatic state in Xist preempts X inactivation choice without RNA stabilization. *Mol Cell* 21(5): 617-628.

Sun, Y., Jiang, X., Chen, S., Fernandes, N., and Price, B.D. 2005. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proceedings of the National Academy of Sciences of the United States of America* 102(37): 13182-13187.

Sun, Y., Xu, Y., Roy, K., and Price, B.D. 2007. DNA damage induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Molecular and cellular biology*.

Sykes, S.M., Mellert, H.S., Holbert, M.A., Li, K., Marmorstein, R., Lane, W.S., and McMahon, S.B. 2006. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol Cell* 24(6): 841-851.

Szyf, M., Rouleau, J., Theberge, J., and Bozovic, V. 1992. Induction of myogenic differentiation by an expression vector encoding the DNA methyltransferase cDNA sequence in the antisense orientation. *J Biol Chem* 267(18): 12831-12836.

Takahashi, Y., Rayman, J.B., and Dynlacht, B.D. 2000. Analysis of promoter binding by the E2F and pRB families in vivo: distinct E2F proteins mediate activation and repression. *Genes Dev* 14(7): 804-816.

Tamburini, B.A. and Tyler, J.K. 2005. Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. *Molecular and cellular biology* 25(12): 4903-4913.

Tan, X. and Wang, J.Y. 1998. The caspase-RB connection in cell death. *Trends in cell biology* 8(3): 116-120.

Tanaka, Y., Naruse, I., Maekawa, T., Masuya, H., Shiroishi, T., and Ishii, S. 1997. Abnormal skeletal patterning in embryos lacking a single Cbp allele: a partial similarity with Rubinstein-Taybi syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 94(19): 10215-10220.

Tang, Y., Luo, J., Zhang, W., and Gu, W. 2006. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol Cell* 24(6): 827-839.

Taubert, S., Gorrini, C., Frank, S.R., Parisi, T., Fuchs, M., Chan, H.M., Livingston, D.M., and Amati, B. 2004. E2F-dependent histone acetylation and recruitment of the Tip60 acetyltransferase complex to chromatin in late G1. *Molecular and cellular biology* 24(10): 4546-4556.

Taylor, S.M. and Jones, P.A. 1982. Changes in phenotypic expression in embryonic and adult cells treated with 5-azacytidine. *J Cell Physiol* 111(2): 187-194.

Telese, F., Bruni, P., Donizetti, A., Gianni, D., D'Ambrosio, C., Scaloni, A., Zambrano, N., Rosenfeld, M.G., and Russo, T. 2005. Transcription regulation by the adaptor protein Fe65 and the nucleosome assembly factor SET. *EMBO Rep* 6(1): 77-82.

Trimarchi, J.M. and Lees, J.A. 2002. Sibling rivalry in the E2F family. *Nature reviews* 3(1): 11-20.

Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M., and Kouzarides, T. 1997. RB and hbrm cooperate to repress the activation functions of E2F1. *Proceedings of the National Academy of Sciences of the United States of America* 94(21): 11268-11273.

Turner, B.M. 2007. Defining an epigenetic code. *Nat Cell Biol* 9(1): 2-6.

Turner, B.M., Birley, A.J., and Lavender, J. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. *Cell* 69(2): 375-384.

Tzouanacou, E., Tweedie, S., and Wilson, V. 2003. Identification of Jade1, a gene encoding a PHD zinc finger protein, in a gene trap mutagenesis screen for genes involved in anteroposterior axis development. *Molecular and cellular biology* 23(23): 8553-8552.

Uchida, C., Miwa, S., Kitagawa, K., Hattori, T., Isobe, T., Otani, S., Oda, T., Sugimura, H., Kamijo, T., Ookawa, K., Yasuda, H., and Kitagawa, M. 2005. Enhanced Mdm2 activity inhibits pRB function via ubiquitin-dependent degradation. *The EMBO journal* 24(1): 160-169.

Umlauf, D., Goto, Y., Cao, R., Cerqueira, F., Wagschal, A., Zhang, Y., and Feil, R. 2004. Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. *Nature genetics* 36(12): 1296-1300.

Valley, C.M., Pertz, L.M., Balakumaran, B.S., and Willard, H.F. 2006. Chromosome-wide, allele-specific analysis of the histone code on the human X chromosome. *Hum Mol Genet* 15(15): 2335-2347.

van der Lugt, N.M., Domen, J., Linders, K., van Roon, M., Robanus-Maandag, E., te Riele, H., van der Valk, M., Deschamps, J., Sofroniew, M., van Lohuizen, M., and et al. 1994. Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. *Genes Dev* 8(7): 757-769.

Vermaak, D. and Wolffe, A.P. 1998. Chromatin and chromosomal controls in development. *Dev Genet* 22(1): 1-6.

Vervoorts, J., Luscher-Firzlaff, J., and Luscher, B. 2006. The ins and outs of MYC regulation by posttranslational mechanisms. *J Biol Chem* 281(46): 34725-34729.

Vervoorts, J., Luscher-Firzlaff, J.M., Rottmann, S., Lilischkis, R., Walsemann, G., Dohmann, K., Austen, M., and Luscher, B. 2003. Stimulation of c-MYC transcriptional activity and acetylation by recruitment of the cofactor CBP. *EMBO Rep* 4(5): 484-490.

von Rotz, R.C., Kohli, B.M., Bosset, J., Meier, M., Suzuki, T., Nitsch, R.M., and Konietzko, U. 2004. The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. *J Cell Sci* 117(Pt 19): 4435-4448.

Wakimoto, B.T. and Hearn, M.G. 1990. The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of *Drosophila* melanogaster. *Genetics* 125(1): 141-154.

Wang, Y., Zhang, W., Jin, Y., Johansen, J., and Johansen, K.M. 2001. The JIL-1 tandem kinase mediates histone H3 phosphorylation and is required for maintenance of chromatin structure in *Drosophila*. *Cell* 105(4): 433-443.

Wang, Z.F., Sirotkin, A.M., Buchold, G.M., Skoultchi, A.I., and Marzluff, W.F. 1997. The mouse histone H1 genes: gene organization and differential regulation. *J Mol Biol* 271(1): 124-138.

Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., and Meaney, M.J. 2004. Epigenetic programming by maternal behavior. *Nat Neurosci* 7(8): 847-854.

Weber, M. and Schubeler, D. 2007. Genomic patterns of DNA methylation: targets and function of an epigenetic mark. *Curr Opin Cell Biol* 19(3): 273-280.

Weiler, K.S. and Wakimoto, B.T. 1995. Heterochromatin and gene expression in *Drosophila*. *Annu Rev Genet* 29: 577-605.

Westphal, T. and Reuter, G. 2002. Recombinogenic effects of suppressors of position-effect variegation in *Drosophila*. *Genetics* 160(2): 609-621.

Wieczorek, E., Brand, M., Jacq, X., and Tora, L. 1998. Function of TAF(II)-containing complex without TBP in transcription by RNA polymerase II. *Nature* 393(6681): 187-191.

Willecke, M., Hamaratoglu, F., Kango-Singh, M., Udan, R., Chen, C.L., Tao, C., Zhang, X., and Halder, G. 2006. The fat cadherin acts through the hippo tumor-suppressor pathway to regulate tissue size. *Curr Biol* 16(21): 2090-2100.

Wolffe, A.P. and Dimitrov, S. 1993. Histone-modulated gene activity: developmental implications. *Crit Rev Eukaryot Gene Expr* 3(3): 167-191.

Wood, A.J. and Oakey, R.J. 2006. Genomic imprinting in mammals: emerging themes and established theories. *PLoS Genet* 2(11): e147.

Woodcock, C.L. 2006. Chromatin architecture. *Curr Opin Struct Biol* 16(2): 213-220.

Wu, H.Y., Hamamori, Y., Xu, J., Chang, S.C., Saluna, T., Chang, M.F., O'Malley, B.W., and Kedes, L. 2005. Nuclear hormone receptor coregulator GRIP1 suppresses, whereas SRC1A and p/CIP coactivate, by domain-specific binding of MyoD. *J Biol Chem* 280(5): 3129-3137.

Wu, X., Bayle, J.H., Olson, D., and Levine, A.J. 1993. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev* 7(7A): 1126-1132.

Wustmann, G., Szidonya, J., Taubert, H., and Reuter, G. 1989. The genetics of position-effect variegation modifying loci in *Drosophila* melanogaster. *Mol Gen Genet* 217(2-3): 520-527.

Wysocka, J., Swigut, T., Xiao, H., Milne, T.A., Kwon, S.Y., Landry, J., Kauer, M., Tackett, A.J., Chait, B.T., Badenhorst, P., Wu, C., and Allis, C.D. 2006. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 442(7098): 86-90.

Xiao, H., Chung, J., Kao, H.Y., and Yang, Y.C. 2003. Tip60 is a co-repressor for STAT3. *J Biol Chem* 278(13): 11197-11204.

Xu, W., Edmondson, D.G., Evrard, Y.A., Wakamiya, M., Behringer, R.R., and Roth, S.Y. 2000. Loss of Gcn5l2 leads to increased apoptosis and mesodermal defects during mouse development. *Nature genetics* 26(2): 229-232.

Xu, W., Edmondson, D.G., and Roth, S.Y. 1998. Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. *Molecular and cellular biology* 18(10): 5659-5669.

Yamamoto, T. and Horikoshi, M. 1997. Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. *J Biol Chem* 272(49): 30595-30598.

Yan, Y., Harper, S., Speicher, D.W., and Marmorstein, R. 2002. The catalytic mechanism of the ESA1 histone acetyltransferase involves a self-acetylated intermediate. *Nat Struct Biol* 9(11): 862-869.

Yang, X.J. 2004. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res* 32(3): 959-976.

Yang, Z., Cool, B.H., Martin, G.M., and Hu, Q. 2006. A dominant role for FE65 (APBB1) in nuclear signaling. *J Biol Chem* 281(7): 4207-4214.

Yeo, M., Lee, S.K., Lee, B., Ruiz, E.C., Pfaff, S.L., and Gill, G.N. 2005. Small CTD phosphatases function in silencing neuronal gene expression. *Science (New York, NY* 307(5709): 596-600.

Yuan, W., Condorelli, G., Caruso, M., Felsani, A., and Giordano, A. 1996. Human p300 protein is a coactivator for the transcription factor MyoD. *J Biol Chem* 271(15): 9009-9013.

Zallen, J.A. 2007. Planar polarity and tissue morphogenesis. *Cell* 129(6): 1051-1063.

Zhang, C.L., McKinsey, T.A., and Olson, E.N. 2002. Association of class II histone deacetylases with heterochromatin protein 1: potential role for histone methylation in control of muscle differentiation. *Molecular and cellular biology* 22(20): 7302-7312.

Zhang, H.S., Postigo, A.A., and Dean, D.C. 1999. Active transcriptional repression by the Rb-E2F complex mediates G1 arrest triggered by p16INK4a, TGFbeta, and contact inhibition. *Cell* 97(1): 53-61.

Zhang, K., Faiola, F., and Martinez, E. 2005. Six lysine residues on c-Myc are direct substrates for acetylation by p300. *Biochemical and biophysical research communications* 336(1): 274-280.

Zhang, W., Deng, H., Bao, X., Lerach, S., Girton, J., Johansen, J., and Johansen, K.M. 2006. The JIL-1 histone H3S10 kinase regulates dimethyl H3K9 modifications and heterochromatic spreading in *Drosophila*. *Development (Cambridge, England)* 133(2): 229-235.

Zhang, Y., Xiong, Y., and Yarbrough, W.G. 1998. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell* 92(6): 725-734.

Zhou, M.I., Wang, H., Foy, R.L., Ross, J.J., and Cohen, H.T. 2004. Tumor suppressor von Hippel-Lindau (VHL) stabilization of Jade-1 protein occurs through plant homeodomains and is VHL mutation dependent. *Cancer research* 64(4): 1278-1286.

Zhou, M.I., Wang, H., Ross, J.J., Kuzmin, I., Xu, C., and Cohen, H.T. 2002. The von Hippel-Lindau tumor suppressor stabilizes novel plant homeodomain protein Jade-1. *J Biol Chem* 277(42): 39887-39898.

Zhou, S., Yang, Y., Scott, M.J., Pannuti, A., Fehr, K.C., Eisen, A., Koonin, E.V., Fouts, D.L., Wrightsman, R., Manning, J.E., and et al. 1995. Male-specific lethal 2, a dosage compensation gene of *Drosophila*, undergoes sex-specific regulation and encodes a protein with a RING finger and a metallothionein-like cysteine cluster. *The EMBO journal* 14(12): 2884-2895.

Zhu, X., Singh, N., Donnelly, C., Boimel, P., and Elefant, F. 2007. The cloning and characterization of the histone acetyltransferase human homolog Dmel\TIP60 in *Drosophila* melanogaster: Dmel\TIP60 is essential for multicellular development. *Genetics* 175(3): 1229-1240.

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EAST CHINA NORMAL UNIVERSITY, Shanghai, P.R. China — B.S., Biochemistry — 1999

Experience

DREXEL UNIVERSITY — 2002-2007

Research Assistant: Studied the function of histone acetyltransferases (i.e. Dmel\TIP60) during development, using *Drosophila* as model system. Techniques included gene cloning, real-time RT-PCR, RNAi, cell culture and transfection, microinjection, phenotype assessment, ELISA, western blot, immunohistochemistry, etc.
Teaching Assistant: Taught in both lab and recitation, collaborated on curriculum and exam development, met with students upon request, and graded all written work, including final exam papers.

SHANGHAI RESEARCH CENTER OF BIOTECHNOLOGY — 1999-2002

Research Assistant: Designed and carried out experiments on erythropoietin (EPO) gene therapy. Performed vector construction, cell culture and transfection, RT-PCR, ELISA, mouse Intramuscular injection, etc.

EAST CHINA NORMAL UNIVERSITY — 1996-1999

Work-study: Did research on a new fibrinolytic enzyme to develop a novel medicine for thrombosis, which has already been approved in the clinical trial in China. Ran immuno-PCR to detect and analyze mutant P53 protein in cancer, which won Daxia Award.

Publications

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- Xianmin Zhu, Zhongmei Chu, Yunlong Hu, Jianfeng Lu, Cheng Liu, Xianming Qu, Yi Zhang. In vitro and in vivo transfection and expression of plasmid-based non-viral vector for erythropoietin gene therapy. Biotechnol. Lett. 2002, 24 (11): 943-947.
- Xianmin Zhu, Yi Zhang. Plasmid-based Gene Therapy, Chemistry of Life, 2002, 22 (6): 583-585.
- Meihua Zhang, Xiaoyan Wang, Zirong Wu, Xianmin Zhu, Pinjie Song. Primary study on immuno-PCR for detection of mutant p53 protein in cancer. Biotechnology. 2000, 10 (5): 9-11.