Thesis for doctoral degree (Ph.D.)
2007

Genome wide analysis of the Ssn6-Tup11/Tup12 co-repressor complex in the fission yeast Schizosaccharomyces pombe

Fredrik Fagerström Billai

Karolinska Institutet

Thesis for doctoral degree (Ph.D.) 2007

Genome wide analysis of the Ssn6-Tup11/Tup12 co-repressor in fission yeast

Fredrik Fagerström Billai

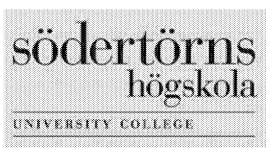
Karolinska Institutet

From the School of Life Sciences, Södertörns Högskola
and the Department of Biosciences and Medical Nutrition
Karolinska Institute, Stockholm Sweden

Genome wide analysis of the Ssn6-Tup11/Tup12 co-repressor complex in the fission yeast *Schizosaccharomyces pombe.*

Fredrik Fagerström Billai

Karolinska Institutet



Stockholm 2007

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by Larserics Digital AB, Sundbyberg, Sweden

© Fredrik Fagerström Billai, 2007
ISBN 978-91-7357-120-3

"Everything has its beauty but not everyone sees it" K'ung-fu-tzu 551 – 479 B.C.

ABSTRACT

In this study, we have investigated the fission yeast Ssn6-Tup11/Tup12 transcriptional co-repressor which is involved in regulation of many genes important for a wide variety of processes. In contrast to the well characterised budding yeast Tup1 protein there are two paralogous proteins present in fission yeast, namely Tup11 and Tup12. We have shown that the two proteins can interact with each other and are expressed at similar levels, which is in line with a reported redundant function. Sequence analysis shows that the intermediate proposed histone interacting domain is highly variable between Tup11 and Tup12 indicating a diversification. Interestingly, we show that *tup11*$^-$ and *tup12*$^-$ mutants have different phenotypes on media containing KCl and $CaCl_2$. Consistent with this functional difference, we identify a number of target genes by genome wide expression profiling that are differentially affected by *tup11*$^-$ and *tup12*$^-$. Many of these genes are Tup12 dependent and correlate with genes that have previously been shown to respond to a range of different environmental stress conditions. The observed different physiological roles of Tup11 and Tup12 can not be explained by differential recruitment of Ssn6 which can interact independently with both Tup11 and Tup12. Most interestingly we show that the Ssn6 protein is essential in fission yeast and therefore must have a distinct role separated from Tup11 and Tup12. Surprisingly, a conditional *ssn6HA-ts* mutant displays the same growth phenotype as *tup12*$^-$, indicating a role in Tup12 dependent stress response. Consistent with the diverse phenotypes of the individual co-repressor proteins, we identify a group of genes that requires Ssn6 for their regulation which is overlapping but distinct from the group of genes that depend on Tup11 or Tup12. Genome wide chromatin immunoprecipitation shows that Ssn6 is almost invariably found in the same genomic locations as Tup11 and/or Tup12. All three co-repressor subunits are generally bound to genes that are selectively regulated by Ssn6 or Tup11/12, and thus, likely in the context of a co-repressor complex containing all three subunits. The co-repressor binds to both the intergenic and coding regions of genes, but differential localization of the co-repressor within genes does not appear to account for the selective dependence of target genes on the Ssn6 or Tup11/12 subunits. Ssn6, Tup11, and Tup12 are preferentially found at genomic locations at which histones are deacetylated, primarily by the Clr6 class I HDAC. A subset of co-repressor target genes, including direct target genes affected by Ssn6 overexpression, is in addition associated with the function of class II (Clr3) and III (Hst4 and Sir2) HDACs. Interestingly, many specific Hst4 repressed ORF targets involved in amino acid biosynthesis are also direct targets for the Ssn6-Tup11/12 co-repressor, suggesting an association with the class III sirtuins which has not been reported previously.

LIST OF PUBLICATIONS

I. **Fagerstrom-Billai F**, Wright AP. Functional comparison of the Tup11 and Tup12 transcriptional co-repressors in fission yeast. Mol Cell Biol. 2005 Jan;25 (2):716-27.

II. **Fagerstrom-Billai F**, Durand-Dubief M, Ekwall K, Wright AP. Individual subunits of the Ssn6-Tup11/12 co-repressor are selectively required for repression of different target genes. Mol Cell Biol. 2007 Feb;27(3):1069-82. Epub 2006 Nov 13.

III. Mickaël Durand-Dubief, Indranil Sinha, **Fredrik Fagerström-Billai**, Carolina Bonilla Anthony Wright, Michael Grunstein, Karl Ekwall. Specific functions for the fission yeast Sirtuins Hst2 and Hst4 in gene regulation and retrotransposon silencing. Manuscript.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Tup1	Tymidine Uptake protein
Ssn6	Suppressor of *snf1* protein
HDAC	Histone Deacetylase
TSA	Trichostatin A
HAT	Histone Acetylase
CRE	Cyclic AMP Response Element
HSE	Heat Shock Element
GRE	Glucocorticoid Resonse Element
TRE	Thyroid hormone Resonse Element
UAS	Upstream Activating Sequence
RNAP	Ribo Nucleic Acid Polymerase
CTD	C Terminal Domain (RNAP)
TBP	TATA Binding Protein
TAFs	Trancription Associated Factors
PIC	Pre-Initiation Complex
ChIP	Chromatin Immunoprecipitation
DBD	DNA Binding Domain
AD	Activation Domain
HLH	Helix Loop Helix
SRB	Suppressor of RNA polymerase *B. subtilis*
SWI/SNF	Switch Sucrose non Fermenting
N-CoR	Nuclear receptor Co-Repressor complex
NuRD	Nucleosome Remodeling
CLR	Cryptic Loci Regulator
SIR	Silent Information Regulator
CYC	Cytochrome c
WD	Trypthophan (W) and Aspartic acid (D)
TPR	Tetra Trico Peptide Repeat
TLE	Transducin like Enhancer of Split
UTY	Ubiquitously Transcribed Y chromosome
UTX	Ubiquitously Transcribed X chromosome
MAPK	Mitogen Activated Protein Kinase
IGR	Intergenic Region
TBZ	Thiabendazole
ORF	Open Reading Frame

1 GENERAL INTRODUCTION

Genes are made of DNA, which is the platform for transcription in all organisms. The flow of genetic information involves transcription of DNA to form RNA followed sequentially by translation of the RNA by a protein synthesis mechanism. The genetic code, specified by triplets of nucleotides in the DNA, is in this way transmitted to proteins, the major machines and structural components of the cell. The shape of the assembled proteins is determined by the amino acid sequence coded by the nucleotide sequence in the DNA. Directed by their amino acid sequence proteins fold into three dimensional structures with different shapes and functions. A high degree of flexibility allows the polypeptide chain to form different motifs composed by a-helices and b-sheet that combine and define the tertiary structure of the protein. Motifs build domains which are responsible for the different functions of the protein. Many protein domains are shared by families of proteins with similar functions and sequence analysis and comparison is therefore important for understanding protein function and evolution. The proteome of a cell consists of the proteins present within a cell with different functions like catalysis, regulation, signal transduction, cell-cycle control, building material, metabolism, stress response and transport among others. Importantly, proteins can also interact non-covalently with other proteins to form multiprotein complexes involved in many of the processes described above. Therefore, investigations of protein interactions are important to determine the context and the function of proteins. The biological function is determined by the surface of the protein which can be modified in many different ways. Changes can be induced by interactions or by modifications. Proteins can bind ligands or be covalently altered by different chemical linkages which can induce dynamic changes in the conformation and function (Alberts, 2002).

1.1 GENE EXPRESSION

Cells need to respond to signals and turn genes on and off to orchestrate the protein composition of the cell. A particular protein in a specific tissue in a certain cell type is needed at a given time while another protein needs to be silenced. Many of the regulatory steps controlling protein output are fundamental for understanding how cells react and respond to different changes in the environment. Regulation of protein levels can occur at different stages. The most important is the first step in the chain, the transcription event which is the major control point of gene expression (Latchman, 1997). The initiation of transcription is a critical step in this process, and is therefore

subjected to complex regulation in eukaryotes. Other regulatory mechanisms which can influence protein function are secondary to this event and involve RNA processing, RNA degradation, protein synthesis, folding and breakdown. In the eukaryotes basal transcription is very low and transcription depends on many regulatory events. External signals need to be funnelled into activators that can initiate transcription and start the elongation process (Alberts, 2002).

1.2 CHROMATIN

Eukaryotic genomes are localised to the cell nucleus and consist of linear DNA molecules packed into chromosomes. The chromosome number and the number of genes differ between species. In the chromosomes the DNA is bundled together with certain proteins called histones into a highly ordered structure called chromatin (Wolffe, 1994a). The DNA binding proteins consist of the different histone variants H2A, H2B, H3 and H4 which form the octameric core nucleosome which 146bp of DNA is wrapped around approximately twice (Fig. 1) (Luger et al., 1997). The histones are highly conserved proteins, reflecting their fundamental role in DNA maintenance (Grunstein, 1992). Other proteins like the linker histone H1 are also involved in the organisation of the chromatin by binding nucleosomes to a more condense structure. The DNA wrapped around the nucleosomes forms a higher order 30nm chromatin fibre which is anchored to the nuclear scaffold of the cell nucleus. The DNA nucleosome structure allows the chromatin to be dynamic and to change into different states. A more relaxed state of the chromatin (euchromatin) is associated with transcriptionally active DNA, while a tense form of the chromatin (heterochromatin) makes the DNA less accessible and therefore transcriptionally inactive (Wolffe, 1994b).

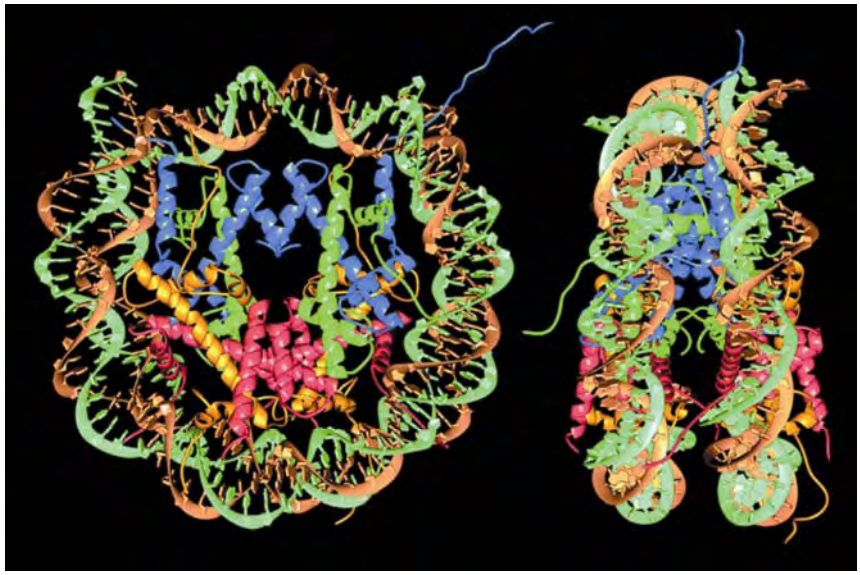


Figure 1. Structure of a nucleosome particle shown with a ribbon diagram from the front (left) and from the side (right). The DNA strands are shown in green and brown. The individual histones are shown in color: H2A in yellow, H2B in red, H3 in blue and H4 in green. With permission from Nature Publishing Group (Luger et al., 1997).

2 TRANSCRIPTIONAL CONTROL

2.1 CONTROL ELEMENTS

The intergenic region (IGR) or non coding DNA between structural genes contain sequence element which can influence gene expression. Some elements are involved in basal transcription and some elements are gene specific and respond to certain input signals. The promotor region is found proximal to the coding region and contain essential sequences for binding RNA polymerases. Together with the start site for transcription these sequences build the core promotor element. One common feature of an eukaryotic core promotor is the TATA box which is an AT-rich consensus sequence found approximately 30bp upstream from the ATG start (Carcamo et al., 1990). However, it has been suggested that the number of TATA box promotors are overestimated and that the majority of human genes instead are TATA less (Gross and Oelgeschlager, 2006). Control elements which are involved in basal transcription can also be found at a longer distance from the start site. Such enhancers or upstream promotor elements allow contact with the promotor region by DNA looping and thereby constrain bound proteins to interact and influence transcription (Fig. 2) (Muller and Schaffner, 1990; Talbert and Henikoff, 2006).

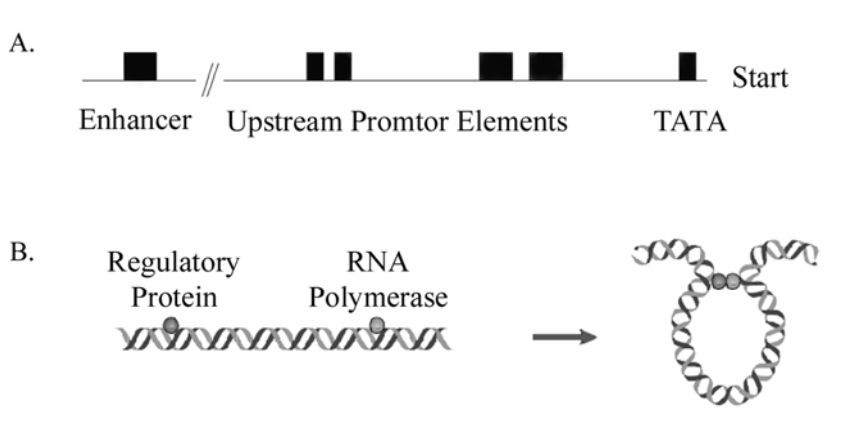


Figure 2. **(A)** Schematic view of a eukaryotic promotor shown with different types of regulatory elements. **(B)** Simplified model showing enhancer function. Transcriptional regulatory proteins can bind at the Upstream Activating Sequence (UAS) and interact with the basal transcriptional machinery by DNA looping. With permission from Nature Publishing Group (Talbert and Henikoff, 2006).

Some upstream regulatory elements that have been described are: heat shock elements (HSE), cyclic AMP response elements (CRE) (Sassone-Corsi, 1995) and hormone induced (GRE and TRE) (Becker et al., 1986; Latchman, 1997). Certain promotor elements can affect transcription in an orientation and position independent manner. Examples of such silencers are found in the mating type loci and the centromere of various yeast species (Laurenson and Rine, 1992).

2.2 RNA POLYMERASES

Three different RNA polymerases (Cramer, 2004; Ishihama et al., 1998) manage the transcription of eukaryotic genes. All are multi subunit protein complexes (10-12 subunits) which are arranged over the core promotor of transcribed genes. RNA polymerase II holoenzyme transcribes the messenger RNA (mRNA) coding for the proteins in eukaryotic cells. RNA polymerase I is involved in transcription of ribosomal RNA (rRNA), which is a component of the ribosome involved in protein synthesis. RNA polymerase III transcribes small RNA (sRNA) and transfer RNA (tRNA) that translate triplets of RNA nucleotides into specific amino acids. The formation of a stable RNA polymerase II (RNAP II) complex has been studied extensively, and typically involves core promotor elements bound by initiation factors and a gene specific upstream activation element (UAS) recognised by regulatory factors (Roeder, 1996; Weil et al., 1979). Initiation at a TATA core promotor starts by binding of the general factor TFIID which contain the sequence recognizing TATA Binding Protein (TBP) and TFIIA over the TATA box (Burley and Roeder, 1996; Werner and Burley, 1997). The TFIID factor also contain a number of stabilising TBP associated factors (TAFs), which have both positive and negative regulatory functions in core promotor binding. TFIID and TFIIA are then further stabilised by binding of TFIIB and TFIIF which allows recruitment of the catalytic RNAP II (Hahn et al., 1989; Leuther et al., 1996). The pre-initiation complex (PIC) is finally formed by binding of TFIIE and TFIIH which induces promotor opening by melting of the DNA (Svejstrup et al., 1996; Zawel et al., 1995). An important step required for elongation of the PIC is the phosphorylation of the C terminal domain (CTD) of the RNAP II subcomplex (Gileadi et al., 1992). Many of the described components are released before departure of the PIC and can be used in pre initiation and assembly of new complexes. The assembly over the TATA box is also affected in many different ways by other regulatory proteins. Factors bound at other upstream elements are also attracted into the vicinity of the core promotor where they can accelerate or repress the assembly of a stable

transcription complex. This regulation is fundamental to coordinate important cellular mechanisms in all eukaryotic organisms from yeast to humans. This complex regulation allows gene expression to be altered and fine tuned depending on the general context of the promotor. In this way, both negative and positive regulators can be integrated to work together.

2.3 DNA BOUND TRANSCRIPTION FACTORS

DNA binding transcription factors target nucleotide sequences in the intergenic region and can thereby serve as a platform for recruitment of the transcriptional machinery and other transcriptional regulators (Nelson, 1995). They have distinct functional domains for DNA binding as well as activation or repression depending on the type (Ptashne, 1988). Several different DNA binding domains (DBD) have been described. (i) The helix-turn-helix motif (HTH), found in many homeodomain proteins, consists of two a-helices that can recognize specific DNA sequences (Kornberg, 1993). (ii) The zinc-finger motif, which belongs to a very large family of eukaryotic DNA binding transcription factors, uses Zn^{2+} ions to stabilise the coordination of cysteines and histidines which form a segment of b-sheet followed by an a-helix pointing out from the protein and contacting the DNA (Pavletich and Pabo, 1991; Rhodes and Klug, 1993). (iii) The basic element DBDs, which are formed by dimers containing a leucine zipper motif or a helix-loop-helix motif. Many important transcription factors like Max, c-Fos, c-Myc, CREB and Gcn4 are found in this group (Ellenberger et al., 1992; Latchman, 1997; Nelson, 1995). Similar to the DBDs, a number of different activation domains have been described, which stimulate transcription across a wide range of species. Such activators are sometimes classified on the basis of their content of amino acids including proline-rich, glutamine-rich and acidic activators (Hahn et al., 1989). The transactivation domain recruits the transcriptional machinery upon activation in a process where the random coiled activation domain is believed to form an alpha helix upon binding to its target protein (McEwan et al., 1996; Radhakrishnan et al., 1997; Uesugi et al., 1997). Mapping of the viral VP16 activation domain, which can activate transcription in mammalian cells, has also revealed indispensable hydrophobic residues important for activation (Cress and Triezenberg, 1991). Even if the major part of the DNA bound transcription factors are activators there is also evidence of DNA bound repressors (Cowell, 1994; Latchman, 1996). A DNA bound repressor can interfere directly with the transcriptional machinery, but it can also act indirectly by masking the regulatory element for positive regulators and other factors that can mediate the effect.

Indirect repression can occur by blocking of an enhancer element or by sequestering of an activator, thereby preventing it from binding the DNA.

2.4 CO-ACTIVATORS AND CO-REPRESSORS

Co-activators and co-repressors are recruited to DNA bound transcription factors to mediate the physical contact with the RNAP II or with other regulatory factors. They differ from general transcription factors since they cannot bind DNA directly. Co-regulators are often members of large protein complexes that are recruited to target sites and affect transcription in different ways. Regulation generally involves interplay between activators and repressors and multiple regulators which can be integrated into enhanceosomes and repressosomes. Some of the most important classes are associated with the TBP-, mediator-, acetylation-, deacetylation-, methylation- and remodeling-complexes that will be further described below (Lee and Young, 1998; Wolffe, 1997; Wu and Grunstein, 2000).

2.5 THE MEDIATOR COMPLEX

In vitro experiments studying transcription in yeast demonstrates the requirement for a large multi component protein complex in association with the RNAP II complex that was found to mediate activator signals to the transcriptional machinery. Independent genetic studies have also identified mutants that can suppress phenotypes caused by mutations in the CTD of the RNAP II. Many of these suppressor components (SRB) are essential for *in vitro* transcription with nuclear extracts. Purification of the Srb/mediator complex has been found to contain additional factors implicated in many different steps in the regulation of RNAP II (Kim et al., 1994; Lee and Young, 2000). It has been shown that the mediator complex has a kinase activity that stimulates phosphorylation of the CTD, which is important for transducing the signal to RNAP II (Lee and Young, 2000; Thompson et al., 1993). The composition of the mediator complex differs between species but they have analogous structural composition, indicating conserved function (Malik and Roeder, 2005). Genetic screens have shown that the mediator participates in activation but also in repression of individual genes since some of the SRB and MED components have a role in repression of target genes (Balciunas and Ronne, 1995; Carlson, 1997) . In conclusion, it is clear that the mediator function as a central control component of transcription, where it can transmit signals to the RNAP II. The large size and the position of the mediator suggest involvement in many more undiscovered functions.

2.6 CHROMATIN MODIFICATIONS

Histones can also influence gene expression by changing the structure of chromatin and the accessibility of elements in the DNA sequences to transcriptional activators and repressors. Hence, nucleosomes have to change their structure in promotors of active genes (Wolffe, 1994a). The ability of the nucleosome to repress initiation of transcription has been shown both *in vitro* and *in vivo* (Grunstein, 1990; Kornberg and Lorch, 1999). The charged lysine rich N-terminal tail, external to the histone fold domain of the histone extends from the nucleosome and plays a key role in transcriptional regulation. Post-translational modifications of the lysine rich tail of the histones like acetylation, deacetylation, methylation, phoshorylation, ubiquitination and sumoylation can thus change the accessibility of the chromatin and affect transcription (Fig. 3) (Lee and Young, 2000).

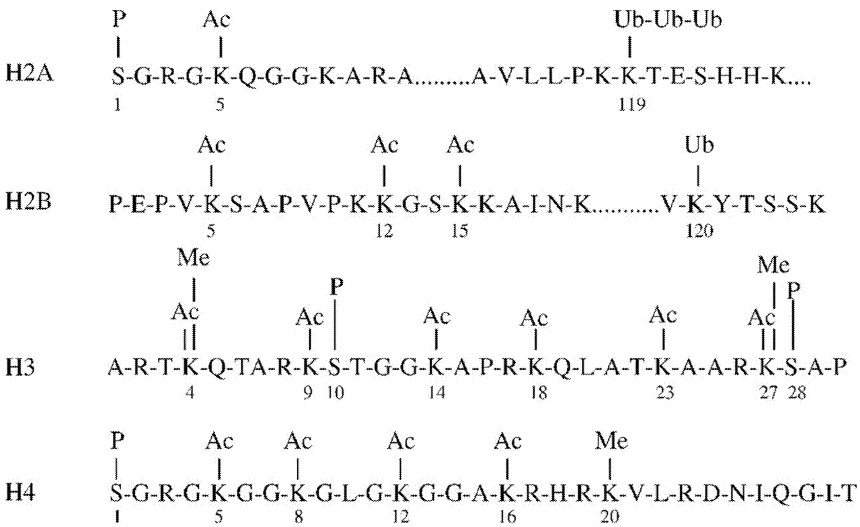


Figure 3. Sites of covalent modifications at N-terminal residues of the nucleosome core histones. The different modifications include: Acetylation (Ac), Phosphorylation (P), Methylation (Me) and Ubiquitination (Ub). With permission from Annual Reviews Publishing (Lee and Young, 2000).

2.7 HISTONE ACETYLATION

Acetylation of the histone tails removes the positive charge from the lysines and is known to correlate with an open chromatin structure and increased gene activity (Allfrey et al., 1964; Kornberg and Lorch, 1999; Wolffe and Pruss, 1996). Histone

acetyltransferases (HATs) transfer acetylgroups from acetyl-coenzyme A to the N-terminal lysine residues. Homologues to the initially cloned *Tetrahymena thermophila* HAT that have been identified include the yeast Gcn5 and p300/CBP in mammalian cells (Bannister and Kouzarides, 1996; Brownell et al., 1996). The HATs do not work individually but are members of multiprotein co-activator complexes. Many different HAT complexes have been identified, such as the Gcn5 containing yeast SAGA and ADA (Grant et al., 1997) and the Esa1 containing NuA4 complex (Allard et al., 1999; Smith et al., 1998). The acetylation has been shown to be highly specific and the targets for SAGA are histones H3 and H2B while NuA4 acetylates histones H4 an H2A (Allard et al., 1999; Grant et al., 1999). In human cells the PCAF, TFTC and STAGA complexes have been characterised (Martinez et al., 1998; Martinez et al., 2001). In some forms HAT complexes also contain subsets of general transcription factors that reportedly contribute to changing the specificity for different genes (Ogryzko et al., 1998). The activity of HAT complexes is dependent on DNA binding gene specific regulators. The selectivity and close association with the Pol II machinery underline the importance of HAT complexes in gene regulation. HATs can also acetylate other substrates than histones. Some DNA binding activators like p53 and GATA (Boyes et al., 1998; Gu and Roeder, 1997), and some GTFs like TFIIE and TFIIF (Imhof et al., 1997) can also be modified by acetylation.

2.8 CHROMATIN REMODELING

Access to the transcriptional template can also be affected by a number of chromatin remodeling factors capable of binding and actively shifting the nucelosomes. Chromatin remodeling factors utilize energy from hydrolysing ATP and have in common the presence of an ATPase motor component (Pazin and Kadonaga, 1997). Remodeling complexes can be classified into different groups based on their composition of subunits, type of remodeling activity and specificity. Two main families of remodeling complexes have been described in yeast namely the SWI/SNF and ISWI complexes. Subtypes of these complexes are also found in higher eukaryotes such as BRAHMA, dSWI/SNF and NURF in *D. melanogaster* and hSWI/SNF and RSF in *H. sapiens* (Vignali et al., 2000). Remodeling complexes have been described to regulate transcriptional activation and interact with HAT co-activators like SAGA suggesting a link between acetylation and remodeling.

2.9 HISTONE DEACETYLATION

In contrast to histone acetylases another important group of enzymes act to remove acetyl groups from the histone N-terminal lysine residues, namely the histone deacetylases (HDAC). Reversible histone acetylation has been observed simultaneously in many different systems including yeast and mammalian cells, where the rapid turnover of histone acetyl levels in cells was found to be in equilibrium with deacetylated histones (Vidali et al., 1972). As mentioned acetylated histones correlate with highly transcribed genes while inactive repressed genes are instead associated with deacetylated histones, indicating a close connection to transcriptional regulation (Chen and Allfrey, 1987). Their roles in transcriptional regulation have been linked to a wide variety of biological activities like cell cycle progression, gene silencing, cell differentiation and DNA replication (Bestor, 1998; Grunicke et al., 1984; Lehrmann et al., 2002; Zhang et al., 2000). The acetylation and deacetylation events are highly specific, and certain lysines of the N-terminal tails of histones H2A, H2B, H3 and H4 are important targets of acetyl modifications by different classes of HDACs (Grunstein, 1997; Rundlett et al., 1996). Just like the HATs the HDACs probably also act on other proteins than histones, providing another level of protein regulation (Bannister and Kouzarides, 1996).

An important breakthrough for the characterisation of HDACs was the finding that exposure to N-butyrate causes accumulation of acetylated histones in HeLa cells (Candido et al., 1978; Riggs et al., 1977). N-butyrate inhibits HDAC activity and causes many different biological effects like induction of differentiation in tumour cell lines and cell cycle arrest (Kruh, 1982). Isolation of more potent inhibitors, like trapoxin and trichostatin A (TSA) revealed stronger effects on cell differentiation and accumulation of acetylated histones (Yoshida et al., 1990). The first isolated HDAC, the mammalian HDAC1, was isolated with trapoxin (Taunton et al., 1996) and was found to co-purify with histone deacetylase activity. Peptide sequence comparison of the HDAC1 sequence identified similarity with the Rpd3 protein, a previously isolated transcriptional regulator with a phenotype of reduced potassium dependency in *S. cerevisiae* (Vidali et al., 1972), demonstrating a conserved and important role for HDACs as key regulators of eukaryotic transcription. After this discovery many other HDACs have been identified that share similarity with the central conserved domain of Rpd3. The eukaryotic HDACs fall into three different groups (Table 1) based on their sequence homology and their sensitivity to TSA. Proteins similar to Rpd3 form the

class I HDACs, including the mammalian HDAC1, HDAC2, HDAC3, HDAC8 and the *S. pombe* Clr6 and Hos2 which are TSA sensitive and share the 300bp long conserved N-terminal HDAC domain. This class have been reported to function by interacting with DNA-binding factors as parts of the yeast Sin3 and mammalian nuclear receptor co-repressor complex (N-CoR) and the RbAp48/RbAp46 containing nucleosome remodeling (NuRD) complexes in transcriptional repression (Lee and Young, 2000; Ng and Bird, 2000).

Table 1. Different classes of eukaryotic histone deacetylases.

	Class I HDACs	**Class II HDACs**	**Class III HDACs**
S. cerevisiae	Rpd3, Hos1, Hos2	Hda1, Hos3	Sir2, Hst1, Hst2, Hst3, Hst4
S. pombe	Clr6, Hos2	Clr3	Sir2, Hst2, Hst4
H. sapiens	HDAC1, HDAC2, HDAC3, HDAC8	HDAC4, HDAC5, HDAC7, HDAC9, HDAC10	SIR1, SIR2, SIR3, SIR4, SIR5, SIR6, SIR7

The second class of HDACs contains the founding member *S. cerevisiae* Hda1, which was found in a biochemical purification together with Hda2 and Hda3, and the *S. pombe* Clr3 protein and the mammalian HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10 (Carmen et al., 1996; Ekwall, 2005; Vogelauer et al., 2000; Wu et al., 2001). The class II HDACs are also TSA sensitive but they form a distinct group based on their C-terminal sequence homology and association with other co-repressors. Mammalian class II HDACs sometimes contain the C-terminal binding protein (CtBP) and the myocyte enhancer (MEF2) binding domains localised to the N-terminal and sometimes also two catalytic deacetylase domains (Bertos et al., 2001). The class II HDACs have reportedly also been differentially expressed in different tissues and localised both to the nucleus and the cytoplasm, in distinction to class I HDACs which are strictly nuclear (Bertos et al., 2001). Like the Class I HDACs the Class II HDACs are associated with large co-repressor complexes, like MEF2 and SMRT that are recruited to target genes by DNA-bound regulators (Bertos et al., 2001).

The class III HDACs include the yeast silent information regulator (Sir2) and the homologues of Sir2 (Hst1-4) which were isolated in *S. cerevisiae* as factors involved in position control of yeast mating type genes (Derbyshire et al., 1996; Freeman-Cook et

al., 1999; Strathern et al., 1982) The Sir2 protein family (Sirtuins) are divergent from the other two classes of HDACs with respect to sequence similarity, TSA sensitivity and NAD dependent deacetylase activity (Landry et al., 2000). The sirtuin family is conserved from yeast to man and the central human SIR2 domain has been shown to be functional in yeast (Freeman-Cook et al., 1999). Many homologues in other species have been identified like the human SIRT1-7 and the *S. pombe* Sir2, Hst2 and Hst4 proteins (Ekwall, 2005; Freeman-Cook et al., 1999; Gray and Ekstrom, 2001) The Sirtuins have been implicated in different processes like chromatin silencing at telomeres and the mating-type loci, chromosome segregation and cell aging (Gray and Ekstrom, 2001). Different Sir containing protein complexes play a major role in maintaining a transcriptionally inactive and heterchromatic state at certain silent regions, for example the silencing complex Rap1/Sir2/Sir3/Sir4 and the distinct Net1 complex which have been identified via biochemical purifications (Blander and Guarente, 2004). The spreading of heterochromatin in *S. cerevisiae* involves Rap1 binding and recruitment of the Sir proteins allowing deacetylation and propagation by oligomerisation (Hoppe et al., 2002; Talbert and Henikoff, 2006) Silencing is reportedly different in *S. pombe* and other higher eukaryotes compared to *S. cerevisiae* and spreading of heterochromatin is thought to be initiated by binding of interfering small RNAs (siRNAs) to methylation complexes which allows Swi6 binding to stabilise the chromatin (Ekwall, 2004; Schramke and Allshire, 2003).

DNA microarray methods in combination with genetically modified yeast strains allow genome wide expression analysis, histoneacetylation profiling and binding mapping of the different yeast HDACs. Gene expression profiling of the *rpd3*, *hda1* and *sir2* mutants in *S. cerevisiae* reveal distinct association with different targets. It was found that *hda1* targets are over represented for genes involved in carbohydrate metabolism, while the *rpd3* mutant targets are highly similar to *sin3* targets and associated with cell cycle regulated genes. In contrast, targets associated with amino acid metabolism were over represented in the *sir2* expression profile (Bernstein et al., 2000). Investigations in *S. pombe* indicate that *clr6-1* controls a large number of genes partially redundant with *clr3* (Hansen et al., 2005; Wiren et al., 2005). Additionally, many clr6 and clr3 affected targets are stress induced and localised to subtelomeric regions in a similar manner to Hda1 in *S. cerevisiae* which has been found to affect distinct subtelomeric chromosomal (HAST) domains induced by stress (Hansen et al., 2005; Robyr et al., 2002; Wiren et al., 2005).

The specificity of the different HDACs towards different histone N-terminal lysine residues has been studied extensively. Different acetylation and deacetylation patterns are important for gene activity and it has been shown that both hyper and hypoacetylated states can activate transcription under certain circumstances. It has been demonstrated that Hos2 deactetylation is required for gene activation (Wang et al., 2002) and that Rpd3 is involved in activation of Hog1 targets (De Nadal et al., 2004). Acetylation profiling with the different *S. cerevisiae* HDAC mutants has determined the *in vivo* specificity and correlates Rpd3 function with low acetylation levels at histones H4-K12, H4-K5 and H3-K8 (Robyr et al., 2002). This correlates with the specificity of the Clr6 homologue in *S. pombe*, where expression profiles of *clr6-1* overlaps mainly with low acetylation levels of H4-K5, and H4-K12 (Wiren et al., 2005). Rpd3 and Clr6 do not appear to be associated with actively transcribed genes and can therefore be classified as repressors in growing cells.

In *S. cerevisiae* Hda1 affects acetylation levels at histones H3-K9, H3-K18 and H2B-K16, while Sir2 shows specificity for H4-K16, H4-K8 and H3-K9 at subtelomeric regions and other silent loci (Robyr et al., 2002). Interestingly, the specificity and the localisation of targets of Hda1 and Clr3 correlates between *S. cerevisiae* and *S. pombe* indicating conserved roles of these class II HDACs (Ekwall, 2005; Wiren et al., 2005). Hos1, Hos2, and Hos3 in *S. cerevisiae* affect the regulation of ribosomal DNA and ribosomal protein genes (Robyr et al., 2002). Global binding studies show that Hos2 has a specific role in the coding region of actively transcribed genes (Wang et al., 2002). Similarly, further studies in *S. pombe* reveal the same important role for gene activation of Hos2 , where low H4-K16 levels in the open reading frames (ORF) of Hos2 target genes correlate with highly expressed genes (Wiren et al., 2005).

Formation of heterochromatic regions by Sir2 is not affected by other HDACs in *S. cerevisiae*. Disruption of *sir2* leads to hyperacetylation of H4-K16 at subtelomeric located gene targets which are not affected by HDACs (Robyr et al., 2002). In *S. pombe* there is instead a correlation between Clr3 and Sir2 activity at silent regions consistent with similarities between silencing phenotypes of *clr3* and *sir2* (Wiren et al., 2005). Other studies indicate that deacetylation of H3-K9 is a common and important function of the eukaryotic Sir2 protein (Robyr et al., 2002; Shankaranarayana et al., 2003) while

the reported H4-K16 specificity of *S. cerevisiae* is not found in *S. pombe* (Wiren et al., 2005).

2.10 METHYLATION, THE HISTONE CODE AND RNA SILENCING.

Histone lysine methyltransferases play an important role in controlling epigenetic modifications of eukaryotic chromatin important for proliferation, differentiation, development and gene expression. The factors involved include the *S. cerevisiae* proteins Set1 and Set2, the *S. pombe* Clr4 and the metazoan Su(var) and SET proteins. Methylation targets of these factors include H3-K9, important for many epigenetic processes associated with transcriptional repression, and H3-K4 which is connected with transcriptionally active chromatin (Lachner and Jenuwein, 2002). Post-translational modifications of histones have been proposed to establish a "code" that extends the genetic DNA information and determines patterns of cellular gene expression (Jenuwein and Allis, 2001). It has been suggested that methylation of lysines has a lower turnover than phosphorylation and acetylation of lysines and therefore functions as a more stable mark of the chromatin state which controls other modifications (Rice and Allis, 2001). Some of these modifications mark the chromatin for binding and spreading of particular proteins involved in the formation of silent chromatin. Often, formation of silent heterochromatin is coupled to RNA silencing, a process in which small RNA molecules induce repression of homologous sequences (Bernstein and Allis, 2005). In RNA silencing doubled stranded RNA molecules are cut to small active pieces by the helicase/RNase like Dicer protein (Bernstein et al., 2001). The small RNAs are then incorporated into RISC like complexes containing the Argonaut protein (Bohmert et al., 1998), which directs the sequence specific repression guided by different specific histone modifications (Bernstein et al., 2001).

3 REGULATION BY REPRESSION

A majority of the genes in eukaryotic genomes are usually transcribed. Monitoring of the internal protein levels in *S. cerevisiae* reveals that approximately 80% of the proteome is represented under normal conditions (Ghaemmaghami et al., 2003). Many classes of proteins were however found to be under represented in the same study, indicating the importance of maintaining negative regulation and silencing. In addition, only a fraction of the transcribed genes are simultaneously on and regulatory control by repressor proteins is therefore significant. Some genes are also oscillatory and subjected to periods of negative regulation under certain conditions and stages of the cell cycle. Genes that need to be induced from basal transcription levels similarly need a fast derepression of negatively acting repressor proteins. Many repressor proteins are evolutionary conserved which indicates the importance of repressors for maintaining cellular integrity. The biological importance is also manifested by a number of diseases and cancers in mammals which are associated with defects in transcriptional repressors. One important well characterised eukaryotic repressor system is the Ssn6-Tup1 co-repressor. Ssn6-Tup1, first described in the budding yeast *Saccharomyces cerevisiae*, provides a very robust repression system that affects a large number of genes by integrating different repression mechanisms. Ssn6 and Tup1 are conserved proteins which can be found not only in budding yeast but also in other ascomycetes, basidiomycetes and cyanobacteria.

3.1 GENETIC SCREENS IDENTIFYING SSN6 AND TUP

Tup1 was first described by spontaneous mutants capable of incorporating deoxythymidine-5´-monophosphate dTMP into the DNA in the presence of aminopterin. The *TUP1* (tymidine uptake) locus was mapped to chromosome III and further analysis revealed that the *tup1* strain also displayed disturbed mating efficiency (Wickner, 1974). In an independent genetic screen using a *cyc1* strain that lacked iso-l-cytochrome c causing defects in utilizing lactate as a carbon source, revertants were identified: *cyc* mutants, which were overproducing iso-2-cytochrome c to compensate for the loss of *CYC1* (Rothstein and Sherman, 1980a; Rothstein and Sherman, 1980b). In yet another study, spontaneous revertants from a strain deficient in maltose growth in the respiratory state were isolated. The revertants, which were insensitive to catabolite repression for maltase, invertase and succinate dehydrogenase, showed a higher than normal concentration of free cAMP during derepression. The mutants were described

to be "flaky" due to their heavy flocculation behaviour when grown in liquid media, and consequently named *FLK1* (Schamhart et al., 1975). In a later study, *umr7-1*, isolated from a genetic screen affecting the mating type specific functions, showed reduced mating ability, defects in sporulation and extreme cell aggregation similar to *flk1* (Lemontt, 1980). Linkage analysis also conferred that *flk1*, *umr7-1*, *cyc9* were allelic with *tup1*. *S. cerevisiae* strains carrying mutations in *SNF1*, which is required for repression of many glucose repressible genes including *SUC2,* are not able to metabolise sucrose by fermentation. Revertants have been isolated and characterisation of the different suppressors of *snf1* into complementation groups identified the *ssn6* (suppressors of *snf1*) gene, which was found to be allelic to the previously characterised *cyc8* (Carlson et al., 1984). The *ssn6* mutant showed constitutive high concentrations of invertase under de-repressed (non-glucose growth) and repressed (glucose growth) conditions and like *tup1* was found to be clumpy, deficient in sporulation and mating and suggested to be a regulatory gene. Later on, ssn6 and *tup1* were investigated together for many glucose-repressible enzymes and displayed similar degree of action (Trumbly, 1986). Complete deletion of the cloned *SSN6* (*CYC8*) gene confirmed previous results with isolated point mutations, but showed more extreme phenotypes. The *ssn6* deletion strain was viable but showed reduced growth rate (Trumbly, 1988). Furthermore, *TUP1* was not essential for viability and observed deletion phenotypes were indistinguishable from phenotypes caused by *SSN6* disruption (Williams and Trumbly, 1990). More recently other Tup homologs have also been identified in different yeast species based on their sequence identity with *S. cerevisiae*. This includes the *K.lactis* and *C.albicans* Tup1 proteins and the *S. pombe* Tup11 and Tup12 proteins (Braun and Johnson, 1997; Mukai et al., 1999).

3.2 SSN6-TUP TARGETS

The similar phenotypes of *tup1* and *ssn6* including mating type defects, flocculation and sporulation defects, suggested a common regulatory role in many diverse pathways. The constitutive expression of target genes in deletion mutants identified the *TUP1* and *SSN6* genes as negative regulators of expression (Trumbly, 1988). Several investigations confirmed that Ssn6-Tup1 regulates repression of many different sets of genes that can be classified according to the different processes they are involved in including, glucose repressed genes (Janoo et al., 2001; Trumbly, 1988), mating type regulated genes (Mukai et al., 1991), DNA damage induced genes (Zhou and Elledge, 1992), oxygen regulated genes (Zitomer and Lowry, 1992), stress induced targets

(Marquez et al., 1998), fatty acid regulated targets (Fujimori et al., 1997), flocculation associated genes (Teunissen et al., 1995), sporulation genes (Friesen et al., 1997) and meiosis related genes (Mizuno et al., 1998) which will be further described below.

3.3 GLUCOSE REPRESSION, HYPOXIA AND FLOCCULATION ASSOCIATED TARGETS

Yeast cells growing on high levels of glucose accumulate high ATP levels and need a mechanism to shut off alternative enzymatic pathways in order to save energy. Glucose repressed genes include gene products involved in: (i) gluconeogenesis such as *FBP1* and *PCK1*, (ii) enzymes that are not needed for fermentative growth which includes mitochondrical enzymes involved in the citric acid cycle and oxidative phosphorylation and (iii) enzymes needed for growth on alternative carbon sources such as the *GAL*, *SUC* and *MAL* genes (Ronne, 1995). Transmission of the glucose repression signal involves the Snf1 and Snf4 proteins, which are part of a multiprotein complex responsible for phosphorylation and deactivation of the DNA binding repressor Mig1 (Carlson et al., 1984; Nehlin and Ronne, 1990; Schuller, 2003). Binding sites for the Mig1 protein have been identified in glucose repressed genes such as *GAL1*, *GAL4*, *SUC2* and several *MAL* genes and disruption of these elements leads to derepression of some of the promotors (Carlson, 1999). As described, both Tup1 and Ssn6 were identified in genetic screens for genes downstream of Snf1. Further analysis has shown that both Tup1 and Ssn6 are required for repression of some Mig1 regulated genes. In addition evidence has been introduced that Ssn6 and Tup1 can interact directly with Mig1 (Treitel and Carlson, 1995).

Similarly, oxygen can function as a messenger to shut off enzyme systems required for non fermentative anaerobic growth. The targets regulated by oxygen are genes involved in respiratory functions and gene products involved in controlling oxidative radicals. The transcriptional control of many so called heme genes are regulated by the heme activation protein complex (Hap), while repression is executed by the transcriptional repressor Rox1 (Zitomer and Lowry, 1992). The effect of *tup1* on the repression of heme genes has been studied, and in a *tup*1 deletion the aerobically repressed *ANB1* gene is expressed constitutively (Zhang et al., 1991). In addition, the fatty acid desaturase gene *OLE1* involved in lipid biosynthesis is negatively regulated by the DNA bound regulator Rox1 (Deckert et al., 1995). As with *ANB1* regulation both

TUP1 and *SSN6* have shown to be required for repression of *OLE1* expression together with the Rox1 transcription factor (Fujimori et al., 1997).

The quality of yeast strains important for brewing depend on their flocculation properties. Flocculation in growing yeast cultures requires the expression of specific proteins that mediate the interaction between cells (Shankaranarayana et al., 2003). The flocculation and cell aggregation genes belong to a family of genes located near the telomeres (Teunissen et al., 1995). Genes that affect flocculation have been identified: *FLO1*, *FLO5* and *FLO8* are regulatory genes involved in the positive control of flocculation. Deletion mutants of the *FLO* genes show similar phenotypes to *tup1* and *ssn6*, and transcript analysis shows a direct role of the Ssn6-Tup1 in this regulation (Teunissen et al., 1995).

3.4 MATING, A-SPECIFIC AND HAPLOID SPECIFIC GENE REGULATION

S. cerevisiae cells differentiate into two different mating types, namely a and a. The two types can mate and form a diploid a/a cell type that can sporulate. The proteins needed to determine the mating type are coded by a single locus, the *MAT* locus, which codes for three different regulatory proteins, a1, a2 and a1. The a1 protein is an activator protein while the a2 and a1 are repressor proteins. The regulatory proteins function together with the Mcm1 protein that is expressed in all cell types. The a2/Mcm1 represses a-typical genes in a haploid cells while a2/a1 represses haploid specific genes without the Mcm1 protein in the diploid state (Johnson and Herskowitz, 1985; Keleher et al., 1988). The non mating phenotype caused by *tup1* in a cells led to investigations to understand the role of Tup1 in the expression of mating type genes. It has been shown that both Ssn6 and the Tup1 proteins are key regulatory proteins involved in repression by a2/Mcm1 and a2/a1. Deletion of *ssn6* was found to cause inappropriate expression of the a-specific a-factor and the *BAR1* protease (Schultz and Carlson, 1987). Both Tup1 and Ssn6 are also required for the repression of the a-specific *MFA2* in a cells and the haploid specific *RME1* in a/a cells (Keleher et al., 1992; Mukai et al., 1991).

3.5 MEIOSIS AND SPORULATION RELATED GENE TARGETS

The *IME1* gene product is important for initiation of meiosis and is negatively controlled by Rme1 and positively regulated by Ime4. Independently, transcription of

IME1 is increased in the *tup1* and the *ssn6* mutants, indicating a role of Ssn6-Tup1 together with unidentified repressors (Mizuno et al., 1998). In response to starvation diploid yeast cells pass through sporulation, a process that requires the expression of the sporulation specific genes *DIT1* and *DIT2* that encode proteins needed for spore wall formation. The repression of *DIT1* and *DIT2* is dependent on the upstream NRE control element, and the regulation is believed to be controlled via the DNA binding factors Nrf and Bf3 and mediated by the co-repressor Ssn6-Tup1 (Bogengruber et al., 1998).

3.6 OSMOTIC STRESS AND DNA DAMAGE INDUCED GENES

Adaptation to changes in the environment is mediated by sensing components and transduction pathways in yeast. The mitogen activated protein kinase (MAPK) system consists of sequentially activated protein kinases that funnel environmental signals to the transcriptional level (Sprague, 1998). During salt stress, the MAPK cascade activates the high-osmolarity glycerol (HOG) pathway, which provides specificity for the response to osmotic stress in *S. cerevisiae*. The Hog1 MAP kinase targets DNA bound transcriptional repressors that bind to cAMP response elements (CRE) sites upstream of several genes involved in osmotic stress response. The CREB homologue Sko1 is normally a DNA bound repressor that recruits Ssn6-Tup1 for repression of target genes like *ENA1*, *GPD1*, *CTT1* and *HAL1* (Marquez et al., 1998; Nehlin and Ronne, 1990; Proft and Serrano, 1999). Upon salt stress Sko1 is phosphorylated by Hog1, which disrupts the interaction with Ssn6-Tup1 and transforms Sko1 into an activator that recruits SAGA histone acetylase and SWI/SNF components to the promotor of Sko1 regulated genes (Proft and Struhl, 2002).

The Crt1 protein in *S. cerevisiae* is a repressor of the DNA damage checkpoint pathway that regulates damage inducible genes like the ribonucleotide reductase *RNR* genes (Huang et al., 1998; Li and Reese, 2000). The Ssn6 and Tup1 co-repressors are important for the repression and are recruited to their target by the Crt1 repressor. Upon DNA damage Ssn6-Tup1 is released together with the repressor.

3.7 REGULATION OF GENES IN *S. POMBE.*

In the related fission yeast S. *pombe*, two Tup1 like homologs have been identified, namely Tup11 and Tup12. Evidence that these proteins function as repressors in similarity with Tup1 has been forwarded (Mukai et al., 1999). Deletion of the *tup11*

and *tup12* genes leads to derepression of the glucose repressed *Fbp1* gene in similar fashion to other glucose repressible gene targets described in *S. cerevisiae* (Janoo et al., 2001). Regulation of the *Fbp1* gene by Tup11 and Tup12 includes signalling through the Sty1 stress induced MAPK pathway in response to glucose starvation and glucose repression by the cAMP dependent protein kinase A (PKA). The *tup11* and *tup12* deletions display redundant phenotypes with respect to *Fbp1* derepression, flocculation, poor growth and highly elongated cells (Janoo et al., 2001). The Sty1 pathway in *S. pombe* is believed to act differently from the related Hog1 pathway in *S. cerevisiae*. In *S. pombe* a large number of different stress conditions induce a similar response in contrast to *S. cerevisiae* were different transduction pathways operate in parallel and direct the proper stress response. The specificity in *S. pombe* is instead believed to be directed by co-repressor components such as Tup11 and Tup12 which direct the proper gene response by relieving repression and function as gatekeepers of the general stress response (Hirota et al., 2004). For example, activation of the intracellular cation transporter *Cta3* gene in response to elevated potassium and calcium levels involves activation by the response regulator Prr1 and the Atf1/Pcr1 transcription factors and derepression by the Tup11 and Tup12 co-repressors (Greenall et al., 2002). Iron repression of the *fio1* gene by the *S. pombe* Tup proteins in complex with the DNA binding regulator Fep1 has also been described (Pelletier et al., 2003).

3.8 THE SSN6-TUP1 COMPLEX: A GENERAL REPRESSOR

After identification of Ssn6 and Tup1 as negative regulators of transcription in genetic screens, cloning and sequencing provided more information on the proteins. The *S. cerevisiae* Tup1 is encoded from a single open reading frame of 2139 bp located on chromosome 3, which translates into a 713 amino acid protein with a molecular mass of 78,2 kDa (Mukai et al., 1991; Trumbly, 1988). Ssn6, on the other hand is a 966 amino acid long protein which is encoded by a 2901 bp open reading frame located on chromosome 2 (Schultz and Carlson, 1987). Immunofluorescence studies of Tup1 and Ssn6 show localisation to the nucleus in both cases (Schultz and Carlson, 1987) . Biochemical analysis of the Ssn6 and Tup1 proteins with raised immunosera indicates an equal expression independent of glucose levels. The two proteins were also found to immunoprecipitate with each other which suggest association in a complex. Under native condition the two proteins co-migrate and associate in a high molecular weight complex (Williams et al., 1991). By determining the sedimentation coefficient in a sucrose gradient the mass of the Ssn6-Tup1 complex was calculated. The complex,

which is believed to contain only Ssn6 and Tup1 subunits, has a mass of 437 kDa. By using Ssn6 free extracts the mass of the complex was estimated to be 306kDa and when Tup1 was excluded the mass was instead calculated to be 100kDa suggesting an unequal composition of the subunits. The proportion suggests a ratio of one Ssn6 subunit to four Tup1 subunits in the complex (Varanasi et al., 1996). It has been shown that the N-terminal part of Tup1 is required for oligomerisation of the Ssn6-Tup1 complex (Tzamarias and Struhl, 1994; Varanasi et al., 1996). The rather large complex mass with many subunits is believed to make the complex more efficient as a repressor by providing multiple interacting surfaces.

The observation that Ssn6-Tup1 forms a complex implied the idea of a general repressor that can repress targets when recruited to the vicinity of any gene. Functional analysis of target genes has shown that repression is interdependent of Ssn6 and Tup1. Experiments with the a2-Mcm1 repressor show that Ssn6 occupancy at the operator is required for repression *in vivo* (Keleher et al., 1992). In the same study experiments with a LexA-Ssn6 fusion showed that Ssn6 can repress target genes when brought to the promotor. However, Keleher et al. also showed that the LexA-Ssn6 repressor was dependent on Tup1 for proper repression, which led to a model of Ssn6-Tup1 repression. In this model the Ssn6-Tup1 complex, which does not have the capability to bind DNA on its own, was believed to be recruited to the promotor by specific DNA bound repressor proteins that did not repress the targets themselves. Instead, Ssn6-Tup1 was believed to provide the repression when positioned at the target gene. Some of the key effectors for recruiting Ssn6-Tup1 have already been described and include: Mig1, a2-Mcm1, Rox1, Rme1, Sko1 and Crt1 among others. According to the model, Ssn6-Tup1 was believed to be released from the promotor during derepression to allow gene activation.

3.9 THE SSN6-TUP1 STRUCTURE

Analysis of the Tup1 protein sequence shows interesting features. The protein is glutamine (Q) rich in the N-terminal and contains blocks of repeated trypthophan (W) and aspartic acid (D) residues. These WD amino acid blocks are found in the C-terminal domain of Tup1, and are repeated seven times with about 40 amino acids in each repeat (van der Voorn and Ploegh, 1992). The WD40 repeat domain was first described in the b-subunit of heterotrimeric G proteins and is shared by many different

protein families and found in proteins like Ste4, Cdc4 and Cdc20 among others (Fong et al., 1986; Varanasi et al., 1996). The X-ray crystal structure of the C-terminal part of Tup1 has been solved (Sprague et al., 2000). The C-terminal domain of Tup1 folds into a seven bladed b-propeller around a central axis with a narrow channel (Fig. 4). Each blade consists of a repeated b-sheet structure with 4 antiparallel strands (Sprague et al., 2000). The contact between the adjacent blades of the propeller consists of mainly hydrophobic residues interacting with each other and giving the propeller rigidity. The structure contains loops which connect the blades between the WD40 repeats. The inserted fragments differ in length and composition and are believed to give flexibility to the structure (Sprague et al., 2000). The surface of the propeller structure is believed to be a general structure important for protein interactions. Point mutations that affect the repression of Mata2 target genes have been isolated (Komachi and Johnson, 1997) and are located on the surface of the 4th blade of the propeller structure. Comparison of sequences of different fungal Tup1 proteins identifies many conserved residues located mainly in the top surface of the propeller structure centred around the channel. The highest variability is found in the bottom surface and in the loops connecting the blades. The structure of the Q-domain, important for complex formation, has not yet been solved but is believed to form an extended independent structure linked to the propeller by an intermediate structure. CD spectroscopy studies indicate that the N-terminal is highly a-helical and diffraction analysis of N-terminal Tup1 crystals shows characteristic coiled-coil fibre pattern (Jabet et al., 2000).

The Ssn6 protein has not been crystallised and less is therefore known about the overall structure of Ssn6. However, sequence analysis identifies a central part of the protein consisting of a 10 times repeated tetratricopeptide (TPR) domain (Schultz et al., 1990). The TPR, which is a 34 amino acid tandem sequence of hydrophobic residues, has been identified in several proteins with various functions (Das et al., 1998). TPR proteins are often assembled into multi protein complexes and different subunits in the anaphase promoting complex (APC) like cdc16, cdc23 and cdc27 and the general transcription factors TFIIIc and p300/CBP contain different numbers of TPR repeats (Das et al., 1998; Gounalaki et al., 2000). The TPR structure is believed to self assemble into a-helices that mediate protein interactions. Analysis of the crystal structure of TPR containing proteins reveals a helical structure consisting of series of antiparallel a-helices and multiple TPR repeat proteins that are predicted to fold into super-helical

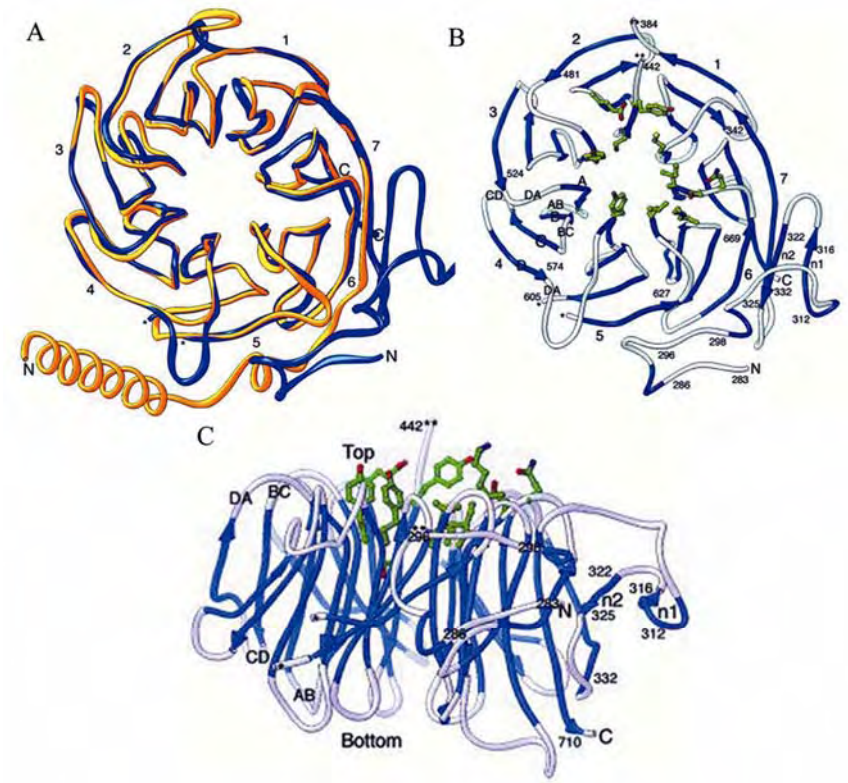


Figure 4. (**A**) Ribbon representation of the WD40 propeller structure of *S.cerevisiae* Tup1 (yellow) aligned with the structure of the Gβ protein (blue). (**B**) Ribbon structure showing the top surface of the C-terminal b-propeller of Tup1. Residues important for interaction with Mata-2 are shown in green (**C**) Ribbon structure showing the C-terminal domain of Tup1 from the side with Mata-2 interacting residues in green. With permission from Nature Publishing Group (Sprague et al., 2000).

Figure 5. (**A**) View of a model showing a TPR helix with 12 TPR motifs. The model indicates that the tandem arranged TPR motifs are organised into a right-handed super-helix with a hollow internal continuous groove that can fit a-helix of a target protein. (**B**) View parallell to the axis of a 8 TPR helix with the amphipathic groove. With permission from Nature Publishing Group (Das et al., 1998).

structures with a groove for target proteins (Fig. 5). The structure of different TPR containing proteins are believed to be very similar and modelling reveals similar folding of the individual TPR repeats (Das et al., 1998). The Ssn6 protein also contains a notable glutamine (Q) and proline (P) rich domain located in the N-terminal. Similar domains have been implicated in transcriptional regulation from yeast to humans. Analysis has shown that such Q-rich domains are likely to form amphipathic coiled-coil structures identified in many types of transcription factors like c-Fos, c-Jun, Maf and c-Myc (Escher et al., 2000) The interaction between the Tup1 and the Ssn6 proteins has been mapped with two-hybrid approaches to the N-terminal parts of the proteins and is mediated by the Q-domain of the Tup1 protein and the three first N-terminal TPR repeats of the Ssn6 protein (Tzamarias and Struhl, 1994; Tzamarias and Struhl, 1995). More specifically, point mutations positioned in TPR1 have been shown to be important for interaction with Tup1. It has been suggested that the flexibility between the TPR helices allows a super-helical structure to form on top of the Tup1 anti-parallell tetramer bundle (Jabet et al., 2000). This would make the outer surface of the Ssn6 TPR repeats available for interaction with DNA bound factors while the propeller surfaces of the Tup1 tetramers are similarly free to interact with transcriptional regulators.

3.10 EVOLUTION OF SSN6 AND TUP HOMOLOGUES

Sequence analysis suggests the presence of Tup1 homologues in *Candida albicans*, *Kluyveromyces lactis*, *Neurospora crassa*, *Schizosaccharomyces pombe* among others among others (Fig. 6A). In fission yeast there are two paralogous TUP genes namely *tup11*$^{+}$ and *tup12*$^{+}$, which are the result of a distant gene duplication event. Gene duplication is of major importance in speciation and thought to be one of the primary driving forces in evolution together with genetic drift (Ohta, 1989). The most common fate of a duplicated gene is deletion of one of the copies, but sometimes diversification of the gene function leads to fixation of the gene pair (Moore and Purugganan, 2003). The Tup protein appears to be single copy in most yeast species, except in *S. pombe* where a duplicated gene pair has remained through evolution. One exception are some close relatives to *S. cerevisiae* that diverged after a whole genome duplication, namely *Saccharomyces castelli* and *Candida glabrata* (Scannell et al., 2006). The duplicated genes in fission yeast do not appear to be the result of whole genome duplication but have another origin. Interestingly, comparison reveals that the total number of

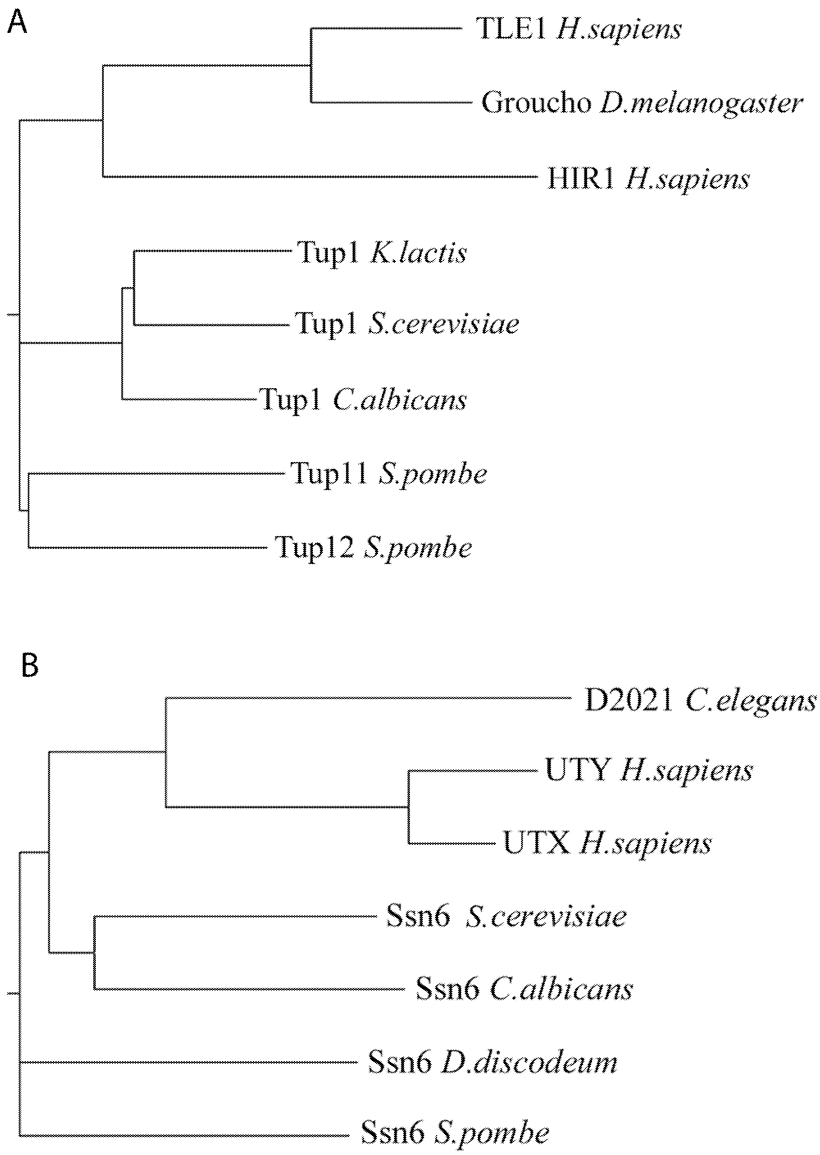


Figure 6. (A) Phylogenetic dendrogram of the Tup1 and TLE1 protein family. **(B)** Phylogenetic dendrogram of the Ssn6 protein family and related UTX and UTY proteins. Full length protein sequences were compared and ordered by their relationship into phylogenetic classes. Both phylogenetic trees were generated with a CLUSTAL W multiple sequence program.

transcription factors is significantly smaller in *S. pombe* compared to other yeast species, indicating a lesser degree of separation from a common ancestor (Beskow and Wright, 2006).

Protein database comparisons between identified Tup proteins show differential domain similarity, where the WD40 repeat domain is the most conserved domain followed by the N-terminal Ssn6 interacting domain. The middle part of the Tup1 protein is the most variable and least conserved part between different yeast species. The 614 aminoacid long Tup11 protein is encoded close to the 586 aminoacid long Tup12 protein on chromosome 1. The overall sequence similarity between Tup11 and Tup12 is above 50 %. Interestingly, orthologues involved in similar repressor function can also be found in metazoans. The WD40 repeat is also present in the *Drosophila groucho* gene product and in the mammalian transducer like enhancer (TLE) protein (Stifani et al., 1992). The Gro/TLE proteins are widely distributed among the vertebrates and invertebrates where they are believed to function as transcriptional co-repressors with homologous function to the Tup1 proteins. In addition to the highly homologous WD40 domain they contain a pronounced N-terminal Q-domain with low similarity to the Tup1 proteins and a highly variable central region that separates the two conserved domains. Evidence that Gro function as an oligomer consisting of four subunits mediated by the N-terminal Q-domain in a similar manner to Tup1 has also been forwarded (Song et al., 2004). In the mammalian TLE group there are at least five different genes encoding an even larger number of proteins, indicating an important role in a wide array of cellular response (Gasperowicz and Otto, 2005; Grbavec et al., 1999). Another member of the eukaryotic WD40 protein family is the HIR proteins, which are co-repressors that regulate histone gene transcription (Sherwood et al., 1993). The Gro/TLE proteins lack an obvious DNA binding domain and are recruited to targets by interactions with sequence specific repressors (Flores-Saaib and Courey, 2000). There is evidence that Gro is required for neurogenesis, eye-formation and sex-determination during development in co-operation with a number of different transcription factors like Notch, Hairy, Runt, Engrailed and Dorsal (Chen and Courey, 2000; de Celis and Garcia-Bellido, 1994; de Celis and Ruiz-Gomez, 1995; Mannervik et al., 1999; Song et al., 2004). The Gro/TLE family also share homology with the so called AES subgroup of proteins which are homologous in the N-terminal Q-domain and partly in the variable central domain but lack the WD40 domain. Interaction or oligomerisation with such a protein are believed to sequester Gro/TLE co-repressors to

inactivate them (Chen and Courey, 2000; Miyasaka et al., 1993). Some of the DNA binding factors that bind Gro/TLE proteins do not always act as repressor proteins but are known to activate specific targets under certain circumstances. This includes findings concerning Dorsal and Runt associated factors which are dependent on the general transcription factor context for activation or repression. Taken together, the functional similarity in combination with the seven C-terminal WD40 and the N-terminal Q-domain clearly identifies the Tup and Gro/TLE proteins as homologous eukaryotic proteins.

Ssn6 homologues are also found in the above mentioned yeast species. Characterisation of Ssn6 function has been performed in *C.albicans* and *S. pombe* but also in the distantly related slime mold *D. discodeum*, were they have been identified as co-repressors together with Tup1 ortholouges (Garcia-Sanchez et al., 2005; Mukai et al., 1999; Saito et al., 1998). Identification of Ssn6 homologues in metazoans is not obvious. The most similar are the ubiquitously transcribed nuclear TPR proteins, UTY and UTX, which escape X-chromosome inactivation. (Greenfield et al., 1998). The UTY protein, which is expressed in all male tissues, has eight TPR repeats positioned in the N-terminal. Sequence alignment of the TPR domain identifies the Ssn6 protein as the best match. Non characterised homologs to UTX and UTY are also found in *C. elegans* and *Drosophila*, which in addition to the TPR domain also contain a Jumanji domain which has been implicated in transcriptional repression and chromatin regulation (Berge-Lefranc et al., 1996; Takeuchi et al., 1995). Similarly to some of the yeast Ssn6 proteins the UTY protein sequence contains glutamine and proline residues indicating further roles in transcriptional regulation. The relationship of the TPR containing Ssn6-like and UTY and UTX proteins can be seen in the phylogenetic tree in Fig. 6B. The Gro/TLE proteins have not been found to be directly coupled to an obvious Ssn6 homolog in the metazoans, but some studies suggest that UTX and UTY are linked to TLE1 and TLE2 (Grbavec et al., 1999). In the same study, it was also shown with the two-hybrid technique that Ssn6 is able to bind TLE1 and TLE2. Taken together, emerging evidence shows that Ssn6 and Tup1 homologues play an important role as transcriptional co-regulators with many diverse roles in fungi and animals.

3.11 FUNCTIONAL DISSECTION OF THE SSN6-TUP COMPLEX

Efforts have been made to map the different functional domains of the two proteins of the complex. By using different truncated hybrid Tup1-LexA constructs tethered

upstream of a functional promotor the repressive function of different domains of Tup1 have been mapped (Tzamarias and Struhl, 1994). The analysis showed that Tup1 contains two independent non-overlapping repression domains. One region was mapped to the N-terminal and the other was found C-terminal of amino acid 288. When a LexA-Ssn6 construct was tested in a *TUP1* deleted background in the same study it was found that Ssn6 had no effect upon transcription by itself. Thus, it was concluded that the ability to repress was brought about by the Tup1 protein and not by Ssn6, which was believed to function as an adaptor protein for the Tup1 tetramer (Tzamarias and Struhl, 1994). Other studies have defined more C-terminal point mutations in the WD40 repeat which are important for the full repression of target genes like *STE2* and *ANB1* (Carrico and Zitomer, 1998). More recently, another study investigated the expression of *MFA2*, *SUC2*, *RNR2* and *HEM13* in different *TUP* deletion mutants. This study support the finding that Tup1 has two independent repressor domains but the C-terminal repressor domain was found to be dominant over the N-terminal since the N-terminal domain was found to repress targets only partially (Zhang et al., 2002). This could be explained by the importance of Tup1 functioning as a tetramer for proper repression. The different Tup1 repressor domains described above can be seen in Fig. 7A.

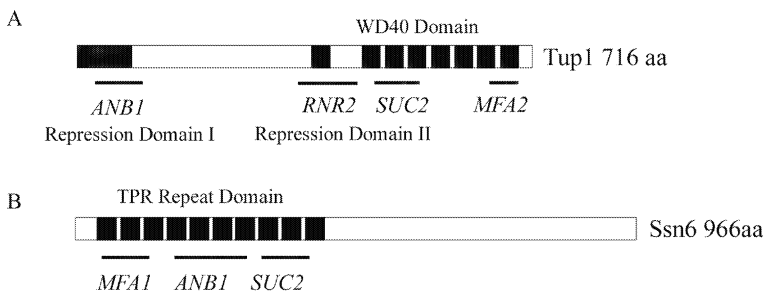


Figure 7. Schematic view of the different functional domains of the Ssn6-Tup proteins. (**A**) Different regions of Tup1 have different impact on oligomerisation, Ssn6 interaction and effect on target gene expression. Black lines indicate the most important repression domains from different studies. Gene names show representative target genes which are derepressed by deletion of indicated positions. (Carrico and Zitomer, 1998; Tzamarias and Struhl, 1994; Zhang et al., 2002). (**B**) Different functional domains of the Ssn6 protein important for repression of indicated target genes (Tzamarias and Struhl, 1995).

In a similar manner the different TPR derivatives of the Ssn6 protein have been evaluated and functionally mapped (Tzamarias and Struhl, 1995). It was reported that the N-Terminal Tup1 interaction domain of Ssn6 is required for proper repression of all targets. The different TPR domains of the Ssn6 protein were found to be differentially important for repression of a selection of different classes of target genes. Repression of the *ANB1* gene involved in DNA damage response via the Rox1 repressor requires TPR repeats 1-7 of Ssn6 while glucose repression of the *SUC2* gene by Mig1 is dependent on TPR repeats 1-3 and 8-10. Fig. 7B summarises the different functional mapping studies described above.

3.12 MECHANISM OF ACTION

Studies have been performed with emphasis on explaining the regulatory mechanism behind Ssn6 and Tup function. Initially, regulation of MAT specific genes was found to be linked to positioning of nucleosomes upstream of the structural gene. Binding of the Mata2 repressor at the a2 operator leads to a distinct shift in the positioning of the nucleosomes in the promotor so that the TATA box is masked by steric hindrance (Shimizu et al., 1991). Similarly, analysis of the roles of Ssn6 and Tup1 at the a-cell specific *STE6* gene shows that both Ssn6 and Tup1 are involved in the re-positioning of nucleosomes. Deletion of *SSN6* and *TUP1* in a-cells leads to disruption of the nucleosome positioning in the promotor (Cooper et al., 1994). In addition, the *ssn6* and *tup1* deletions show distinct patterns of nucleosome positioning which correlates with their relative effect upon gene expression and they are therefore believed to play different roles in the establishment of repressive chromatin. Nucleosome positioning at the *SUC2* gene is altered in a manner directly dependent on the Snf2/Swi2 and Snf5 factors when cells are shifted from the repressed state (glucose) to derepressed state (low glucose) (Hirschhorn et al., 1992). This change in chromatin structure cannot be observed in an *ssn6* or a *tup1* deleted background indicating a similar role of Ssn6 and Tup1 as in *STE6* chromatin remodeling (Gavin and Simpson, 1997).

The finding that mutations in histone H3 can suppress mutations in *SNF2/SWI2* affecting the chromatin structure (Prelich and Winston, 1993) and that mutations in the histone H4 amino terminal affect gene expression of a-cell specific genes (Roth et al., 1992) suggests a link between organisation of nucleosomes, transcription and interaction with histones (Cooper et al., 1994). Interestingly, Tup1 has subsequently been shown to bind the amino termini of histones H3 and H4 directly, indicating a role

in repression mediated by histone interactions and chromatin organisation. The histone interaction domain has been mapped to the central part of Tup1 which is rich in proline and glutamine residues and overlaps with the repression domain of Tup1 (Edmondson et al., 1996; Tzamarias and Struhl, 1994). Tup1 was not found to interact with histones H2A and H2B in this study. Most interestingly, Tup1 interacts with underacetylated forms of histones H3 and H4 which is consistent with the correlation of histone deacetylation with transcriptional repression. In addition it was also shown that mutations in H3 and H4 amino termini affect Ssn6 and Tup1 repression targets in a synergistic manner. Both an a-specific reporter and a DNA damage induced reporter was found to be derepressed by these H3 and H4 mutations (Edmondson et al., 1996).

Studies with *in vitro* transcription systems indicate that *a2* repression is dependent on the expression of Ssn6 and Tup1 for full repression which means that Ssn6 and Tup1 can act by an alternative mechanism independent of chromatin assembly, most likely by interaction with the basal transcriptional machinery (Komachi et al., 1994). The observed degree of the *in vitro* repression was however not comparable with the *in vivo* repression which indicates exclusion of important factors in *in vitro* systems. Many glucose repressed and a-specific genes have also been found to be regulated by basal transcriptional components of the mediator complex. Genes like Rgr1 involved in the repression of *SUC2* (Sakai et al., 1988) and Sin4 (Wahi and Johnson, 1995) involved in a-specific gene regulation have been identified by genetic screens. Similarly genetic screens have identified suppressors affecting the derepression of *SUC2* in *snf1* deleted cells, namely the mediator component *SRB8* (*MED12, SSN5*) and the *SRB10* (*SSN3*) and *SRB11* (*SSN8*) which were isolated together with *SSN6*. Srb10/11 is a cyclin-dependent kinase pair associated with the carboxy-terminal domain (CTD) of RNA polymerase II and Srb8 is a subunit of the RNA polymerase II mediator complex (Balciunas and Ronne, 1995; Kuchin and Carlson, 1998). Experiments have shown that Srb10/11 alleviates repression by Ssn6 and Tup1 which establish a functional link between the co-repressor and the RNA polymerase machinery (Kuchin and Carlson, 1998; Zaman et al., 2001). Interaction studies have also confirmed the two different models in various experiments and many of the mediator components and histone associated proteins have shown to interact directly with Tup1 and Ssn6 biochemically by immunoprecipitation or by two hybrid methods. Mediator components like Rgr1, Med2, Med3, Hrs1 and Sin4, which are important or required for Tup1 repression, can interact with the Tup1 protein in a GST pull-down assay (Han et al., 2001;

Papamichos-Chronakis et al., 2000). Similarly another study has shown that the Srb7 component of the mediator binds Tup1 both *in vivo* with the two hybrid method and *in vitro* in a GST pull-down assay (Gromoller and Lehming, 2000). In yet another study, using the two hybrid method, the Hrs1 component of the mediator was found to activate transcription independently together with both Ssn6 and Tup1. The described interaction was also confirmed *in vitro* with pull down assays and was shown to be important for full repression of Hrs1 reporter genes (Papamichos-Chronakis et al., 2000).

The function of Tup1 and Ssn6 has also been found to be connected with gene activation under certain circumstances. Initial experiments indicate that a LexA-Ssn6 construct can activate transcription of a LacZ reporter when it is transcribed in a *tup1* or a *rgr1* deleted background and that Ssn6 also is important for activation of the endogenous citrate synthase gene *CIT2*, which suggest a dual role for Ssn6 in gene regulation (Conlan et al., 1999). It has further been demonstrated that the co-repressor complex is associated with target genes like the *GAL1* and *SUC2* genes both during repression and activation. Experiments suggest that the Tup1/Ssn6 complex facilitates the recruitment of components of the SAGA complex important for activation via the Cti6 protein which interconnects Ssn6 with Gcn5 of the SAGA complex (Papamichos-Chronakis et al., 2002). More recently, Ssn6 has also been identified as coactivator of Gcn4 activated targets involved in amino acid biosynthesis (Kim et al., 2005). Taken together there is substantial evidence present that both Ssn6 and Tup1 form interactions with subunits of the RNA polymerase II which are important for the regulation of Ssn6 and Tup1 targeted genes. Most interestingly some studies also reveal a role in derepression and activation by the Ssn6-Tup complex that is thought to be important for correct response of expression targets during changes in the environment.

The interaction of Ssn6 and Tup1 with histone H3 and H4 has been suggested to provide a mechanism for spreading of chromatin in line with the described silencing mechanism at telomeres and the mating type loci by the Sir proteins. By using chromatin immunoprecipitation methods Tup1 has been shown to be distributed from the promotor along the entire coding region of the *STE6* gene (Ducker and Simpson, 2000; Edmondson et al., 1996). By investigating Ssn6 and Tup1 mediated repression in HDAC deleted strains interesting findings have been put forward. The repression of the endogenous *SUC2* and a-cell specific gene *MFA2* in a-cells was severely affected in a

multiple HDAC deleted background. The effect on repression was only seen in a *rpd3*, *hos1* and *hos2* deleted background which indicates a functional redundancy of these class I HDACs (Watson et al., 2000). In addition, chromatin immunoprecipitated from the same promotors of the HDAC mutants reveals that the acetylation status of both H3 and H4 was increased. Most interestingly the investigated HDACs were also found to interact directly with Ssn6 and Tup1 proteins *in vivo* and *in vitro*. The N-terminal TPR domain of Ssn6 was found to interact weakly with both Rpd3 and Hos2 using the two hybrid assay and the same proteins interacted reciprocally in immunoprecipitation experiments. The experiments were performed with yeast cell extract expressing fusion proteins and did not exclude Ssn6 or Tup1 as indirect or direct interactors. In a more fundamental study both Tup1 and Ssn6 were found to interact independently with Rpd3 and Hos2 was found to interact independently with Ssn6 via the TPR repeats 1-7 (Davie et al., 2003). Evidence that class II HDACs can interact with Tup1 in similar fashion has also been observed. Investigation of the *ENA1* promotor, implicated in osmotic stress regulation by Sko1 and Ssn6-Tup1 (Marquez et al., 1998), indicates that Hda1 specifically deacetylates histone H3 and H2B terminals with a sharp peak over the TATA box. The H2B acetylation levels were found to be unaffected in a *tup1* deleted strain but H3 acetylation levels were severely affected and comparable to a *hda1* deletion indicating a role for Tup1 in recruiting Hda1 to the *ENA1* promotor (Wu et al., 2001). Expression from the *ENA1* reporter also shows that both Hda1 and Tup1 are required for full repression. In the same study disruption of *rpd3* was found to affect H4 acetylation in the coding region but had no affect upon the *ENA1* reporter expression. Indeed, the Tup1 protein could also interact by immunoprecipitation with Hda1 as well as with the other class II HDAC Hda3 found in complex with Hda1 (Wu et al., 2001). In summary the described findings have led to two proposed parallel co-operating models for Ssn6 and Tup1 repression. One involves chromatin structure and modifications, and a second involves direct interaction with the RNA polymerase II.

3.13 MICROARRAY STUDIES INVOLVING SSN6-TUP.

Expression microarray analysis suggests that the Tup1 transcriptional repressor is involved in regulating as many as 3% of all yeast genes in *S. cerevisiae* when grown in rich media containing glucose as carbon source (DeRisi et al., 1997) A number of genes that were Tup1 repressed were in addition induced by depletion of glucose. Thus, repression by Tup1 appears to be highly specific at individual gene targets since the right intracellular signals in response to environmental changes can accurately relive

repression. Few studies have addressed the changes in expression patterns due to the deletion of ssn6 and/or *tup1* with a global microarray approach. The study performed by DeRisi et al. did not include the *ssn6* expression profile. Only one additional study has included both *tup1* and the *ssn6* deletion in *S. cerevisiae*. In a systematic approach with 300 different expression profiles the *ssn6* and *tup1* profiles clustered together in a very similar profile and were therefore suggested to be virtually identical (Hughes et al., 2000). However, the cluster used for this conclusion only contained a selection of the most significantly regulated genes in the different profiles (n=568 genes). The rest of the targets were not analysed as thoroughly and further investigation reveals that ssn6 and tup1 shares a large co-regulated group of targets but there are also an equally large group of distinct targets that are only regulated by one of the proteins. This conclusion is also strengthened by findings in the pathogenic fungus *C. albicans*, where deletion mutants of *ssn6* and *tup1* display differences in morphological phenotypes (Hwang et al., 2003). A global comparison also shows that the expression profiles of *ssn6* and *tup1* in *C. albicans* are different with respect to regulation of hypha specific genes, but most similar with respect to genes involved in carbon metabolism (Garcia-Sanchez et al., 2005). Recently the genomic distribution of Tup1 has been monitored by global chromatin immunoprecipitation (ChIP) both under glucose and glucose depleted growth (Buck and Lieb, 2006). The analysis was performed with respect to binding of the activator-repressor protein Rap1 and results suggest that Tup1 restricts the binding of Rap1 in the presence of glucose by recruitment of Hda1. During glucose depletion Tup1 remains at the promotor but does not impair binding of Rap1. Genome wide deacetylation maps of Rpd3, Hda1 and Hos1 have been tested for correlation with *tup1* expression targets extracted from the Hughes et al. study (Robyr et al., 2002) Interestingly, the best genome wide correlation with *tup1* was found for Hda1 H3-K18 which also overlaps with subtelomeric domains. The Hda1 affected subtelomeric regions (HAST) contain many genes important for physiological transitions and response to stress. Sir3 mediated Tup1 repression by telomeric looping has been reported (Zaman et al., 2002). Heterochromatin regions formed by interactions with Sir2, Sir3 and Sir4 are also found at subtelomeric regions but do not correlate with regions affected by Hda1 and Tup1 in *S. cerevisiae* (Robyr et al., 2002) Many interesting conclusions with respect to mechanism and affected targets of Ssn6-Tup can be drawn from these studies, but further global studies are necessary to fully investigate Ssn6-Tup action in the metazoans.

4 COMMENTS ON METHODS

The methods used in this thesis have been fully described in detail in the enclosed separate papers. Here, I will focus on describing the model organism, *Schizosaccharomyces pombe,* and the microarray methods involved in generating expression profiles and binding localisation maps for the Ssn6-Tup11/12 co-repressor.

4.1 FISSION YEAST AS A MODEL ORGANISM

The simple unicellular fission yeast *Schizosaccharomyces pombe* is a very suitable model system for studying a wide range of diverse important biological functions including: metabolism, intracellular signaling, gene expression, cell cycle and morphogenesis among others. It has been emerging as a true contender and a complement to the much more characterized budding yeast *Saccharomyces cerevisiae.* The complete sequence of its genome contains 13.8 Mbp and 4824 protein coding genes divided into three chromosomes (Wood et al., 2002). The genome is similar in size to the *S. cerevisiae* genome but it is separated by longer intergenic regions and longer centromere structures. Calculations based on sequence comparisons of the fission and budding yeast genomes have estimated that the two species separated from each other approximately 400 million years ago (Sipiczki, 2000). In eukaryotic genomes a large proportion of the coding genes belong to multigene families that have evolved through gene duplication. Interestingly, a majority of the protein families identified in higher eukaryotes are also represented in *S. pombe* which implies that the transition to a multicellular organism does not require new protein families to any large extent. There are thus many advantages of using *S. pombe* as a model organism. Obvious reasons are the simple maintenance, fast growth and relatively ease of genetic modification that is connected to keeping laboratory fission yeast strains. Among the protein coding genes about one third has been characterized and many are highly conserved with other yeasts and multicellular eukaryotes. In addition, many of the *S. pombe* genes are related to genes connected to human diseases to a greater extent than reported for budding yeast.

4.2 GENOME ANALYSIS USING MICROARRAY

New tools have recently emerged which make it possible to analyse the expression of many genes in parallel and to uncover potential gene targets that depend on genotype or environmental factors. The use of microarrays developed from fully sequenced

genomes of a model organism allows to collect "fingerprints" of the transcriptome from different mutants, tissues, growth conditions, stress or other changes of circumstances (Lockhart and Winzeler, 2000). Two principal types of microarrays are used; (i) *in situ* synthesised oligonucleotide micrroarrays or one color arrays were biotin labelled fragments are hybridised to the array or (ii) spotted two color arrays were PCR amplified fragments are spotted to the array and hybridised with Cy3 and Cy5 labelled cDNA (Lockhart and Winzeler, 2000). Traditionally, one of the most important applications for microarrays is expression profiling where cDNA fragments isolated from RNA extractions are synthesised to DNA by Reverse Transcriptase PCR (RT-PCR) and labelled and hybridised to the arrays. When using microarrays a sample is always compared to a control. In one color experiments, the sample and control are hybridised to two separate identical arrays. In two color experiments, the sample and control are labelled differently with Cy3 and Cy5 and the fragments are allowed to hybridise to the same array under competition. Chromatin immunoprecipitation (ChIP) methods can be used in combination with microarrays to characterise the binding sites of a DNA or chromatin bound protein. Prior to hybridisation isolated chromatin fragment are subjected to shearing by sonication to achieve appropriate resolution size in the analysis, which is around 1kb for yeast applications. Normally, expression microarrays with open reading frames (ORFs) are not used for mapping of chromatin binding proteins. Instead the use of tiling microarrays with a complete genomic coverage or two color arrays with intergenic sequences (IGRs) allows mapping of the promotor regions. The intensity of the precipitated samples, the IP, is compared with the input control DNA. Thus, in a ChIP microarray experiments the ratios for the enriched elements are compared to all other non enriched elements in the experiment. With the microarray technique very large data sets are collected which demands statistical treatment for quality control and filtration of data. Computer based normalisation methods remove systematic variation from the microarray data. Two color data from mutant expression analysis in this thesis work have been normalised with locally weighted scatterplot smoothing (Lowess) normalisation, which is a technique for fitting a smoothing curve to the data (Yang et al., 2002). After quality control, the dataset is subjected to filtration and removal of background noise to find significant positives. Depending on the purpose, different methods based on fold change, sample variation and coherence can be used individually or in combination to identify differentially regulated genes. Chromatin binding profiles are preferably not analysed with these methods. Instead, ChIP microarray data is often normalised by

median percentile ranking approaches, which makes magnitude and scale of the ratios less important (Buck and Lieb, 2004). Classification or comparisons of selected significant targets from different experiments are important. One method, based on the hypergeometric distribution test, calculates the probability of selecting two genelists in a genome by chance. More sophisticated methods classify samples in different groups of experiments by their class prediction based on the distance or the similarity between samples. Such clustering methods can cluster up and down regulated genes from different experiments into common groups and are important for assigning regulatory classes involved in the same functional groups. Most importantly, filtrated interesting gene lists can also be analysed for significantly enriched gene ontology (GO) categories among a group of genes which can be valuable for classification of gene targets. The combination of expression array and genomic binding data provides an opportunity to identify direct targets to understand transcriptional networks.

5 RESULTS AND DISCUSSION

5.1 AIMS OF THE STUDY

The main aim of this study has been to investigate gene regulation processes important for understanding general mechanisms and evolution of eukaryotic transcriptional regulators. For this purpose, we have studied the fission yeast *Schizosaccharomyces pombe* Ssn6-Tup11/Tup12 transcriptional co-repressor involved in the regulation of target genes important for glucose repression and stress regulation among others.

We wanted to study conserved and specific characteristics of the individual fission yeast Ssn6, Tup11 and Tup12 proteins in order to gain further knowledge of Ssn6-Tup co-repressor function. The first issue to address was if the differences between the individual co-repressor genes influence different functional roles of the encoded proteins. We therefore wanted to investigate the cellular localisation patterns, the expression levels and interaction properties of the individual Ssn6, Tup11 and Tup12 proteins.

We have also been interested to investigate the physiological roles of the fission yeast Ssn6 and Tup proteins. We therefore wanted to characterise mutants affecting the individual co-repressor proteins under different growth conditions. Furthermore, we wanted to study the influence on gene expression and identify specific gene targets and we therefore aimed to characterise different Ssn6, Tup11 and Tup12 mutants with microarray expression profiling.

Another question was to understand whether genes that are selectively dependent on co-repressor subunits also show differential recruitment of Ssn6, Tup11 and Tup12. Consequently, we wanted to characterise the genome wide localisation of the individual proteins by global chromatin immunoprecipiation. In order to investigate interactions of the Ssn6-Tup11/Tup12 co-repressor with the *S. pombe* histone deacetylases, we have correlated genome wide interactions of co-regulated transcripts and co-localisations in chromatin binding studies.

5.2 PAPER I: FUNCTIONAL COMPARISON OF THE TUP11 AND TUP12 TRANSCRIPTIONAL CO-REPRESSORS IN FISSION YEAST.

Here, we have investigated the function of the fission yeast Tup11 and Tup12 proteins. By sequence comparison, we identified highly variable sequences in the proposed histone interacting intermediate M-domain, indicating a diversification of the proteins. We therefore wanted to analyse the cellular roles with respect to expression levels, localisation and complex formation. We found that both proteins are similarly expressed and thus likely to be functionally important. Coimmunoprecipitation data suggest that Tup11 and Tup12 do interact and we therefore wanted to analyse the degree of co-localisation between Tup11 and Tup12 by immunofluorescence microscopy analysis. Results indicate a high degree of nuclear co-localisation but also some uniquely localised regions of Tup11 and Tup12 and we therefore cannot exclude the possibility of certain pools of co-repressors containing exclusively Tup11 and Tup12.

Next, we wanted to investigate how Tup11 and Tup12 influence growth phenotypes and we therefore analysed *tup11* and *tup12* deletion strains on different media containing KCl and $CaCl_2$. Most interestingly, the *tup12* strain showed a more severe phenotype similar to the double deletion than the *tup11* strain. The specificity of the *tup12* phenotype was also tested by complementation. A Tup12 expressing plasmid could reverse the observed phenotype which could not be changed by a Tup11 expressing plasmid clearly showing that the two proteins have different functional roles in response to salt stress. To identify gene targets that could explain the functional difference the *tup11* and *tup12* deletion strains were subjected to expression profiling both under normal conditions (YES) and under stress (YES 1M KCl). Altogether, we detected 24 genes that were significantly changed >2 fold under normal conditions and 18 genes that were significantly changed >2 fold under stress induction in the *Δ11/Δ12* expression profile. The majority of the affected targets were derepressed in the *tup12* deletion compared to the *tup11* deletion which reflects the more drastic phenotype of the *tup12* deletion under these conditions. Identified targets were also validated by RT-PCR which clearly confirmed the microarray results and identified the Tup12 protein as the major repressor.

To analyse if the diverse phenotypes were due to differential binding of the fission yeast Ssn6 protein we performed co-immunoprecipitation. Results suggest that both

Tup11 and Tup12 can interact independently with the fission yeast Ssn6 protein, indicating the existence of an Ssn6-Tup11/12 complex in fission yeast.

We wanted to investigate whether the Ssn6 protein is involved in the regulation of Tup12 dependent targets and we generated an Ssn6 deletion strain. Interestingly, the Ssn6 protein is essential in fission yeast, but a conditional *ssn6-HA-ts* strain shows similar growth phenotypes to *tup12* on salt media, indicating a role in Tup12 dependent stress response. To confirm this behaviour, the *ssn6HA* mutant was investigated by RT-PCR which showed that Tup12 dependent targets were similarly derepressed in the *ssn6HA* ts strain. By comparing the extracted Tup12 targets with described gene ontology categories we found an over representation of genes induced by environmental stress, which was confirmed by the presence of ATF/CRE binding sites in the promotor regions. Virtually all the Tup12 dependent genes were found to be induced by stress indicating an important role of Tup12 in mediating repression of stress genes. Stress signalling in *S. pombe* is different from *S. cerevisiae* and appears to be funnelled uniquely by the Sty1 protein, demonstrating the demand for specific regulators that control the response. The major conclusion from these studies is thus that Tup12 has evolved a specific function as a stress regulator together with the Ssn6 protein. Consistent with the microarray experimental design we did not identify previously reported redundant targets of Tup11 and Tup12 (Janoo et al., 2001). It is therefore also possible that Tup11 and Tup12 act redundantly at other gene targets involved in glucose repression.

5.3 PAPER II: INDIVIDUAL SUBUNITS OF THE SSN6-TUP11/12 CO-REPRESSOR ARE SELECTIVELY REQUIRED FOR REPRESSION OF DIFFERENT TARGET GENES.

In this study we show that the Ssn6 protein is essential in fission yeast, and therefore must have a distinct role from Tup11 and Tup12. This non conserved feature of the fission yeast Ssn6 protein indicates important regulatory functions and to investigate this matter further we compared the roles of Ssn6 versus Tup11 and Tup12 with global gene expression experiments. For this purpose we used a conditional *ssn6HA*-ts strain and a *tup11,tup12* double deletion strain and performed microarray expression analysis. We also included a strain were Ssn6 was overexpressed, which affected a large number of up and down regulated targets. The nature of these targets is however highly uncertain since squelching of the Tup subunits or other effects not can be excluded.

Comparison of the three expression profiles reveals significant overlaps, specifically between the up-regulated targets in the *ssn6-HA* and the *tup11,tup12* profiles. Interestingly there is also a large number of distinct targets that are individually affected by *ssn6-HA* or *tup11,tup12,* reflecting the diverse phenotypes of Ssn6 and Tup11/12 in fission yeast.

To test whether the genes that are selectively dependent on co-repressor subunits also show differential recruitment of Ssn6 and Tup11/12 we characterised the genome wide localisation of the individual proteins by global chromatin immunoprecipiation. Generally, there was a significant correlation of co-repressor binding targets with the identified up regulated *ssn6-HA* or *tup11,tup12* expression targets and we isolated many direct co-repressor targets. Altogether about one third of the isolated expression targets were also bound by the co-repressor.

The binding data indicate that the co-repressor subunits are localised with both IGR an ORF regions with a high degree of overlap in binding between the different subunits. The few positions of unique binding of any co-repressor subunit were closely investigated, but we found no evidence for individual binding of Ssn6 without Tup11/12 at any position. To investigate the individual binding localisation with a higher resolution we used tiling arrays with a resolution of 250bp. The tiling array profiles show a remarkably similar binding to identified direct targets of all the individual subunits, indicating the existence of stable Ssn6-Tup11/12 complex in fission yeast. Interestingly, the co-repressor was found to be preferentially associated with the promotor region but also bound to the ORFs. The association of the Ssn6-Tup complex with coding regions has not been reported before and the conclusion of this finding is uncertain. Interestingly, the Srb8-11 proteins of the mediator subcomplex have also been reported to influence transcription inside coding regions in both fission and budding yeast (Andrau et al., 2006; Zhu et al., 2006).

To characterise whether the Ssn6-Tup11/12 co-repressor is involved in co-regulation with HDACs as reported in *S. cerevisiae*, we clustered Ssn6-Tup11/12 targets with previous data for wt acetylation levels and mutant HDAC expression (Wiren et al., 2005). Regions of low acetylation levels of H3 and H4 modifications generally correlated with co-repressor binding indicating association with HDAC activity. Clustering of Ssn6-Tup11/12 bound target genes with respect to mutant expression

levels identified Clr6 as the primary HDAC involved in regulation of Ssn6 and Tup11/12 affected genes. Interestingly, the Ssn6 overexpression profile is more similar to the *clr3* and *hst4* expression profiles, which implicates a role of the Ssn6-Tup11/12 co-repressor in HDAC class II and III regulation.

To test if co-repressor targets depend on any specific HDAC for deacetylation we next compared the mutant HDAC histone acetylation for a range of sites with binding targets that are affected by *ssn6HA-ts*, *tup11D, tup12D*, or Ssn6 overexpression (except for *hst4* which was not available). Results show that Ssn6 and Tup11/12 targets significantly overlap with Clr6 deacetylation at several sites while Ssn6 overexpression targets are instead deacetylated mainly by Clr3 and Sir2. Thus, we observe the same selective separation of Ssn6 and Tup11/12 target genes as observed in the expression profiles. The overlaps with class II and class III HDACs are based on Ssn6 overexpression data which have been used as a complement to the Ssn6-HA data. It is possible that the effect we observe with the partly functional Ssn6-HA is mainly redundant with Tup11/12 while Ssn6 overexpression identifies additional Ssn6 targets not affected by Ssn6-HA. The Ssn6 overexpression data is therefore of uncertain nature but indubitably well correlated with co-repressor binding. Interestingly, many direct Ssn6 overexpression targets are up-regulated which implicates a role in activation as observed in *S. cerevisiae* (Conlan et al., 1999).

We conclude that Ssn6 and Tup11/12 targets are mainly deacetylated and regulated by Clr6 in fission yeast which is in line with observations in *S. cerevisiae* where the class I Rpd3 protein has also been associated with Tup1-Ssn6 (Watson et al., 2000). Many of the co-repressor binding targets we observe are also found within the coding regions. Interestingly, similar findings have also been reported for Hos2 and Rpd3 (Florens et al., 2006; Wang et al., 2002; Wiren et al., 2005). The finding that Ssn6 is associated with class II and III HDACs is strengthened by genome wide studies in *S. cerevisiae* were expression targets affected by Ssn6-Tup1 are mainly deacetylated by the Clr3 analog Hda1 at subtelomeric regions (Robyr et al., 2002). Based on our findings and previous investigations we therefore propose that the individual subunits of Ssn6-Tup11/12 regulate selective targets associated with different classes of HDACs.

5.4 PAPER III: SPECIFIC FUNCTIONS FOR THE FISSION YEAST SIRTUINS HST2 AND HST4 IN GENE REGULATION AND RETROTRANSPOSON SILENCING.

We wanted to investigate the genome wide functional relationship and define the roles of the fission yeast class III HDACs. We therfore performed expression profiling of *hst2* and compared the results with previous data for the other *S. pombe* HDACs (Fagerstrom-Billai et al., 2007; Wiren et al., 2005). Analysis of the data sets indicates that upregulated *hst4* targets significantly overlaps with both *clr3* and *clr6-1* repressed targets. Interestingly, the *hst4* expression profile also correlates with expressed non silenced gene targets indicating a role in down tuning the expression of active genes. This is in contrast to the *sir2* and *hst2* expression profiles which mainly affect silenced gene targets. We conclude that the *S. pombe* sirtuins clearly have repressive roles but affect distinct sets of non-overlapping genes. Interestingly, the role of Hst4 which mainly acts on expressed genes in the ORF is similar to the reported function of Hos2 in both fission and budding yeast (Wang et al., 2002; Wiren et al., 2005) However, analysis of affected expression targets show that Hos2 mainly is associated with activation of distinct targets not affected by *hst4*. Comparison with gene ontolgy categories for the different expression groups reveals diverse belonging of the *hst2* and *hst4* repressed targets. The hst4 targets are significantly enriched for categories involved in amino acid biosynthesis and the hst2 targets are enriched for transporter and microtubule cytoskeleton categories.

To investigate the direct roles of Hst2 and Hst4 in deacetylation we analysed the mutant specific binding to a set of different H3 and H4 acetylation modifications by global ChIP. The analysis indicates that all three sirtuin mutants affect acetylation levels in IGR and ORF regions. Interestingly, *hst4* and *sir2* showed opposite effects upon acetylation. While *sir2* affected predominantly silent genes in IGR regions *hst4* was found to mainly change acetylation of transcribed genes in the ORF regions.

Next, the genome wide binding patterns for Hst4 and Hst2 was determined with global ChIP. By comparing the results with previous binding data for Sir2 we found a significant number of common targets which includes all the fission yeast silent heterochromatic regions previously associated with Clr3 binding (Wiren et al., 2005). Apart from the common binding sites, each sirtuin also had a large number of distinct binding locations, indicating independent association at some targets genes. We

isolated direct targets that had distinct binding and also were affected in the expression profiles. Interestingly, these direct targets were enriched in the same categories as described for the expression groups and we therefore conclude that Hst4 and Hst2 repress genes involved in distinct cellular functions.

Previous investigations of expression profiles and binding locations have shown that Sir2 act co-operatively at silent locations together with the class II HDAC Clr3, via deacetylation of H3-K14 and H3-K9 (Bjerling et al., 2002; Wiren et al., 2005). To determine the deacetylation specificity in this study we compared IGR and ORF regions bound by the sirtuins with lists of region affected by mutant acetylation. Again, we independently correlated Sir2 with H3-K9 deacetylation in the IGR and ORF region. However, when the distinct Sir2 targets were analysed we instead found a correlation with H4-K16 in IGR and H4-K12 in ORF regions.

Binding of Hst4 was instead correlated with Hst4 deacetylation at H3-K14 in the IGR and the ORF region. No large difference was observed between the distinct targets and the total number of targets bound by Hst4, but we note a slightly higher correlation with deacetylation of H3-K9 for the total number of targets in the ORF region. Both the total number of fragments and the distinct fragments bound by Hst2 matched best with H3-K14 deacetylation both for IGR and ORF regions.

Expression and acetylation data of *hst4* indicates role of Hst4 in down tuning expressed genes in the ORF. To further characterise such targets, we isolated a set of Tf2 elements from overlapping profiles of Hst4 ORF binding and *hst4* expression of highly transcribed genes, suggesting a specific role for Hst4 in retrotransposon regulation. Validation by Northern blotting and RT-PCR also shows that *hst4*$^{+}$ is required for correct Tf2 RNA processing. This is the first report of transposon silencing in fission yeast. Related mechanisms which involve the RNAi machinery in plants have previously been reported (Lippman et al., 2003). Expression profiles of RNAi components involved in heterochromatic silencing did not involve regulation of the TF2 elements (Hansen et al., 2005) which indicates an alternative mechanism for Hst4 directed transposon RNA processing in fission yeast.

Interestingly, all of the Hst4 directly repressed ORF targets which are involved in amino acid biosynthesis are also direct targets for the Ssn6-Tup11/12 co-repressor

complex and are affected by Ssn6 overexpression, suggesting that Ssn6 could be involved in targeting of the class III HDAC Hst4.

In this study, localisation shown with immunofluoresence micoscopy indicates that Hst2 has a shuttling role previously observed for the budding yeast orthologue Hst2 (Lamming et al., 2005). To further understand the functional role of Hst2 we characterised the role of Hst2 in heterochromatin silencing. Interestingly, *hst2*$^{+}$ was found to be important for rDNA silencing in a similar manner to *sir2*$^{+}$ (Shankaranarayana et al., 2003) and for the centromeric silencing of the outer repeats (*Otr*) together with both *sir2*$^{+}$ and *hst4*$^{+}$ (Freeman-Cook et al., 2005; Freeman-Cook et al., 1999). This co-operative role of the sirtuins is also observed in double deletion strains where phenotypic analysis indicates synthetic genetic interactions. Specifically, sensitivity to the microtubule destabilizing drug TBZ, shows that *hst2* and *hst4* interacts synthetically since a double mutant shows impaired growth compared to the single mutants, suggesting a role in microtubule function for Hst2. This is in line with the observed gene ontology categories affected by *hst2*.

To summarise, we conclude that the fission yeast sirtuin proteins have adapted redundant functions but have also evolved specific regulatory functions which is specifically reflected by the effect upon distinct regulatory targets involved in different gene ontology categories, distinct *in vivo* deacetylation specificities and different specific roles within the IGR and ORF regions.

5.5 UNPUBLISHED RESULTS

We have analysed Ssn6 interactions with the fission yeast HDACs by using the 2-hybrid technique (Fields and Song, 1989). Most interestingly, full length Hos2, Clr6 and Clr3 can restore growth together with a partial Ssn6 protein containing the central TPR domain (unpublished data) (Fig. 8). The strongest interaction appears to be between the Clr3 protein and the TPR domain followed by Clr6 and Hos2. The interaction with the Clr3 protein has also been validated and the Ssn6 protein can interact with Clr3 *in vivo* by immunoprecipitation (data not shown). Together with presented microarray results and previous results in *S. cerevisiae* this shows that the Ssn6 association with HDACs is an evolutionary conserved feature and that Ssn6 can interact with both class I and class II HDACs in fission yeast.

As described, direct targets of Ssn6 have also been shown to correlate with Sir2 and Hst4 targets, which suggest a functional overlap. To test if the sirtuins can interact with the Ssn6 protein we therefore performed 2-hybrid using the full length Hst4, Hst2 and Sir2 together with the Ssn6 TPR domain. However, we have not been able to restore growth with any class III proteins, which indicates that the sirtuins may not be directly bound by the Ssn6-Tup11/12 co-repressor.

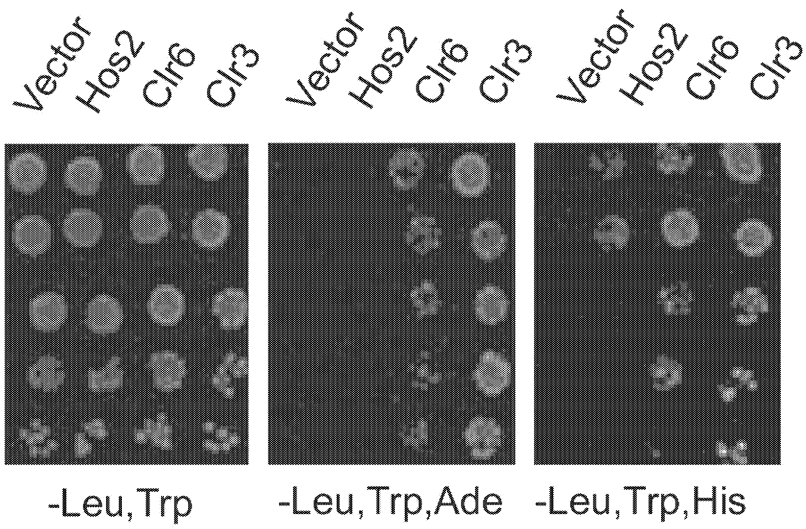


Figure 8. Yeast cells spotted in 5 fold dilutions growing on amino acid depleted media. Cells with positive 2-Hybrid interactions grow on depleted –Leu, Trp,Ade and –Leu, Trp, His reporter media. The TPR domain of Ssn6 is fused to the Gal4 DNA binding domain interacts with the different indicated fission yeast HDACs which are fused to the Gal4 activation domain (Fields and Song, 1989).

5.6 CONCLUDING REMARKS

The most important findings presented in this thesis reflect the apparent different roles of the individual subunits of the Ssn6-Tup11/12 co-repressor. The observed phenotypes of the characterised deletion strains are remarkably diverse and with genome expression profiling we have identified a number of genes that are differentially affected by defects in Tup11, Tup12 and Ssn6. The results suggest that the Ssn6-Tup11/12 co-repressor is involved in the regulation of many genes affected by environmental stress conditions. In fission yeast, stress transduction is maintained via the common Sty1 pathway and it is likely that the different subunits of the Ssn6-

Tup11/12 co-repressor have important roles for controlling the right response. In addition we have also found a common and selective group of Ssn6-Tup11/12 regulated genes involved in different categories such as conjugation, meiosis, carbohydrate metabolism, ion transport and amino acid metabolism. Genome wide binding studies of the co-repressor with high resolution tiling arrays show a striking consistency in binding of the different subunits. The selective repression is thus not likely due to recruitment of individual subunits, and we conclude that the Ssn6-Tup11/12 constitution is highly stable. Consistent with previous findings, our results also suggest that Ssn6-Tup11/12 is recruited to the upstream region of regulated genes, but most interestingly we also note significant binding to the coding region, similar to Srb8-11 occupancy in fission yeast. It is thus likely that the interplay between Ssn6-Tup1 and the mediator is a conserved feature. Our study shows that Ssn6-Tup11/12 function is conserved with respect to HDAC function as described in *S. cerevisiae*. Genome wide analysis clearly correlates the class I Clr6 with Ssn6-Tup11/12 function in fission yeast, but we also note an association with the class II Clr3 and the class III Sir2 and Hst4 HDACs. Further studies would be necessary to determine the exact requirement for the described factors. Binding studies in mutant backgrounds would be an important tool for performing such experiments. The development of partial Ssn6 deletions could possibly also be important for targeting of additional Ssn6 specific genes and to find out more about the essential Ssn6 protein.

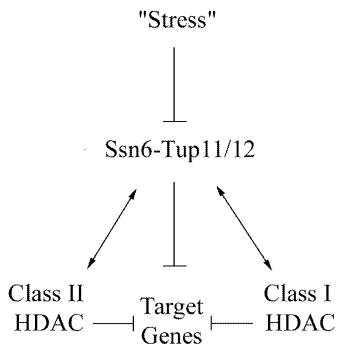


Figure 9. Simplified model of Ssn6-Tup11/12 function in fission yeast showing repression of a target genes in co-operation with class I and class II HDACs. Stress signals induce the activity of pathways that interfere with Ssn6-Tup11/12 activity for fast derepression.

6 ACKNOWLEDGEMENTS

I would like to express my honest and deepest gratitude to all the people who have contributed to this work. All are included but my special concern goes to the following friends and colleagues:

My supervisor, Professor Anthony Wright, for introducing me to the world of science with remarkable patience and optimism. Thank you for encouraging and guiding me on the long and winding way. Karl Ekwall, my co-supervisor, thank you for showing me many useful fission yeast tricks. You have been co-operative and supporting and always found the time for questions and discussions.

Special thanks to all the AWR lab group members: Thank you, Anna, Azadeh, Chiounan, Helmi, Monica, Yongtao and Rachel for always being so helpful, concerning, nice, and friendly. You have all been great and I wish you all my best. Many thanks to all the people who have come and gone during the years of my graduate studies: Thanks to Elisabeth, Jonas and Stefan for always having the time to help and answer questions. I would also like to thank all the present and previous members of the KEK lab group: Carolina, Ingela, Indranil, Mikael and Marianna, Rebecca, Pernilla and specially Julian Walfridsson, for always shining with the right spirit and "raising the ceiling" at the department. Henrik Appelgren, believe it or not, a truly nice and honest "AIKare". Thanks for fixing all the derby tickets.

Thanks to everybody at Södertörns Högskola for framing the superb environment and all the people I have forgotten to mention at the Department of Biosciences at Novum.
I would also like to thank everybody at BEA for bringing me on and helping me to start my new position. Thank you: Karin, Johan, Malin, Marika and David for being so easy going and friendly.

My mother , Marita, for endless support and immediate stand-ins at difficult times. Thank you for always being so positive.

And last, my very special thanks to my family: Anne, Linn, Kim and Dante. Without your support I could never have achieved this. You have been the lights that have guided me during these years. Thank you.

7 REFERENCES

Alberts, Bruce A.J., Julian Lewis, Martin Raff, Keith Roberts, Peter Walter (2002) *Molecular Biology of the Cell.*

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. *Embo J*, **18**, 5108-5119.

Allfrey, V.G., Faulkner, R. and Mirsky, A.E. (1964) Acetylation and Methylation of Histones and Their Possible Role in the Regulation of Rna Synthesis. *Proc Natl Acad Sci U S A*, **51**, 786-794.

Andrau, J.C., van de Pasch, L., Lijnzaad, P., Bijma, T., Koerkamp, M.G., van de Peppel, J., Werner, M. and Holstege, F.C. (2006) Genome-wide location of the coactivator mediator: Binding without activation and transient Cdk8 interaction on DNA. *Mol Cell*, **22**, 179-192.

Balciunas, D. and Ronne, H. (1995) Three subunits of the RNA polymerase II mediator complex are involved in glucose repression. *Nucleic Acids Res*, **23**, 4421-4425.

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

Becker, P.B., Gloss, B., Schmid, W., Strahle, U. and Schutz, G. (1986) In vivo protein-DNA interactions in a glucocorticoid response element require the presence of the hormone. *Nature*, **324**, 686-688.

Berge-Lefranc, J.L., Jay, P., Massacrier, A., Cau, P., Mattei, M.G., Bauer, S., Marsollier, C., Berta, P. and Fontes, M. (1996) Characterization of the human jumonji gene. *Hum Mol Genet*, **5**, 1637-1641.

Bernstein, B.E., Tong, J.K. and Schreiber, S.L. (2000) Genomewide studies of histone deacetylase function in yeast. *Proc Natl Acad Sci U S A*, **97**, 13708-13713.

Bernstein, E. and Allis, C.D. (2005) RNA meets chromatin. *Genes Dev*, **19**, 1635-1655.

Bernstein, E., Caudy, A.A., Hammond, S.M. and Hannon, G.J. (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature*, **409**, 363-366.

Bertos, N.R., Wang, A.H. and Yang, X.J. (2001) Class II histone deacetylases: structure, function, and regulation. *Biochem Cell Biol*, **79**, 243-252.

Beskow, A. and Wright, A.P. (2006) Comparative analysis of regulatory transcription factors in Schizosaccharomyces pombe and budding yeasts. *Yeast*, **23**, 929-935.

Bestor, T.H. (1998) Gene silencing. Methylation meets acetylation. *Nature*, **393**, 311-312.

Bjerling, P., Silverstein, R.A., Thon, G., Caudy, A., Grewal, S. and Ekwall, K. (2002) Functional divergence between histone deacetylases in fission yeast by distinct cellular localization and in vivo specificity. *Mol Cell Biol*, **22**, 2170-2181.

Blander, G. and Guarente, L. (2004) The Sir2 family of protein deacetylases. *Annu Rev Biochem*, **73**, 417-435.

Bogengruber, E., Eichberger, T., Briza, P., Dawes, I.W., Breitenbach, M. and Schricker, R. (1998) Sporulation-specific expression of the yeast DIT1/DIT2 promoter is controlled by a newly identified repressor element and the short form of Rim101p. *Eur J Biochem*, **258**, 430-436.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M. and Benning, C. (1998) AGO1 defines a novel locus of Arabidopsis controlling leaf development. *Embo J*, **17**, 170-180.

Boyes, J., Byfield, P., Nakatani, Y. and Ogryzko, V. (1998) Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature*, **396**, 594-598.

Braun, B.R. and Johnson, A.D. (1997) Control of filament formation in Candida albicans by the transcriptional repressor TUP1. *Science*, **277**, 105-109.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell*, **84**, 843-851.

Buck, M.J. and Lieb, J.D. (2004) ChIP-chip: considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics*, **83**, 349-360.

Buck, M.J. and Lieb, J.D. (2006) A chromatin-mediated mechanism for specification of conditional transcription factor targets. *Nat Genet*, **38**, 1446-1451.
Burley, S.K. and Roeder, R.G. (1996) Biochemistry and structural biology of transcription factor IID (TFIID). *Annu Rev Biochem*, **65**, 769-799.
Candido, E.P., Reeves, R. and Davie, J.R. (1978) Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell*, **14**, 105-113.
Carcamo, J., Maldonado, E., Cortes, P., Ahn, M.H., Ha, I., Kasai, Y., Flint, J. and Reinberg, D. (1990) A TATA-like sequence located downstream of the transcription initiation site is required for expression of an RNA polymerase II transcribed gene. *Genes Dev*, **4**, 1611-1622.
Carlson, M. (1997) Genetics of transcriptional regulation in yeast: connections to the RNA polymerase II CTD. *Annu Rev Cell Dev Biol*, **13**, 1-23.
Carlson, M. (1999) Glucose repression in yeast. *Curr Opin Microbiol*, **2**, 202-207.
Carlson, M., Osmond, B.C., Neigeborn, L. and Botstein, D. (1984) A suppressor of SNF1 mutations causes constitutive high-level invertase synthesis in yeast. *Genetics*, **107**, 19-32.
Carmen, A.A., Rundlett, S.E. and Grunstein, M. (1996) HDA1 and HDA3 are components of a yeast histone deacetylase (HDA) complex. *J Biol Chem*, **271**, 15837-15844.
Carrico, P.M. and Zitomer, R.S. (1998) Mutational analysis of the Tup1 general repressor of yeast. *Genetics*, **148**, 637-644.
Chen, G. and Courey, A.J. (2000) Groucho/TLE family proteins and transcriptional repression. *Gene*, **249**, 1-16.
Chen, T.A. and Allfrey, V.G. (1987) Rapid and reversible changes in nucleosome structure accompany the activation, repression, and superinduction of murine fibroblast protooncogenes c-fos and c-myc. *Proc Natl Acad Sci U S A*, **84**, 5252-5256.
Conlan, R.S., Gounalaki, N., Hatzis, P. and Tzamarias, D. (1999) The Tup1-Cyc8 protein complex can shift from a transcriptional co-repressor to a transcriptional co-activator. *J Biol Chem*, **274**, 205-210.
Cooper, J.P., Roth, S.Y. and Simpson, R.T. (1994) The global transcriptional regulators, SSN6 and TUP1, play distinct roles in the establishment of a repressive chromatin structure. *Genes Dev*, **8**, 1400-1410.
Cowell, I.G. (1994) Repression versus activation in the control of gene transcription. *Trends Biochem Sci*, **19**, 38-42.
Cramer, P. (2004) Structure and function of RNA polymerase II. *Adv Protein Chem*, **67**, 1-42.
Cress, W.D. and Triezenberg, S.J. (1991) Critical structural elements of the VP16 transcriptional activation domain. *Science*, **251**, 87-90.
Das, A.K., Cohen, P.W. and Barford, D. (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *Embo J*, **17**, 1192-1199.
Davie, J.K., Edmondson, D.G., Coco, C.B. and Dent, S.Y. (2003) Tup1-ssn6 interacts with multiple class I histone deacetylases in vivo. *J Biol Chem*, **278**, 50158-50162.
de Celis, J.F. and Garcia-Bellido, A. (1994) Modifications of the notch function by Abruptex mutations in Drosophila melanogaster. *Genetics*, **136**, 183-194.
de Celis, J.F. and Ruiz-Gomez, M. (1995) groucho and hedgehog regulate engrailed expression in the anterior compartment of the Drosophila wing. *Development*, **121**, 3467-3476.
De Nadal, E., Zapater, M., Alepuz, P.M., Sumoy, L., Mas, G. and Posas, F. (2004) The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. *Nature*, **427**, 370-374.
Deckert, J., Perini, R., Balasubramanian, B. and Zitomer, R.S. (1995) Multiple elements and auto-repression regulate Rox1, a repressor of hypoxic genes in Saccharomyces cerevisiae. *Genetics*, **139**, 1149-1158.
Derbyshire, M.K., Weinstock, K.G. and Strathern, J.N. (1996) HST1, a new member of the SIR2 family of genes. *Yeast*, **12**, 631-640.
DeRisi, J.L., Iyer, V.R. and Brown, P.O. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science*, **278**, 680-686.

Ducker, C.E. and Simpson, R.T. (2000) The organized chromatin domain of the repressed yeast a cell-specific gene STE6 contains two molecules of the corepressor Tup1p per nucleosome. *Embo J*, **19**, 400-409.
Edmondson, D.G., Smith, M.M. and Roth, S.Y. (1996) Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. *Genes Dev*, **10**, 1247-1259.
Ekwall, K. (2004) The roles of histone modifications and small RNA in centromere function. *Chromosome Res*, **12**, 535-542.
Ekwall, K. (2005) Genome-wide analysis of HDAC function. *Trends Genet*, **21**, 608-615.
Ellenberger, T.E., Brandl, C.J., Struhl, K. and Harrison, S.C. (1992) The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted alpha helices: crystal structure of the protein-DNA complex. *Cell*, **71**, 1223-1237.
Escher, D., Bodmer-Glavas, M., Barberis, A. and Schaffner, W. (2000) Conservation of glutamine-rich transactivation function between yeast and humans. *Mol Cell Biol*, **20**, 2774-2782.
Fagerstrom-Billai, F., Durand-Dubief, M., Ekwall, K. and Wright, A.P. (2007) Individual subunits of the Ssn6-Tup11/12 co-repressor are selectively required for repression of different target genes. *Mol Cell Biol.* **27**, 1069-82.
Fields, S. and Song, O. (1989) A novel genetic system to detect protein-protein interactions. *Nature*, **340**, 245-246.
Florens, L., Carozza, M.J., Swanson, S.K., Fournier, M., Coleman, M.K., Workman, J.L. and Washburn, M.P. (2006) Analyzing chromatin remodeling complexes using shotgun proteomics and normalized spectral abundance factors. *Methods*, **40**, 303-311.
Flores-Saaib, R.D. and Courey, A.J. (2000) Analysis of Groucho-histone interactions suggests mechanistic similarities between Groucho- and Tup1-mediated repression. *Nucleic Acids Res*, **28**, 4189-4196.
Fong, H.K., Hurley, J.B., Hopkins, R.S., Miake-Lye, R., Johnson, M.S., Doolittle, R.F. and Simon, M.I. (1986) Repetitive segmental structure of the transducin beta subunit: homology with the CDC4 gene and identification of related mRNAs. *Proc Natl Acad Sci U S A*, **83**, 2162-2166.
Freeman-Cook, L.L., Gomez, E.B., Spedale, E.J., Marlett, J., Forsburg, S.L., Pillus, L. and Laurenson, P. (2005) Conserved locus-specific silencing functions of Schizosaccharomyces pombe sir2+. *Genetics*, **169**, 1243-1260.
Freeman-Cook, L.L., Sherman, J.M., Brachmann, C.B., Allshire, R.C., Boeke, J.D. and Pillus, L. (1999) The Schizosaccharomyces pombe hst4(+) gene is a SIR2 homologue with silencing and centromeric functions. *Mol Biol Cell*, **10**, 3171-3186.
Friesen, H., Hepworth, S.R. and Segall, J. (1997) An Ssn6-Tup1-dependent negative regulatory element controls sporulation-specific expression of DIT1 and DIT2 in Saccharomyces cerevisiae. *Mol Cell Biol*, **17**, 123-134.
Fujimori, K., Anamnart, S., Nakagawa, Y., Sugioka, S., Ohta, D., Oshima, Y., Yamada, Y. and Harashima, S. (1997) Isolation and characterization of mutations affecting expression of the delta9- fatty acid desaturase gene, OLE1, in Saccharomyces cerevisiae. *FEBS Lett*, **413**, 226-230.
Garcia-Sanchez, S., Mavor, A.L., Russell, C.L., Argimon, S., Dennison, P., Enjalbert, B. and Brown, A.J. (2005) Global roles of Ssn6 in Tup1- and Nrg1-dependent gene regulation in the fungal pathogen, Candida albicans. *Mol Biol Cell*, **16**, 2913-2925.
Gasperowicz, M. and Otto, F. (2005) Mammalian Groucho homologs: redundancy or specificity? *J Cell Biochem*, **95**, 670-687.
Gavin, I.M. and Simpson, R.T. (1997) Interplay of yeast global transcriptional regulators Ssn6p-Tup1p and Swi-Snf and their effect on chromatin structure. *Embo J*, **16**, 6263-6271.
Ghaemmaghami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K. and Weissman, J.S. (2003) Global analysis of protein expression in yeast. *Nature*, **425**, 737-741.

Gileadi, O., Feaver, W.J. and Kornberg, R.D. (1992) Cloning of a subunit of yeast RNA polymerase II transcription factor b and CTD kinase. *Science*, **257**, 1389-1392.
Gounalaki, N., Tzamarias, D. and Vlassi, M. (2000) Identification of residues in the TPR domain of Ssn6 responsible for interaction with the Tup1 protein. *FEBS Lett*, **473**, 37-41.
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**, 1640-1650.
Grant, P.A., Eberharter, A., John, S., Cook, R.G., Turner, B.M. and Workman, J.L. (1999) Expanded lysine acetylation specificity of Gcn5 in native complexes. *J Biol Chem*, **274**, 5895-5900.
Gray, S.G. and Ekstrom, T.J. (2001) The human histone deacetylase family. *Exp Cell Res*, **262**, 75-83.
Grbavec, D., Lo, R., Liu, Y., Greenfield, A. and Stifani, S. (1999) Groucho/transducin-like enhancer of split (TLE) family members interact with the yeast transcriptional co-repressor SSN6 and mammalian SSN6-related proteins: implications for evolutionary conservation of transcription repression mechanisms. *Biochem J*, **337 (Pt 1)**, 13-17.
Greenall, A., Hadcroft, A.P., Malakasi, P., Jones, N., Morgan, B.A., Hoffman, C.S. and Whitehall, S.K. (2002) Role of fission yeast Tup1-like repressors and Prr1 transcription factor in response to salt stress. *Mol Biol Cell*, **13**, 2977-2989.
Greenfield, A., Carrel, L., Pennisi, D., Philippe, C., Quaderi, N., Siggers, P., Steiner, K., Tam, P.P., Monaco, A.P., Willard, H.F. and Koopman, P. (1998) The UTX gene escapes X inactivation in mice and humans. *Hum Mol Genet*, **7**, 737-742.
Gromoller, A. and Lehming, N. (2000) Srb7p is a physical and physiological target of Tup1p. *Embo J*, **19**, 6845-6852.
Gross, P. and Oelgeschlager, T. (2006) Core promoter-selective RNA polymerase II transcription. *Biochem Soc Symp*, 225-236.
Grunicke, H., Csordas, A., Helliger, W., Hauptlorenz, S., Loidl, A., Multhaup, I., Zwierzina, H. and Puschendorf, B. (1984) Depression of histone acetylation by alkylating antitumor agents: significance for antitumor activity and possible biological consequences. *Adv Enzyme Regul*, **22**, 433-446.
Grunstein, M. (1990) Nucleosomes: regulators of transcription. *Trends Genet*, **6**, 395-400.
Grunstein, M. (1992) Histones as regulators of genes. *Sci Am*, **267**, 68-74B.
Grunstein, M. (1997) Histone acetylation in chromatin structure and transcription. *Nature*, **389**, 349-352.
Gu, W. and Roeder, R.G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell*, **90**, 595-606.
Hahn, S., Buratowski, S., Sharp, P.A. and Guarente, L. (1989) Yeast TATA-binding protein TFIID binds to TATA elements with both consensus and nonconsensus DNA sequences. *Proc Natl Acad Sci U S A*, **86**, 5718-5722.
Han, S.J., Lee, J.S., Kang, J.S. and Kim, Y.J. (2001) Med9/Cse2 and Gal11 modules are required for transcriptional repression of distinct group of genes. *J Biol Chem*, **276**, 37020-37026.
Hansen, K.R., Burns, G., Mata, J., Volpe, T.A., Martienssen, R.A., Bahler, J. and Thon, G. (2005) Global effects on gene expression in fission yeast by silencing and RNA interference machineries. *Mol Cell Biol*, **25**, 590-601.
Hirota, K., Hasemi, T., Yamada, T., Mizuno, K.I., Hoffman, C.S., Shibata, T. and Ohta, K. (2004) Fission yeast global repressors regulate the specificity of chromatin alteration in response to distinct environmental stresses. *Nucleic Acids Res*, **32**, 855-862.
Hirschhorn, J.N., Brown, S.A., Clark, C.D. and Winston, F. (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev*, **6**, 2288-2298.
Hoppe, G.J., Tanny, J.C., Rudner, A.D., Gerber, S.A., Danaie, S., Gygi, S.P. and Moazed, D. (2002) Steps in assembly of silent chromatin in yeast: Sir3-

independent binding of a Sir2/Sir4 complex to silencers and role for Sir2-dependent deacetylation. *Mol Cell Biol*, **22**, 4167-4180.
Huang, M., Zhou, Z. and Elledge, S.J. (1998) The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. *Cell*, **94**, 595-605.
Hughes, T.R., Marton, M.J., Jones, A.R., Roberts, C.J., Stoughton, R., Armour, C.D., Bennett, H.A., Coffey, E., Dai, H., He, Y.D., Kidd, M.J., King, A.M., Meyer, M.R., Slade, D., Lum, P.Y., Stepaniants, S.B., Shoemaker, D.D., Gachotte, D., Chakraburtty, K., Simon, J., Bard, M. and Friend, S.H. (2000) Functional discovery via a compendium of expression profiles. *Cell*, **102**, 109-126.
Hwang, C.S., Oh, J.H., Huh, W.K., Yim, H.S. and Kang, S.O. (2003) Ssn6, an important factor of morphological conversion and virulence in Candida albicans. *Mol Microbiol*, **47**, 1029-1043.
Imhof, A., Yang, X.J., Ogryzko, V.V., Nakatani, Y., Wolffe, A.P. and Ge, H. (1997) Acetylation of general transcription factors by histone acetyltransferases. *Curr Biol*, **7**, 689-692.
Ishihama, A., Kimura, M. and Mitsuzawa, H. (1998) Subunits of yeast RNA polymerases: structure and function. *Curr Opin Microbiol*, **1**, 190-196.
Jabet, C., Sprague, E.R., VanDemark, A.P. and Wolberger, C. (2000) Characterization of the N-terminal domain of the yeast transcriptional repressor Tup1. Proposal for an association model of the repressor complex Tup1 x Ssn6. *J Biol Chem*, **275**, 9011-9018.
Janoo, R.T., Neely, L.A., Braun, B.R., Whitehall, S.K. and Hoffman, C.S. (2001) Transcriptional regulators of the Schizosaccharomyces pombe fbp1 gene include two redundant Tup1p-like corepressors and the CCAAT binding factor activation complex. *Genetics*, **157**, 1205-1215.
Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science*, **293**, 1074-1080.
Johnson, A.D. and Herskowitz, I. (1985) A repressor (MAT alpha 2 Product) and its operator control expression of a set of cell type specific genes in yeast. *Cell*, **42**, 237-247.
Keleher, C.A., Goutte, C. and Johnson, A.D. (1988) The yeast cell-type-specific repressor alpha 2 acts cooperatively with a non-cell-type-specific protein. *Cell*, **53**, 927-936.
Keleher, C.A., Redd, M.J., Schultz, J., Carlson, M. and Johnson, A.D. (1992) Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell*, **68**, 709-719.
Kim, S.J., Swanson, M.J., Qiu, H., Govind, C.K. and Hinnebusch, A.G. (2005) Activator Gcn4p and Cyc8p/Tup1p are interdependent for promoter occupancy at ARG1 in vivo. *Mol Cell Biol*, **25**, 11171-11183.
Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H. and Kornberg, R.D. (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell*, **77**, 599-608.
Komachi, K. and Johnson, A.D. (1997) Residues in the WD repeats of Tup1 required for interaction with alpha2. *Mol Cell Biol*, **17**, 6023-6028.
Komachi, K., Redd, M.J. and Johnson, A.D. (1994) The WD repeats of Tup1 interact with the homeo domain protein alpha 2. *Genes Dev*, **8**, 2857-2867.
Kornberg, R.D. and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell*, **98**, 285-294.
Kornberg, T.B. (1993) Understanding the homeodomain. *J Biol Chem*, **268**, 26813-26816.
Kruh, J. (1982) Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol Cell Biochem*, **42**, 65-82.
Kuchin, S. and Carlson, M. (1998) Functional relationships of Srb10-Srb11 kinase, carboxy-terminal domain kinase CTDK-I, and transcriptional corepressor Ssn6-Tup1. *Mol Cell Biol*, **18**, 1163-1171.
Lachner, M. and Jenuwein, T. (2002) The many faces of histone lysine methylation. *Curr Opin Cell Biol*, **14**, 286-298.
Lamming, D.W., Latorre-Esteves, M., Medvedik, O., Wong, S.N., Tsang, F.A., Wang, C., Lin, S.J. and Sinclair, D.A. (2005) HST2 mediates SIR2-independent life-span extension by calorie restriction. *Science*, **309**, 1861-1864.

Landry, J., Sutton, A., Tafrov, S.T., Heller, R.C., Stebbins, J., Pillus, L. and Sternglanz, R. (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci U S A*, **97**, 5807-5811.
Latchman, D.S. (1996) Inhibitory transcription factors. *Int J Biochem Cell Biol*, **28**, 965-974.
Latchman, D.S. (1997) Transcription factors: an overview. *Int J Biochem Cell Biol*, **29**, 1305-1312.
Laurenson, P. and Rine, J. (1992) Silencers, silencing, and heritable transcriptional states. *Microbiol Rev*, **56**, 543-560.
Lee, T.I. and Young, R.A. (1998) Regulation of gene expression by TBP-associated proteins. *Genes Dev*, **12**, 1398-1408.
Lee, T.I. and Young, R.A. (2000) Transcription of eukaryotic protein-coding genes. *Annu Rev Genet*, **34**, 77-137.
Lehrmann, H., Pritchard, L.L. and Harel-Bellan, A. (2002) Histone acetyltransferases and deacetylases in the control of cell proliferation and differentiation. *Adv Cancer Res*, **86**, 41-65.
Lemontt, J.F. (1980) Genetic and physiological factors affecting repair and mutagenesis in yeast. *Basic Life Sci*, **15**, 85-120.
Leuther, K.K., Bushnell, D.A. and Kornberg, R.D. (1996) Two-dimensional crystallography of TFIIB- and IIE-RNA polymerase II complexes: implications for start site selection and initiation complex formation. *Cell*, **85**, 773-779.
Li, B. and Reese, J.C. (2000) Derepression of DNA damage-regulated genes requires yeast TAF(II)s. *Embo J*, **19**, 4091-4100.
Lippman, Z., May, B., Yordan, C., Singer, T. and Martienssen, R. (2003) Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification. *PLoS Biol*, **1**, E67.
Lockhart, D.J. and Winzeler, E.A. (2000) Genomics, gene expression and DNA arrays. *Nature*, **405**, 827-836.
Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, **389**, 251-260.
Malik, S. and Roeder, R.G. (2005) Dynamic regulation of pol II transcription by the mammalian Mediator complex. *Trends Biochem Sci*, **30**, 256-263.
Mannervik, M., Nibu, Y., Zhang, H. and Levine, M. (1999) Transcriptional coregulators in development. *Science*, **284**, 606-609.
Marquez, J.A., Pascual-Ahuir, A., Proft, M. and Serrano, R. (1998) The Ssn6-Tup1 repressor complex of Saccharomyces cerevisiae is involved in the osmotic induction of HOG-dependent and -independent genes. *Embo J*, **17**, 2543-2553.
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**, 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. *Mol Cell Biol*, **21**, 6782-6795.
McEwan, I.J., Dahlman-Wright, K., Ford, J. and Wright, A.P. (1996) Functional interaction of the c-Myc transactivation domain with the TATA binding protein: evidence for an induced fit model of transactivation domain folding. *Biochemistry*, **35**, 9584-9593.
Miyasaka, H., Choudhury, B.K., Hou, E.W. and Li, S.S. (1993) Molecular cloning and expression of mouse and human cDNA encoding AES and ESG proteins with strong similarity to Drosophila enhancer of split groucho protein. *Eur J Biochem*, **216**, 343-352.
Mizuno, T., Nakazawa, N., Remgsamrarn, P., Kunoh, T., Oshima, Y. and Harashima, S. (1998) The Tup1-Ssn6 general repressor is involved in repression of IME1 encoding a transcriptional activator of meiosis in Saccharomyces cerevisiae. *Curr Genet*, **33**, 239-247.
Moore, R.C. and Purugganan, M.D. (2003) The early stages of duplicate gene evolution. *Proc Natl Acad Sci U S A*, **100**, 15682-15687.

Mukai, Y., Harashima, S. and Oshima, Y. (1991) AAR1/TUP1 protein, with a structure similar to that of the beta subunit of G proteins, is required for a1-alpha 2 and alpha 2 repression in cell type control of Saccharomyces cerevisiae. *Mol Cell Biol*, **11**, 3773-3779.
Mukai, Y., Matsuo, E., Roth, S.Y. and Harashima, S. (1999) Conservation of histone binding and transcriptional repressor functions in a Schizosaccharomyces pombe Tup1p homolog. *Mol Cell Biol*, **19**, 8461-8468.
Muller, H.P. and Schaffner, W. (1990) Transcriptional enhancers can act in trans. *Trends Genet*, **6**, 300-304.
Nehlin, J.O. and Ronne, H. (1990) Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. *Embo J*, **9**, 2891-2898.
Nelson, H.C. (1995) Structure and function of DNA-binding proteins. *Curr Opin Genet Dev*, **5**, 180-189.
Ng, H.H. and Bird, A. (2000) Histone deacetylases: silencers for hire. *Trends Biochem Sci*, **25**, 121-126.
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**, 35-44.
Ohta, T. (1989) Role of gene duplication in evolution. *Genome*, **31**, 304-310.
Papamichos-Chronakis, M., Conlan, R.S., Gounalaki, N., Copf, T. and Tzamarias, D. (2000) Hrs1/Med3 is a Cyc8-Tup1 corepressor target in the RNA polymerase II holoenzyme. *J Biol Chem*, **275**, 8397-8403.
Papamichos-Chronakis, M., Petrakis, T., Ktistaki, E., Topalidou, I. and Tzamarias, D. (2002) Cti6, a PHD domain protein, bridges the Cyc8-Tup1 corepressor and the SAGA coactivator to overcome repression at GAL1. *Mol Cell*, **9**, 1297-1305.
Pavletich, N.P. and Pabo, C.O. (1991) Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 A. *Science*, **252**, 809-817.
Pazin, M.J. and Kadonaga, J.T. (1997) SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein-DNA interactions? *Cell*, **88**, 737-740.
Pelletier, B., Beaudoin, J., Philpott, C.C. and Labbe, S. (2003) Fep1 represses expression of the fission yeast Schizosaccharomyces pombe siderophore-iron transport system. *Nucleic Acids Res*, **31**, 4332-4344.
Prelich, G. and Winston, F. (1993) Mutations that suppress the deletion of an upstream activating sequence in yeast: involvement of a protein kinase and histone H3 in repressing transcription in vivo. *Genetics*, **135**, 665-676.
Proft, M. and Serrano, R. (1999) Repressors and upstream repressing sequences of the stress-regulated ENA1 gene in Saccharomyces cerevisiae: bZIP protein Sko1p confers HOG-dependent osmotic regulation. *Mol Cell Biol*, **19**, 537-546.
Proft, M. and Struhl, K. (2002) Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. *Mol Cell*, **9**, 1307-1317.
Ptashne, M. (1988) How eukaryotic transcriptional activators work. *Nature*, **335**, 683-689.
Radhakrishnan, I., Perez-Alvarado, G.C., Parker, D., Dyson, H.J., Montminy, M.R. and Wright, P.E. (1997) Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: a model for activator:coactivator interactions. *Cell*, **91**, 741-752.
Rhodes, D. and Klug, A. (1993) Zinc fingers. *Sci Am*, **268**, 56-59, 62-55.
Rice, J.C. and Allis, C.D. (2001) Histone methylation versus histone acetylation: new insights into epigenetic regulation. *Curr Opin Cell Biol*, **13**, 263-273.
Riggs, M.G., Whittaker, R.G., Neumann, J.R. and Ingram, V.M. (1977) n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature*, **268**, 462-464.
Robyr, D., Suka, Y., Xenarios, I., Kurdistani, S.K., Wang, A., Suka, N. and Grunstein, M. (2002) Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell*, **109**, 437-446.
Roeder, R.G. (1996) The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem Sci*, **21**, 327-335.
Ronne, H. (1995) Glucose repression in fungi. *Trends Genet*, **11**, 12-17.

Roth, S.Y., Shimizu, M., Johnson, L., Grunstein, M. and Simpson, R.T. (1992) Stable nucleosome positioning and complete repression by the yeast alpha 2 repressor are disrupted by amino-terminal mutations in histone H4. *Genes Dev*, **6**, 411-425.

Rothstein, R.J. and Sherman, F. (1980a) Dependence on mating type for the overproduction of iso-2-cytochrome c in the yeast mutant CYC7-H2. *Genetics*, **94**, 891-898.

Rothstein, R.J. and Sherman, F. (1980b) Genes affecting the expression of cytochrome c in yeast: genetic mapping and genetic interactions. *Genetics*, **94**, 871-889.

Rundlett, S.E., Carmen, A.A., Kobayashi, R., Bavykin, S., Turner, B.M. and Grunstein, M. (1996) HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc Natl Acad Sci U S A*, **93**, 14503-14508.

Saito, J., Kon, T., Nagasaki, A., Adachi, H. and Sutoh, K. (1998) Dictyostelium TRFA homologous to yeast Ssn6 is required for normal growth and early development. *J Biol Chem*, **273**, 24654-24659.

Sakai, A., Shimizu, Y. and Hishinuma, F. (1988) Isolation and characterization of mutants which show an oversecretion phenotype in Saccharomyces cerevisiae. *Genetics*, **119**, 499-506.

Sassone-Corsi, P. (1995) Transcription factors responsive to cAMP. *Annu Rev Cell Dev Biol*, **11**, 355-377.

Scannell, D.R., Byrne, K.P., Gordon, J.L., Wong, S. and Wolfe, K.H. (2006) Multiple rounds of speciation associated with reciprocal gene loss in polyploid yeasts. *Nature*, **440**, 341-345.

Schamhart, D.H., Ten Berge, A.M. and Van De Poll, K.W. (1975) Isolation of a catabolite repression mutant of yeast as a revertant of a strain that is maltose negative in the respiratory-deficient state. *J Bacteriol*, **121**, 747-752.

Schramke, V. and Allshire, R. (2003) Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science*, **301**, 1069-1074.

Schuller, H.J. (2003) Transcriptional control of nonfermentative metabolism in the yeast Saccharomyces cerevisiae. *Curr Genet*, **43**, 139-160.

Schultz, J. and Carlson, M. (1987) Molecular analysis of SSN6, a gene functionally related to the SNF1 protein kinase of Saccharomyces cerevisiae. *Mol Cell Biol*, **7**, 3637-3645.

Schultz, J., Marshall-Carlson, L. and Carlson, M. (1990) The N-terminal TPR region is the functional domain of SSN6, a nuclear phosphoprotein of Saccharomyces cerevisiae. *Mol Cell Biol*, **10**, 4744-4756.

Shankaranarayana, G.D., Motamedi, M.R., Moazed, D. and Grewal, S.I. (2003) Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. *Curr Biol*, **13**, 1240-1246.

Sherwood, P.W., Tsang, S.V. and Osley, M.A. (1993) Characterization of HIR1 and HIR2, two genes required for regulation of histone gene transcription in Saccharomyces cerevisiae. *Mol Cell Biol*, **13**, 28-38.

Shimizu, M., Roth, S.Y., Szent-Gyorgyi, C. and Simpson, R.T. (1991) Nucleosomes are positioned with base pair precision adjacent to the alpha 2 operator in Saccharomyces cerevisiae. *Embo J*, **10**, 3033-3041.

Sipiczki, M. (2000) Where does fission yeast sit on the tree of life? *Genome Biol*, **1**, REVIEWS1011.

Smith, E.R., Eisen, A., Gu, W., Sattah, M., Pannuti, A., Zhou, J., Cook, R.G., Lucchesi, J.C. and Allis, C.D. (1998) ESA1 is a histone acetyltransferase that is essential for growth in yeast. *Proc Natl Acad Sci U S A*, **95**, 3561-3565.

Song, H., Hasson, P., Paroush, Z. and Courey, A.J. (2004) Groucho oligomerization is required for repression in vivo. *Mol Cell Biol*, **24**, 4341-4350.

Sprague, E.R., Redd, M.J., Johnson, A.D. and Wolberger, C. (2000) Structure of the C-terminal domain of Tup1, a corepressor of transcription in yeast. *Embo J*, **19**, 3016-3027.

Sprague, G.F., Jr. (1998) Control of MAP kinase signaling specificity or how not to go HOG wild. *Genes Dev*, **12**, 2817-2820.

Stifani, S., Blaumueller, C.M., Redhead, N.J., Hill, R.E. and Artavanis-Tsakonas, S. (1992) Human homologs of a Drosophila Enhancer of split gene product define a novel family of nuclear proteins. *Nat Genet*, **2**, 343.
Strathern, J.N., Klar, A.J., Hicks, J.B., Abraham, J.A., Ivy, J.M., Nasmyth, K.A. and McGill, C. (1982) Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the MAT locus. *Cell*, **31**, 183-192.
Svejstrup, J.Q., Vichi, P. and Egly, J.M. (1996) The multiple roles of transcription/repair factor TFIIH. *Trends Biochem Sci*, **21**, 346-350.
Takeuchi, T., Yamazaki, Y., Katoh-Fukui, Y., Tsuchiya, R., Kondo, S., Motoyama, J. and Higashinakagawa, T. (1995) Gene trap capture of a novel mouse gene, jumonji, required for neural tube formation. *Genes Dev*, **9**, 1211-1222.
Talbert, P.B. and Henikoff, S. (2006) Spreading of silent chromatin: inaction at a distance. *Nat Rev Genet*, **7**, 793-803.
Taunton, J., Hassig, C.A. and Schreiber, S.L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science*, **272**, 408-411.
Teunissen, A.W., van den Berg, J.A. and Steensma, H.Y. (1995) Transcriptional regulation of flocculation genes in Saccharomyces cerevisiae. *Yeast*, **11**, 435-446.
Thompson, C.M., Koleske, A.J., Chao, D.M. and Young, R.A. (1993) A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. *Cell*, **73**, 1361-1375.
Treitel, M.A. and Carlson, M. (1995) Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein. *Proc Natl Acad Sci U S A*, **92**, 3132-3136.
Trumbly, R.J. (1986) Isolation of Saccharomyces cerevisiae mutants constitutive for invertase synthesis. *J Bacteriol*, **166**, 1123-1127.
Trumbly, R.J. (1988) Cloning and characterization of the CYC8 gene mediating glucose repression in yeast. *Gene*, **73**, 97-111.
Tzamarias, D. and Struhl, K. (1994) Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. *Nature*, **369**, 758-761.
Tzamarias, D. and Struhl, K. (1995) Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. *Genes Dev*, **9**, 821-831.
Uesugi, M., Nyanguile, O., Lu, H., Levine, A.J. and Verdine, G.L. (1997) Induced alpha helix in the VP16 activation domain upon binding to a human TAF. *Science*, **277**, 1310-1313.
Wahi, M. and Johnson, A.D. (1995) Identification of genes required for alpha 2 repression in Saccharomyces cerevisiae. *Genetics*, **140**, 79-90.
van der Voorn, L. and Ploegh, H.L. (1992) The WD-40 repeat. *FEBS Lett*, **307**, 131-134.
Wang, A., Kurdistani, S.K. and Grunstein, M. (2002) Requirement of Hos2 histone deacetylase for gene activity in yeast. *Science*, **298**, 1412-1414.
Varanasi, U.S., Klis, M., Mikesell, P.B. and Trumbly, R.J. (1996) The Cyc8 (Ssn6)-Tup1 corepressor complex is composed of one Cyc8 and four Tup1 subunits. *Mol Cell Biol*, **16**, 6707-6714.
Watson, A.D., Edmondson, D.G., Bone, J.R., Mukai, Y., Yu, Y., Du, W., Stillman, D.J. and Roth, S.Y. (2000) Ssn6-Tup1 interacts with class I histone deacetylases required for repression. *Genes Dev*, **14**, 2737-2744.
Weil, P.A., Luse, D.S., Segall, J. and Roeder, R.G. (1979) Selective and accurate initiation of transcription at the Ad2 major late promotor in a soluble system dependent on purified RNA polymerase II and DNA. *Cell*, **18**, 469-484.
Werner, M.H. and Burley, S.K. (1997) Architectural transcription factors: proteins that remodel DNA. *Cell*, **88**, 733-736.
Wickner, R.B. (1974) Mutants of Saccharomyces cerevisiae that incorporate deoxythymidine-5'-monophosphate into deoxyribonucleic acid in vivo. *J Bacteriol*, **117**, 252-260.
Vidali, G., Boffa, L.C. and Allfrey, V.G. (1972) Properties of an acidic histone-binding protein fraction from cell nuclei. Selective precipitation and deacetylation of histones F2A1 and F3. *J Biol Chem*, **247**, 7365-7373.
Vignali, M., Hassan, A.H., Neely, K.E. and Workman, J.L. (2000) ATP-dependent chromatin-remodeling complexes. *Mol Cell Biol*, **20**, 1899-1910.

Williams, F.E. and Trumbly, R.J. (1990) Characterization of TUP1, a mediator of glucose repression in Saccharomyces cerevisiae. *Mol Cell Biol*, **10**, 6500-6511.
Williams, F.E., Varanasi, U. and Trumbly, R.J. (1991) The CYC8 and TUP1 proteins involved in glucose repression in Saccharomyces cerevisiae are associated in a protein complex. *Mol Cell Biol*, **11**, 3307-3316.
Wiren, M., Silverstein, R.A., Sinha, I., Walfridsson, J., Lee, H.M., Laurenson, P., Pillus, L., Robyr, D., Grunstein, M. and Ekwall, K. (2005) Genomewide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. *Embo J*, **24**, 2906-2918.
Vogelauer, M., Wu, J., Suka, N. and Grunstein, M. (2000) Global histone acetylation and deacetylation in yeast. *Nature*, **408**, 495-498.
Wolffe, A.P. (1994a) Nucleosome positioning and modification: chromatin structures that potentiate transcription. *Trends Biochem Sci*, **19**, 240-244.
Wolffe, A.P. (1994b) Transcription: in tune with the histones. *Cell*, **77**, 13-16.
Wolffe, A.P. (1997) Transcriptional control. Sinful repression. *Nature*, **387**, 16-17.
Wolffe, A.P. and Pruss, D. (1996) Targeting chromatin disruption: Transcription regulators that acetylate histones. *Cell*, **84**, 817-819.
Wood, V., Gwilliam, R., Rajandream, M.A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., Basham, D., Bowman, S., Brooks, K., Brown, D., Brown, S., Chillingworth, T., Churcher, C., Collins, M., Connor, R., Cronin, A., Davis, P., Feltwell, T., Fraser, A., Gentles, S., Goble, A., Hamlin, N., Harris, D., Hidalgo, J., Hodgson, G., Holroyd, S., Hornsby, T., Howarth, S., Huckle, E.J., Hunt, S., Jagels, K., James, K., Jones, L., Jones, M., Leather, S., McDonald, S., McLean, J., Mooney, P., Moule, S., Mungall, K., Murphy, L., Niblett, D., Odell, C., Oliver, K., O'Neil, S., Pearson, D., Quail, M.A., Rabbinowitsch, E., Rutherford, K., Rutter, S., Saunders, D., Seeger, K., Sharp, S., Skelton, J., Simmonds, M., Squares, R., Squares, S., Stevens, K., Taylor, K., Taylor, R.G., Tivey, A., Walsh, S., Warren, T., Whitehead, S., Woodward, J., Volckaert, G., Aert, R., Robben, J., Grymonprez, B., Weltjens, I., Vanstreels, E., Rieger, M., Schafer, M., Muller-Auer, S., Gabel, C., Fuchs, M., Dusterhoft, A., Fritzc, C., Holzer, E., Moestl, D., Hilbert, H., Borzym, K., Langer, I., Beck, A., Lehrach, H., Reinhardt, R., Pohl, T.M., Eger, P., Zimmermann, W., Wedler, H., Wambutt, R., Purnelle, B., Goffeau, A., Cadieu, E., Dreano, S., Gloux, S., Lelaure, V., Mottier, S., Galibert, F., Aves, S.J., Xiang, Z., Hunt, C., Moore, K., Hurst, S.M., Lucas, M., Rochet, M., Gaillardin, C., Tallada, V.A., Garzon, A., Thode, G., Daga, R.R., Cruzado, L., Jimenez, J., Sanchez, M., del Rey, F., Benito, J., Dominguez, A., Revuelta, J.L., Moreno, S., Armstrong, J., Forsburg, S.L., Cerutti, L., Lowe, T., McCombie, W.R., Paulsen, I., Potashkin, J., Shpakovski, G.V., Ussery, D., Barrell, B.G. and Nurse, P. (2002) The genome sequence of Schizosaccharomyces pombe. *Nature*, **415**, 871-880.
Wu, J. and Grunstein, M. (2000) 25 years after the nucleosome model: chromatin modifications. *Trends Biochem Sci*, **25**, 619-623.
Wu, J., Suka, N., Carlson, M. and Grunstein, M. (2001) TUP1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. *Mol Cell*, **7**, 117-126.
Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J. and Speed, T.P. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res*, **30**, e15.
Yoshida, M., Kijima, M., Akita, M. and Beppu, T. (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J Biol Chem*, **265**, 17174-17179.
Zaman, Z., Ansari, A.Z., Koh, S.S., Young, R. and Ptashne, M. (2001) Interaction of a transcriptional repressor with the RNA polymerase II holoenzyme plays a crucial role in repression. *Proc Natl Acad Sci U S A*, **98**, 2550-2554.
Zaman, Z., Heid, C. and Ptashne, M. (2002) Telomere looping permits repression "at a distance" in yeast. *Curr Biol*, **12**, 930-933.
Zawel, L., Kumar, K.P. and Reinberg, D. (1995) Recycling of the general transcription factors during RNA polymerase II transcription. *Genes Dev*, **9**, 1479-1490.

Zhang, H.S., Gavin, M., Dahiya, A., Postigo, A.A., Ma, D., Luo, R.X., Harbour, J.W. and Dean, D.C. (2000) Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. *Cell*, **101**, 79-89.

Zhang, M., Rosenblum-Vos, L.S., Lowry, C.V., Boakye, K.A. and Zitomer, R.S. (1991) A yeast protein with homology to the beta-subunit of G proteins is involved in control of heme-regulated and catabolite-repressed genes. *Gene*, **97**, 153-161.

Zhang, Z., Varanasi, U. and Trumbly, R.J. (2002) Functional dissection of the global repressor Tup1 in yeast: dominant role of the C-terminal repression domain. *Genetics*, **161**, 957-969.

Zhou, Z. and Elledge, S.J. (1992) Isolation of crt mutants constitutive for transcription of the DNA damage inducible gene RNR3 in Saccharomyces cerevisiae. *Genetics*, **131**, 851-866.

Zhu, X., Wiren, M., Sinha, I., Rasmussen, N.N., Linder, T., Holmberg, S., Ekwall, K. and Gustafsson, C.M. (2006) Genome-wide occupancy profile of mediator and the Srb8-11 module reveals interactions with coding regions. *Mol Cell*, **22**, 169-178.

Zitomer, R.S. and Lowry, C.V. (1992) Regulation of gene expression by oxygen in Saccharomyces cerevisiae. *Microbiol Rev*, **56**, 1-11.