View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Noncoding RNAs and Intranuclear Positioning in Monoallelic Gene Expression

Pok Kwan Yang[1,2,3] and Mitzi I. Kuroda[1,2,3,*]
[1] Howard Hughes Medical Institute
[2] Harvard-Partners Center for Genetics and Genomics, Brigham and Women's Hospital
[3] Department of Genetics, Harvard Medical School
Boston, Massachusetts 02115, USA
*Correspondence: mkuroda@genetics.med.harvard.edu
DOI 10.1016/j.cell.2007.01.032

Mammalian X inactivation, imprinting, and allelic exclusion are classic examples of monoallelic gene expression. Two emerging themes are thought to be critical for monoallelic expression: (1) noncoding, often antisense, transcription linked to differential chromatin marks on otherwise homologous alleles and (2) physical segregation of alleles to separate domains within the nucleus. Here, we highlight recent progress in identifying these phenomena as possible key regulatory mechanisms of monoallelic expression.

Most genes are expressed from both alleles in diploid organisms. However, there are intriguing phenomena in which genes are only expressed from a single allele. Sex chromosome dosage compensation in mammals is the classic example of monoallelic expression. To equalize the expression of X-linked genes, which differ in copy number in males (XY) and females (XX), the majority of genes on one of the female X chromosomes are transcriptionally silenced in a process called X chromosome inactivation (XCI). Mammalian imprinting is another well-known example of monoallelic expression. A small set of genes are expressed only from paternally inherited alleles, while another select group of genes is expressed only from maternally inherited alleles. The significance of imprinting is well documented since disruption in monoallelic expression of imprinted genes often leads to genetic disorders. Another example of monoallelic expression is allelic exclusion, which occurs in specialized cell types such as B-lymphocytes or olfactory neurons. This process generally excludes gene expression from all but one of a family of alleles in order to obtain strict cell-type specificity during differentiation.

Regulation of monoallelic expression is clearly epigenetic, as homologous alleles follow dissimilar, heritable fates within a shared nucleus. In these three systems, the establishment of epigenetic inheritance may depend on asymmetry in DNA methylation, replication timing, chromatin structure, noncoding RNAs (ncRNAs), and nuclear positioning (reviewed in Goldmit and Bergman, 2004). The role of DNA methylation in imprinting is well established (reviewed in Verona et al., 2003; Smith et al., 2004). Replication timing is linked to differential gene expression, although cause and effect are difficult to discern (reviewed in Goldmit and Bergman 2004). This review will focus on the increasing body of work featuring the potential roles for ncRNAs, chromatin structure, and the effects of nuclear spatial organization on monoallelic expression.

Antisense Transcription and/or Transcripts: Tools for Establishing Epigenetic Marks?

A series of recent studies utilizing high-resolution tiling arrays and various methods of tagging expressed transcripts followed by large-scale sequencing detected an unexpectedly large number of unannotated transcripts, suggesting that most of the mammalian genome is transcribed (reviewed in Frith et al., 2005; Carninci, 2006; Mendes Soares and Valcarcel, 2006). A majority of these unannotated transcripts are putative ncRNAs, some of which are natural antisense transcripts. Transcriptome analysis of the sense/antisense (S/AS) pairs revealed frequent concordant regulation of expression (Katayama et al., 2005). SAGE analysis of the S/AS pairs demonstrated that they are coexpressed and/or inversely expressed in the same tissue more frequently than expected by chance (Chen et al., 2005). Further, in both prokaryotes and eukaryotes, antisense transcripts are clearly capable of regulating gene expression (reviewed in Wagner and Simons, 1994; Terryn and Rouze, 2000; Ogawa and Lee, 2002). These lines of evidence suggest that sense-antisense regulation may be relatively common in mammals. In mammalian dosage compensation, in which one of the two X chromosomes is silenced in females, antisense transcription of ncRNAs plays important roles during the establishment of X inactivation. In addition, antisense transcription units are present in several well-documented examples of imprinting. The mechanisms underlying the regulation of gene expression by antisense transcription units and/or transcripts remain unresolved. Recent

literature suggests an intimate relationship between the establishment of epigenetic modifications and antisense transcription in systems that express genes in a monoallelic manner.

Xist and *Tsix* Influence Chromatin Modification

In random XCI, the silencing of the X chromosome is controlled by the X *chromosome* inactivation center (*Xic*), an important *cis* regulatory region on the X (reviewed in Plath et al., 2002; Heard and Disteche, 2006). *Xic* contains elements mediating various processes that include counting the number of X chromosomes, choice of which X will be inactivated, and initiation and silencing of genes on X (reviewed in Avner and Heard 2001; Plath et al., 2002; Heard and Disteche, 2006). Several critical elements within the *Xic* are genes that produce noncoding transcripts. The *Xist* (X i*nactive* s*pecific* t*ranscript*) noncoding transcript is required for silencing of the inactive X chromosome (Penny et al., 1996; Marahrens et al., 1997). Transcription of *Tsix*, the noncoding antisense transcript of *Xist*, protects the active X chromosome from inactivation, implicating *Tsix* in regulation of *Xist* (Lee et al., 1999a; Lee and Lu, 1999; Sado et al., 2001). Noncoding transcription of *Xite* (X-*inactivation* i*ntergenic* t*ranscription* e*lements*), an additional region of the *Xic,* also influences counting and choice (Ogawa and Lee, 2003). Studies have shown that *Xist* expression is required for establishing epigenetic marks on the inactive X chromosome (Heard et al., 2001; Plath et al., 2003; Silva et al., 2003; de Napoles et al., 2004; Fang et al., 2004; Kohlmaier et al., 2004; Okamoto et al., 2004). More recently, several papers propose that antisense transcription of *Tsix* alters epigenetic chromatin marks that are likely to be involved in *Xist* regulation (Navarro et al., 2005; Sado et al., 2005; Navarro et al., 2006; Sun et al., 2006). Thus, one model is that *Xist* and *Tsix* RNAs recruit protein complexes required for DNA and histone modifications.

It has been known that the inactive X chromosome (Xi) is characterized by various epigenetic marks such as hypoacetylation of histones H3 and H4, enrichment of an H2A variant, macro-H2A.1, and DNA methylation (reviewed in Plath et al., 2002; Heard and Disteche, 2006). These modifications have been proposed to function in maintaining the inactive state of Xi, since they appear late in the silencing process. In contrast, *Xist* expression is early and initiates the transcriptional silencing of most X-linked genes. The mechanisms by which *Xist* accomplishes silencing remain unclear. However, experiments using mammalian embryonic stem (ES) cell lines, which induce *Xist* expression and X inactivation upon differentiation, are beginning to reveal the precise molecular events correlating with X inactivation. Areas of particular interest are histone modifications that may function as epigenetic marks. Methylation of histone H3 at Lys-9 was demonstrated to be an early mark on the X chromosome during Xi even though HP1 does not associate with the inactive X (Heard et al., 2001). More recently, the Polycomb group (PcG) complex PRC2 Eed-Ezh2, which methylates histone H3 on lysine 27 (H3-K27) (reviewed in Cao and Zhang [2004]; see also the Review by B. Schuettengruber et al., page 735 of this issue), was discovered to associate transiently with the future Xi early, during, and immediately after *Xist* expression (Wang et al., 2001; Mak et al., 2002; Plath et al., 2003; Silva et al., 2003; Kohlmaier et al., 2004). This transient association was dependent on *Xist* expression and accompanied by the methylation of H3-K27 (Plath et al., 2003; Kohlmaier et al., 2004). In mouse ES cells, when an autosomal *Xist* transgene was induced, H3-K27 trimethylation (H3-K27me3) and H4-K20 monomethylation (H4-K20me1) were shown to associate with the *Xist*-expressing chromosome (Kohlmaier et al., 2004). Furthermore, a reduction of H3K4 di- and trimethylation, marks generally associated with active chromatin, was observed upon *Xist* expression (Heard et al., 2001; O'Neill et al., 2003; Kohlmaier et al., 2004).

Components of PRC1, another PcG complex, also have been documented to associate transiently with the Xi in ES and in extraembryonic lineage stem cells (de Napoles et al., 2004; Kalantry et al., 2006). Recently, Schoeftner et al. (2006) demonstrated that inducible *Xist* expression on an autosome also leads to the recruitment of PRC1. The association of PRC1 is presumed to monoubiquitinate lysine 119 of histone H2A on the *Xist*-expressing chromosome, providing yet another repressive chromatin mark (de Napoles et al., 2004; Kalantry et al., 2006; Schoeftner et al., 2006). These experiments demonstrate that the act of *Xist* transcription or the transcript itself can recruit different complexes to modify histones on the inactive X.

Surprisingly, these repressive histone modifications are not sufficient for silencing (Plath et al., 2003; Kohlmaier et al., 2004; Kalantry and Magnuson, 2006; Schoeftner et al., 2006). In ES cells, PcG complexes and their targeted modifications associate with chromosomes expressing an inducible mutant *Xist* RNA incapable of silencing (Kohlmaier et al., 2004; Schoeftner et al., 2006). Moreover, random XCI is observed in mouse embryos lacking endogenous and maternally contributed embryonic ectoderm development (EED) proteins, demonstrating that EED is not essential for XCI in the embryo (Kalantry and Magnuson, 2006). Therefore, it is possible that functionally redundant silencing marks are utilized in X inactivation, or alternatively, that the precise molecular events that causes initial silencing of the X chromosome remain to be discovered.

The antisense *Tsix* gene regulates the expression of *Xist*, and its deletion leads to the selective inactivation of the mutant X chromosome (Lee and Lu, 1999; Luikenhuis et al., 2001; Sado et al., 2001). Recently, the ability of *Tsix* to alter chromatin structure along the *Xist*/*Tsix* locus was illustrated in several new studies, suggesting a mechanism for how *Xist* could be regulated (Navarro et al., 2005; Sado et al., 2005; Navarro et al., 2006; Sun et al., 2006). *Xist* expression normally is accompanied in *cis* by the monoallelic association of H3-K4me2 and the preinitiation complex at the *Xist* promoter (Navarro et al., 2005). Navarro et al., (2006) further compared the profiles of histone modifications at the *Xist* 5′ region between wild-type and

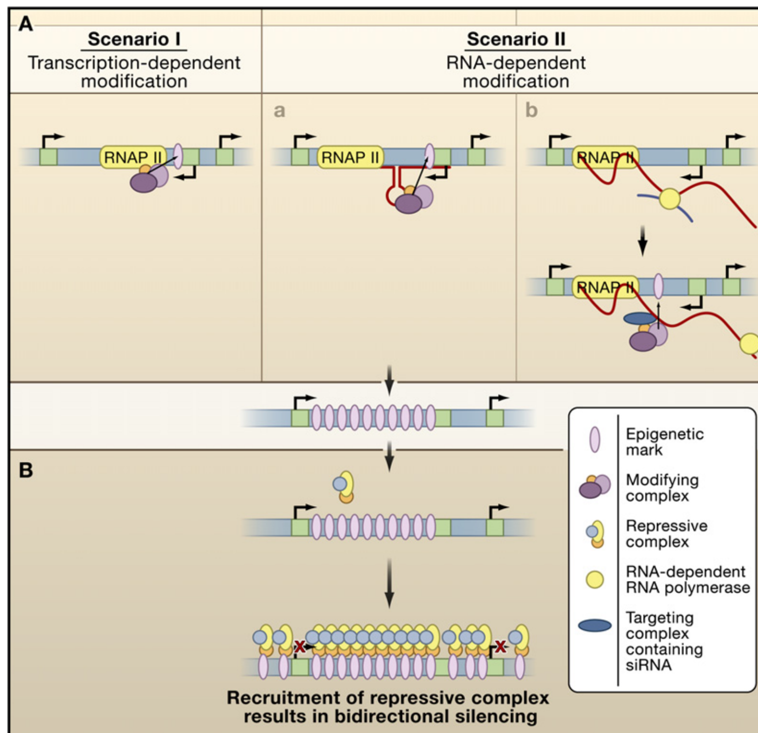

Figure 1. Establishment of Epigenetic Modifications by Antisense Transcription
(A) Scenario I depicts transcription-dependent epigenetic modifications. During antisense transcription, histone-modifying complexes and/or DNA methyltransferase are recruited by the transcriptional machinery. These enzymes then place epigenetic marks at the transcribed locus. Scenario II depicts RNA-dependent epigenetic modification. In IIa, during antisense transcription, the transcribed RNA forms a structure that is recognized by modifying complex(es). The complex(es), recruited by the RNA, then place(s) epigenetic marks on the locus. Scenario IIb depicts a gene silencing dependent on components of the RNAi machinery and RNA-dependent RNA polymerase. Antisense transcript recruits the polymerase, which then generates complementary RNA. RNase III then processes the resulting double-stranded RNA into siRNA molecules. The siRNA molecules are incorporated into a complex that mediates the recruitment of modifying enzymes.
(B) Once epigenetic marks are established, they recruit repressive complexes, such as PRC1, to the locus. The repressive complex then spreads along the chromosome through protein-protein interactions to silence neighboring genes.

mutant male ES cells in which *Tsix* is prematurely truncated. In mutants, the level of repressive marks, DNA methylation and H3-K9me3, dramatically decreased at the *Xist* 5′ region, while the level of H3-K4me2, H3-K4me3, H3K9 acetylation, and H4 acetylation increased when compared to wild-type (Navarro et al., 2006). These modification changes were also observed upstream of *Xist* in mouse embryos in which *Tsix* transcription was truncated (Sado et al., 2005). The results suggest that the presence of *Tsix* transcription and/or transcript normally represses active chromatin modifications at the *Xist* promoter in *cis*, coincident with repression of *Xist* expression on the active X chromosome. Analyses of the dynamics of chromatin changes detected at the *Xist*/*Tsix* locus during the initial establishment of *Xist* expression suggest a more complex regulatory pathway. Sun et al. (2006) found that prior to and during differentiation of ES cells, H3-K27 trimethylation, a mark usually associated with silencing, increases at the *Xist*-promoter region on a *Tsix*-deletion chromosome. This unexpected association of a silencing mark with the promoter of the *Xist* allele that is destined to be expressed is a transient effect and was not detected by Navarro et al. (2006). However, as XCI proceeds, the promoter of the expressed allele of *Xist* acquires the active chromatin marks normally associated with open chromatin (Sun et al., 2006).

Although it is not known which chromatin changes might be directly influenced by *Tsix* expression, it is clear that *Tsix* influences epigenetic modifications at the *Xist* locus. This could be accomplished either by antisense transcription, through the association of modifying complexes with the RNA polymerase machinery (Figure 1A, scenario I), or by the association of the transcript with modifying complexes (Figure 1A, scenario IIa). Although the RNA-interference machinery has not been implicated in the mammalian examples we cite in this review, recent work in *S. pombe* suggests the possibility of further complexity in the second scenario (Buhler et al., 2006). As shown in Figure 1A, scenario IIb, an RNA-dependent RNA polymerase could target an ncRNA such as *Tsix* or *Xist* during transcription, synthesizing double-stranded RNA to attract the chromatin-silencing machinery strictly in *cis* (Buhler et al., 2006).

Antisense Transcription in Imprinting: Bidirectional Silencing of Neighboring Genes

Imprinting is a phenomenon in which only one of the two alleles of a gene is expressed, dictated by its paternal or maternal origin. There are approximately 70 imprinted genes in mammals, and inappropriate expression of most of these genes affects fetal development and placental function (reviewed in Reik et al., 2001; Tycko and Efstratiadis, 2002). Imprinted genes often exist in clusters and contain imprinting control regions (ICRs) that are differentially marked in gametogenesis (reviewed in Mann et al., 2000). ICRs are characterized by differentially methylated regions (DMRs), which are methylated CpG islands that determine whether or not the gene located in *cis* will be expressed (reviewed in Verona et al., 2003). Most imprinted clusters also contain at least one imprinted ncRNA (http://www.mgu.har.mrc.ac.uk/research/imprinting/index.html), suggesting that these RNAs could play a functional

role. Four of the six well-characterized imprinting clusters, *Igf2r*, *Kcnq1*, *Gnas*, and *Pws*, contain an ncRNA in the antisense orientation of one of the silenced genes. Identifying a specific role for an RNA as opposed to the DNA that encodes it can be difficult. However, in two cases, the truncation of *Air* and *Kcnq1ot1* antisense transcripts within *Igf2r* and *Kcnq1*, respectively, has enabled this distinction to be made. In these two cases, it is clear that transcription of the RNAs is critical for the imprinted expression of the genes along these clusters (Sleutels et al., 2002; Mancini-Dinardo et al., 2006). Ironically, the first ncRNA implicated in imprinting, *H19*, seems to be dispensible for imprinting of *Igf2*, as long as *cis*-acting regulatory regions remain intact (Jones et al., 1998). In the remaining examples, the data are suggestive but not yet definitive that the expression of antisense transcripts is important in determining the expression of the imprinted protein-coding genes (Williamson et al., 2004. 2006). For an overview on noncoding RNAs in gene silencing, see Review by M. Zaratiegui et al., page 763 in this issue.

The *Igf2r* (*insulin growth factor 2 receptor*) imprinted cluster is the most thoroughly documented example of antisense transcription mediating the silencing of neighboring genes. The noncoding, antisense transcript *Air* is expressed from a promoter located in intron 2 of the *Igf2r* gene on the paternally repressed allele (Sleutels et al., 2002), while the maternal copy of the promoter is nonfunctional due to DNA methylation. Even though the *Air* transcript overlaps only the *Igf2r* gene, the deletion of the *Air* promoter results in the derepression of *Igf2r* and *Slc22a2* and *Slc22a3*, two genes that do not overlap with *Air* (Wutz et al., 1997; Zwart et al., 2001; Sleutels et al., 2002).

Premature termination of imprinted *Air* transcription also leads to loss of silencing of *Igf2r*, *Slc22a2*, and *Slc22a3*, suggesting that the transcription of *Air* or the transcript itself is responsible for the gene silencing (Sleutels et al., 2002). How could this silencing be accomplished? An interesting result obtained in mouse primary cultured neurons might explain the underlying silencing mechanism (Yamasaki et al., 2005). In these neurons, which biallelically express *Igf2r*, *Air* is not expressed. In the absence of *Air* expression, the promoter regions of both *Igf2r* alleles display similar levels of chromatin modifications including DNA hypomethylation, histone H3 and H4 acetylation, and H3K4-me2. In contrast, the promoter region of *Igf2r* in cultured glial cells that express *Igf2r* in a monoallelic manner displays differential levels of these modifications between the maternal and paternal chromosomes. This observation suggests that like *Tsix*, *Air* antisense transcription or *Air* transcript may establish repressive histone modifications at the *Igf2r* promoter.

Another well-studied imprinted cluster containing an antisense transcript is the *Kcnq1* domain on mouse chromosome 7. The primary basis for imprinting is differential DNA methylation. The paternal repression of the imprinted *Kcnq1* domain is controlled by the ICR, KvDMR1, which consists of a CpG island located in the intron of the *Kcnq1* gene that is methylated on the maternal allele (Fitzpatrick et al., 2002). The unmethylated paternal KvDMR1 gives rise to an ncRNA *Kcnq1ot1* (Lee et al., 1999b; Smilinich et al., 1999). Recently, expression of this RNA has been implicated in Polycomb-based silencing in *cis*, which was observed to play a role in the repression of several genes along the *Kcnq1* cluster (Mager et al., 2003; Lewis et al., 2004, 2006; Umlauf et al., 2004). In mouse extraembryonic tissues, for instance, the silencing of some genes along the paternal chromosome involves PcG complex, Eed-Ezh2, and repressive marks, H3-K27me3 and H3-K9me2 (Lewis et al., 2004; Umlauf et al., 2004).

Recent studies suggest that recruitment of the PcG complex to the paternal chromosome depends on the transcription of *Kcnq1ot1*. First, an episomal construct consisting of the KvDMR1 ICR flanked by two reporter genes demonstrated that the expression of *Kcnq1ot1* is able to silence gene expression bidirectionally (Thakur et al., 2004; Kanduri et al., 2006). The length of the RNA being transcribed in the construct is proportionally related to the degree of silencing: the longer the transcript, the stronger the repression of the flanking genes (Kanduri et al., 2006). Furthermore, the acquisition of repressive histone modifications depends strongly on transcription elongation. This idea is further supported by a recent observation in mice expressing a truncated form of *Kcnq1ot1* RNA (Mancini-Dinardo et al., 2006). Repression of the paternal allele normally depends on the transcription of *Kcnq1ot1*, and premature transcription termination of the noncoding transcript results in derepression of paternally silenced genes (Mancini-Dinardo et al., 2006). Therefore, transcription of the *Kcnq1ot1* ncRNA may recruit a histone-modifying complex to mediate the silencing of imprinted genes (Figure 1A). This recruitment could either depend on transcription (scenario I) or the transcript (scenario IIa & IIb). Once the histone modifications are established, they could then recruit repressive complexes to the locus (Figure 1B). These complexes could repress the expression of the genes bidirectionally in the cluster, possibly by a "spreading" mechanism.

The *Gnas*-imprinting cluster on mouse chromosome 2 consists of maternally, paternally, and biallelically expressed transcripts (Peters et al., 1999). Three DMRs have been identified in the *Gnas* cluster (Kelsey et al., 1999; Peters et al., 1999; Liu et al., 2000; Coombes et al., 2003). Two of the DMRs are maternally methylated, and one of these is located at the promoter of *Gnasxl* and *Nespas* (Coombes et al., 2003). *Gnasxl* and *Nespas*, a noncoding transcript (Wroe et al., 2000), are both paternally expressed. *Nespas* is located in the middle of the cluster and is an antisense transcript of the gene *Nesp*, a maternally expressed transcript coding for a neuroendocrine secretory protein (Ischia et al., 1997; Peters et al., 1999). Targeted deletion of the DMR abolishes the expression of *Nespas* (Williamson et al., 2006). Surprisingly, it affected the imprinted expression of the genes along the cluster bidirectionally. This effect is reminiscent of the ICRs of the *Igf2r* and *Kcnq1* imprinting clusters. Remarkably, the

deletion of the *Nespas/Gnasxl* DMR affected the methylation pattern of the two other DMRs in the clusters (Williamson et al., 2006). One of these DMRs is comprised of a promoter region for another ncRNA, *Exon 1A*, which is important for expression of *Gnas* in certain tissues (Williamson et al., 2004). How these ncRNAs play a role in the regulation of imprinted gene expression remains to be answered. The transcription or the transcript of *Nespas* and *Exon 1A* could play a role in the stable repression of some of the genes.

Antisense transcription clearly plays a role in the establishment of histone modifications in some imprinting clusters. However, it is important to note that differential DNA methylation at imprinting clusters is often the initial signal governing allele-specific expression (reviewed in Brannan and Bartolomei, 1999). In most scenarios, establishment of differential histone modifications is an important downstream regulatory process to implement the initial asymmetry established in the maternal or paternal germline by DNA methylation.

Chromatin Modification and Antisense Transcription in the IgH Region of B Lymphocytes

Monoallelic expression is important for the proper function of the mammalian immune system. Functional genes encoding the antigen receptors of B and T lymphocytes are produced by recombination events during differentiation (reviewed in Bassing et al., 2002; Johnson et al., 2005). One example of monoallelic expression is the production of the murine immunoglobulin heavy chain (IgH), whose locus is comprised of several hundred variable (V_H) gene segments, 16 diversity (D_H) gene segments, and 4 joining (J_H) gene segments (Chevillard et al., 2002). Unique V_H, D_H, and J_H segments at the locus are selected and recombined to give rise to a functional IgH gene (reviewed in Bassing et al., 2002). Once the heavy chain is expressed, recombination is inhibited at the IgH locus, resulting in allelic exclusion to ensure the production of only one type of antibody molecule per B cell clone (reviewed in Bassing et al., 2002).

The recombination of V_H, D_H, and J_H segments is a tightly controlled, ordered event (reviewed in Johnson et al., 2005). The joining of the D_H and J_H is the first step of the recombination process (reviewed in Bassing et al., 2002). Prior to V_H -DJ_H recombination, the V_H locus becomes hyperacetylated on histones H4 and H3 (Chowdhury and Sen, 2001; Johnson et al., 2003), presumably generating a more open chromatin structure to be accessed by the recombinase. Once the fully functional VDJ_H allele is assembled, there is decreased histone acetylation at the rest of the V_H locus (Chowdhury and Sen, 2001; Johnson et al., 2003), suggesting that histone modification contributes to the maintenance of allelic exclusion. In addition, several studies have implicated a role for H3K9 methylation at the IgH locus during B cell differentiation (Morshead et al., 2003; Johnson et al., 2004).

A study recently found that antisense transcription could also play a role in the ordered joining of V_H-DJ_H segments (Bolland et al., 2004). Antisense transcription is detected extensively across the genic and intergenic DNA of the V_H locus. It is strand specific and occurs prior to or during the V_H-DJ_H rearrangement but is rapidly downregulated after VDJ_H recombination. The function of the transient antisense transcription remains unclear. Since it only occurs during or after DJ_H recombination, its primary function is probably not to establish gene silencing of the V_H region prior to recombination. It is also unlikely to be used to set up epigenetic marks on one allele, since it is biallelically expressed. Bolland et al. (2004) have proposed that the antisense transcription remodels the V_H region on both alleles, thereby facilitating downstream events required for V_H-DJ_H recombination. This model is similar to a proposal that *Tsix* initially renders the *Xist* locus epigenetically equivalent prior to the onset of random XCI (Navarro et al., 2005). Antisense transcription in the V_H region may provide an equal opportunity for each V_H gene to be recombined.

The molecular functions of antisense transcription appear pivotal to the mechanisms governing mammalian dosage compensation, genomic imprinting, and perhaps allelic exclusion. Their molecular functions could be varied, but they are all coupled to the establishment of epigenetic chromatin marks. The antisense transcription or the antisense transcript could recruit modifying and/or remodeling complexes to regulate neighboring genes. In cases such as *Kcnq1ot1* antisense transcription, it could attract PRC2 to establish histone modifications that then recruit PRC1 to assist in the silencing of neighboring genes. In other cases, such as *Tsix* transcription, it could attract or activate DNA and histone methyltransferases to establish modifications at the *Xist* locus. In another example, antisense transcription at the V_H region of the B cell IgH locus could recruit remodeling complexes to open the chromatin of the V_H locus for recombination. Distinguishing effects of transcription from functions of the resulting transcripts and going beyond correlative evidence for the function of histone modifications at these loci remain a significant challenge.

Spatial Organization within the Nucleus: A Mechanism for Mutually Exclusive Gene Expression?

Spatial organization of the genome within the nucleus may play a critical role in the regulation of gene expression. In the interphase nucleus, chromosomes occupy distinct regions termed chromosome territories, which are nonrandomly organized within the nuclear space (reviewed in Cremer and Cremer, 2001; Parada et al., 2004). The functional role of nonrandom positioning of the chromosome territories is unclear, but it could promote the efficiency of gene expression/silencing by creating specialized nuclear subcompartments. The position of genes relative to their resident chromosome territory may also influence their transcriptional status (Mahy et al., 2002a) as location in the interior of the chromosome territory generally correlates with silencing, while location at the periphery generally correlates with transcription, with notable exceptions

(Mahy et al., 2002b). In X inactivation, the two X chromosomes occupy clearly distinct territories, correlating with their dissimilar expression states (Bacher et al., 2006; Xu et al., 2006). Within the Xi, the silencing of X-linked genes is further linked to their position relative to the chromosome territory (Chaumeil et al., 2006; Clemson et al., 2006). In imprinting, the spatial organization of DMRs and enhancer elements of the *Igf2*/*H19*-imprinting cluster is correlated with regulation of monoallelic expression of *Igf2* and *H19* (Murrell et al., 2004; Kurukuti et al., 2006). In addition, the spatial organization between chromosomes appears important for mutually exclusive expression of alleles in mouse olfactory neuron receptor cells. Here, we will discuss how nuclear architecture and spatial location and organization of chromosomes are implicated in control of X inactivation, genomic imprinting, and allelic exclusion.

The Role of X-X Interaction in X Inactivation

In random XCI, females must count the number of X chromosomes and choose only one to keep active. The counting/choosing mechanism depends on elements of the *Xic* (Lee and Lu, 1999; Ogawa and Lee, 2003). It has been proposed that there is communication between the two X chromosomes to allow counting and then mutually exclusive expression of *Xist* and *Tsix*. This notion is supported by two recent reports that study the proximity of X chromosomes during the establishment of X inactivation. Using imaging and statistical analyses, the *Xic* regions of the two X chromosomes were found to associate transiently prior to and/or during the initial stages of *Xist* expression (Bacher et al., 2006; Xu et al., 2006). Partial deletion of the *Xic* leads to a disruption in the counting mechanism and the absence of colocalization of the two *Xics*, confirming that elements within the *Xic* are important for counting. Further, Xu et al. (2006) mapped regions within *Tsix* and *Xite* required for *Xic* colocalization. Using chromosome conformation capture (3C), they also showed that these regions of the two X chromosomes physically interact, demonstrating at the molecular level that homologous X chromosomes pair transiently at the onset of X inactivation.

The spatial location and organization of the X chromosomes within the nucleus is likely very important for their transient association. How are regions of the *Xic* initially brought within close proximity to physically interact? The analysis of Xu et al. (2006) suggest that *Tsix* and *Xite* RNAs could play a role. However, the interaction is transient (Bacher et al., 2006; Xu et al., 2006). The dissociation could be due to sequestration of the *Xic* into a silent X-chromosome territory during the establishment of X inactivation (Chaumeil et al., 2006). Expressed X-linked genes tend to reside in the periphery of the X chromosome (Chaumeil et al., 2006; Clemson et al., 2006). During the establishment of X inactivation, *Xist* RNA expression correlates with the exclusion of RNA polymerase followed by gene relocation to the interior of the Xi chromosome territory, forming a silent nuclear compartment (Chaumeil et al., 2006).

Intrachromosomal Interaction: Regulation of Imprinting

The *Igf2*/*H19* region on chromosome 7 is an example of genomic imprinting that may depend on the spatial genome organization in the nucleus for monoallelic expression of *Insulin growth factor 2* (*Igf2*) (reviewed in Leighton et al., 1996; Verona et al., 2003; Reik et al., 2004). An ICR at the 5′ region of *H19* is essential for the imprinted expression of *H19* and *Igf2*, located more than 80 kb upstream (Thorvaldsen et al., 1998). It contains a DMR, which in its unmethylated state is bound by CTCF, a DNA binding protein that acts as an insulator in the *Igf2*/*H19* imprinting region (Bell and Felsenfeld 2000; Hark et al., 2000; Kanduri et al., 2000; Szabo et al., 2000). On the paternal chromosome, the ICR is hypermethylated, preventing the binding of CTCF. Enhancers located downstream of *H19* are proposed to associate with a DMR near the *Igf2* promoter, leading to paternal expression of *Igf2* (reviewed in Reik et al., 2004). On the maternal chromosome, the ICR is hypomethylated and bound by CTCF, which prevents the association of the enhancer with the DMR. Instead, the enhancer associates with the promoter of *H19*, leading to maternal expression of the noncoding *H19* transcript. The physical intrachromosomal interactions between these regions have recently been confirmed by 3C (Murrell et al., 2004), suggesting that these loci are in close proximity relative to each other. These results demonstrated that *Igf2*/*H19* DMRs and enhancers, though located more than 80 kb apart, are dynamically organized within the chromosome territory to promote their association.

Recent studies using variations of 3C further suggest an extensive network of intra- and interchromosomal interactions at the *H19* ICR region (Ling et al., 2006; Zhao et al., 2006). Zhao et al. (2006) developed circular chromosomal conformation capture (4C) to determine the possible intra- and interchromosomal interactions of a specific element on a genome-wide scale. Using the method to search for sequences associated with the *H19* ICR, they found 114 unique sequences dispersed throughout the autosomes, several of which interact primarily with the maternally inherited *H19* ICR, suggesting that CTCF plays a role in these interactions. Interestingly, a number of imprinted domains were determined to interact with the *H19* ICR using 4C and 3C, demonstrating a possible spatially organized network for epigenetic regulation.

Using another variation of 3C, Ling et al. (2006) discovered two additional sequences that interact with *H19* ICR, which are distinct from the sequences identified by Zhao et al. (2006). One of the two sequences is located at a gene-poor region on chromosome 6, while the other is in the intergenic region between genes *Wsb1* and *Nf1* on chromosome 11. In cells with reduced expression of CTCF, which binds to the maternal ICR, the long-range interaction between *Igf2*/*H19* and *Wsb1*/*Nf1* was abolished,

suggesting that the association is dependent on CTCF. Despite biallelic expression of both genes, low levels of CTCF reduced the expression of *Nf1* and *Wsb1* from only the paternal chromosome.

Though speculative, genes in different imprinted clusters could be organized into specific nuclear subcompartments that regulate gene expression in a coordinated fashion. Factors that recruit genes to specific compartments would also play an important role. For example, the ICR of *Igf2/H19* and the *Nf1/Wsb1* loci seem to localize to the same nuclear compartment through interaction with CTCF, which has been proposed to tether insulator elements to the nucleolar surface (Yusufzai et al., 2004). Besides directing physical association between genes, the CTCF insulator may also play a role in transcriptional activation, as demonstrated by reduced expression of *Nf1* and *Wsb1* upon knocking down CTCF. Insulator elements have been suggested to play a dual role in transcriptional activation and the organization of independent chromatin domains (reviewed in Capelson and Corces 2004), and the CTCF insulator seems to be involved in both events. It remains puzzling, however, that the reduction in *Nf1* and *Wsb1* expression is allelic specific even though these genes are not known to be imprinted.

Monoallelic Expression of Olfactory Receptors

Each murine olfactory neuron expresses only one odorant receptor (OR) gene from a repertoire of more than 1300 genes, producing one type of receptor per neuron (Chess et al., 1994; Malnic et al., 1999). Recent findings suggest that a single enhancer element may govern the expression of different OR genes through intra- and interchromosomal interactions (Serizawa et al., 2003; Lomvardas et al., 2006). A *cis*-acting enhancer element *H*, 70 kb upstream of a cluster of OR genes, was first demonstrated to activate the expression of single OR genes in the linked cluster (Serizawa et al., 2003). Deletion of the *H* region abolished the expression of any of the linked OR genes, while its addition restored expression in transgenic mice. After the production of a functional OR receptor, a negative-feedback mechanism inhibited the expression of other OR genes. Serizawa et al. (2003) proposed that the enhancer element *H* could interact intrachromosomally at the promoter of only one OR gene per nucleus to activate expression.

Subsequently, Lomvardas et al. (2006) demonstrated that enhancer element *H* not only associates with OR genes on the same chromosome, it can also associate in *trans* with OR genes on other chromosomes. DNA *f*luorescence *in* situ *h*ybridization (FISH) showed that *H* DNA colocalizes with OR genes located on different chromosomes, suggesting an interchromosomal interaction between the *H* element and OR gene promoters. Furthermore, DNA/RNA FISH showed *H* DNA colocalizing with expressed OR RNAs. These physical inter- and intrachromosomal associations between the *H* region and different OR genes were confirmed by 3C. Interestingly, in single cells, only one of the alleles of *H* is methylated, indicating

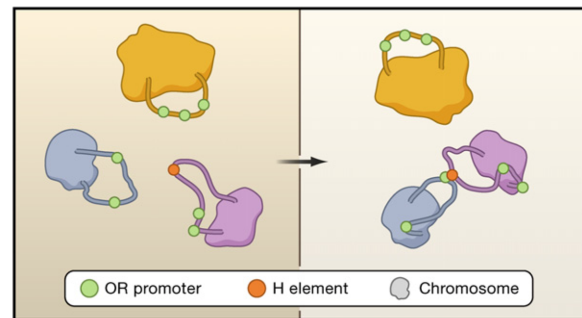

Figure 2. Competition for a Shared Regulatory Element by Murine OR Genes
OR genes on different chromosomes compete for enhancer H, a regulatory element. Once a gene is "chosen" and expressed, a negative signal is provided to prevent the expression of other OR genes. The genes that are not expressed could be repositioned in the nuclear periphery or sequestered into the interior of chromosomal territories.

that there is only one functional *H* to activate one OR gene. These observations suggest that the mutually exclusive expression of a large family of OR genes on different chromosomes may be controlled through a remarkable competition for physical interaction with a common regulatory element. One can imagine that once a negative-feedback signal is generated upon the production of a receptor, the nonselected OR genes can reposition from an active domain to a transcriptionally silent domain within the nucleus (Figure 2).

Gene regulation through subnuclear compartmentalization can also occur in other examples of allelic exclusion. For example, it has been proposed that during the development of pro B cells, the IgH locus can reposition within the nucleus to permit recombination and expression of a recombined VDJ_H gene (Kosak et al., 2002).

We have provided specific examples from X inactivation, imprinting, and allelic exclusion that demonstrate that a dynamic nuclear architecture may be critical to regulate monoallelic expression. Clearly, genes that are expressed in a mutually exclusive and monoallelic manner could take advantage of distinct nuclear subcompartments. Regulation by compartmentalization may be quite general, as genes that participate in a binary developmental decision during T cell development also exhibit mutually exclusive interchromosomal interactions and repositioning behavior (Spilianakis et al., 2005). However, the mechanism and factors that promote the reorganization or the movement of genes and chromatin in the nucleus remain to be determined. Could epigenetic marks play a role? In *Drosophila*, PcG proteins form discrete nuclear bodies, and it has been proposed that PcG-targeted genes can be recruited to these loci (Ficz et al., 2005; Grimaud et al., 2006; see also Review by B. Schuettengruber et al., page 735 in this issue). An appealing possibility is that RNA-directed epigenetic modifications, intra- and interchromosomal interactions, and nuclear positioning are linked processes that act together to promote

strikingly different expression states of homologous alleles residing within the same nucleus.

ACKNOWLEDGMENTS

We thank M. Bartolomei, M. Gelbart, A. Gortchakov, J. Lee, and the reviewers for helpful comments. We thank J. Racine for assistance with the figures. P.-K. Yang and M.I. Kuroda are supported by the Howard Hughes Medical Institute.

REFERENCES

Avner, P., and Heard, E. (2001). X-chromosome inactivation: counting, choice and initiation. Nat. Rev. Genet. *2*, 59–67.

Bacher, C.P., Guggiari, M., Brors, B., Augui, S., Clerc, P., Avner, P., Eils, R., and Heard, E. (2006). Transient colocalization of X-inactivation centres accompanies the initiation of X inactivation. Nat. Cell Biol. *8*, 293–299.

Bassing, C.H., Swat, W., and Alt, F.W. (2002). The mechanism and regulation of chromosomal V(D)J recombination. Cell *109*, S45–S55.

Bell, A.C., and Felsenfeld, G. (2000). Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature *405*, 482–485.

Bolland, D.J., Wood, A.L., Johnston, C.M., Bunting, S.F., Morgan, G., Chakalova, L., Fraser, P.J., and Corcoran, A.E. (2004). Antisense intergenic transcription in V(D)J recombination. Nat. Immunol. *5*, 630–637.

Brannan, C.I., and Bartolomei, M.S. (1999). Mechanisms of genomic imprinting. Curr. Opin. Genet. Dev. *9*, 164–170.

Buhler, M., Verdel, A., and Moazed, D. (2006). Tethering RITS to a nascent transcript initiates RNAi- and heterochromatin-dependent gene silencing. Cell *125*, 873–886.

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*, 155–164.

Capelson, M., and Corces, V.G. (2004). Boundary elements and nuclear organization. Biol. Cell *96*, 617–629.

Carninci, P. (2006). Tagging mammalian transcription complexity. Trends Genet. *22*, 501–510.

Chaumeil, J., Le Baccon, P., Wutz, A., and Heard, E. (2006). A novel role for Xist RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced. Genes Dev. *20*, 2223–2237.

Chen, J., Sun, M., Hurst, L.D., Carmichael, G.G., and Rowley, J.D. (2005). Genome-wide analysis of coordinate expression and evolution of human cis-encoded sense-antisense transcripts. Trends Genet. *21*, 326–329.

Chess, A., Simon, I., Cedar, H., and Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene expression. Cell *78*, 823–834.

Chevillard, C., Ozaki, J., Herring, C.D., and Riblet, R. (2002). A three-megabase yeast artificial chromosome contig spanning the C57BL mouse Igh locus. J. Immunol. *168*, 5659–5666.

Chowdhury, D., and Sen, R. (2001). Stepwise activation of the immunoglobulin mu heavy chain gene locus. EMBO J. *20*, 6394–6403.

Clemson, C.M., Hall, L.L., Byron, M., McNeil, J., and Lawrence, J.B. (2006). The X chromosome is organized into a gene-rich outer rim and an internal core containing silenced nongenic sequences. Proc. Natl. Acad. Sci. USA *103*, 7688–7693.

Coombes, C., Arnaud, P., Gordon, E., Dean, W., Coar, E.A., Williamson, C.M., Feil, R., Peters, J., and Kelsey, G. (2003). Epigenetic properties and identification of an imprint mark in the Nesp-Gnasxl domain of the mouse Gnas imprinted locus. Mol. Cell. Biol. *23*, 5475–5488.

Cremer, T., and Cremer, C. (2001). Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat. Rev. Genet. *2*, 292–301.

de Napoles, M., Mermoud, J.E., Wakao, R., Tang, Y.A., Endoh, M., Appanah, R., Nesterova, T.B., Silva, J., Otte, A.P., Vidal, M., et al. (2004). Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. Dev. Cell *7*, 663–676.

Fang, J., Chen, T., Chadwick, B., Li, E., and Zhang, Y. (2004). Ring1b-mediated H2A ubiquitination associates with inactive X chromosomes and is involved in initiation of X inactivation. J. Biol. Chem. *279*, 52812–52815.

Ficz, G., Heintzmann, R., and Arndt-Jovin, D.J. (2005). Polycomb group protein complexes exchange rapidly in living Drosophila. Development *132*, 3963–3976.

Fitzpatrick, G.V., Soloway, P.D., and Higgins, M.J. (2002). Regional loss of imprinting and growth deficiency in mice with a targeted deletion of KvDMR1. Nat. Genet. *32*, 426–431.

Frith, M.C., Pheasant, M., and Mattick, J.S. (2005). The amazing complexity of the human transcriptome. Eur. J. Hum. Genet. *13*, 894–897.

Goldmit, M., and Bergman, Y. (2004). Monoallelic gene expression: a repertoire of recurrent themes. Immunol. Rev. *200*, 197–214.

Grimaud, C., Bantignies, F., Pal-Bhadra, M., Ghana, P., Bhadra, U., and Cavalli, G. (2006). RNAi components are required for nuclear clustering of Polycomb group response elements. Cell *124*, 957–971.

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*, 486–489.

Heard, E., and Disteche, C.M. (2006). Dosage compensation in mammals: fine-tuning the expression of the X chromosome. Genes Dev. *20*, 1848–1867.

Heard, E., Rougeulle, C., Arnaud, D., Avner, P., Allis, C.D., and Spector, D.L. (2001). Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation. Cell *107*, 727–738.

Ischia, R., Lovisetti-Scamihorn, P., Hogue-Angeletti, R., Wolkersdorfer, M., Winkler, H., and Fischer-Colbrie, R. (1997). Molecular cloning and characterization of NESP55, a novel chromogranin-like precursor of a peptide with 5-HT1B receptor antagonist activity. J. Biol. Chem. *272*, 11657–11662.

Johnson, K., Angelin-Duclos, C., Park, S., and Calame, K.L. (2003). Changes in histone acetylation are associated with differences in accessibility of V(H) gene segments to V-DJ recombination during B-cell ontogeny and development. Mol. Cell. Biol. *23*, 2438–2450.

Johnson, K., Pflugh, D.L., Yu, D., Hesslein, D.G., Lin, K.I., Bothwell, A.L., Thomas-Tikhonenko, A., Schatz, D.G., and Calame, K. (2004). B cell-specific loss of histone 3 lysine 9 methylation in the V(H) locus depends on Pax5. Nat. Immunol. *5*, 853–861.

Johnson, K., Shapiro-Shelef, M., Tunyaplin, C., and Calame, K. (2005). Regulatory events in early and late B-cell differentiation. Mol. Immunol. *42*, 749–761.

Jones, B.K., Levorse, J.M., and Tilghman, S.M. (1998). Igf2 imprinting does not require its own DNA methylation or H19 RNA. Genes Dev. *12*, 2200–2207.

Kalantry, S., and Magnuson, T. (2006). The Polycomb group protein EED is dispensable for the initiation of random X-chromosome inactivation. PLoS Genet. *2*, e66.

Kalantry, S., Mills, K.C., Yee, D., Otte, A.P., Panning, B., and Magnuson, T. (2006). The Polycomb group protein Eed protects the inactive X-chromosome from differentiation-induced reactivation. Nat. Cell Biol. *8*, 195–202.

Kanduri, C., Pant, V., Loukinov, D., Pugacheva, E., Qi, C.F., Wolffe, A., Ohlsson, R., and Lobanenkov, V.V. (2000). Functional association of

CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. Curr. Biol. *10*, 853–856.

Kanduri, C., Thakur, N., and Pandey, R.R. (2006). The length of the transcript encoded from the Kcnq1ot1 antisense promoter determines the degree of silencing. EMBO J. *25*, 2096–2106.

Katayama, S., Tomaru, Y., Kasukawa, T., Waki, K., Nakanishi, M., Nakamura, M., Nishida, H., Yap, C.C., Suzuki, M., Kawai, J., et al. (2005). Antisense transcription in the mammalian transcriptome. Science *309*, 1564–1566.

Kelsey, G., Bodle, D., Miller, H.J., Beechey, C.V., Coombes, C., Peters, J., and Williamson, C.M. (1999). Identification of imprinted loci by methylation-sensitive representational difference analysis: application to mouse distal chromosome 2. Genomics *62*, 129–138.

Kohlmaier, A., Savarese, F., Lachner, M., Martens, J., Jenuwein, T., and Wutz, A. (2004). A chromosomal memory triggered by Xist regulates histone methylation in X inactivation. PLoS Biol. *2*, E171.

Kosak, S.T., Skok, J.A., Medina, K.L., Riblet, R., Le Beau, M.M., Fisher, A.G., and Singh, H. (2002). Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science *296*, 158–162.

Kurukuti, S., Tiwari, V.K., Tavoosidana, G., Pugacheva, E., Murrell, A., Zhao, Z., Lobanenkov, V., Reik, W., and Ohlsson, R. (2006). CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. Proc. Natl. Acad. Sci. USA *103*, 10684–10689.

Lee, J.T., Davidow, L.S., and Warshawsky, D. (1999a). Tsix, a gene antisense to Xist at the X-inactivation centre. Nat. Genet. *21*, 400–404.

Lee, J.T., and Lu, N. (1999). Targeted mutagenesis of Tsix leads to nonrandom X inactivation. Cell *99*, 47–57.

Lee, M.P., DeBaun, M.R., Mitsuya, K., Galonek, H.L., Brandenburg, S., Oshimura, M., and Feinberg, A.P. (1999b). Loss of imprinting of a paternally expressed transcript, with antisense orientation to KVLQT1, occurs frequently in Beckwith-Wiedemann syndrome and is independent of insulin-like growth factor II imprinting. Proc. Natl. Acad. Sci. USA *96*, 5203–5208.

Leighton, P.A., Saam, J.R., Ingram, R.S., and Tilghman, S.M. (1996). Genomic imprinting in mice: its function and mechanism. Biol. Reprod. *54*, 273–278.

Lewis, A., Green, K., Dawson, C., Redrup, L., Huynh, K.D., Lee, J.T., Hemberger, M., and Reik, W. (2006). Epigenetic dynamics of the Kcnq1 imprinted domain in the early embryo. Development *133*, 4203–4210.

Lewis, A., Mitsuya, K., Umlauf, D., Smith, P., Dean, W., Walter, J., Higgins, M., Feil, R., and Reik, W. (2004). Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. Nat. Genet. *36*, 1291–1295.

Ling, J.Q., Li, T., Hu, J.F., Vu, T.H., Chen, H.L., Qiu, X.W., Cherry, A.M., and Hoffman, A.R. (2006). CTCF mediates interchromosomal colocalization between Igf2/H19 and Wsb1/Nf1. Science *312*, 269–272.

Liu, J., Yu, S., Litman, D., Chen, W., and Weinstein, L.S. (2000). Identification of a methylation imprint mark within the mouse Gnas locus. Mol. Cell. Biol. *20*, 5808–5817.

Lomvardas, S., Barnea, G., Pisapia, D.J., Mendelsohn, M., Kirkland, J., and Axel, R. (2006). Interchromosomal interactions and olfactory receptor choice. Cell *126*, 403–413.

Luikenhuis, S., Wutz, A., and Jaenisch, R. (2001). Antisense transcription through the Xist locus mediates Tsix function in embryonic stem cells. Mol. Cell. Biol. *21*, 8512–8520.

Mager, J., Montgomery, N.D., de Villena, F.P., and Magnuson, T. (2003). Genome imprinting regulated by the mouse Polycomb group protein Eed. Nat. Genet. *33*, 502–507.

Mahy, N.L., Perry, P.E., and Bickmore, W.A. (2002a). Gene density and transcription influence the localization of chromatin outside of chromosome territories detectable by FISH. J. Cell Biol. *159*, 753–763.

Mahy, N.L., Perry, P.E., Gilchrist, S., Baldock, R.A., and Bickmore, W.A. (2002b). Spatial organization of active and inactive genes and noncoding DNA within chromosome territories. J. Cell Biol. *157*, 579–589.

Mak, W., Baxter, J., Silva, J., Newall, A.E., Otte, A.P., and Brockdorff, N. (2002). Mitotically stable association of polycomb group proteins eed and enx1 with the inactive x chromosome in trophoblast stem cells. Curr. Biol. *12*, 1016–1020.

Malnic, B., Hirono, J., Sato, T., and Buck, L.B. (1999). Combinatorial receptor codes for odors. Cell *96*, 713–723.

Mancini-Dinardo, D., Steele, S.J., Levorse, J.M., Ingram, R.S., and Tilghman, S.M. (2006). Elongation of the Kcnq1ot1 transcript is required for genomic imprinting of neighboring genes. Genes Dev. *20*, 1268–1282.

Mann, J.R., Szabo, P.E., Reed, M.R., and Singer-Sam, J. (2000). Methylated DNA sequences in genomic imprinting. Crit. Rev. Eukaryot. Gene Expr. *10*, 241–257.

Marahrens, Y., Panning, B., Dausman, J., Strauss, W., and Jaenisch, R. (1997). Xist-deficient mice are defective in dosage compensation but not spermatogenesis. Genes Dev. *11*, 156–166.

Mendes Soares, L.M., and Valcarcel, J. (2006). The expanding transcriptome: the genome as the 'Book of Sand'. EMBO J. *25*, 923–931.

Morshead, K.B., Ciccone, D.N., Taverna, S.D., Allis, C.D., and Oettinger, M.A. (2003). Antigen receptor loci poised for V(D)J rearrangement are broadly associated with BRG1 and flanked by peaks of histone H3 dimethylated at lysine 4. Proc. Natl. Acad. Sci. USA *100*, 11577–11582.

Murrell, A., Heeson, S., and Reik, W. (2004). Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. Nat. Genet. *36*, 889–893.

Navarro, P., Page, D.R., Avner, P., and Rougeulle, C. (2006). Tsix-mediated epigenetic switch of a CTCF-flanked region of the Xist promoter determines the Xist transcription program. Genes Dev. *20*, 2787–2792.

Navarro, P., Pichard, S., Ciaudo, C., Avner, P., and Rougeulle, C. (2005). Tsix transcription across the Xist gene alters chromatin conformation without affecting Xist transcription: implications for X-chromosome inactivation. Genes Dev. *19*, 1474–1484.

O'Neill, L.P., Randall, T.E., Lavender, J., Spotswood, H.T., Lee, J.T., and Turner, B.M. (2003). X-linked genes in female embryonic stem cells carry an epigenetic mark prior to the onset of X inactivation. Hum. Mol. Genet. *12*, 1783–1790.

Ogawa, Y., and Lee, J.T. (2002). Antisense regulation in X inactivation and autosomal imprinting. Cytogenet. Genome Res. *99*, 59–65.

Ogawa, Y., and Lee, J.T. (2003). Xite, X-inactivation intergenic transcription elements that regulate the probability of choice. Mol. Cell *11*, 731–743.

Okamoto, I., Otte, A.P., Allis, C.D., Reinberg, D., and Heard, E. (2004). Epigenetic dynamics of imprinted X inactivation during early mouse development. Science *303*, 644–649.

Parada, L.A., Sotiriou, S., and Misteli, T. (2004). Spatial genome organization. Exp. Cell Res. *296*, 64–70.

Penny, G.D., Kay, G.F., Sheardown, S.A., Rastan, S., and Brockdorff, N. (1996). Requirement for Xist in X chromosome inactivation. Nature *379*, 131–137.

Peters, J., Wroe, S.F., Wells, C.A., Miller, H.J., Bodle, D., Beechey, C.V., Williamson, C.M., and Kelsey, G. (1999). A cluster of oppositely imprinted transcripts at the Gnas locus in the distal imprinting region of mouse chromosome 2. Proc. Natl. Acad. Sci. USA *96*, 3830–3835.

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 *300*, 131–135.

Plath, K., Mlynarczyk-Evans, S., Nusinow, D.A., and Panning, B. (2002). Xist RNA and the mechanism of X chromosome inactivation. Annu. Rev. Genet. *36*, 233–278.

Reik, W., Dean, W., and Walter, J. (2001). Epigenetic reprogramming in mammalian development. Science *293*, 1089–1093.

Reik, W., Murrell, A., Lewis, A., Mitsuya, K., Umlauf, D., Dean, W., Higgins, M., and Feil, R. (2004). Chromosome loops, insulators, and histone methylation: new insights into regulation of imprinting in clusters. Cold Spring Harb. Symp. Quant. Biol. *69*, 29–37.

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

Sado, T., Wang, Z., Sasaki, H., and Li, E. (2001). Regulation of imprinted X-chromosome inactivation in mice by Tsix. Development *128*, 1275–1286.

Schoeftner, S., Sengupta, A.K., Kubicek, S., Mechtler, K., Spahn, L., Koseki, H., Jenuwein, T., and Wutz, A. (2006). Recruitment of PRC1 function at the initiation of X inactivation independent of PRC2 and silencing. EMBO J. *25*, 3110–3122.

Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y., and Sakano, H. (2003). Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. Science *302*, 2088–2094.

Silva, J., Mak, W., Zvetkova, I., Appanah, R., Nesterova, T.B., Webster, Z., Peters, A.H., Jenuwein, T., Otte, A.P., and Brockdorff, N. (2003). Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes. Dev. Cell *4*, 481–495.

Sleutels, F., Zwart, R., and Barlow, D.P. (2002). The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature *415*, 810–813.

Smilinich, N.J., Day, C.D., Fitzpatrick, G.V., Caldwell, G.M., Lossie, A.C., Cooper, P.R., Smallwood, A.C., Joyce, J.A., Schofield, P.N., Reik, W., et al. (1999). A maternally methylated CpG island in KvLQT1 is associated with an antisense paternal transcript and loss of imprinting in Beckwith-Wiedemann syndrome. Proc. Natl. Acad. Sci. USA *96*, 8064–8069.

Smith, R.J., Arnaud, P., and Kelsey, G. (2004). Identification and properties of imprinted genes and their control elements. Cytogenet. Genome Res. *105*, 335–345.

Spilianakis, C.G., Lalioti, M.D., Town, T., Lee, G.R., and Flavell, R.A. (2005). Interchromosomal associations between alternatively expressed loci. Nature *435*, 637–645.

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*, 617–628.

Szabo, P., Tang, S.H., Rentsendorj, A., Pfeifer, G.P., and Mann, J.R. (2000). Maternal-specific footprints at putative CTCF sites in the H19 imprinting control region give evidence for insulator function. Curr. Biol. *10*, 607–610.

Terryn, N., and Rouze, P. (2000). The sense of naturally transcribed antisense RNAs in plants. Trends Plant Sci. *5*, 394–396.

Thakur, N., Tiwari, V.K., Thomassin, H., Pandey, R.R., Kanduri, M., Gondor, A., Grange, T., Ohlsson, R., and Kanduri, C. (2004). An antisense RNA regulates the bidirectional silencing property of the Kcnq1 imprinting control region. Mol. Cell. Biol. *24*, 7855–7862.

Thorvaldsen, J.L., Duran, K.L., and Bartolomei, M.S. (1998). Deletion of the H19 differentially methylated domain results in loss of imprinted expression of H19 and Igf2. Genes Dev. *12*, 3693–3702.

Tycko, B., and Efstratiadis, A. (2002). Genomic imprinting: piece of cake. Nature *417*, 913–914.

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. Nat. Genet. *36*, 1296–1300.

Verona, R.I., Mann, M.R., and Bartolomei, M.S. (2003). Genomic imprinting: intricacies of epigenetic regulation in clusters. Annu. Rev. Cell Dev. Biol. *19*, 237–259.

Wagner, E.G., and Simons, R.W. (1994). Antisense RNA control in bacteria, phages, and plasmids. Annu. Rev. Microbiol. *48*, 713–742.

Wang, J., Mager, J., Chen, Y., Schneider, E., Cross, J.C., Nagy, A., and Magnuson, T. (2001). Imprinted X inactivation maintained by a mouse Polycomb group gene. Nat. Genet. *28*, 371–375.

Williamson, C.M., Ball, S.T., Nottingham, W.T., Skinner, J.A., Plagge, A., Turner, M.D., Powles, N., Hough, T., Papworth, D., Fraser, W.D., et al. (2004). A cis-acting control region is required exclusively for the tissue-specific imprinting of Gnas. Nat. Genet. *36*, 894–899.

Williamson, C.M., Turner, M.D., Ball, S.T., Nottingham, W.T., Glenister, P., Fray, M., Tymowska-Lalanne, Z., Plagge, A., Powles-Glover, N., Kelsey, G., et al. (2006). Identification of an imprinting control region affecting the expression of all transcripts in the Gnas cluster. Nat. Genet. *38*, 350–355.

Wroe, S.F., Kelsey, G., Skinner, J.A., Bodle, D., Ball, S.T., Beechey, C.V., Peters, J., and Williamson, C.M. (2000). An imprinted transcript, antisense to Nesp, adds complexity to the cluster of imprinted genes at the mouse Gnas locus. Proc. Natl. Acad. Sci. USA *97*, 3342–3346.

Wutz, A., Smrzka, O.W., Schweifer, N., Schellander, K., Wagner, E.F., and Barlow, D.P. (1997). Imprinted expression of the Igf2r gene depends on an intronic CpG island. Nature *389*, 745–749.

Xu, N., Tsai, C.L., and Lee, J.T. (2006). Transient homologous chromosome pairing marks the onset of X inactivation. Science *311*, 1149–1152.

Yamasaki, Y., Kayashima, T., Soejima, H., Kinoshita, A., Yoshiura, K., Matsumoto, N., Ohta, T., Urano, T., Masuzaki, H., Ishimaru, T., et al. (2005). Neuron-specific relaxation of Igf2r imprinting is associated with neuron-specific histone modifications and lack of its antisense transcript Air. Hum. Mol. Genet. *14*, 2511–2520.

Yusufzai, T.M., Tagami, H., Nakatani, Y., and Felsenfeld, G. (2004). CTCF tethers an insulator to subnuclear sites, suggesting shared insulator mechanisms across species. Mol. Cell *13*, 291–298.

Zhao, Z., Tavoosidana, G., Sjolinder, M., Gondor, A., Mariano, P., Wang, S., Kanduri, C., Lezcano, M., Singh Sandhu, K., Singh, U., et al. (2006). Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat. Genet. *38*, 1341–1347.

Zwart, R., Sleutels, F., Wutz, A., Schinkel, A.H., and Barlow, D.P. (2001). Bidirectional action of the Igf2r imprint control element on upstream and downstream imprinted genes. Genes Dev. *15*, 2361–2366.