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Analyses of Chd1p, a chromatin-modifying factor

by

Hien G. Tran

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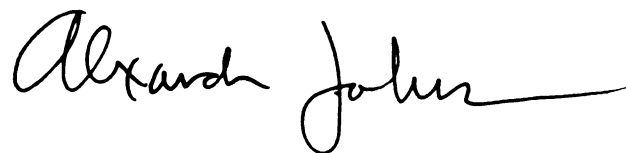Fax: +44 207-269-3482
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Abstract

Analyses of Chd1p, a chromatin modifying factor

Hien G. Tran

Chromatin structure is an integral component of transcriptional control. Nucleosomes inhibit the ability of transcription factors to bind to their regulatory sequences and antagonize initiation by RNA polymerase. Chromatin structure is regulated by chromatin modifying factors that modify histones or alter histone-DNA interactions to either facilitate or impair transcription. In this thesis, I have characterized the function of the chromatin remodeling factor Chd1p in budding yeast. CHD proteins are well conserved, with members found from yeast to humans. However, their cellular roles are not known. To uncovered the function of Chd1p, I implemented genetic, genomic, and biochemical approaches. Through a synthetic lethal screen, *SWI/SNF* genes were determined to have genetic interactions with *CHD1*. These results implicated a role for Chd1p in chromatin structure and transcription. To examine the role of Chd1p in chromatin structure, Chd1p was purified from yeast and assayed for nucleosome remodeling activities. Purified Chd1p was shown to alter the structure of mononuclesomes in an ATP-dependent manner. Genome-wide expression profiling showed that the expression of 2-4% of yeast genes are affected in the absence of Chd1p. To further access the role of Chd1p in transcription, chromatin immunoprecipitation/microarray analyses was carried out to identify genes directly

regulated by Chd1p. Genes encoding ribosomal proteins and translation components were determined to be preferentially precipitated by Chd1p. In addition, Chd1p was selectively localized to the coding regions of these genes and was enriched along the entire open reading frame. These findings indicate that Chd1p acts in transcription elongation. To better understand how Chd1p activity and recruitment may be regulated, an affinity purification of proteins that interact with the chromo domain of Chd1p was performed. Three proteins with links to transcription and chromatin structure have been identified. Genetic and genomic analyses of one Chd1p-interacting protein, Crp1p, has been carried out. Collectively, the work presented in this thesis has determined that Chd1p functions of as a chromatin modifying factor with a role in transcription elongation.

Alexander John

Table of Contents

List of Tables

List of Figures

The proper control of gene expression is critical for all biological processes. Cellular growth, differentiation, and metabolism all depend on the orchestrated transcription of genes. Consequently, much of the past and current biological research has focused on the understanding of transcriptional regulatory mechansims. In the past decade, early studies concentrated on the constitution and activity of the transcription apparatus. In addition, attention was placed on the molecular events leading to gene activation and repression mediated by transcription factors and co-regulators. Recently, there has been a shift towards elucidating the role of chromatin and chromatin modifying activities in the control of gene expression. In this introduction, we will review the current understanding of eukaryotic (class II) transcription, with an emphasis on factors that regulate chromatin structure.

THE TEMPLATE

Promoter and regulatory sequence elements

Sequence features located at the promoter region of genes regulate transcription by the RNA polymerase II (RNA pol II) machinery. This region contains the core promoter element-the transcription initiation site and the TATA box-and additional regulatory sequences (Lee and Young, 2000) Almost all protein-coding genes contain a TATA box found 25-120 base pairs upstream of the start site. The TATA box is bound by the TATA-binding protein (TBP), a component of the general transcription apparatus, and is required for transcription of these genes. Upstream of the TATA box are sequences recognized and bound by transcriptional regulators. These include upstream activating sequences (UAS) and enhancers that are bound by activators. The UASs are typically found near the start site while enhancers can function up to 85 kilobases away

2

and act in an orientation independent manner (Blackwood and Kadonaga, 1998). Transcriptional repressors bind to Upstream Repressing Sequences (URSs), which are found proximal to the promoter, and to silencer elements, which can function in a position and orientation independent fashion (Guarente, 1999; Hanna-Rose and Hansen, 1996). Together, these regulatory sequences play a fundamental role in the control of gene expression.

Chromatin

In eukaryotic cells, genes and their regulatory sequences are not readily accessible as a naked DNA template. Rather, DNA is packaged into chromatin, a nucleo-protein structure that facilitates the storage and transmission of genetic material (Kornberg and Lorch, 1999). In its simplest form, chromatin can be depicted as a linear array consisting of a repeating unit, the nucleosome, separated by linker DNA. From this linear array, chromatin can be organized into a range of higher order structures generated by inter-nucleosomal interactions. It is not clear how the more convoluted chromatin structures are produced. However, the molecular assembly of the nucleosome has been well characterized.

The nucleosome core particle is composed of 146 base pairs of DNA wrapped 1 3/4 left-handed superhelical turns around a histone octomer (Kornberg and Lorch, 1999). The pattern of DNA-histone interactions in this complex consists of minor groove contacts between DNA and the histone octomer at every 10 base pairs (Luger et al., 1997) The octomer is composed of a central histone H3/H4 tetramer flanked by two H2A/H2B dimers. Histones all have a similar polypeptide structure (the histone fold) consisting of a long central α helix and adjoining shorter helices and loops that interact

3

with DNA (Arents and Moudrianakis, 1995; Kornberg and Lorch, 1999). At the amino

terminus of histones are 15-30 unstructured amino acid residues known as the histone

tails. These unstructured tails protrude from the nucleosome core particle (Luger et al.,

1997) and are substrates for various histone modifying enzymes (see below).

The packaging of DNA into chromatin creates a physical barrier that hinders the

ability of RNA polymerases to recognize promoter elements. *In vitro* studies have shown

that a chromatin template prevents both bacterial and eukaryotic RNA polymerases from

initiating transcription (Knezetic and Luse, 1986; Lorch et al., 1987). When histone

synthesis is disrupted in cells, genes that are normally inactive are subsequently

expressed due to the loss of nucleosomes (Han and Grunstein, 1988). In addition, the

promoter of active genes is often found to be nuclease-sensitive, an indication that

nucleosomes have been displaced from the region (Kornberg and Lorch, 1999). These

findings reveal that chromatin can present a significant obstacle for RNA pol II

transcription. In later sections, the cellular mechanisms that are used to overcome

chromatin-mediated inhibition of transcription will be described, along with how

chromatin structure has been utilized as a mode of transcriptional control.

THE TRANSCRIPTION APPARATUS

RNA polymerase II

The core RNA polymerase II from yeast and humans have been purified and

shown to be highly similar (Lee and Young, 2000). Each polymerase is composed of 12

conserved proteins, with most of the human subunits capable of functionally substituting

for the yeast counterparts. The largest component of RNA pol II contains a carboxy-

terminal domain (CTD) consisting of multiple heptapeptide repeats (Tyr-Ser-Pro-Thr-

4

Ser-Pro-Ser) (Corden, 1990). The CTD and its phosporylation state have a central regulatory role during transcription initiation, elongation, and activation (Dahmus, 1996) (Lee and Young, 2000). A hypophosphorylation CTD is correlated with an initiating Pol II while an elongating complex contains a hyperphosphorylated CTD. In addition, factors that mediate transcriptional activation are associated with the CTD (see below).

General transcription factors

Purified core complex can synthesize RNA in a DNA-dependent manner but is unable to specifically recognize promoter sequences. The addition of general transcription factors (GTFs)-TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH-enables promoter specific binding of RNA pol II (Conaway and Conaway, 1997; Hampsey, 1998). *In vitro* studies with RNA pol II and the GTFs have produced a working model for transcription initiation (Lee and Young, 2000). In the first step, the transcription apparatus binds to the promoter and unwinds 12-15 base pairs of promoter DNA (open complex formation). Next, RNA pol II enters the abortive initiation phase, in which the polymerase repeatedly initiates transcription and releases short RNA molecules consisting of a few phosphodiester bonds. The polymerase eventually shifts away from abortive initiation and generates longer RNA molecules (promoter clearance). At many promoters, polymerase stalls 25-30 base pairs away from the start site. At this point, the polymerase makes a transition to a fully elongating form and extends away from the promoter (promoter escape).

For many of the GTFs, their specific roles in transcription initiation have been characterized (Lee and Young, 2000). The TFIID complex, which includes TBP and TBP-associated factors (TAFs), recognizes and binds to promoter sequences. The TBP

subunit binds to the TATA-box and the TAFs interact with neighboring sequences to provide promoter selectivity. TFIIA stabilizes TBP-DNA interactions and TFIIB is involved in the selection of the transcription start site. The TFIIH complex has multiple activities and roles in transcription initiation. A helicase subunit is involved in promoter opening and escape while a cyclin/kinase pair can phosphorylate the CTD and may facilitate elongation. TFIIE has a role in melting the promoter and in stimulating TFIIH activities. TFIIF has been shown to suppress abortive transcription and thereby, stimulate productive transcription.

Mediator

In a reconstituted system, RNA polymerase II and the GTFs are capable of basal, but not activated transcription. This observation led to the discovery of the Mediator, a protein complex that confers the ability of the transcription apparatus to respond to activators (Flanagan et al., 1991). The yeast Mediator is composed of 20 subunits, many of which were previously identified as transcriptional regulators (Myers et al., 1998). Corresponding complexes have also been purified from mammals, and the composition of these complexes is similar to yeast Mediator (Ito et al., 1999).

The Mediator is believed to have a general role in eukaryotic transcription by acting to transduce signals from transcriptional activators and other regulatory factors to RNA polymerase and components of the transcription apparatus (Kornberg, 1999) (Lee and Young, 2000). Mediator has been shown to co-purify with RNA polymerase and to bind to the CTD (Kim et al., 1994; Koleske and Young, 1994; Myers et al., 1998; Thompson et al., 1993). Many Mediator subunits are essential and affect the expression of almost all protein-coding genes *in vivo* (Holstege et al., 1998; Thompson et al., 1993;

Thompson and Young, 1995). Some subunits are involved in activation and make direct contacts with transcriptional activators (Koh et al., 1998; Myers et al., 1999). Other Mediator members are required for the complete repression of certain genes (Carlson, 1997). The Mediator is thought to be dedicated to transcription initiation, since it is not associated with an elongating polymerase (Svejstrup et al., 1997).

Holoenzyme

A form of RNA polymerase has been purified from yeast that is competent for activated transcription *in vitro* (Kim et al., 1994; Koleske and Young, 1994). This complex, the holoenzyme, contains RNA polymerase, a subset of GTFs, and the Mediator. This finding was significant because all of the components required for activated transcription were pre-assembled, suggesting that transcription initiation *in vivo* does not require a stepwise addition of multiple transcription-related complexes. In fact, it has been proposed that the holoenzyme may be recruited to most promoters in yeast [Koleske, 1995 #1580.

REGULATORY FACTORS AND MECHANISMS

Transcription initiation can be separated into two stages: relief of chromatin inhibition and formation of a functional transcription apparatus on promoters (Kornberg, 1999). Transcriptional regulators (activators and repressors) have roles in both of these processes. Some recruit chromatin modifying complexes to promoters while others interact with components of the transcription machinery.

Activators

A typical activator contains two distinguishable domains: a sequence-specific DNA binding module and an activation domain that enhances the activity of the

transcription machinery (directly, or indirectly) (Ptashne and Gann, 1997; Triezenberg, 1995). An activator usually controls the expression of multiple genes, enabling coordinate regulation of genes in common processes or pathways. Different activators can stimulate the expression of certain genes, allowing for combinatorial control of these genes (Lee and Young, 2000).

One class of activators potentiates transcription by recruiting chromatin modifying complexes to the promoter. Positioned nucleosomes at the promoter can significantly inhibit transcription, presumably by preventing the polymerase complex from binding to the core promoter elements (Kornberg and Lorch, 1999). To alleviate this repression, histone modifying enzymes and nucleosome altering activities are brought to promoter regions by transcription factors (Fry and Peterson, 2001; Lee and Young, 2000)(see below). At some genes, activator recruitment of both types of chromatin modifying factors is required for proper expression (Agalioti et al., 2000; Cosma et al., 1999). These remodeling activities are presumed to either disrupt higher order chromatin structures or to displace, or shift, nucleosomes masking the TATA box (Fry and Peterson, 2001; Kornberg, 1999).

Subsequent to chromatin reconfiguration at the promoter, it is thought that activators can also stimulate transcription by recruiting the transcription apparatus (Kornberg, 1999). In vitro studies have detected interactions between activation domains and various members of the transcription machinery (Barberis and Gaudreau, 1998; Burley and Roeder, 1996; Lee and Young, 2000). Additionally, fusion proteins in which the activation domains of activators are replaced by components of the transcription apparatus are able to substitute for the intact activators *in vivo* (Barberis et al., 1995;

8

Chatterjee and K., 1995; Farrell et al., 1996). This result suggests that transcription

factors can function by binding to UAS sequences and sequestering the transcription

machinery to promoters. Early models of gene induction depicted a stepwise addition of

general transcription factors at promoters-a process facilitated by activators-leading to the

assembly of a fully competent transcription complex (Buratowski, 1994). However, with

the identification of the Mediator and the holoenzyme, it is now thought that activation is

propagated by activator-Mediator interactions and can occur as a single recruitment step

(Kornberg, 1999; Lee and Young, 2000).

Repressors

Repressors employ varying mechanisms to inhibit transcription initiation. Like

activators, they can interact with chromatin modifying activities and with components of

the transcription machinery. In addition, they can antagonize transcription by interacting

with activators, competing for binding sites, or by generating repressive structural

domains.

Some repressors function through their interactions with the TATA-binding

protein. Mot1p impairs transcription by displacing TBP from DNA in an ATP dependent

manner (Auble et al., 1997). The NC2 repressor binds to TBP on promoter DNA and

prevents the formation of a transcription initiation complex (Gadbois et al., 1997). In

addition to TBP, interactions between repressors and other constituents of the

transcription assembly have been detected. Tup1p, a member of the SSN6/TUP1

corepressor complex, has been shown to have an association with Srb7p and Srb10p,

components of the holoenzyme (Gromoller and Lehming, 2000; Zaman et al., 2001). It

9

has been proposed that one mode of SSN6/TUP1 repression is through its interaction with the transcription apparatus (Smith and Johnson, 2000; Zaman et al., 2001).

Another class of repressors functions by interfering with activators, either by direct interactions or through competition for activator binding sites (Lee and Young, 2000). The Hsp90p protein binds to Hsf1p and prevents the assembly of Hsf1p trimers, the activating complex that induces the expression of heat shock genes (Zou et al., 1998). The Gal4p activator is disabled when Gal80p is bound to its activation domain (Leuther and Johnston, 1992; Ma and Ptashne, 1987). The Acr1p repressor has overlapping binding sites with the ATF/CREB activator and can inhibit transcription by binding to those sites (Vincent and Struhl, 1992).

Some negative regulators impede transcription by restructuring chromatin organization. Repression by Ume6p is mediated by the recruitment of both a histone deacetylase and a nucleosome remodeling factor to the promoters of sporulation genes (Goldmark et al., 2000; Kadosh and Struhl, 1997). The SIR proteins are involved in silencing at the mating type loci and telomeres and are thought to function by forming repressive, nucleo-protein structures (Moazed, 2001). *Drosophila* and fission yeast, HP1 proteins inhibit transcription by generating heterochromatin or heterochromatin-like domains (Jenuwein and Allis, 2001).

CHROMATIN MODIFIERS

It is becoming apparent that chromatin modifying activities play an integral role in transcription control. Proteins that modulate chromatin structure exert positive and negative effects on transcription, both globally and at specific genes. Two main types of chromatin regulators have been characterized: covalent modifier of histones and

10

nucleosome remodeling complexes. In many instances, the concerted actions of both activities are required for proper expression of genes.

The Histone code

Histones can be modified by several different mechanisms, including acetylation, methylation, phosporylation, and ubiquination (Jenuwein and Allis, 2001; Lee and Young, 2000). The lysine and serine residues on histone tails are the primary substrates for these covalent alterations. It has been proposed that different combinations of amino acid modifications serve as a "histone code" (Jenuwein and Allis, 2001). That is, the a specific pattern of histone modification dictates the structural configuration of chromatin and thereby, determines the transcriptional state of a particular chromosomal regions. This model predicts that histone modifying enzymes should have prominent roles in transcription. In addition, the model suggests that acetylated or methylated residues can function as specific markers for the binding of regulatory factors. The findings of recent investigations described below provide support for the histone code hypothesis.

Histone acetyltransferases

Early studies on chromosome and chromatin structure showed that there was a correlation between histone acetylation and transcriptional activity (Kornberg and Lorch, 1999). In isolated nuclei and partially purified chromatin, transcriptional activity was associated with multiply acetylated residues on histone tails. In contrast, at transcriptionally dormant heterochromatic regions, there was an absence of acetylation of histone tail residues important for transcription. Furthermore, transcription of inducible genes in yeast was abrogated when lysine residues in histone H4 were substituted with arginine (which removes acetylation sites).

11

A key discovery finally confirmed the functional link between acetylation and transcription; it was determined that the yeast Gcn5p transcriptional coactivator had similarity to a *Tetrahymena* histone acetyltransferase (HAT) (Brownell et al., 1996). Further analyses revealed that the chromatin around the promoter of Gcn5p regulated genes had an increase level of acetylated histones upon induction of these genes (Kuo et al., 1998). Moreover, mutations in the catalytic domain of Gcn5p abolished Gcn5p-mediated activation and the observed acetylation effects at the promoter (Kuo et al., 1998).

Additional HATs have been identified in yeast and mammals, many of which are transcriptional coactivators and members of large protein complexes (Lee and Young, 2000). Among the yeast HATs are Esa1p, an essential protein involved in the transcription of genes encoding ribosomal proteins, and Elp3p, a protein associated with the elongating RNA polymerase II (Lee and Young, 2000; Reid et al., 2000; Wittschieben et al., 1999). In mammals, the p300/CBP coactivator-a regulator of multiple cellular processes-has HAT activity that is closely associated with its role in transcription (Ogryzko et al., 1996). A general transcription factor subunit, $TAF_{II}250$, has also been shown to possess HAT capabilities (Mizzen et al., 1996). The demonstration that a variety of transcriptional regulators are HATs established the role of histone acetylation as a general mechanism of transcription control.

Though it is assumed that HAT activity stimulates transcription by perturbing chromatin, the structural effects of histone acetylation on the nucleosome appears to be minimal. The substrates of the HATs are the lysine residues in amino-terminal tails of histones. These tails project away from the core of the nucleosome particle and do not

12

participate in histone-histone interactions (Luger et al., 1997). Therefore, it is unlikely that acetylation of the histone tails dramatically compromises the integrity or organization of the nucleosome (Fry and Peterson, 2001; Kornberg and Lorch, 1999). In fact, studies have shown that hyperacetylated tails do not disrupt nucleosome assembly or significantly effect the salt stability or hydrodynamic properties of the core particle (Hansen et al., 1998).

Rather than affecting intra-nucleosomal interactions, the acetylation of histones may alter nucleosome-nucleosome contacts, leading to the disassembly or restructuring of higher order chromatin configurations (Fry and Peterson, 2001; Kornberg and Lorch, 1999). The tail domain has been observed to promote the self-association of oligonucleosome arrays *in vitro* (Schwarz et al., 1996). Furthermore, the acetylation level of tail residues has been shown to directly effect the folding of nucleosome arrays into more complex arrangements (Tse et al., 1998). Increasing the acetylation state of histones diminishes intermolecular and intramolecular interactions of nucleosomal arrays and abolishes the formation of chromatin fibers *in vitro*. Crystallography studies of the nucleosome support the idea that histone tails are involved inter-nucleosomal interactions, as a histone H4 tail from one nucleosome is seen to make contact with a H2A-H2B dimer on an adjacent nucleosome (Luger et al., 1997).

In addition to their likely role in disrupting inter-nucleosomal interactions, HATs may function to modulate the activity of transcriptional regulators and to recruit coactivators to promoters. HATs have been demonstrated to acetylate non-histone substrates, including the transcription factor p53 (Gu and Roeder, 1997) and the general transcription factors TFIIE and TFIIF (Imhof et al., 1997). Two observations suggest that

13

the p53 acetylation is physiologically relevant: acetylated p53 has an enhanced DNA binding activity, and upon stimulation of function, p53 displays increased acetylation (Liu et al., 1999; Sakaguchi et al., 1998). Histone acetylation may also serve to target nucleosome remodeling complexes to promoters. *In vitro* studies have demonstrated that HAT activity stabilizes the binding of the SWI/SNF remodeling complex to the promoter (Hassan et al., 2001). Analysis of the enhanceosome assembly at the *IFN-β* locus has produced similar results, showing that hGCN5 activity facilitates hSWI/SNF recruitment to the promoter (Agalioti et al., 2000). The stable association of SWI/SNF at the promoter may be attributed to a direct association of the complex with acetylated histones. A component of the complex (Swi2) contains a bromodomain, a protein motif that has been shown to bind to acetylated histone tails (Dhalluin et al., 1999; Jacobson et al., 2000).

Histone acetyltransferases can act over large genomic regions or at specific genes. In vertebrates, transcriptionally active genes often reside in large chromosomal domains (up to 100 kilobases) characterized by elevated acetylation of histone tails and increased DNase I sensitivity (Hebbes et al., 1994; Kornberg and Lorch, 1999). The increased acetylation state encompasses both transcribed and intergenic regions, indicative of a broadly acting HAT activity. HATs also exhibit more discrete, localize actions via interactions with DNA binding activators. Recruitment of p300/CBP to the *IFN-β* locus by the enhanceosome leads to an increased acetylation of 2-3 nucleosomes proximal to the promoter (Parekh and Maniatis, 1999). It is unclear if the local acetylation is additive to, or occurs in place of the domain-wide acetylation of histones (Kornberg and Lorch, 1999).

Histone deacetylases

Since acetyltransferases are generally positive acting transcriptional regulators, proteins that reverse HAT activities should have an inhibitory effect on gene expression. Indeed, when the first histone deacetylase (HDAC) was purified, it was found to have sequence similarity to yeast Rpd3p, a negative regulator of transcription (Taunton et al., 1996). Rpd3p has subsequently been shown to possess deacetylase activity and deletion of the *RPD3* gene results in hyperacetylation of histone H3 and H4 tails (Rundlett et al., 1996). Additional histone deacetylases have been purified from yeast and mammals, with many having functional links to transcriptional repression (Lee and Young, 2000). Like HATs, most deacetylases have been purified as subunits of large complexes and are recruited to promoters by DNA binding proteins (Lee and Young, 2000). HDAC activity is thought to repress transcription by facilitating local or regional formation of chromatin folding that hinder transcription (Grant, 2001).

ATP-dependent chromatin remodeling factors

As discussed above, acetylation of histone tails may promote transcription by unraveling condense chromatin fibers. However, this modification is unlikely to perturb the structure of the nucleosome (Kornberg and Lorch, 1999). Since the core particle inhibits the binding of RNA polymerase and activators to nucleosomal DNA, another type of chromatin modifying activity is required to alter nucleosomes and provide access to the DNA template. These activities have been identified and are commonly referred to as chromatin remodeling factors. As expected, remodeling proteins enhance the expression of genes, but can also act to repress transcription.

The genetic analyses of the SWI/SNF genes initiated the proposition and identification of remodeling factors. SWI/SNF genes regulate the expression of a subset of genes in yeast, including genes involved in mating type switching and sugar metabolism (Winston & Carlson, 1992). It was observed that mutations in genes encoding histones and chromatin components could suppress swi/snf mutations. This led to the proposal that some SWI/SNF proteins may function by counteracting chromatin-mediated repression of transcription (Winston and Carlson, 1992). When these proteins were purified, they were shown to exist in a large complex consisting of 11 subunits, of which 7 were SWI/SNF proteins (Cairns et al., 1994). More importantly, *in vitro* studies demonstrated that this complex could alter the structure of nucleosomes in an ATP dependent manner (Cote et al., 1994).

After the identification of the SWI/SNF complex, many additional remodeling factors have been purified from yeast and mammals (Vignali et al., 2000). Based on the characteristics of their catalytic subunit, these activities can be categorized into three groups: SWI2/SNF2, ISWI, and CHD/Mi-2 (Vignali et al., 2000). Complexes in the SWI2/SNF2 family include yeast SWI/SNF and RSC, Drosophila Brahma, and human BRG1. Members of this group all contain an ATPase subunit belonging to the Swi2 family of proteins. The ISWI group consists of yeast ISW1, ISW2, Drosophila NURF, CHRAC, ACF, and human RSF and hACF. The catalytic subunit of this group belong to the ISWI family, a set of protein with an ATPase domain related Swi2p. Finally, the CHD/Mi-2 group is composed of yeast Chd1p, Drosophila NuRD, Xenopus Mi-2, and human CHD3/CHD4 complexes. The catalytic member of these activities belongs to the

CHD family, a class of proteins containing a Swi2-like ATPase and the chromo domain (see below).

Though SWI/SNF functions as a coactivator, other remodeling factors have been shown to act as negative regulators. The ISW proteins repress the expression of sporulation genes and RSC negatively regulates *CHA1* in yeast (Goldmark et al., 2000; Moreira and Holmberg, 1999). The Drosophila CHD complex interacts with the transcriptional repressors Hunchback and Polycomb (Kehle et al., 1998). In mammals, the CHD3/CHD4 complex is thought to inhibit transcription due to its association with a histone deacetylase (Xue et al., 1998; Zhang et al., 1998). In addition to transcription, remodeling proteins have been implicated in other processes influenced by chromatin structure, including DNA replication, repair, and recombination (Fyodorov and Kadonaga, 2001).

Similar to histone modifying enzymes, remodeling activities can be recruited to specific genes by transcriptional regulators. Purified SWI/SNF has been observed to interact with a variety of transcriptional activators (Fry and Peterson, 2001). In vivo, Swi5p is required for SWI/SNF binding to the promoter of the HO gene (Cosma et al., 1999). IWS1 is recruited to sporulation genes by Ume6p, and interactions between CHD complexes and transcription factors have also been detected (see above).

It is unclear how remodeling factors alter nucleosome and chromatin conformations in cells. A number of biochemical assays have provide insight into possible mechanisms of action *in vivo*. In early *in vitro* assays, it was observed that SWI/SNF complexes increased the accessibility of nucleosomal DNA to nuclease digestion by utilizing the energy from ATP hydrolysis. This result suggested that

SWI/SNF activity disrupted histone-DNA contacts (Lee and Young, 2000). In later

studies, it was shown that a remodeling factor could displace a nucleosome completely

from one DNA molecule and transfer the particle to another DNA template (Lorch et al.,

1999). Other investigations revealed that ISWI complexes enable "sliding" of the histone

octomer along a single DNA molecule (Hamiche et al., 1999; Langst et al., 1999).

Recent experiments have uncovered a potential biomechanical mechanism for

nucleosome remodeling. SWI/SNF, ISWI, and CHD complexes can induce superhelical

torsion in naked and nucleosomal DNA in an ATP dependent manner (Gavin et al., 2001;

Havas et al., 2000). This alteration of DNA topology increased the sensitivity of

nucleosomal DNA to nucleases without displacement or sliding of the core particle.

Together, these studies suggest that in vivo, remodeling activities can reposition

nucleosomes or modulate the accessibility of nucleosomal DNA by disrupting histone-

DNA interactions. These effects can potentiate transcription by exposing DNA elements

previously shielded by chromatin, thereby facilitating activator or RNA polymerase

binding to promoter elements. On the other hand, remodeled nucleosomes can hinder

transcription by masking TATA or UAS sequences.

CHD PROTEINS

As stated above, many ATP-dependent nucleosome modifying activities exist in

eukaryotes. Some of these factors are highly conserved and have homologues in yeast,

plants, and humans. Yet, for proteins such as the CHD family, little is known about their

cellular functions. To better understand the role of chromatin remodeling activities in

cells, and of the CHD proteins in particular, we initiated a study on the *S. cerevisiae*

Chd1p.

Identification of CHD proteins

CHD1 was initially and serendipitously identified in mice, in a screen for a factor (κY) that binds to immunoglobulin promoters (Delmas et al., 1993). However, binding analysis determined that CHD1 was not κY and that it bound to the immunoglobulin promoters due to its sequence-selective DNA-binding capability. Sequence analysis revealed that CHD1 contains two domains that have become the hallmark of the CHD family: a Swi2-like helicase/ATPase and a chromo domain, a motif found in many chromatin associated proteins. The CHD protein was subsequently named in reference to these features (chromo domain, helicase/ATPase, DNA-binding). CHD proteins have now been identified in various organisms. There are multiple homologues in humans, flies, worms, plants and one member in budding yeast (Woodage et al., 1997).

CHD domains and functional implications

The presence of the Swi2-like ATPase implicates a role for CHD proteins in transcription and chromatin modification. Indeed, purified CHD3/4 proteins from mammals have been demonstrated to alter nucleosomes in an ATP-dependent fashion (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998). The physiological function of this activity is not known. However, there is evidence suggesting that CHD proteins may have a role in transcription elongation. The mammalian CHD1 has been shown to have a two-hybrid interaction with SSRP1, a component of FACT –a complex that facilitates transcription elongation through chromatin templates (LeRoy et al., 1998; Orphanides et al., 1999). Moreover, in yeast, there is a genetic interaction between *CHD1* and *POB3*, which encodes a component of yFACT (Costa and Arndt, 2000; Orphanides et al., 1999).

CHD proteins are members of the chromo domain family of proteins, another indication that they may have a role in chromatin organization. The chromo domain was originally identified as a stretch of 37 amino acids that was similar between the Drosophila proteins Polycomb and HP1 (Paro and Hogness, 1991). HP1 is involved in position effect variegation, a form of gene silencing mediated by the formation of heterochromatin (Eissenberg et al., 1990). The Polycomb protein is a transcriptional repressor and is thought to act by generating heterochromatin-like regions (Messmer et al., 1992). Other chromo domain proteins also have roles in processes involving chromatin. The *Drosophila* chromo domain proteins MOF and MSL3 proteins are localized to the X chromosome where they act as dosage compensation factors (Lucchesi, 1999). In *S. pombe*, chromo domain proteins are involved in centromere function and silencing at the mating type loci, and both the centromere and the mating loci resemble heterochromatin (Ekwall et al., 1995; Ekwall et al., 1996).

Because all chromo domain proteins characterized have chromatin related functions, it has been proposed that the chromo domain acts as a localization determinant. Recent studies on the binding properties of the chromo domain have uncovered a possible mechanism for targeting chromo domain proteins to their specific chromosomal sites. The chromo domains of HP1 and Swi6 (the S. pombe homologue of HP1) have been shown to bind specifically to methylated lysine 9 on histone H3 tails (Bannister et al., 2001). In addition, localization analyses demonstrated that Swi6 associates with heterochromatic regions containing methylated H3 Lys[9] and that deletion of an H3 Lys[9] methylase abolishes Swi6 recruitment to these sites (Bannister et al., 2001; Nakayama et al., 2001). These findings strongly suggest that HP1 proteins are localized to

heterochromatin via chromo domain interactions with histone H3 methylated at lysine 9. It does not appear, however, that all chromo domain proteins possess methyl-lysine binding capabilities. The chromo domains of Polycomb, SUV39H1, and Mi-2 (a CHD protein) do not bind to methyl-lysine residues (Bannister et al., 2001). Additional types of chromo domain interactions, (e.g. RNA-chromo domain (Akhtar et al., 2000)) are likely to be involved in the recruitment of chromo domain proteins to specific chromosomal locations.

Like other chromo domain proteins, CHD proteins also display a distinguishable localization pattern. *Drosophila* CHD1 is enriched at puffed and interband regions of polytene chromosomes, regions associated with active transcription and extended chromatin structure (Stokes, d., PNAS, 1996). A similar observation was seen with the human CHD1 protein in that the protein is specifically excluded from heterochromatic bodies (Stokes, D, MCB, 1995). These results led to the proposal that CHD proteins are involved in the formation of "open" chromatin structures and function to facilitate transcription (Stokes et al., 1996).

In contrast, other lines of evidence suggest that CHD proteins act as transcriptional repressors. CHD3/4 proteins have been purified in a complex with a histone deacetylase, implicating a role for CHD proteins as negative regulators (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998). In plants, CHD3 represses the expression of *LEC1*, a gene involved in embryo development (Ogas et al., 1999). Furthermore, a chd1 deletion strain is resistant to 6-azauracil (a drug that inhibits transcription elongation), suggesting that CHD1 impedes transcription (Woodage et al., 1997).

21

It is unclear then, how CHD proteins regulate transcription. In addition, little is known about their *in vivo* activities. To clarify the role of CHD proteins in transcription, and to obtain a better understanding of their cellular functions, we initiated a study on the S. cerevisiae protein Chd1p. Since Chd1p is the only CHD protein in yeast, this organism presents a unique system to dissect CHD function. In addition, analysis of CHD in higher eukaryotes has been limited by biochemical or genetic obstacles. The tractability of the yeast system allows for multiple approaches to assess the function of Chd1p.

In Chapter 1, we describe a genomic analysis of the role of Chd1p in transcription, a genetic screen to identify interacting genes, and a biochemical purification and characterization of Chd1p. In chapter 2, we discuss a genomic examination of Chd1p distribution and the identification of specific targets of regulation. In chapter 3, we describe the identification of Chd1p interacting proteins and genetic and genomic analyses of one such protein, Crp1p.

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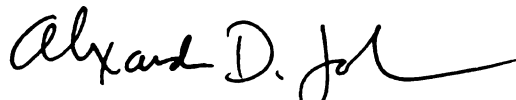
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CHAPTER 1:

The chromo domain protein Chd1p from budding yeast is an ATP-dependent chromatin-modifying factor

This chapter is a reprint of the materials as it appears in EMBO (2000), volume 19, pages 2323-2331. Vishy Iyer contributed to the experiments described in Figure 1A. David Steger performed the experiments described in Figures 4 and 5 and wrote parts of Section 4 in the Results. Hien Tran carried out the experiments summarized in Figures 1, 2, and 3 and wrote the remainder of the paper. Alexander Johnson directed and supervised the research.

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ABSTRACT

CHD proteins are members of the chromo domain family, a class of proteins involved in transcription, DNA degradation, and chromatin structure. In higher eukaryotes, two distinct subfamilies of CHD proteins exist, CHD1 and CHD3/4. Analyses carried out *in vitro* indicate that the CHD3/4 proteins may regulate transcription via alteration of chromatin structure. However, little is known about the role of CHD proteins *in vivo*, particularly the CHD1 subfamily. To better understand the cellular function of CHD proteins, we initiated a study on the Chd1p protein from budding yeast. Using genomic DNA arrays, we identified genes whose expression is affected by the absence of Chd1p. A synthetic lethal screen uncovered genetic interactions between *SWI/SNF* genes and *CHD1*. Biochemical experiments utilizing Chd1p purified from yeast showed that it reconfigures the structure of nucleosome core particles in a manner distinct from the SWI/SNF complex. Taken together, these results suggest that Chd1p functions as a nucleosome remodeling factor, and that Chd1p may share overlapping roles with the SWI/SNF complex to regulate transcription.

INTRODUCTION

Chromo domain proteins are found in all eukaryotes (Cavalli and Paro, 1998; Koonin *et al.*, 1995). The chromo domain was originally recognized as a 37 amino acid segment in the *Drosophila* Polycomb protein that shared close sequence similarity with a portion of the heterochromatin-associated protein, HP1 (Paro and Hogness, 1991). Polycomb and HP1 were believed to act as regulators of transcription via formation of a higher order chromatin structure, hence the name "chromo domain." Other chromo domain proteins, defined as having sequence similarity to this 37 amino acid segment, include Pdd1p, a *Tetrahymena* protein involved in programmed DNA degradation (Madireddi *et al.*, 1996), and SWI6p, a protein required for the maintenance of heterochromatin-like regions in *S. pombe* (Ekwall *et al.*, 1995). Though chromo domain proteins have been studied for over a decade, little was known about their molecular mode of action until recently.

A subset of chromo domain proteins, the CHD family, consists of proteins sharing three sequence features: a chromo domain, an ATPase/helicase, and a DNA binding segment, although not all proteins termed CHD have this last domain (Delmas *et al.*, 1993; Woodage *et al.*, 1997). CHD proteins are well conserved, with members found from yeast to plants to mammals. In higher eukaryotes, up to four distinct CHD genes are present in the genome; for example, the human genome includes CHD1, CHD2, CHD3, and CHD4 (Woodage *et al.*, 1997). The human CHD3 and CHD4 proteins have recently been shown to co-purify with each other and with the human histone deacetylase complex, HDAC (Tong *et al.*, 1998; Xue *et al.*, 1998; Zhang *et al.*, 1998). A *Xenopus* CHD protein related to the human CHD3/4 proteins has also been purified with a deacetylase complex

(Wade *et al.*, 1998). Additionally, the human CHD3/4 complex was shown to have nucleosome remodeling activity. These results suggest that at least some CHD proteins may be involved in altering the chromatin environment around genes. A model was proposed in which the activity of CHD3/4 increased access to the histones, allowing the deacetylase to modify the histone tails and subsequently leading to greater compaction of nucleosomal structure and inhibition of transcription (Zhang *et al.*, 1998). Further support for a role of CHD3/4 proteins in transcriptional repression comes from the study of the *Drosophila* dMi-2 protein, a relative of the human CHD3 and CHD4 proteins. dMi-2 was found to interact in a two hybrid screen with Hunchback, a protein required for the repression of homeotic genes, and mutations in dMi-2 affect both Hunchback and Polycomb mediated repression (Kehle *et al.*, 1998).

In contrast, work on the *Drosophila* DmCHD1 suggests that it is involved in gene activation. Null mutations of DmCHD1 have not been reported but, using immunofluorescence, the DmCHD1 protein was localized to puffs and interband regions on polytene chromosomes, areas generally associated with active transcription (Stokes *et al.*, 1996). One possible explanation for the dichotomy of these results is that the CHD genes have diverged, such that different classes of CHD genes have different cellular roles. In fact, phylogenetic analysis of the CHD family revealed that the CHD3/4 class of genes is in a subfamily distinct from that of the CHD1 members (Woodage *et al.*, 1997).

The genome of the budding yeast *S. cerevisiae* encodes a single CHD protein, Chd1p, which most closely resembles the CHD1 subgroup from more complex eukaryotes (Woodage *et al.*, 1997). *CHD1* null mutations in yeast are viable but have subtle phenotypes when grown under special conditions (Jin *et al.*, 1998; Tsukiyama *et al.*, 1999;

Woodage *et al.*, 1997). For example, a *chd1Δ* strain is resistant to high concentrations of

6-azauracil (6AU), a pyrimidine analog that is toxic to wild type cells. Since RNA pol II

and elongation factor SII mutants are sensitive to 6AU, it was proposed that CHD1 may

formally act as a negative regulator of transcription (Woodage *et al.*, 1997). Taken

together, the work from mammals, flies, and yeast does not yet provide a clear

understanding of the genetic roles of the CHD1 subgroup of proteins or how these protein

function biochemically.

To obtain a better understanding of the CHD proteins, and of the function of the

CHD1 subfamily in particular, we have chosen to study the *CHD1* gene of *S. cerevisiae*.

Since biochemical and genetic experimentation in this organism are feasible, we applied

both approaches simultaneously to this problem. Among other features, our investigation

has revealed that Chd1p is a nucleosome remodeling factor that is biochemically distinct

from the well characterized SWI/SNF complex but may share overlapping functions with

SWI/SNF *in vivo*.

MATERIALS AND METHODS

Strains, media, reagents

Strains yHT148 (S288CMATα ura3-52 lys2-801 ade2-101 leu2-Δ1 his3-Δ200

trp1-Δ1 + pRS314) and yHT147 (S288C MATα chd1Δ::TRP1 ura3-52 lys2-801 ade2-101

leu2-Δ1 his3-Δ200 trp1-Δ1) were used for the DNA array and for the RNA Northern blot

procedures. Strain yHT153 (S288C MATα 6MYC::6HIS::CHD1::TRP1 ura3-52 lys2-

801 ade2-101 leu2-Δ1 his3-Δ200 trp1-Δ1) was used for the chromatin

immunoprecipitation experiment. All three strains were grown in SD-Trp media (0.67%

Bacto-yeast nitrogen base without amino acids, 2% dextrose, 2% Bacto-agar,

supplemented amino acids without tryptophan) and harvested at an OD660 of 2.0.

Strains yHT40 (*W303 MATa ura3 ade2-1 leu2-3 his3-11 trp1-1 chd1Δ::LEU2 +*

pAJ741) and yHT42 (*W303 MATα ura3 ade2-1 leu2-3 his3-11 trp1-1 chd1Δ::TRP1 +*

pAJ741) were used for the synthetic lethal screen. pAJ741 contains the *CHD1, ADE3,*

and *URA3* genes on a 2μ plasmid. Cells were grown on prespo plates (0.8% Bacto-yeast

extract, 0.3% Bacto peptone, 10% dextrose, 2% Bacto agar), or 5FOA plates (0.7% yeast

nitrogen without amino acids, 2% agar, 0.001% uracil, 0.08% 5FOA, 0.1% supplemented

amino acids) during analysis of the mutants.

Strains for analyses of *swi1*, *swi2*, and *swi4* mutants: CY258 (*MATα*

swi1Δ::LEU2 ura3-Δ99 lys2-801 ade2-101 leu2-Δ1 his3-Δ200),

CY521(*MATα swi4Δ::HIS3 ura3-52 lys2-801 ade2-101 leu2-Δ1 his3-Δ200*) and CY26

derivatives (*MATa chd1Δ::HIS3 or chd1Δ::LEU2 ura3-52 lys2-801 ade2-101 leu2-Δ1*

his3-Δ200 trp1-Δ1), yHT2568 (*MATα swi2Δ::HIS3 ura3-52 lys2-801 ade2-101 leu2-Δ1*

his3-Δ200 HO::TRP1), yHT2540 (*MATa chd1Δ::LEU2 ura3-52 lys2-801 ade2-101 leu2-*

Δ1 his3-Δ200 HO::TRP1)

Strain yHT149 (*MATa 6MYC::6HIS::CHD1::TRP1ura3-52 lys2-801 leu2-Δ1*

his3-Δ200 trp1 pep4Δ::HIS3 prb1-Δ1.6R) was used for the purification of Chd1p and

grown in 2X YEPD (2% Bacto-yeast extract, 4% Bacto-peptone, 4% dextrose).

p*CHD1* plasmids all contain the *CHD1* gene derived from a genomic *CHD1*

lambda clone obtained form the American Type Culture Collection (ATCC), (Olson *et*

al., 1986). The genomic library used to clone *SWC3* was also obtained from the ATCC

(Rose *et al.*, 1987; Thrash *et al.*, 1985).

Anti-MYC (9E10) antibodies and anti-Chd1p antibodies were obtained from

Covance. Anti-Chd1p polyclonal antibodies were generated using an N-terminal

fragment of Chd1p (amino acids 122-300) fused to glutathione S-transferase as an

antigen.

DNA micro array

Poly A+ RNA was purified from total RNA using oligo dT resin (Invitrogen).

Hybridizations and array analyses were carried out as previously described (DeRisi *et al.*,

1997).

Northern Blots

O.5 microgram of poly A+ RNA was loaded per sample on an agarose-

formaldehyde gel and electrophoresis was carried out in 1X MOPS buffer. The RNA

was transferred to a GeneScreen membrane (Dupont) and radiolabeled DNA probes were

hybridized to the membrane at 65 degrees Celcius for 12 hours. The membrane was

washed with detergent and transcript signals were quantified using a Molecular Dynamics PhosphorImager.

Chromatin immunoprecipitation

Crosslinking, cell lysis, sonication to shear chromatin, and immunprecipitation were performed essentially as previously described (Aparicio *et al.*, 1997) with the following modifications. Formaldehyde crosslinking was quenched after 1 to 15 minutes and chromatin was sonicated 7x12 seconds to produce fragments ranging from 100-600 base pairs. For PCR reactions, an equal molar amount of primers for *ACT1* and regulated genes were added in addition to either total DNA or immunoprecipitated DNA.

Synthetic lethal screen

Strains yHT40 or yHT42 were grown in 5 ml of SD-URA to an OD660 of 0.5. The cells were harvested and washed twice with water, resuspended in water, and sonicated. The cells were then were mutagenized by UV irradiation at five different doses. A small aliquot of cells at each dose was plated (in the dark) to determine the percent viability of each pool. The remaining master stock was stored in the dark at 4 ° C. Cells from three pools with viability percentages of 72%, 21%, and 14% were chosen for the screen. A total of about 75,000 cells were plated onto prespo plates, at a density of 300-400 cells per plate, and grown at room temperature. Colonies that turned red and did not sector were chosen for restreaking. Those that remained red were restreaked again. 50 mutants remained after the second restreak and were transformed with a p*CHD1* plasmid (either *TRP1* or *LEU2* marked) and simultaneously streaked on 5FOA plates as well. Mutants that sectored after the introduction of a second p*CHD1* plasmid and did not grow on 5FOA when harboring only the original p*CHD1*, *URA3* plasmid

(pAJ741) were selected for further analysis (3 mutants met this criteria, *swc1*, *swc2*, *swc3*, and all originated from the 21% viability pool). All three mutants were backcrossed three times and mated to each other to perform complementation tests. Diploids were tested for both wild type growth and the ability to sector.

SWC1 and *SWC2* were cloned via complementation of the slow growth and sectoring phenotypes of the *swc1* and *swc2* mutations by a *SWI2* plasmid (pBD10, pBD3). To verify that *SWC1* and *SWC2* are indeed *SWI2*, an allelism test was carried out. The *URA3* gene was integrated at the 3' UTR (~ 500 base pairs downstream of the termination codon) of the *SWI2* locus of a *chd1Δ* haploid cell and mated to a *swc1 chd1Δ* mutant. After sporulation and tetrad dissection, the haploids were analyzed. *swc1*, (slow growing colonies) always segregated away from the *URA3* gene in a 2:2 ratio (out of 21 tetrad dissections). In addition, a *chd1Δ* haploid (yHT2540) and a *swi2Δ* haploid (yHT2568) were mated, the diploid sporulated, and spores analyzed for the double mutant phenotype. No double mutant spores were ever recovered (from 28 tetrad dissections). However, transformation of a *CHD1* plasmid (pAJ741) into the diploid and subsequent sporulation and dissection showed that cells with genomic copies of *CHD1* and *SWI2* deleted were viable when harboring the pAJ741 plasmid, revealing that the synthetic interaction between *CHD1* and *SWI2* is indeed *CHD1* dependent. The same dependence on p*CHD1* for spore viability was seen with the *chd1Δ swi1Δ* strain. (Note: It has been observed that the *TRP1* allele *trp1-Δ1* makes *swi/snf* strains sicker than *trp1-1* or *TRP1* strains. The strains used in all of our manipulations of *swi2Δ* and *swi2/swc1/swc2* were either *trp1-1* or *TRP1* [and not *trp1-Δ1*] and synthetic interactions between *chd1Δ* and *swc1/2* as well as *swi2Δ* were observed for both *trp1-1* and *TRP1*

strains.) To clone *SWC3*, a YCp50 genomic library was electroporated into a *swc3*

mutant and plasmids that complemented the slow growth defect were isolated and

sequenced. By subcloning and retransformation of candidate *SWC3* genes, it was

determined that *ALR1* was the only gene on the rescuing plasmids that complemented the

swc3 mutation. *SWC3* was confirmed to be *ALR1* by the ability of high magnesium

media to complement the *swc3* slow growth defect and by construction of an *alr1Δ*

chd1Δ strain and demonstration that the double mutant was synthetically sick.

Purification

24 liters of strain yHT149 was grown in 2X YEPD and harvested at an OD660 of

6. The cell pellet (~400g) was washed with water and resuspended in 200 ml of 3X lysis

buffer (750 mM NaCl, 150 mM HEPES pH 7.6, 30% glycerol, 0.3% nonidet P 40 [NP-

40], 30 mM Mg(OAc)2, 3 mM EDTA) and frozen in a dry ice/ethanol bath for storage.

The cell paste was thawed and β-mercaptoethanol (BME) and protease inhibitors were

added to the following final concentrations: 10mM BME, 1 mM PMSF, 2 mM

benzamidine, 1ug/ml pepstatin, leupeptin, and bestatin. 350 ml Biospec bead beating

chambers were used to lyse cells. Cells were agitated for 30 seconds, with a 90 second

cooling period, 20 times. The lysate was spun at 9k for 20 minutes and the supernatant

was extracted with a 500 mM NaCl incubation step (30 minutes with stirring). The

extract was clarified with a 35K spin in a ultracentrifuge (Beckman L8-55M). The

supernatant was then diluted to a final [NaCl] of 240 mM.

The extract was loaded onto a 500 ml BioRex-70 column (Biorad). The column

was washed with 240 mM and 290 mM NaCl buffer A (20 mM HEPES pH 7.6, 10%

glycerol, 0.1% NP-40, 0.1 mM EDTA, 1 mM PMSF, 2 mM benzamidine, 1ug/ml

46

pepstatin, leupeptin, and bestatin, 10 mM BME) . Chd1p was eluted off the column with

450 mM NaCl buffer A in 50 ml fractions. To one 50 ml peak fraction, 2.5 ml of Ni-

NTA (Qiagen) resin was added, in addition to imidazole to 1 mM final concentration.

The slurry was nutated for 1.5 hours and packed into a column. The Ni-NTA column

was washed with 1 mM, 2 mM, and 4 mM imidazole buffer B (70 mM NaCl, 25 mM Tris

pH 8.0, 10% glycerol, 0.1% NP-40, 0.1 mM EDTA, 1 mM PMSF, 2 mM benzamidine,

1ug/ml pepstatin, leupeptin, and bestatin, 10 mM BME). 200 mM imidazole buffer B

was used to elute Chd1p off the column. Peak fractions were pooled and loaded onto a

0.75 ml DEAE-Sepharose column (Amersham-Pharmacia). The column was washed

with 70 mM NaCl and 120 mM NaCl buffer C (50 mM Tris pH 8.0, 10% glycerol, 0.1%

NP-40, 0.1 mM EDTA, 1 mM PMSF, 2 mM benzamidine, 1ug/ml pepstatin, leupeptin,

and bestatin, 1 mM dithiolthreitol). Chd1p was eluted with 170 mM NaCl buffer C.

Peak fractions were then loaded onto a 1 ml HiTrap SP column (Amersham-Pharmacia).

After washes with 170 mM NaCl and 325 mM NaCl buffer D (50 mM HEPES pH 7.6,

10% glycerol, 0.1% NP-40, 0.1 mM EDTA, 1 mM PMSF, 2 mM benzamidine, 1ug/ml

pepstatin, leupeptin, and bestatin, 1 mM dithiolthreitol), Chd1p was eluted off the column

with 375 mM NaCl buffer D.

Nucleosome reconstitution, DNase I analysis, and EMSA

An end labeled ScaI-AvaII fragment of 172 bp from a sea urchin 5S rRNA gene

was assembled into nucleosome core particles with purified HeLa nucleosomes as

previously described (Steger and Workman, 1997). DNase I and EMSA reactions were

performed in 20 µl with 10 mM HEPES pH 7.8, 50 mM KCl, 4 mM $MgCl_2$, 5 mM DTT,

0.5 mM PMSF, 0.25 mg/ml BSA and 5% glycerol. Reactions were incubated at 30°C for

30 min. Samples with 5S nucleosome core particles contained ~1 to 2 fmol of reconstituted probe and donor nucleosomes to give a total of 0.4 pmol of nucleosomes. Reactions with histone-free DNA contained the same amounts of mock-reconstituted probe DNA and nucleosomes as used for the mononucleosome reactions. The amount of Chd1p required to reconfigure all of the 5S nucleosome core particles was determined to be roughly stoichiometric with the total amount of nucleosomes in the reaction. DNase I digestion and gel electrophoresis were performed as described previously (Steger and Workman, 1997).

ATPase assay

Reactions were carried out under the same conditions used for the DNase I protection analysis with the addition of 0.5 mM ATP and 1 μCi [γ-^{32}P]ATP. After 30 min at 30°C, reactions were terminated by the addition of 1 μl of 0.5 M EDTA. A 1 μl sample from each reaction was spotted onto a polyethyleneimine cellulose plate for thin layer chromatography. Chromatography was carried out in 50 mM HCl to separate ATP and free phosphate.

RESULTS

Genome wide analysis of CHD1 function

Since preliminary work in *Drosophila* and yeast suggest that *CHD1* may be a transcriptional regulator, we carried out a genome-wide analysis of transcription, using DNA microarray technology (Derisi *et al.*, 1997), to examine the possible involvement of *CHD1* in this process. We purified mRNA from wild type and *chd1* deletion strains grown in minimal media. Fluorescent probes from the two mRNA samples were synthesized and hybridized to a DNA microarray representing the yeast genome, and

differential effects on transcript levels of ~ 6,000 genes were monitored. In two

independent experiments, the expression of approximately 2% - 4% of the genes in the

genome was affected by the absence of *CHD1* by a factor of 2.0 or greater (complete data

set available upon request). 23 genes were consistently affected by the loss of *CHD1* in

both experiments (Figure 1A). The differences between the two array experiments are

most likely due to minor differences in the growth conditions of the cells and in the

quality of the RNA. Northern blot analyses verified that a selected subset of genes are

differentially expressed in the wild type and deletion strains (Figure 1B). The Northern

blots (using RNA from a third independent preparation) also indicate that genes affected

in only one of the two array hybridizations are indeed affected by the absence of *CHD1*

(*CAR1* & *LYS9* in Figure 1B). The results from the DNA array experiment are consistent

with the idea that *CHD1* is a regulator of transcription.

In an effort to determine which genes may be directly regulated by *CHD1*, we

performed a series of chromatin immunoprecipitation experiments. Briefly, Myc::Chd1p

was cross-linked to DNA *in vivo* by treating cells with formaldehyde. A variety of cross-

linking times, ranging from 1 to 15 minutes, were used in an attempt to eliminate non-

specific cross-linking. The cells were harvested, lysed, and the chromatin was extracted

and sheared by sonication to produce DNA fragments with an average size of about 400

base pairs. Myc antibodies coupled to beads were added to the sheared chromatin to

precipitate DNA that had been cross-linked to Chd1p. After removing cross-linked

proteins, the DNA was extracted and analyzed using the polymerase chain reaction (PCR).

Primers directed against the promoter region of *CHD1*-regulated genes as well as primers

for a gene (*ACT1*) not affected by *CHD1* were added together in the PCR reactions.

Chromatin immunoprecipitations carried out in extracts derived from a

MYC::CHD1 strain precipitated at least 500 fold more DNA than a control extract in

which Chd1p is not tagged with Myc (unpublished data, and see Figure 1C), showing that

Chd1p can be very effectively cross-linked to DNA. However, the immunoprecipitation

step did not preferentially precipitate DNA from genes regulated by *CHD1* compared to

the control gene (Figure 1C). The *ACT1* gene was detected along with *SNZ1*, *LYS9*, and

YGL258W genes in the Chd1p precipitated DNA pool. The most likely interpretation of

this result is that Chd1p is an abundant protein that is bound to chromatin throughout the

genome, and whose absence affects the expression of only certain genes.

The results from the chromatin immunoprecipitation experiment prompted us to

obtain an estimate of the concentration of Chd1p in cells. The western blot signal of

Chd1p in titrations of whole cell extract was compared to the signal of Chd1p in titrations

of purified Chd1p (data not shown and see below). Using this technique, we estimate that

there are about 1,000 molecules of Chd1p per cell. This amount corresponds to about one

Chd1p molecule for every 12,500 base pairs (one every 6 genes) or about one molecule

for every 50 nucleosomes.

Genetic interactions with SWI/SNF genes

Given that the DNA array results support a role for *CHD1* in transcription, we

attempted to genetically uncover the cellular function of Chd1p responsible for its affect

on gene expression. A synthetic lethal screen was undertaken to identify genes that may

share redundant or overlapping functions with *CHD1*. Since a *CHD1* deleted strain is

viable and has only subtle phenotypes, it is possible that other genes may be able to

substitute for the loss of *CHD1* function. A synthetic lethal screen is a common method

50

used to uncover mutations in a second gene that will require the cell to maintain a wildtype copy of the gene being studied in order to survive (Bender and Pringle, 1991). A colony sectoring assay was implemented to visually identify mutants of interest by their inability to lose a wildtype copy of *CHD1*.

The starting strain was deleted for the genomic copy of *CHD1* and carried a plasmid containing a wildtype copy of *CHD1* and *ADE3*. When grown on low adenine plates, cells form red colonies when they maintain the *CHD1*, *ADE3* plasmid. Because *CHD1* is not essential, cells can lose the plasmid and form red colonies with white sectors. After UV mutagenesis, 75,000 cells were plated on low adenine plates and colonies that stayed completely red, indicating that these cells cannot lose the *CHD1* plasmid, were chosen for further analysis. Three mutants remained after subsequent testing and were named *swc1*, *swc2*, and *swc3* for synthetically sick with *chd1*. We observed that the *swc* mutants are not synthetically lethal with *chd1*; rather, they grow very slowly without a functional copy of *CHD1* and hence, have a growth advantage when they maintain the *CHD1* plasmid (Figure 2A).

swc1 and *swc2* form one complementation group and *swc3* the other. *SWC3* was determined to be *ALR1* by transformation of the *swc3* mutant with a genomic library and isolation of plasmid sequences that complemented the slow growth defect of *swc3*. A disruption of the *ALR1* gene and construction of an *alr1Δ chd1Δ* double mutant verified the synthetic sickness phenotype (unpublished data). *ALR1* codes for a magnesium transporter (MacDiarmid and Gardner, 1998) and is not an essential gene in the *W303* strain (unpublished data). We speculate that *CHD1* may regulate another component of the magnesium uptake/metabolism pathway that when eliminated, leads to cell death in an

51

alr1 background. The data from the DNA microarray experiment cannot be used to identify this gene because of strain differences. We used a *W303* strain in our screen and an *S288C* strain for the array experiment; there are significant differences in the phenotype of an *alr1* deletion between the two strains.

swc1 and *swc2* were complemented by transformation with a *SWI2* plasmid. A *chd1Δ swi2Δ* double mutant was constructed and found to be inviable (Figure 2B). The inviability of the double mutant, in contrast to the synthetic sickness phenotype of the mutants generated from the screen, could be due to the strain differences or to the possibility that the *swc1* and *swc2* mutations are reduction of function rather than null alleles of *SWI2*. *swc*1, *swc2*, and *swc3* were all transformed with a *SWI2* plasmid but only *swc1* and *swc2* were complemented by the plasmid. *SWC1(2)* was confirmed to be an allele of *SWI2* by an allelism test (see Materials and Methods for details).

The genetic interaction between *CHD1* and *SWI2* suggests that they may have similar and redundant functions as regulators of transcription via chromatin alteration (Workman and Kingston, 1998). Swi2p is the ATPase "engine" of the SWI/SNF remodeling complex and is required for the in vitro nucleosome remodeling activity of the complex (reviewed in Travers, 1999). It is possible that Chd1p's biochemical activity may overlap with that of the entire SWI/SNF complex, and not simply with Swi2p. To test this idea, we checked for a genetic interaction between *CHD1* and a gene coding for a different component of the SWI/SNF complex, *SWI1* (Cairns et al., 1994; Peterson et al., 1994). A *chd1Δ swi1Δ* double mutant is also synthetically lethal (Figure 2B), implying that Chd1p shares functions with the SWI/SNF complex as a whole. As a control, we also looked for interactions with another *SWI* gene, *SWI4*, which does not code for a protein

that is part of the SWI/SNF complex. A *swi4Δ chd1Δ* double mutant is viable (Figure 2D), demonstrating that *CHD1* interacts specifically with genes encoding subunits of the SWI/SNF remodeling complex.

To further investigate the genetic interaction between *CHD1* and *SWI2*, we compared the genome-wide expression profile of a *chd1Δ* to a *snf2Δ(swi2Δ)* strain (*snf2* micro-array data generously provided by P. Sudersanam, V. Iyer, P. Brown, and F. Winston). The expression of about 35 genes were similarly affected (fold difference > 1.8.) by the absence of *CHD1* and *SNF2*. Because the growth conditions and strain background were different for the *chd1* and *snf2* strains used in the microarray experiments, the comparison between the two data sets is not ideal, and this estimate of the overlap may not be completely accurate. Still, this result is consistent with the possibility that there is a small subset of genes coordinately regulated by both *CHD1* and *SWI2*. It is possible that one, or more, essential gene requires both *CHD1* and *SWI2* for proper expression and the synthetic lethality between the two mutants is due to the lack of expression of this vital gene(s). In any case, the evidence indicates that *CHD1* and *SWI2* have overlapping, but not superimposed, functions in the cell.

Purification of Chd1p

Because *chd1* is synthetically lethal with *swi2*, and because the two proteins show sequence similarity (Eisen *et al.*, 1995), we tested whether Chd1p possesses biochemical activities resembling those of Swi2p. To this end, Chd1p was purified from yeast . The purification was facilitated by tagging Chd1p with a 6(Myc)::6(His) fragment at the N-terminus. We know that tagged Chd1p is functional in the cell because the hybrid *CHD1* gene fully complemented a *chd1Δ* mutation (monitored by the ability of

MYC::HIS::CHD1 to complement a *chd1 swi2* mutant) when integrated into the yeast genome and transcribed from its own promoter. The expression level of the tagged protein is similar to the wild type protein (unpublished data).

Using column chromatography to fractionate yeast whole cell extract and western analysis to identify Chd1p containing fractions, Myc-tagged Chd1p was purified to near homogeneity (Figure 3). The silver stained gel shows that Chd1p is the predominant protein in the purest fractions and that no other proteins co-purified with Chd1p in stoichiometric amounts (Figure 3B). We believe that the faint bands in these fractions corresponding to proteins that are contaminants because these same proteins elute off the SP column at distinct peaks several fractions after the peak Chd1p fractions. Chd1p obtained from the first column eluted from a Superose-6 gel filtration column with a nominal size of about 340 kD (Figure 3C). Chd1p from the SP-Sepharose fractions eluted from the Superose-6 column with a similar apparent size (unpublished data). These results suggest that the purified Chd1p is either a monomer with a large stokes radius or a dimer. Also, during our purification, westerns blots of early columns showed that there was no heterogeneity in the elution profile of Chd1p, suggesting that there was only one major form of Chd1p in our extracts. However, we cannot rule out the possibility that native Chd1p exists within the cell as a larger protein complex that disassembles during our cell lysis procedure.

Chd1p alters the structure of nucleosome core particles in an ATP-dependent manner distinct from the SWI/SNF complex

To begin a functional analysis of purified Chd1p, ATPase assays were conducted in the presence of a range of potential substrates. As shown in figure 4, the ability of

Chd1p to hydrolyze ATP is stimulated by free DNA and nucleosomal DNA, but not core

histones. In this respect, the ATPase activity resembles that of Swi2p-related subunits,

which are also stimulated by free DNA and nucleosomal DNA (Laurent *et al.*, 1993;

Cairns *et al.*, 1996).

Complexes containing Swi2p-related subunits have been demonstrated to disrupt

histone-DNA contacts within nucleosome core particles assembled with a sea urchin 5S

rRNA gene fragment (for examples, see Owen-Hughes *et al.*, 1996; Cairns *et al.*, 1996).

Disruption of the core particles is detected by a change in accessibility to DNase I

digestion, and requires the energy released from ATP hydrolysis. To address whether

Chd1p can remodel nucleosomes, we added purified Chd1p to 5S nucleosome core

particles, and examined the resulting DNase I cleavage pattern. Because the 5S rDNA

sequence used for this analysis adopts a specific rotational position relative to the

underlying octamer, DNase I predominantly cuts 5S mononucleosomes at sites

approximately 10 base pairs apart (Figure 5A, lane A). In the presence of ATP, but not in

its absence, treatment with Chd1p generated protection from DNase I cleavage at several

regions of the DNA, and in addition, produced sites of hypersensitivity flanking these

regions (Figure 5A, compare lane B with C, L with M). In contrast, purified SWI/SNF

increased the number of sites attacked by DNase I to produce a more uniform cleavage

pattern throughout the DNA (Figure 5A, compare lane C with D). These data reveal that

the ATP-dependent changes conferred by Chd1p are distinct from those of SWI/SNF.

We also determined that ATP-γ-S and AMP-PMP, which are two

nonhydrolyzable forms of ATP, did not enable purified Chd1p to alter core particle

structure (Figure 5A, lanes N & O). Because Chd1p is able to bind ATP-γ-S, as

demonstrated by the observation that ATP-γ-S can competitively inhibit Chd1p ATPase activity (Figure 4, lane 10), these results indicate that the remodeling requires energy released from ATP hydrolysis. To confirm that Chd1p was responsible for the ATP-dependent alterations rather than other proteins in the purified fraction, we challenged the remodeling activity with antibodies raised against Chd1p purified from *E. coli*. Changes to 5S mononucleosome structure were completely inhibited by the addition of anti-Chd1p polyclonal serum but not by preimmune serum (Figure 5A, compare lane P with Q). Moreover, fractions immuno-depleted of Chd1p produced little or no change to 5S core particle structure (data not shown). These results firmly establish that Chd1p alters the structure of 5S nucleosome core particles through interactions requiring ATP hydrolysis.

There are several possible explanations for the extended regions of DNase I protection observed in Chd1p-remodeled nucleosomes. First, Chd1p could be a sequence-specific DNA binding protein and be responsible for the protected regions. Second, Chd1p could recognize a specific feature of the remodeled nucleosome and remain bound following ATP hydrolysis. Third, the protected regions could result from changes in the path of the DNA around the histone core. Although Chd1p can bind naked DNA (Figure 5B, lane 2), it shows no specific protection of naked 5S rDNA (Figure 5A, lanes F-J). These results support the conclusion of earlier studies that Chd1p can bind DNA non-specifically (Delmas *et al.*, 1993). Furthermore, this binding does not require ATP (see below, and Fig 5B, lanes 4 &5). To explore whether Chd1p remains bound to the nucleosome following remodeling, we monitored nucleosome remodeling assays by electrophoretic mobility shift analysis (EMSA) rather than DNase I digestion. Little or no shift in the mobility of the 5S nucleosome core particles was detected upon addition of

Chd1p (Figure 5B, lanes 4 and 5). The shifted bands in these lanes migrate similarly to

Chd1p-DNA complexes, and likely represent Chd1p-DNA complexes formed from the

small amount of free DNA in the 5S nucleosome preparation. The simplest explanation

for these results is that Chd1p does not remain stably bound to the 5S nucleosome

following remodeling. Alternatively, it is possible that Chd1p does remain bound to the

nucleosome but the interaction is not stable to electrophoresis or that Chd1p remains

bound but doesn't produce an observable shift in the mobility of the nucleosome. In any

case, because neither the amount or mobility of nucleosome core particles in the reaction

with Chd1p and ATP change, Chd1p must alter nucleosome structure without removing

histones from the DNA.

DISCUSSION

CHD proteins are highly conserved between yeast, flies, worms, and mammals. Using genetic and biochemical approaches, we have uncovered the cellular function of Chd1p from budding yeast: it is a nucleosome remodeling factor with a likely role as a regulator of transcription.

CHD1 effects on genome wide expression

The results of the DNA array experiments show that *CHD1* has both positive and negative effects on transcription and that about 2-4% of all genes are affected by the absence of *CHD1*. Northern blot analyses of a subset of affected genes confirm the expression differences seen in the microarray experiments. The SWI/SNF complex has recently been described as being both a positive and negative regulator of genes (Holstege *et al.*, 1998; Murphy *et al.*, 1999), and it is becoming more clear that nucleosome remodeling factors may not simply unmask promoter regions to help initiate transcription but may also alter chromatin structure to inhibit transcription.

Chd1p can be readily crosslinked to DNA in cells, but we were not able to detect preferential association of Chd1p to genes whose expression it affects. One interpretation of this result is that Chd1p is distributed throughout the genome but, under the conditions observed, affects the expression of a subset of genes. Another possibility is that Chd1p is only transiently present at certain promoters, and once Chd1p has remodeled those promoters, it is no longer needed at that region to maintain the altered state.

A connection to SWI/SNF function

Using a broad synthetic lethal screen, we have determined that *CHD1* genetically interacts with *SWI2* and that cells require one or the other for viability. In addition, our

genetic analyses have also shown that a *chd1Δ swi1Δ* strain is inviable, revealing that *CHD1* interacts with at least two genes encoding components of the SWI/SNF complex. (It has also been observed that *chd1* is synthetically lethal with *snf6*, which encodes a third component of the SWI/SNF complex, [E. Haswell, personal communication].) These results complement the *in vitro* remodeling data (see below) and strongly suggest that Chd1p functions *in vivo* to alter chromatin structure. The fact that Chd1p may have a similar role to the SWI/SNF complex would explain why neither is essential in cells.

Recently, *CHD1* has also been shown to genetically interact with *ISW* genes. Under stress conditions, a *chd1 isw1 isw2* strain is inviable (Tsukiyama *et al.*, 1999). Because ISW proteins are also components of remodeling factors, *CHD1*'s genetic interaction with *SWI/SNF* and *ISW* genes suggests that all four of these chromatin remodeling components share some overlapping functions in yeast.

Purification of Chd1p

We have purified Chd1p to near homogeneity using four chromatographic steps. In our most purified fractions, no other proteins appeared in stoichiometric levels with Chd1p, and no other proteins appeared to co-purify with Chd1p in the final column steps. The apparent size of Chd1p after the first purification step and after the last purification step remained approximately the same. In addition, an immunoprecipitation experiment performed on the BioRex-70 fraction did not reveal any proteins that specifically precipitated with Chd1p in stoichiometric amounts (unpublished data). These results strongly suggest that the form of Chd1p purified was not associated with other proteins and argues against the possibility that a Chd1p complex disassembled between chromatographic steps. The human CHD1 protein is believed to exist in a large complex

(Kelley *et al.*, 1999), and the human CHD3 and CHD4 proteins have also been found to be associated with a multiprotein complex (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998). It is possible that the yeast Chd1p is a simpler version of its counterpart in more complex organisms; alternatively, it is possible that the yeast Chd1p exists in a complex which is unstable to lysis conditions used in our purification.

Chd1p is a remodeling factor

We have demonstrated that Chd1p, using the energy provided by ATP hydrolysis, alters the structure of reconstituted mononucleosomes. We do, however, see a noticeable difference between Chd1p's activity and that of other remodeling activities. SWI/SNF-like activities increase cutting by DNase I throughout the DNA template whereas Chd1p allows increased accessibility in some locations but causes protection of specific sites in other locations. Both the increased accessibility and the protection are dependent on Chd1p and require both nucleosomal DNA and ATP hydrolysis. It is possible that the protection arises from Chd1p remaining bound following the remodeling step. Alternatively, this protection may reflect repositioning of the underlying histones with the DNA (Hamiche et al., 1999; Langst et al., 1999; Whitehouse et al., 1999).

Active fractions of Chd1p from our purification are predominantly composed of the Chd1p polypeptide. Although other remodeling factors have been purified as multi-protein complexes, the SWI2-like catalytic subunit of these remodeling complexes on its own can alter nucleosomes similarly to that of the intact complexes (Corona et al., 1999; Phelan et al., 1999). Thus, although it is a possible that Chd1p functions within a loose multiprotein complex *in vivo*, it may simply act as a single polypeptide as we observed *in vitro*.

The determination that Chd1p perturbs mononucleosomes assigns the first biochemical function for CHD1 proteins. Previous experiments, and other experiments in this paper, have provided clues that Chd1p may have a role in transcription and chromatin structure. Our results demonstrate that Chd1p can indeed remodel chromatin and that this activity is probably responsible for regulating gene expression .

ACKNOWLEDGEMENTS

FIGURE LEGENDS

Figure 1-1. Analysis of *CHD1*'s effect on genome wide expression.

A) Table of genes whose expression is affected by 2-fold or greater in both microarrays. The fold column represents the average between experiments. Arrows pointing up signify that the genes had higher expression in the deletion strain and arrows pointing down indicate that the genes had decreased expression in the deletion strain. B) Northern blot of genes identified from genomic DNA array analysis. Four genes affected by the absence of *CHD1*, as determined by the array experiment, show similar expression differences when analyzed by RNA Northern blots. C) PCR analysis of DNA cross-linked to Chd1p. PCR reactions were carried out using either total DNA from whole cell extracts (WCE) or chromatin immunoprecipitated DNA (Ch-IP), and primers targeted against genes affected by the absence of *CHD1*. Primers for the ACT1 gene, which is unaffected by the loss of *CHD1*, were also added to the reactions. The right panel shows a control reaction with DNA from an untagged strain.

Figure 1

A

Gene	Fold
YOR387C	↑ 9.5
YGL258W	↑ 9.1
SSA2	↑ 2.9
YHR087W	↑ 2.6
SNZ1	↓ 3.0
CEM1	↓ 3.0
ARG4	↓ 2.9
YHR214C	↓ 2.9
HIS5	↓ 2.7
ARG1	↓ 2.7
CPA2	↓ 2.6
HIS4	↓ 2.6
YGL117W	↓ 2.6
YKL218C	↓ 2.5
YEL071W	↓ 2.5
ADE12	↓ 2.5
YOL119C	↓ 2.4
YCR101C	↓ 2.3
YJL200C	↓ 2.3
LEU1	↓ 2.2
YER175C	↓ 2.2
YDR391C	↓ 2.2

B

|	ACT1		CAR1		LYS9		SNZ1		YGL 258W	
	WT	Δ	WT	Δ	WT	Δ	WT	Δ	WT	Δ
FOLD										
Northern	1.0		2.8		3.0		3.8		13	
Array	1.0		2.2		2.8		3.0		9.1	

C

ACT1 —
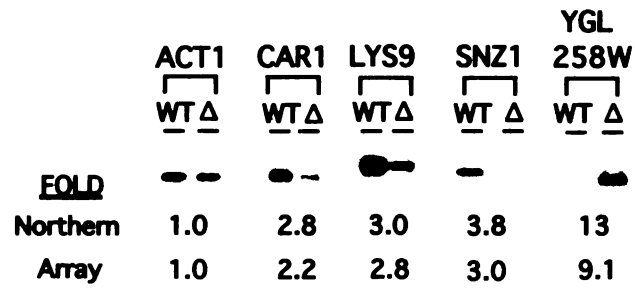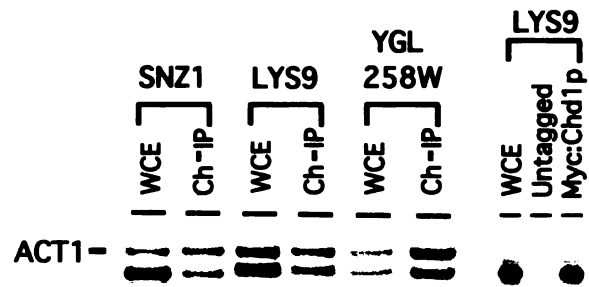
Figure 1-2. A *chd1* mutation is synthetically sick/lethal with *swi /snf* mutations.

A) A *chd1Δ* strain is severely sick when combined with a *swc1/2 (swi2)* or *swc3 (alr1)*

mutation. B) A *chd1Δ swi2Δ* double mutant and a *chd1Δ swi1Δ* double mutant (C) are

both unable to survive on 5FOA when forced to lose a wildtype copy of *CHD1* on a

plasmid containing the *URA3* gene (pAJ741). All strains in B) and C) possess the

pAJ741 plasmid before being streaked on 5FOA plates. D) A *swi4Δ chd1Δ* strain is

viable.

Figure 2

C

chd1Δ

swi1Δ

WT

swi1Δ
chd1Δ

D

chd1Δ

swi4Δ

WT

swi4Δ
chd1Δ
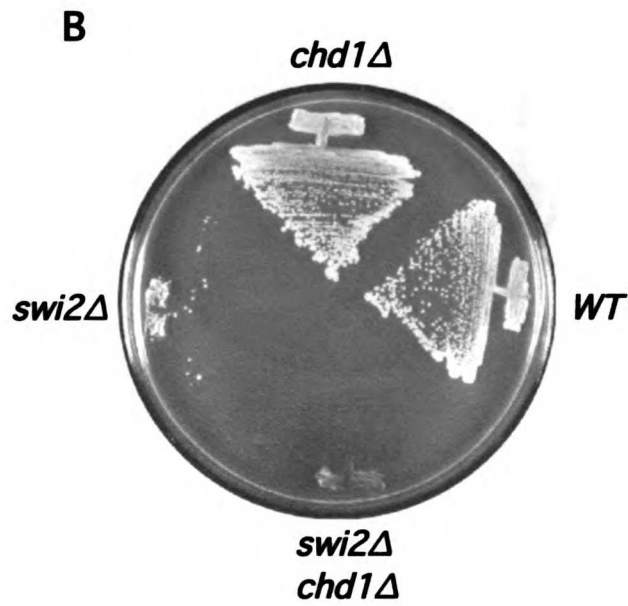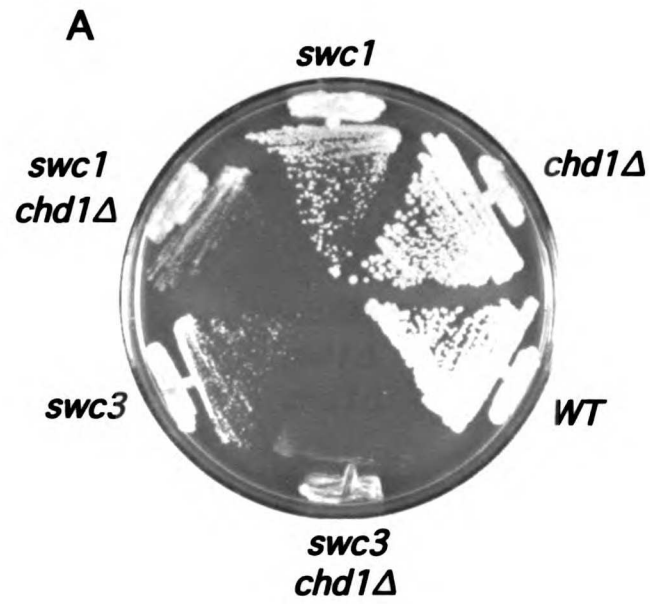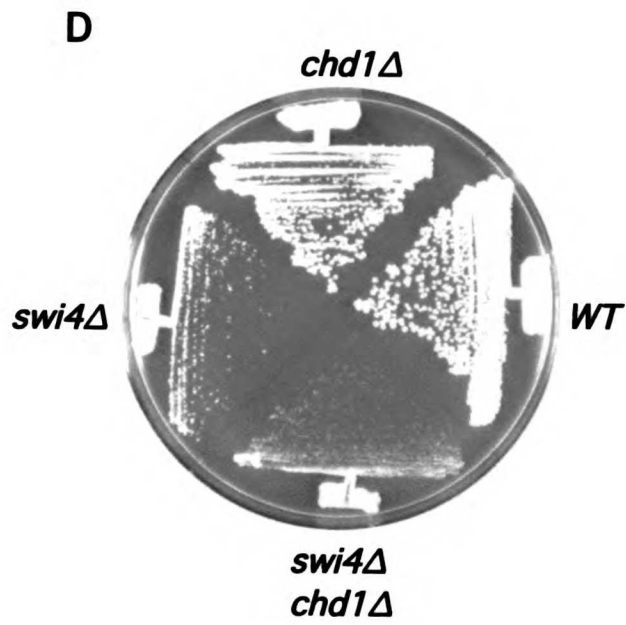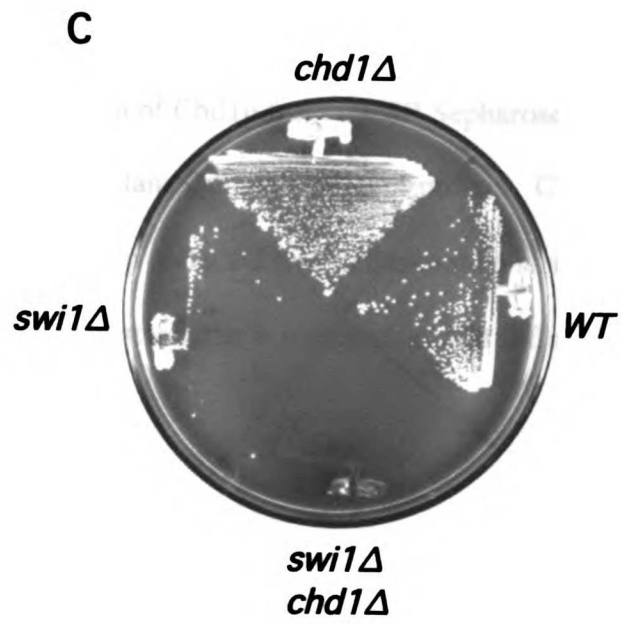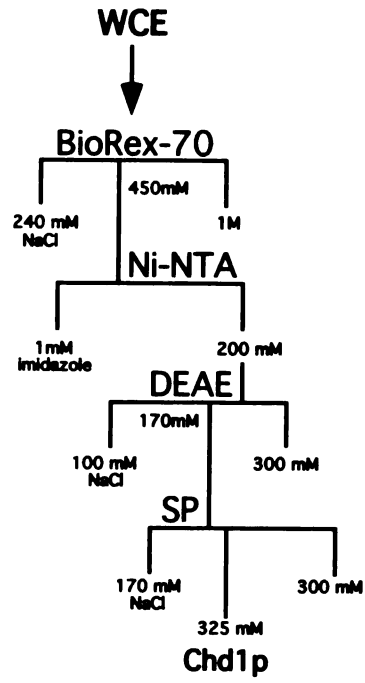
Figure 1-3. Purification of Chd1p.

A) Schematic flowchart of the chromatographic steps used to purify Chd1p. B) Silver stained gel of the most pure fraction of Chd1p from the SP-Sepharose column. Lane 1 contains 1 μl (~15 nanograms) and lane 2 contains 5 μl of protein. C) Western blot showing the elution profile of Chd1p collected from a Superose 6 sizing column. The elution profile of molecular weight standards is indicated as 670 (kDa), 158, 44, and 17.

Figure 3

A

WCE

↓

BioRex-70

450mM

240 mM NaCl   1M

Ni-NTA

1mM imidazole   200 mM

DEAE

170mM

100 mM NaCl   300 mM

SP

170 mM NaCl   325 mM   300 mM

Chd1p

B

175 —   —Chd1p

83 —

62 —

48 —

70

C

Void   *670*
 1   2   3   4   5   6   7   8   9 10 11 12 13 14

              158   *44*   *17*
15 16 17 18 19 20 21 22 23 24 25 26 27 28

Figure 1-4. Chd1p possesses DNA, and nucleosome, stimulated ATPase activity.

ATPase assays contained purified Chd1p (2 μl) and 1 pmol of nucleosomes (N) or the

equivalent amount of DNA (D) or core histones (H). As competitors, unlabeled ATP and

ATP-γ-S were added to the indicated reactions at a 5-fold molar excess over the standard

ATP concentration. The arrow points to the signal derived from free phosphate.
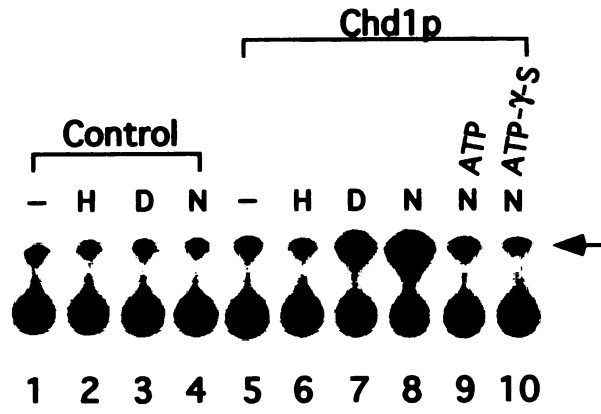
Figure 4

Figure 1-5. Chd1p alters nucleosome core particle structure. A) Purified Chd1p (0.5 μl) or purified SWI/SNF was incubated with mock-reconstituted 5S DNA (DNA) or nucleosome-assembled 5S DNA (Nucl. DNA) and subjected to DNase I protection analysis. Where indicated, ATP and its respective analogs were added to a final concentration of 1 mM. To challenge Chd1p activity, 1 μl of anti-Chd1p polyclonal serum (α-Chd1p) or pre-immune serum (pre-imm) was incubated with Chd1p for 5 min prior to the addition the 5S nucleosome core particles. Brackets and arrows indicate DNase I protected regions and DNase I hypersensitive sites, respectively, that are generated by Chd1p activity on mononucleosomes. B) Reactions identical to those in A were subjected to EMSA.

Figure 5

A

B

Chd1p-DNA, Nucl., DNA gel
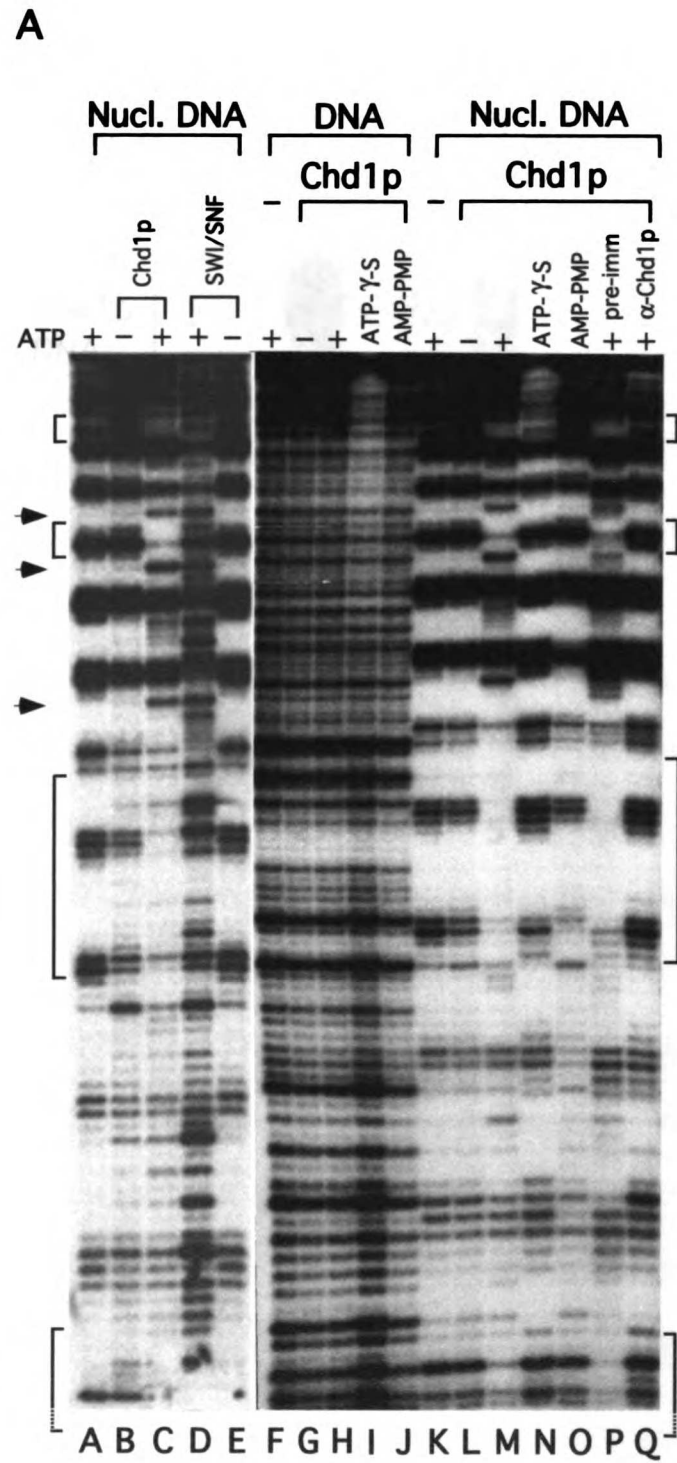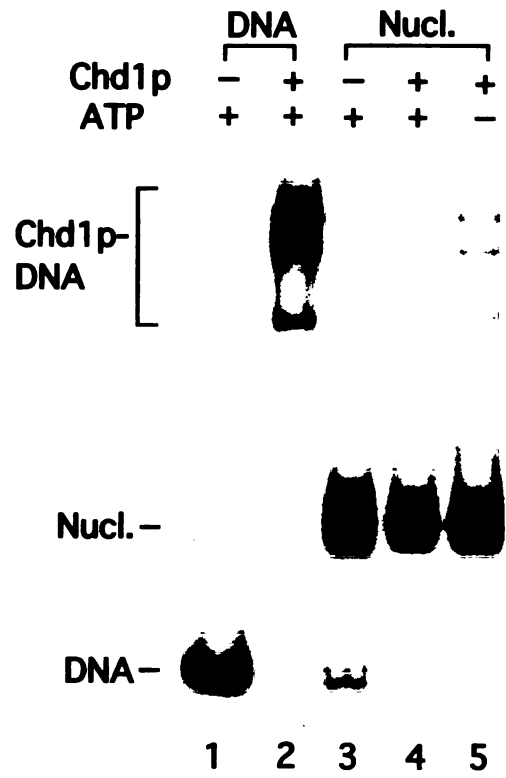
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CHAPTER 2:

A role for Chd1p in transcription elongation

ABSTRACT

CHD proteins have a likely role in transcription. However, genes that are directly regulated by Chd1p have not been identified. In addition, it is not known how CHD proteins function to control the expression of genes. To better understand the role of Chd1p in transcription, chromatin immunoprecipitation (ChIP)/microarray analyses was carried out to map the genomic distribution of Chd1p and to identify specific sites of action. Chd1p was found to be preferentially associated with genes encoding ribosomal proteins (RP) and *TEF2*, a translation elongation factor. ChIP/PCR analyses revealed that Chd1p is selectively associated with the coding regions of these genes. Detailed mapping by PCR showed that Chd1p is enriched along the entire length of the open reading frame and not at promoters or regions downstream of the coding sequences. In addition, Chd1p is only preferentially associated with *TEF2* when the gene is actively expressed. Together, these results indicate that Chd1p is indeed a transcriptional regulator that that it has a role in transcription elongation.

INTRODUCTION

Chromatin remodeling factors are found in all eukaryotes. These proteins alter

the structure of nucleosomes by perturbing the interactions between histones and DNA

(Kornberg and Lorch, 1999; Lee and Young, 2000). One consequence of these actions is

the reconfiguration of chromatin architecture around genes and other DNA sequences

which are exposed to remodeling complexes. Local nucleosome remodeling can lead to

either the stimulation or suppression of transcriptional activity. Though it is clear that

remodeling complexes have roles in transcription, the precise targets of most nucleosome

modifying activities have yet to be determined. Additionally, the precise manner in

which these activities controls transcription remains to be characterized.

The CHD remodeling proteins are thought to function as transcriptional

regulators. *Drosophila* CHD1 is localized to regions of active transcription while the

CHD3 protein interacts with the transcription factor Hunchback (Kehle et al., 1998;

Stokes et al., 1996). In mammals, the CHD3/4 proteins associate with histone

deacetylases, suggesting that they have a role in transcriptonal repression (Tong et al.,

1998; Xue et al., 1998; Zhang et al., 1998). Furthermore, genomic expression profiling

showed that the transcript levels of 2-4% of total yeast genes were affected in the absence

of Chd1p (Tran et al., 2000). Though these observations indicate that the CHD proteins

regulate transcription, genes that are directly regulated by CHD proteins have not been

identified.

In an effort to identify direct targets of CHD1 control, we examined the

localization of Chd1p on yeast chromosomes by chromatin immunoprecipitation (Ch-IP)

and microarray profiling. An initial attempt to identify genes regulated by Chd1p had

85

also employed microarray analyses and examined the changes in mRNA levels (Tran et al., 2000). However, this approach does not allow for a clear determination of which genes are directly regulated by Chd1p. In contrast to expression analysis, Ch-IP combined with microarray hybridizations surveys the physical distribution of Chd1p on chromosomes and can more effectively identify direct targets of Chd1p activity. The characterization of sites bound by Chd1p in living cells will also reveal cellular processes regulated by Chd1p and provide insight into possible modes of Chd1p transcriptional control.

MATERIALS AND METHODS

Strains

yHT151 (W303, Mat a, Myc::(His)6::CHD1::TRP1, ura3, trp1-1, leu2-3, his3-11, ade2-11, can1-100) and yHT190 (W03, Mat a, Myc::(His)6::CHD1::TRP1, crp1Δ::LEU2) were used for the ChIP/micro array analyses. ChIP/PCR analyses was carried out with yHT151, except for the analyses of Rap1 binding site deletion of the TEF2 promoter, in which ChIP was performed with yHT192 (W303, Mat a, Rap1Δ::TEF2::URA3).

Chromatin immunoprecipitations

Strains were grown in 250 ml of YEPD to an OD660 of 1. Formahdehyde was added to a final concentration of 1% to fix cells. The crosslinking was quenched after 20 minutes by addition of glycine to a final concentration of 125 mM. Cells were harvested and washed twice with 20 mM Tris pH 7.6, 150 mM NaCl. The cell pellet was aliquoted into 4 fractions, and lysed in 1 ml of buffer A (140 mM NaCl, 50 mM Hepes pH 7.6, 1% Triton X-100, 0.1% Na-Deoxycholate, 1 mM EDTA, 1 mM PMSF, 1 mM benzamidine, 1ug/ul of leupeptin, bestatin, and pepstatin.) and 1 ml of glass beads, in a biospec beadbeater (6 times at full power, 40 second bursts with 2 minute on ice). Cell lysate was spun down at 8,000 RPM (in Eppendorf 5417) and the chromatin-containing pellet was resuspended in 500 ul of buffer A. Chromatin was sheared by sonication (6 pulses of 15 seconds at a power setting of 2.5, 100% duty cycle, 2 minute ice bath in between, with a Branson 450 sonifier). The sample was spun at 13,000 RPM for 5 minutes. The supernatant was spun again at 15,000 RPM for 15 minutes.

The supernatant was then removed and 5 ul of anti-Myc monclonal antibodies

(Covance) was added. The antibodies were incubated for 4 hours, after which 50 ul of

protein G-sepharose beads were added and the incubated with the extract for an

additional hour. The beads were washed with 1 ml of buffer A twice (10 minutes each

wash), buffer A with 500 mM NaCl, once with buffer B (10 mM Tris pH 8.0, 0.25M

LiCl, 0.5% NP-40, 0.5% Na-Deoxycholate, 1 mM EDTA), and once with Tris pH 8.0, 10

mM EDTA. The precipitate was eluted off the beads with 1% SDS, Tris pH 8.0, 10 mM

EDTA at 65°C for 30 miutes. To reverse the crosslinking, the elution sample was

incubated at 65°C for 6-12 hours. Proteinase K was added (100 ug) and the sample was

incubated at 37°C for 2 hours. Phenol/chloroform extractions and ethanol precipitations

were carried out to purify the DNA. The DNA sample was then treated with RNAse A

(10 ug) for 1 hour at 37°C. The DNA was purified using a Qiagen kit for subsequent use

in microarray and PCR analyses.

Microarray analyses

The DNA obtained from two independent ChIP experiments (one with yHT 151

and one with yHT 190) was amplified in three consecutive rounds before being

hybridized to microarrays. Round A: 7 ul of DNA, 2 ul of sequenase buffer (5X) (US

Biochemicals), and 1 ul of primer A (5'-GTTTCCCAGTCACGATCNNNNNNNNN)

was incubated at 94°C for 2 minutes and 10°C for 5 minutes. At 10°C, 5.05 ul of a

reaction mix was added (1 ul 5x sequenase buffer, 1.5 ul 3 mM dNTPs, 0.75 ul 0.1 M

DTT, 1.5 ul 0.5 mg/ml BSA, 0.3 ul Sequenase). The following thermal cycling was then

carried out: ramp to 37°C over 8 minutes, hold at 37°C for 8 minutes, go to 94°C for 2

minutes, go to 10°C for 5 minutes while adding 1.2 ul of diluted Sequenase (dilute 4 fold

with dilution buffer), ramp to 37°C over 8 minutes. Add 43.75 ul of water to each reaction. Round B: To 15 ul of Round A DNA, add 8 ul of 25 uM MgCl2, 10 ul of 10X PCR buffer, 2 ul of 25 mM dNTPs, 1 ul of primer B (5'-GTTTCCCAGTCACGATC), 1 ul Taq, and 63 ul water. The following thermal cycling was carried out: 92°C for 30 seconds, 40°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute; 15 cycles. Round C: same as for Round B except 2ul of 25 mM dATP, 25 mM dGTP, 25 mM dCTP, 10 mM dTTP, and 15 mM aa-dUTP were used instead of the standard dNTPs. Coupling, hybridization, and analyses were carried out as in Tran et. al., 2000.

RESULTS

Chd1p associates with coding sequences

In an attempt to uncover the genomic localization of Chd1p, and to identify genes that are directly regulated by this remodeling factor, chromatin immunoprecipitation followed by microarray analyses (Ch-IP/CHIP) was carried out. This approach has been taken to examine the global distribution of several transcription factors in the yeast genome (Iyer et al., 2001; Ren et al., 2000). Strains containing a (Myc)6::Chd1p fusion were grown in rich media, harvested and treated with formaldehyde to covalently crosslink proteins to chromatin. After cell lysis, the chromatin was purified and sonicated to generate a population of DNA fragments with a size spectrum of 200-800 base pairs. Chd1p was specifically precipitated from the chromatin fraction using Myc antibodies, and DNA coupled to Chd1p was isolated. After reversing the crosslinking, the DNA was amplified and labeled with a fluorescent dye. This DNA was hybridized together with a genomic DNA reference sample to microarrays containing both the coding and intergenic regions of the yeast genome.

Two independent experiments were performed, and DNA sequences that were preferentially precipitated by Chd1p in both experiments (by a factor of 2.5 or greater relative to the reference sample) are shown in Table 1. Of the sixteen loci listed, five corresponded to intergenic regions and nine to the coding regions of genes. The majority of these genes have a role in protein synthesis, encoding ribosomal components (*RPLs*), a translation elongation factor (*TEF2*), or a protein involved in rRNA processing (*NOP1*). In addition, for each of the two array profiles, about 25% of the 40 highest ranking non-intergenic ORFs correspond to genes encoding components of the translation apparatus.

Because it is known that genes involved in protein synthesis are coordinately regulated (Warner, 1999), the microarray results suggest that Chd1p may also participate in the transcriptional control of these genes. Therefore, we focused our analyses on the localization of Chd1p to these sites.

To verify the recruitment of Chd1p to genes detected by the Ch-IP/CHIP experiment, quantitative PCR was performed. Independent PCR reactions were carried out, using equal amounts of either Ch-IP DNA or total input DNA, and primers encompassing the promoter regions of *TEF2*, *RPL19A*, and *RPL8A*. The promoter regions were initially chosen for analysis because regulators of genes encoding ribosomal proteins (RP) have been crosslinked primarily to these sequences (Reid et al., 2000). The PCR results, however, show that there was no enrichment of promoter sequences in the Chd1p precipitated DNA (Figure 2-1A, lanes 1,3, & 4). In contrast, when the PCR was performed using primers directed towards the coding region, a significantly greater amount of product was generated in the reactions containing Ch-IP DNA versus reactions containing total DNA (Figure 2-1A, compare lanes 1 & 2, Figure 2-1B). Quantitation of the PCR products from the coding sequence reactions revealed that approximately five fold more DNA was amplified in reactions containing Ch-IP DNA. The coding region for *ACT1*, a gene unaffiliated with protein synthesis, was not precipitated by Chd1p to the same extent as seen for *TEF2* or *RPL8A* (Figure 2-1A, lane 5).

To more comprehensively map the distribution of Chd1p at the *TEF2* gene, PCR reactions were carried out using a series of primer pairs spanning the locus, from 900 base pairs upstream of the initiating ATG to 542 base pairs downstream of the stop codon (Figure 2-2). The PCR analyses show that Chd1p is preferentially crosslinked along the

91

entire coding region of *TEF2*. Chd1p does not appear to be coupled as efficiently to sequences upstream or downstream of the open reading frame. In particular, Chdp1 is not enriched at Rap1p sites. These results are in contrast to that seen for known activators of *TEF2* and RP genes (Reid et al., 2000). In addition, the distinct localization of the protein to the transcribed regions of genes implicates a role for Chd1p in transcription elongation.

Chd1p is specifically recruited to RP genes

Because genes encoding ribosomal proteins are coordinately regulated (Reid et al., 2000; Warner, 1999) and because many RP loci were enriched in the Ch-IP/CHIP analyses, we examined the general recruitment of Chd1p to this class of genes. PCR reactions were carried out to investigate the presence of Chd1p at an additional eight RP genes, some of which were not identified by the microarray experiment. The coding region of all RP genes assayed were preferentially precipitated by Chd1p (Figure 2-3A). Depending on the gene, the enrichment of the ORF sequences varied from 2-5 fold. The association of Chd1p at all of the RP loci tested suggests that the protein is likely to be recruited to a majority of the genes in this family.

Genes encoding ribosomal subunits are highly expressed in rapidly growing cells (Warner, 1999). It is possible that Chd1p may be broadly localized to actively transcribed genes, and not specifically to RP genes. Therefore, we examined GAL and heat shock response genes that are robustly expressed when cells are grown in galactose or at high temperatures, respectively. To analyze GAL genes (*GAL1, GAL2*), Ch-IP was carried out with cells grown in glucose media and in galactose. For investigation of heat shock genes (*HSP12,SSA4*), Ch-IP was performed on cells before and after a heat pulse.

PCR analyses of the Chd1p precipitated DNA show that the association of Chd1p at *GAL1*,*GAL2*, *HSP12*, and *SSA4* is not enhanced under inducing conditions (Figure 3B and 3C). Rather, for *HSP12* and *SSA4*, we observed a decrease in the amount of Chd1p coupled to the coding regions after heat shock. The determination that Chd1p is not globally enriched at highly expressed genes indicates that Chd1p is specifically recruited to the RP coding sequences.

Transcription dependent localization of Chd1p

To characterize the functional significance of Chd1p recruitment, we examined the dependence of Chd1p localization on transcriptional activity. The chromatin immunoprecipitation experiments described thus far have been performed under growth conditions in which protein synthesis-related genes are highly expressed. In order to assess the occupancy of Chd1p at these genes when they are minimally transcribed, Ch-IP was carried out with cells grown at 30°C and briefly shifted to 42°C. RP (and *TEF2*) gene expression is repressed as part of the cellular response to heat shock (Warner, 1999). PCR analyses reveal that although Chd1p is efficiently coupled to*TEF2* at 30°C, its coupling is much reduced at 42°C (Figure 2-3C). Therefore, the association of Chd1p with the*TEF2* gene correlated with the elevated transcription of the gene.

In a different approach designed to decrease transcription at *TEF2*, Rap1p binding sites were removed from its promoter. Rap1p is the primary activator of *TEF2* and RP genes (Warner, 1999). A plasmid containing the*TEF2* promoter with the Rap1 sites deleted was integrated into the genome at the *TEF2* locus. Because of the nature of the integration event, two copies of *TEF2* were generated. One copy contains an intact promoter but has a truncated coding region. The other contains a promoter without

Rap1p sites and includes the complete coding sequence of *TEF2* (Figure 2-4A). Ch-

IP/PCR analyses show that Chd1p is preferentially crosslinked to the truncated*TEF2*

copy possessing an intact promoter but not at the *TEF2* locus lacking Rap1 binding sites

(Figure 2-4B). This result, as well as that described above, shows that Chd1p is recruited

to *TEF2* only when the gene is transcriptionally active.

DISCUSSION

Previous studies have indicated that the CHD family of proteins have chromatin remodeling activity and play a role in transcription. However, their precise mode of regulation and molecular sites of action have not been clearly defined. In this study, we have identified a class of genes to which Chd1p is specifically recruited. Additionally, our analyses have provided further support for the involvement of Chd1p in transcription elongation.

Chd1p is specifically associated with RP genes

Employing chromatin immunoprecipitation, genomic microarrays, and PCR, the specific localization of Chdp1 to a subset of chromosomal sites has been determined. Chd1p is enriched at the coding sequences of genes encoding translation components, mainly ribosomal proteins. There are 137 RP genes and they are coordinately regulated such that changes in growth conditions and nutrient availability produce a concomitant shift in their expression (Warner, 1999). Rap1p is the primary activator of RP genes and almost all RP genes contain Rap1p binding sites in their promoters (Lascaris et al., 1999). Recently, the Esa1p protein, a histone acetyltransferase, has also been shown to be a regulator of RP genes (Reid et al., 2000). Like Rap1p, Esa1p can be crosslinked to the promoter regions of RP genes and depletion of Esa1p leads to a decrease in RP transcription. The specific recruitment of Chd1p to RP genes suggests that the protein may act in conjunction with Rap1p and Esa1p to coordinately regulate the expression of protein synthesis factors.

Chd1p is recruited to actively transcribed RP genes

To assess the functional role of Chd1p at RP genes, we examined the effect of transcription on Chd1p localization. When RP expression is repressed (by heat shock or deletion of Rap1 sites), Chd1p is no longer enriched at these genes. These results suggest that Chd1p is a positive regulator of RP transcription. Chd1p though, is not required for RP transcription because a *chd1* deletion strain is viable and does not have a noticeable growth defect (Tran et al., 2000; Woodage et al., 1997). Therefore, the protein may act to enhance the expression or to facilitate the induction of RP genes. In previous expression analyses of a *chd1Δ* strain (Tran et al., 2000), RP transcription was apparently unaffected. However, the cells in those experiments were grown in minimal media and harvested in late log phase, conditions in which RP activity is diminished (Warner, 1999). As a result, the absence of Chd1p would not have produced a noticeable defect in RP transcription.

In contrast to the RP loci, active transcription may antagonize the association of Chd1p with heat shock response genes. We observed that the level of Chd1p at the coding region of *HSP12* and *SSA4* were significantly lower at 42°C than at 30°C. Since both of these genes are induced when cells are shifted to high temperatures, these results suggest that Chd1p may function as a repressor of heat shock genes at 30°C.

Because Chd1p is an abundant protein with non-specific DNA binding properties, it may be globally, and randomly, distributed throughout the genome (Stokes and Perry, 1995; Tran et al., 2000). However, Chd1p may still be recruited to particular sites (e.g. RP genes) while being displaced from others (e.g. heat shock genes). Rap1p or Esa1p activity may selectively localize Chd1p to RP genes. Both transcriptional activators and histone acetyltransferases have been demonstrated to facilitate the binding of remodeling

factors to chromatin templates (Agalioti et al., 2000; Cosma et al., 1999; Goldmark et al., 2000; Hassan et al., 2001). Furthermore, it has been shown that the chromo domain of HP1 can bind preferentially to histone tails methylated at lysine 9 (Bannister et al., 2001). The modification of histones at RP and heat shock genes may positively or negatively affect the binding of Chd1p (via the chromo domain) at these loci and contribute to the specificity of Chd1p localization. It is also possible that Chd1p is a component of, or generally associated with RNA polymerase. As a result, Chd1p may be globally and preferentially crosslinked to heavily transcribed genes. Chd1p, however, is not enriched at GAL and heat shock genes under inducing conditions, suggesting that it is specifically recruited to RP genes. Because GAL and heat shock genes are not as abundantly transcribed as RP genes, even under inducing conditions, it remains a possibility that Chd1p generally associates with elongating polymerases.

A role for Chd1p in elongation

Utilizing Ch-IP/PCR, we have mapped the distribution of Chd1p at the *TEF2* locus. Unlike other regulators of RP related genes, Chd1p is predominantly associated with the coding region, and not enriched at the promoter. One explanation for the coupling of Chd1p to transcribed sequences is that Chd1p may function in transcription elongation. Several lines of evidence support this idea. A *chd1* deletion strain is resistant to 6-azauracil, an inhibitor of transcription elongation (Woodage et al., 1997). The mammalian Chd1 protein interacts with SSRP1 (Kelley et al., 1999), a component of the FACT complex that facilitates transcription elongation through chromatin templates (LeRoy et al., 1998; Orphanides et al., 1999). The *S. cerevisiae* homologue of FACT, composed of Spt16p and Pob3p, has also been purified as a complex and genetically,

SPT16 interacts with genes encoding elongation components (Brewster et al., 1998; Orphanides et al., 1999). In addition, the growth defect of a *pob3* mutant is suppressed by mutations in *CHD1* (Costa and Arndt, 2000). These findings, together with the results of our study, strongly suggest that Chd1p acts as in transcription elongation. Because Chd1p has chromatin remodeling capabilities, it may assist RNA polymerase transcribe through nucleosomal barriers. The SWI/SNF complex has been observed to facilitate RNA polymerase transcribe through chromatin imposed pause sites (Brown et al., 1996).

Chd1p appears to have both positive and negative effects on transcription. Genome wide expression analysis of *CHD1* has shown that an equal number of genes were affected negatively or positively in the absence of *CHD1* (Tran et al., 2000). At the RP loci, Chd1p apparently acts as an activator while at the heat shock genes, Chd1p may inhibit transcription. This observation is consistent with previous studies on Chd1p. In *Drosophila* Chd1 has been localized to puffs and interband regions of polytene chromosomes, areas associated with active transcription (Stokes et al., 1996). In yeast, the 6-azauracil resistance of a *chd1* deletion strain has implicated Chd1p as an inhibitor of transcription (Woodage et al., 1997). It is not uncommon for transcriptional regulators to exert opposing effects on gene expression. The Rap1p protein is an activator of RP genes but also functions as a repressor (Warner, 1999). The Spt4/Spt5 complex, found in yeast and humans, is a translation elongation factor that both facilitates and hinders transcription (Hartzog et al., 1998; Wada et al., 1998). Therefore, Chd1p can conceivably have dual and opposing roles in regulating gene activity.

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99

Table 1: Summary of chromatin-IP/microarray analyses

LOCI	FOLD #	DESCRIPTION
*TEF2**	5.3	Translation elongation factor
*RPL19A**	3.7	Ribosomal protein
iYNL339C	3.7	Intergenic
iYJL148W	3.5	Intergenic
NOP1	3.2	rRNA processing, 35S
*RPL8A**	3.2	Ribosomal protein
ERG1	3.2	Sterol metabolism
YIL082W	3.1	Unknown
iSNR190	3.1	Intergenic
iYGRCdelta18	3.0	Intergenic
SRO9	2.9	Actin filament organization
RET2	2.9	Vesicle coat component
iYLRCdelta18	2.9	Intergenic
TOM71	2.7	Translocase component
*RPL26B**	2.7	Ribosomal protein
*RPL33A**	2.5	Ribosomal protein

The fold value is the average from two experiments and represents the ratio of Chd1p precipitated DNA versus total genomic DNA (as determined by the ratio of Cy5/Cy3 fluorescence.) Only loci with a fold value of 2.5 or higher are shown.

* Regulated by Rap1p

FIGURE LEGENDS

Figure 2-1. Chd1p associates with the coding region of genes identified by

ChIP/microarray analyses. (A) ChIP/PCR analyses show that Chd1p is not enriched at

the promoters of *TEF2*, *RPL19A*, and *RPL8A*. Instead, Chd1p is enriched in the coding

regions of *TEF2* (lane 2) and *RPL8A* (B). "T" represents total DNA input and "IP"

signifies Chd1p precipitated DNA.

Figure 2-1

A
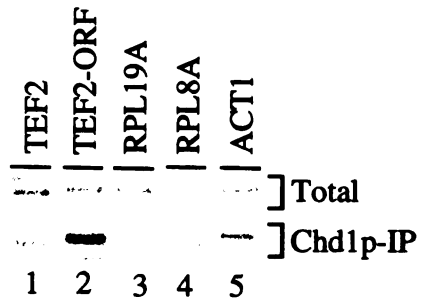
B
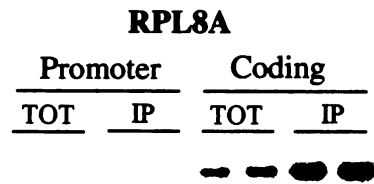
Figure 2-2. Chd1p is enriched along the entire coding sequence of *TEF2*. ChIP/PCR

analyses was carried out using primers spanning the entire *TEF2* locus. The results

demonstrate that Chd1p is preferentially associated along the entire open reading frame

of *TEF2*, but not at the promoter region or downstream of the ORF.

Figure 2-2
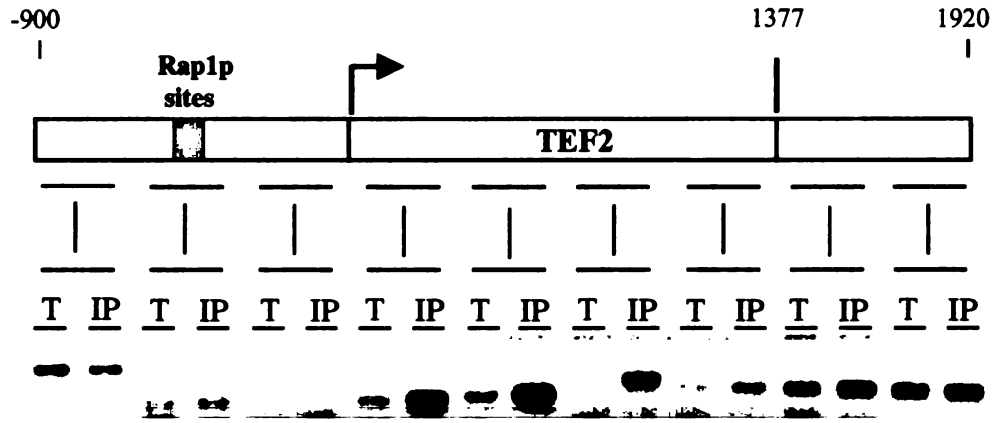
Figure 2-3. Chd1p is specifically recruited to RP genes and not generally to highly

expressed genes. (A) ChIP/PCR analyses indicates that Chd1p is enriched at the coding

regions of an additional eight RP genes examined. The fold enrichment of RP genes in

Chd1p precipitated DNA versus total DNA varies from 2-5 fold. (B) Chd1p is not

preferentially localized to GAL genes under inducing conditions. ChIP was performed

on cell grown in either glucose (GLU) or galactose (GAL). (C) Chd1p is not enriched at

actively transcribed heat shock genes and is no longer enriched at *TEF2* after heat shock.

ChIP was carried out using cells grown at 30 degrees and cells heat shocked (HS) at 42

degrees for 20 minutes.
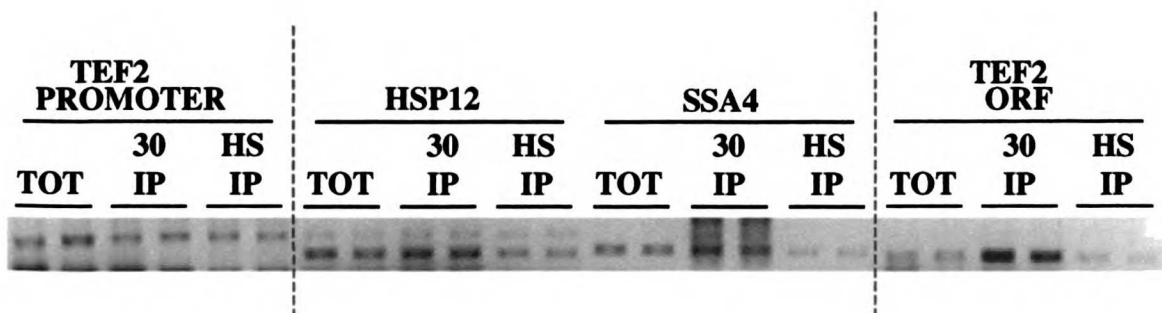
Figure 2-3

A
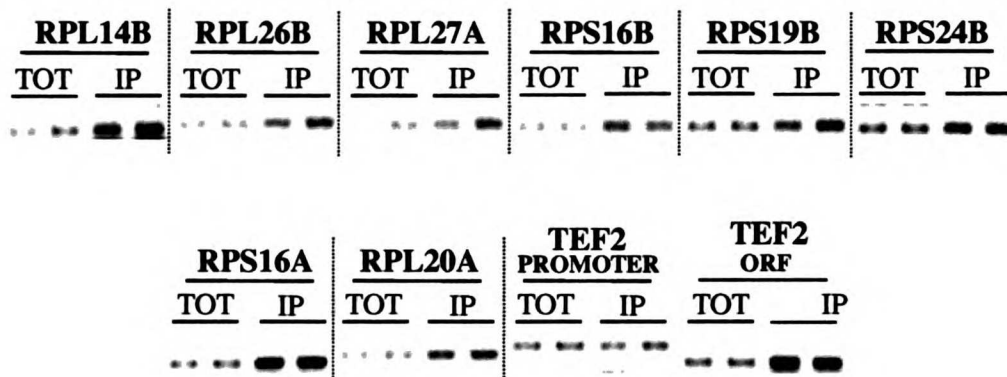
B
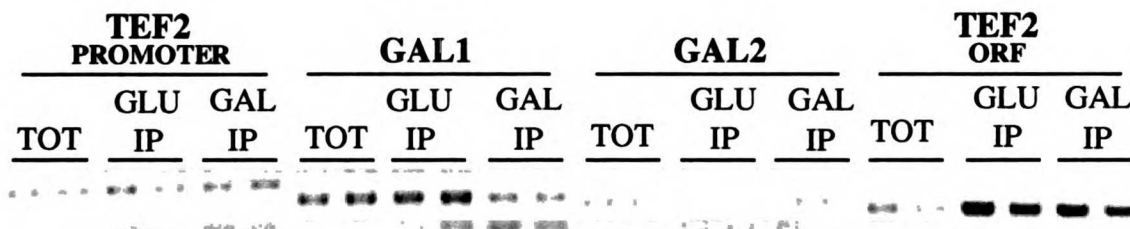
C

Figure 2-4 Deletion of Rap sites diminishes Chd1p association at *TEF2*. (A) Schematic diagram of the *TEF2* locus after integration of a Rap1 site deletion plasmid. Two copies of *TEF2* are generated after the integration event; one contains an intact promoter region with a truncated ORF while the other contains a promoter with the Rap1 site deleted and a truncated ORF. (B) ChIP/PCR analyses show that deletion of Rap1 sites decreases Chd1p binding to the coding region of *TEF2* (segment 3). Chd1p is still preferentially associated with the *TEF2* ORF downstream of an intact promoter (segment 2).

Figure 2-4
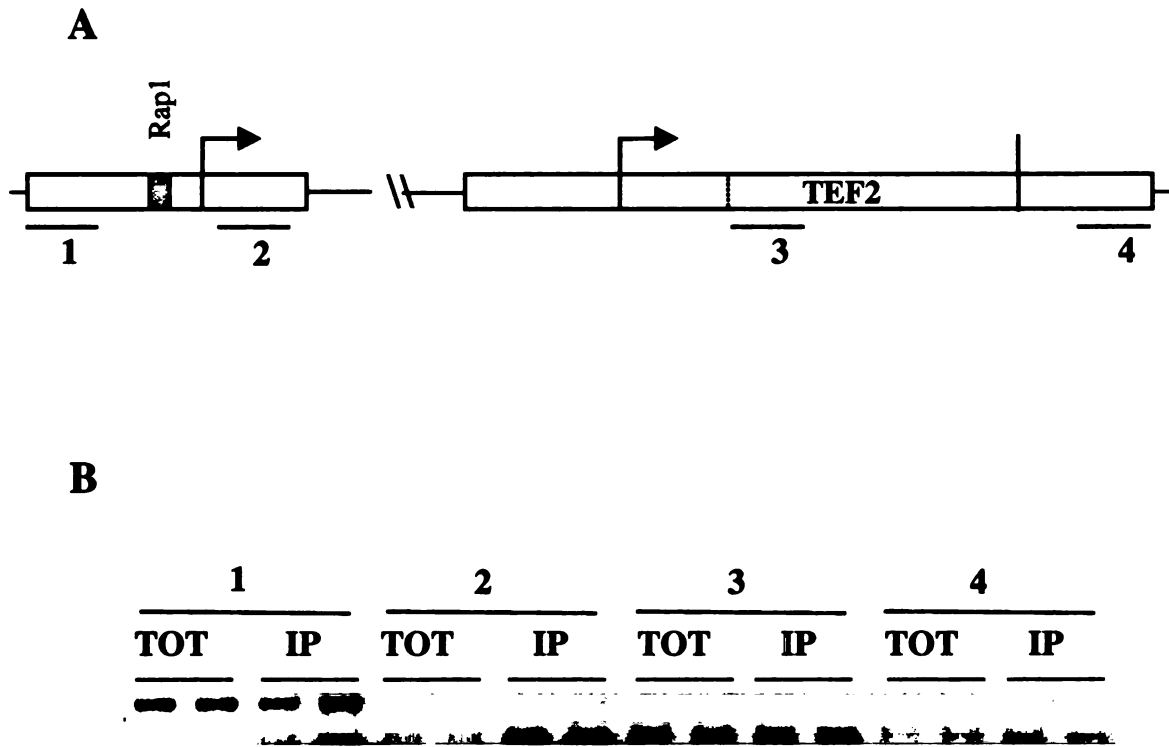
A

B

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CHAPTER 3:

Identification of Chd1p-interacting proteins

ABSTRACT

Chromatin modifying factors are recruited to specific sites through their interactions with transcription factors and co-regulators. Additionally, their biochemical activities are modulated by interacting proteins. To better understand the regulation of Chd1p localization and activity, an affinity purification was performed utilizing a chromo domain fragment of Chd1p. Three proteins were identified that were differentially retained on the chromo domain column and not on a control matrix. The proteins were determined to be Hsl7p, Lhp1p, and Yil105p (renamed Crp1p) by mass spectrometry. All three proteins have been implicated in transcription or chromatin structure. The association between Chd1p and Crp1p has been verified by co-immunoprecipitation experiments. Genetic and genomic analyses of *CRP1* has been carried out and a subset of genes that may be coordinately regulated by Chd1p and Crp1p have been identified.

INTRODUCTION

Nucleosome modifying activities are recruited to specific chromosomal sites where they alter the local structure of chromatin. These factors are brought to particular loci through their interaction with DNA binding proteins and transcriptional co-regulators. Swi5p mediates SWI/SNF binding at the HO promoter (Cosma et al., 1999) while Ume6p targets ISWI and Rpd3p to sporulation genes (Goldmark et al., 2000). The *Drosophila* CHD3 protein has been shown to interact with Hunchback, a transcriptional repressor (Kehle et al., 1998). However, little is known about how other CHD proteins are recruited to their sites of action. For example, it is not known how Drosophila CHD1 is localized to puffs and interband regions of polytene chromosomes or how the distribution of yeast Chd1p is regulated.

It is likely that the chromo domain of CHD proteins play a role in their selective localization. The chromo domain of mammalian CHD1 is required for its proper association with chromatin (Kelley et al., 1999). The chromo domains of other proteins have also been shown to be required for their specific localization. Mutations in the chromo domain of Polycomb abolishes its proper recruitment to chromosomal sites (Messmer et al., 1992). In *S. pombe*, the heterochromatin binding domain of Swi6 has been mapped to its chromo domain. In addition, the chromo domain can confer protein specific localization properties. In the incorporation of the Polycomb chromo domain into the HP1 protein mislocalizes the fusion protein to Polycomb specific locations (Platero et al., 1995).

Recent studies have revealed two distinct molecular interactions involving the chromo domain that may function in directing chromo domain proteins to their sites of

action. MOF, a *Drosophila* dosage compensation factor, can bind non-coding RNA via the chromo domain (Akhtar et al., 2000). This association with RNA has been proposed to be necessary for proper MOF localization. The mammalian HP1 chromo domain protein has been shown to bind specifically to methylated lysine 9 on histone H3 peptides and to full length H3 (Bannister et al., 2001). In competition experiments with H3 peptides methylated at lysine 9, HP1 is displaced from the nucleus (where it is normally bound to chromatin). These results suggest that the selective localization of HP1 is determined by the methyl-lysine binding capability of the chromo domain.

The generality of the RNA and methyl-lysine binding properties for chromo domain proteins is not clear. The chromo domain of Polycomb, SUV391H1, and Mi2 (CHD3) do not bind methylated lysine peptides (Bannister et al., 2001). Therefore, it is possible that not all chromo domain proteins possess methyl-lysine (or non-coding RNA) binding properties. Instead, certain chromo domains may promote interactions with non-histone proteins. Chromo domain proteins have been purified as components of multiprotein complexes. It is possible that the chromo domain may mediate interactions with members of these complexes. Additionally, a motif closely related to the chromo domain, the chromo shadow domain, has been demonstrated to interact with transcription factors by the two-hybrid assay (Bannister et al., 2001).

To further characterize the function of the chromo domain, and to investigate how Chd1p is localized, we initiated an affinity purification utilizing the chromo domain of the *S. cerevisiae* Chd1p. The identification of associating factors will provide further insight into the different type of protein interactions mediated by the chromo domain. In

116

addition, the characterization of these proteins may uncover the mechanism by which

Chd1p is selectively recruited.

MATERIAL AND METHODS

Strains

The SF10 (BJ5459, Mat a, ura3-52, trp1, leu2Δ1, his3Δ200, pep4::HIS3, prb1Δ1.6R, can1) strain was used for the affinity purification. Strains yHT149 (SF10, Myc::His::CHD1::TRP1) and yHT 150 (SF10, Myc::His::CHD1::TRP1, HA::CRP1::URA3) were used for the co-immunoprecipitation experiments. For tetrad analyses, yHT69 (W303, Mat a/α, chd1Δ::HIS3/+, crp1Δ::LEU2/+, ura3/ura3, trp1-1/trp1-1, leu2-3/leu2-3, his3-11/his3-11, ade2-11/ade2-11, can1-100/can1-100), yHT88 (W303, a/α, crp1Δ::LEU2/+, ynl047cΔ::URA3/+, ade2/ade2, ade3/ade3), yHT138 (W303, a/α, pGal::HA::CRP1::URA3/+, ynl047cΔ::LEU2/+), and yHT70 (W303, a/α, chd1Δ::HIS3/+, ynl047cΔ::URA3/+) diploids were sporulated and dissected. For the expression profiling, RNA was obtained from strains yHT175 (W303, Mat α, leu2::LEU2), yHT177 (W303, Mat α, chd1d::LEU2), and yHT65 (W303, Mat α, crp1d::LEU2).

Affinity chromatography

A chromo domain fragment consisting of amino acids 122-300 fused to glutathione S-transferase (GST) (pAJ737), was expressed in DH5-α and purified utilizing glutathione beads (Sigma). 5 mg of the fusion protein and 5 mg of GST were crosslinked separately to 1 ml of Affigel 10 resin (Biorad). Two 1 ml columns were generated from the protein:affigel matrix. Whole cell yeast extract (SF10) was prepared from a 3 L culture (similar to Tran et al.) and applied simultaneously to both columns (in buffer A: 75-150 mM NaCl, 50 mM Hepes pH 7.6, 10% glycerol, 0.5% Triton-X 100, 10 mM Mg(Oac)2, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 5 mM benzamidine, 1 ug/ml

leupeptin, 1ug/ml pepstatin, 1 ug/ml bestatin). The columns were washed with 20 ml of

buffer A. Proteins were eluted by the application of 1 M NaCl buffer A (without Triton-

X 100) followed by 1 M urea buffer A in 1 ml fractions. 100 ul of each fraction was

TCA precipitated and the sample was analyzed by SDS-PAGE. To determine the protein

identity of p90, p80, and p40, the proteins were excised from a coomassie stained

acrylamide gel and sequenced by mass spectrometry (Yates, j, j. protein chem., 1997).

Co-immunoprecipitations

Cells were grown in YEPD to an OD660 of 2 and harvested. The cell pellet was

resuspended in 400 ul of lysis buffer (200 mM NaCl, 50 mM Hepes pH 7.6, 10%

glycerol, 0.5% NP-40, 1 mM EDTA, 1 mM PMSF, 2 mM benzamidine, 1 ug/ml

leupeptin, 1ug/ml pepstatin, 1 ug/ml bestatin). 700 ul of glass beads were added to the

resuspension and cells were lysed by agitation in an Eppendorf mixer for 40 minutes.

The lysate was clarified by a 5 minute spin at 14, 000 rpm in an Eppendorf 5417C

microcentrifuge. The supernatant was removed and spun again for 15 minutes. 200 ul of

the supernatant was then diluted into lysis buffer (without NaCl) to a final volume of 500

ul at 80-105 mM NaCl. 25 ul of beads with coupled anti-HA or anti-Myc antibodies

were added to the diluted supernatant and incubated for 3-4 hours. The beads were

washed 3X with lysis buffer, 1X with lysis buffer without glycerol and 1X with 50 mM

Hepes pH7.6, 150 mM NaCl, and 1 mM EDTA. Proteins bound to the beads were

analyzed by Western blot analyses.

Micro array expression profiling

Total RNA was isolated from cells grown in 200 ml of YEPD to an OD660 of 1

or in SD (-LEU) to an OD660 of 2. Poly A+ RNA was purified from total RNA using

oligo dT resin (Invitrogen). Hybridizations and analyses were carried out as previously

described (DeRisi, 1997, Science).

RESULTS

Interactions with the chromo domain

An affinity chromatography purification was carried out utilizing a chromo domain of fragment Chd1p (see Materials and methods). In a previous purification, Chd1p did not associate with any other proteins in stoichiometric amounts. It is possible, however, that a Chd1p complex may have disassembled during cell lysis. Also, components that transiently or conditionally interact with Chd1p *in vivo* may associate with Chd1p in non-stoichiometric amounts and therefore, may not have been identified. Affinity chromatography is a sensitive experimental approach that can detect low affinity interactions as well as interactions with minimally expressed proteins.

A translational fusion containing the chromo domain and glutathione S-transferase (GST)was expressed in *E. coli*, purified, and coupled to an Affigel resin (BioRad). Two columns were prepared, one with the chromo domain fusion and one with a GST control (Figure 3-1A). Whole cell yeast extract was applied over both columns simultaneously. The columns were washed with low salt and bound proteins were eluted with 1M NaCl. The protein samples were then precipitate and analyzed by SDS-PAGE.

Three proteins were noticeably, and differentially retained on the chromo domain column and not on the GST control (Figure 3-1B). These proteins were designated p90, p80, and p40 based on their predicted molecular weights. To determine their identity, the proteins were resolved on an acrylamide gel, excised, and sequenced by mass spectrometry (courtesy of David Schieltz, Univ. of Washington). Peptide sequences were

obtained for each of the excised bands, with the majority matching the protein sequences of Hsl7p (p90), Yil105p (p80), and Lhp1p (p40) (Table2).

Hsl7p is a negative regulator of Swe1p, a protein kinase that phosphorylates the Cdc28p cell cycle regulator (Ma et al., 1996), and Ste20p, a protein kinase that regulates the MAPK and other signal transduction pathways (Fujita et al., 1999). Lhp1p is required for tRNA processing and binds to the 3' end of nascent polymerase III transcripts (Yoo and Wolin, 1994; Yoo and Wolin, 1997)Yil105Cp is an uncharacterized protein that is similar to Ask10p (33% identity), a putative DNA binding transcriptional activator (Page et al., 1996). The similarity to Ask10p suggests that Yil105Cp may function as a transcription factor and that its interaction with Chd1p may regulate the recruitment of Chd1p to specific sites. Therefore, we focused our investigation on Yil105Cp.

To verify the association between Chd1p and Yil105p, renamed Crp1p (Chromo domain interacting protein), co-immunoprecipitation experiments were carried out. Whole cell extracts were obtained from a strain containing Myc::Chd1p and HA::Crp1p. Chd1p was precipitated from the extracts using Myc antibodies coupled to protein A sepharose beads. Western blot analyses show that Crp1p co-precipitates with Chd1p (Figure 3-2). Conversely, in immunoprecipitations with anti HA antibodies, Chd1p is also present with Crp1p in the precipitant. In immunoprecipitations using HA antibodies and extracts containing untagged Crp1p, Chd1p was not precipitated. These results demonstrate that Chd1p and Crp1p physically associate in yeast extracts.

Genetic analyses of *CRP1*

Given that Chd1p and Crp1p can physically interact, we sought to determine if there was also a genetic interaction between *CHD1* and *CRP1*. A *crp1* deletion strain was generated, and the mutant had no significant growth defect (Figure 3-3A). A *chd1Δ crp1Δ* double mutant also displayed wild type growth (Figure 3-3A). This finding indicates that there in terms of growth, there is no synthetic interactions between *chd1* and *crp1*.

Since cells do not require *CRP1* for viability, it is possible that other genes may have redundant or overlapping functions with *CRP1* and can compensate for its absence. In addition to Ask10p, two other proteins are encoded in the yeast genome that share sequence identity with Crp1p: Ynl047p (53%), and Ypr15p (34%). Because of the high sequence identity between Crp1p and Ynl047p, it is possible that the two proteins have very similar functions. Therefore, we examined the phenotype of *ynl047Δ* and *ynl047Δ crp1Δ* mutants. When *YNL047C* is deleted, cells are viable (Figure 3-3B). However, a *crp1Δ ynl047cΔ* mutant is inviable, as determined by tetrad analysis of a *CRP1/crp1Δ, YNL047C/ynl047cΔ* diploid (Figure 3-3B). To confirm the synthetic lethal interactions, the *CRP1* genes was placed under the control of a galactose inducible promoter (pGAL). A *pGAL::CRP1/CRP1,YNL047C/ynl047cΔ* diploid was sporulated and tetrads were dissected onto either glucose (GAL promoter is repressed) or galactose plates. In glucose media, *pGAL::CRP1 ynl047cΔ* haploids were inviable (Figure 3-3C). In contrast, the *pGAL::CRP1 ynl047cΔ* cells were able to germinate on galactose plates (Figure 3-3C). These results show that the expression of either *CRP1* or *YNL047C* is required for viablility and suggest that the two genes have overlapping functions.

123

Because Chd1p physically interacts with Crp1p, it is possible that *CHD1* may also have overlapping roles with *YNL047C*. Most chromatin remodeling activities exist as multiprotein assemblies and removal of one protein can abolish the functional activity of the entire complex *in vivo*. If Chd1p and Crp1p are components of a nucleosome modifying complex, and if their functions are interdependent, *chd1* should also have synthetic lethal interactions with *ynl047c*. However, unlike a *crp1Δ ynl047cΔ* strain, tetrad analysis shows that a *chd1Δ,ynl047cΔ* double mutant is viable (Figure 3-3D). This result indicates that Chd1p and Crp1p activities are not completely co-dependent.

Expression profiling of *CRP1*

To further characterize the significance of the physical interaction between Crp1p and Chd1p, we examined the genomic expression profile of a *crp1* mutant by microarray analysis. The similarity to Ask10p and the association with Chd1p indicates that Crp1p has a likely role in transcription. Moreover, since many remodeling activities are recruited to specific genes by transcription factors, expression profiling of *crp1* and *chd1* mutants may uncover genes regulated by both Crp1p and Chd1p. The identification of common target genes will subsequently enable a more detailed analyses of the functional relationship between these two proteins. The following section describes the preliminary results of an expression profile comparison between *crp1* and *chd1* mutants.

crp1Δ, *chd1Δ*, and wild type strains ere grown in two different growth conditions, minimal and rich media. mRNA was isolated from the three strains and utilized as templates to synthesize cDNA. The cDNA derived from the *crp1Δ* or *chd1Δ* sample, along with the wild type reference cDNA, were coupled to fluorescent dyes and hybridized onto microarrays containing ~ 6000 yeast genes. Table 3 lists genes whose

expression were either elevated or decreased by at least two fold in both *crp1*Δ and *chd1*Δ strains. For growth in minimal media, 19 genes had an increase in transcription while 18 genes had a decrease. For growth in rich media, only three genes were affected in both mutants and all three had an increase in transcript levels in the deletion strain versus the wild type cells. These results present a set of genes that may be coordinately regulated by Chd1p and Crp1p and suggest that the two proteins together have a more pervasive role in transcription during growth in minimal media.

We also compared the microarray results from the two different experiments in order to identify genes or processes that are regulated by Chd1p and Crp1p in both growth conditions. *LEU2* is the only gene whose expression is similarly affected in *chd1*Δ and *crp1*Δ strains in both experiments. However the *LEU2* gene was used as a selectable marker during the construction of *chd1* and *crp1* deletion mutants. Therefore, its expression in the mutant strains may be altered from that of the wild type cells (which have *LEU2* integrated at the endogenous locus) due to position and integration effects.

In addition to *LEU2*, the expression of heat shock genes was affected in both growth conditions. In minimal media, *HSP42*, and *SSE2* transcription was enhanced in *crp1* and *chd1* mutants while in rich media, *HSP12* expression was elevated in the mutant strains. These results suggest that Chd1p and Crp1p are negative regulators of heat shock genes. Consistent with these findings, localization studies on Chd1p have shown that Chd1p is displaced from heat shock genes under inducing conditions.

DISCUSSION

To gain insight into chromo domain mediated interactions, and to better understand how Chd1p activity is regulated, we carried out an affinity purification of proteins that interact with a Chd1p chromo domain fragment. Three proteins were found to associate with the chromo domain region of Chd1p, including Crp1p, a putative transcription factor. Genetic analyses indicate that *CRP1*, together with a homolog, have essential roles in yeast. In addition, genomic analysis has uncovered genes that may be coordinately controlled by Chd1p and Crp1p. This study has led to the identification of a Chd1p interacting factor that may act in conjunction with Chd1p to regulate transcription.

Identification of chromo domain interacting proteins

Using affinity chromatography purification, we have identified three proteins, Hsl7p, Lhp1p, and Yil105p (Crp1p) that interact with a chromo domain fragment from Chd1p.. *HSL7* is required for viability when cells harbor mutations in histone genes. Recently, a study has shown that Hsl7p possesses methyltransferase activity and can methylate histone H2A and H4 in vitro (Lee et al., 2000). This finding raises the possibility that Hsl7p may target Chd1p to specific chromosomal sites via the binding of Chd1p to histone H2A and H4 methylated by Hsl7p. This model can account for observation that the chromo domain of CHD3 does not bind to methylated histone H3 peptides.

Lhp1p is involved in Pol III transcription while Crp1p has similarity to a putative transcriptional regulator. Therefore, Hsl7p, Lhp1p, and Crp1p all have functional links to either chromatin structure or transcription. Because Chd1p also has roles in these processes, it is possible that all three proteins interact with Chd1p *in vivo*. In fact, using a

co-immunoprecipitation assay, Crp1p has been shown to associate with Chd1p in yeast extracts. These results strongly suggest that Chd1p and Crp1p interact in cells. Also, these findings reveal only the second known chromo domain-protein interaction.

Genetic and genomic analyses of *CRP1*

Crp1p has no clear homologue in other organism but has similarity to *S. cerevisiae* proteins. Crp1p shares sequence identity with two uncharacterized proteins, Ynl047p (53%) and Ypr115p (34%), and with Ask10p (33%), a putative transcription factor. The high identity of Crp1p to Ynl047p suggests that the two proteins may have redundant or overlapping roles. Indeed, genetic analyses have demonstrated that *crp1Δ* and *ynl047cΔ* are synthetically lethal. Also, Crp1p and Ynl047p have been shown to interact by the two-hybrid assay (Uetz et al., 2000). Though Chd1p and Crp1p physically interact, *chd1* does not have synthetic lethal interactions with *ynl047c*, indicating that Chd1p and Crp1p functions are not entirely interdependent.

The similarity to Ask10p implicates a role for Crp1p as a transcription factor. It is possible that the interaction between Chd1p and Crp1p may mediate the recruitment of Chd1p to specific sites. It has been shown that chromatin modifying factors can be selectively targeted to certain genes by their interactions with transcription factors. Our previous analyses have shown that Chd1p can be recruited to genes encoding ribosomal proteins (Chapter 2). Crp1p, though, is not required for Chd1p localization at these genes (Chapter 2 and our unpublished data)

Genomic analyses of Crp1p activity, however, have uncovered a subset of genes that may be co-regulated by Chd1p and Crp1p. Preliminary results from microarray analyses show that the expression of at least 40 genes are similarly affected by the

absence of either *CHD1* or *CRP1*. In particular, heat shock genes appear to be negatively regulated by Chd1p and Crp1p. This result is consistent with the findings from chromatin immunoprecipitation (Ch-IP) experiments showing that the crosslinking of Chd1p to heat shock genes is diminished when these genes are actively transcribed. Additional Ch-IP analyses will clarify the roles of Chd1p and Crp1p at heat shock genes and at other genes that may be directly regulated by these two proteins.

ACKNOWLEDGEMENTS

Table 2: Peptide sequences of chromo domain interacting proteins

PROTEIN	SEQUENCE
Hsl7p (p90)	KALEPSNELPR
	KVLNSNSNHQFLLQEDSR (4)
	RTPSYVLNR
	RHEDLEEDYPEVHVR
Yil105Cp (p80)	KADSYESMMSWFDNLK
	RDPNFLLPNLPMR
	KILTSTSNIQDK
	KILTSTSNIQDKYK
	RLSHAVQFPFFSIENQYQPSSQEDK
	KQLQEENFLHEAFDNLETSGAELEK
	RSPLVILVPTAAQPTDILAAR
	KYQFDPLTYEIK
Lhp1p (p40)	KLGEINQVR
	RRVPLDLTAAR
	RSSEILEVSADGENVK
	KSYSNDDESNEILSYEGK
	KNDGWVPISTIATFNR
	RNSFAVIEFTPEVLDR
	RTLAVMNFPHEDVEASQIPELQENLEAFFK

Table 3: Summary of *chd1*Δ and *crp1*Δ expression analyses *

Experiment	Gene	Description
Minimal media: Genes with elevated transcription in both *chd1*Δ and *crp1*Δ strains	ALD4	Ethanol utilization
	CAR1	Arginine metabolism
	USV1	Unknown
	GPD1	Glycerol metabolism
	LEU2	Leucine biosynthesis
	YML128C	Unkown
	YCR061W	Unknown
	YJL048C	Unknown
	YMR187C	Unkown
	HSP42	Heat shock reponse
	YHL021C	Unknown
	GLC3	Cell wall biogenesis
	HXK1	Glycolysis
	YERO67W	Unknown
	YHR181W	Unknown
	YDL023C	Unknown
	SSE2	Heat shock reponse
	YNL274C	Unknown
	POR1	Mitochondrial transport protein
Minimal Media: Genes with decreaed activity in both *chd1*Δ and *crp1*Δ strains	NOG1	Viral propagation
	NIP1	Cell wall biogenesis
	YOL141W	Unkown
	FKS3	Unkown
	YDR412W	Unkown
	MEU1	Glucose derepression
	YPL012W	Unkown
	RPB3	RNA pol II subunit
	CIN4	GTP binding protein, mitosis
	RPS30B	Ribosomal protein
	SLK19	Synthetic lethal with kar3, mitosis
	YML108W	Unkown
	ELM1	Pseudohyphal growth
	SKI2	mRNA decay
	SMF2	Putative manganese transporter
	YPL205C	Unkown
	RTT102	Regulator of Ty1 transposition
	YFR026C	Unkown
Rich media: Genes with elevated transcription in both *chd1*Δ and *crp1*Δ Strains	HSP12	Heat shock reponse
	LEU2	Leucine biosynthesis
	YCLX10C	Unkown

* Only genes with fold differences > or = to 2 are shown

131

FIGURE LEGENDS

Figure 3-1. Affinity purification of Chd1p interacting proteins. (A) Schematic diagram

of the purification method. The chromo domain fragment (amino acids 122-300)

contains the chromo domain (a.a. ~ 197-234) and flanking sequences fused to GST. (B)

Three proteins-p90, p80 and p40-were differentially retained on the chromo domain

column and not on the GST control. The gel is a silver stained gel with samples from the

1M NaCl elutions.

Figure 3-1

A
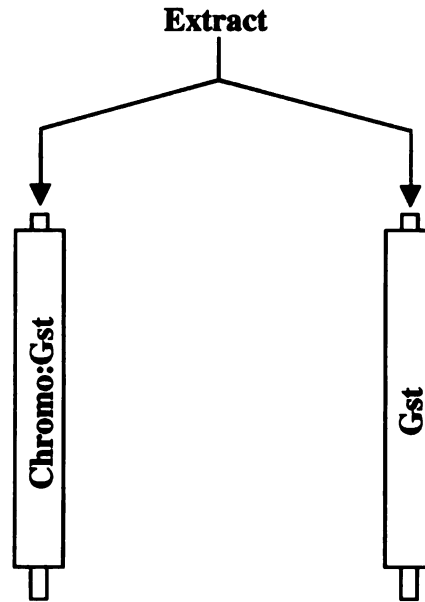
1) Apply whole cell extract
2) Wash with 75-150 mM NaCl
3) Elute with 1M NaCl
4) TCA precipitate
5) SDS-PAGE
6) Silver stain

B
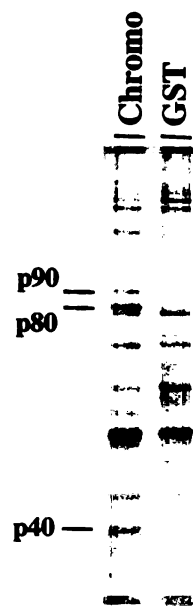
Figure 3-2. Crp1p co-immunoprecipitates with Chd1p. Extract from a Myc::CHD1,

HA::CRP1 strain was immunoprecipitated with HA antibodies (top left panel). Western

analysis with Myc antibodies shows that Chd1p co-precipitates with Crp1p. In the

converse experiment, when immunoprecipitation were performed with Myc antibodies,

Crp1p is present in the precipitant (bottom panel). In an untagged Crp1p strain,

immunoprecipitations with HA antibodies does not precipitate Chd1p (top right panel).
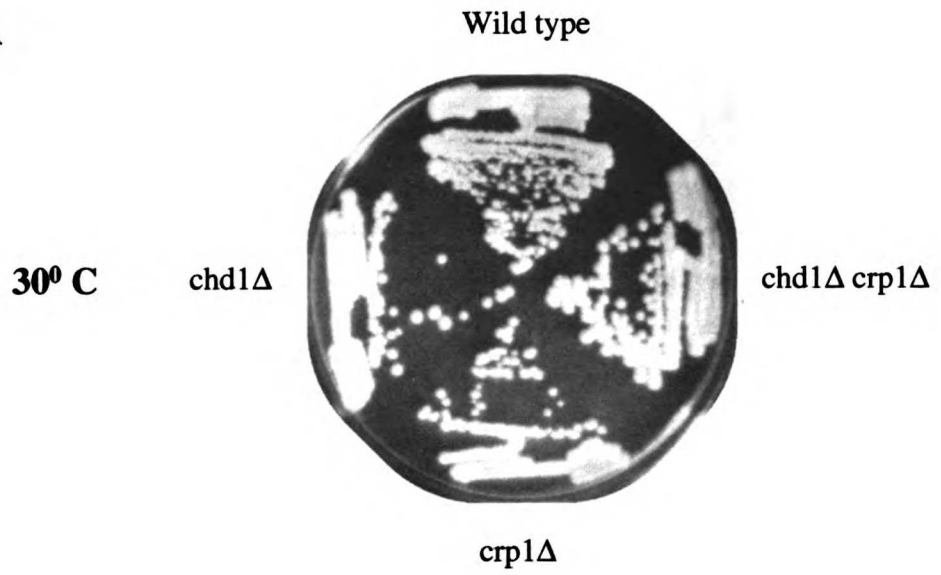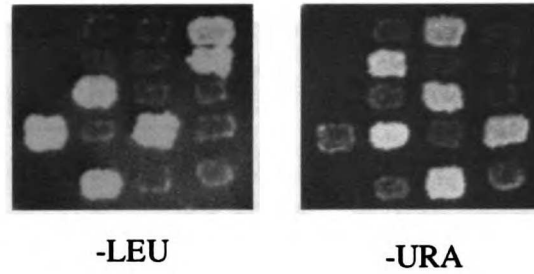
Figure 3-2
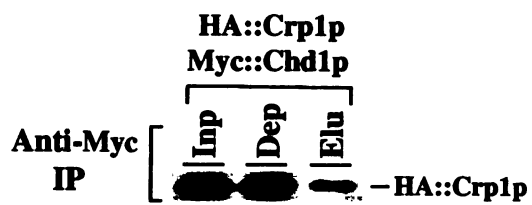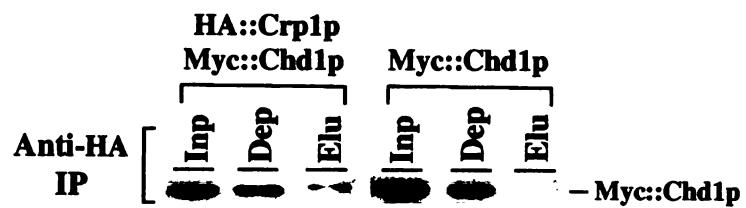
Figure 3-3. Genetic analyses of *CRP1* and *YNL047C*. (A) *crp1* deletions strains are viable and *crp1* does not have synthetic interactions with *chd1*. At 37° C, *crp1* strains have a slight growth defect. (B) Tetrad analyses show that *crp1 ynl047c* double mutants are inviable. Only single mutants and wild type haploids are recovered after dissection of a *crp1/+, ynl047c/+* diploid. (C), (D) *pGal::CRP1 ynl047c* haploids are viable when CRP1 is expressed. Tetrad dissection demonstrates that *pGal::CRP1 ynl047c* spores are inviable when dissected onto glucose media, but are viable when grown on galactose media. (E) There is no synthetic interaction between *CHD1* and *YNL047C*. Tetrad analysis shows that *chd1 ynl047c* double mutants are viable.
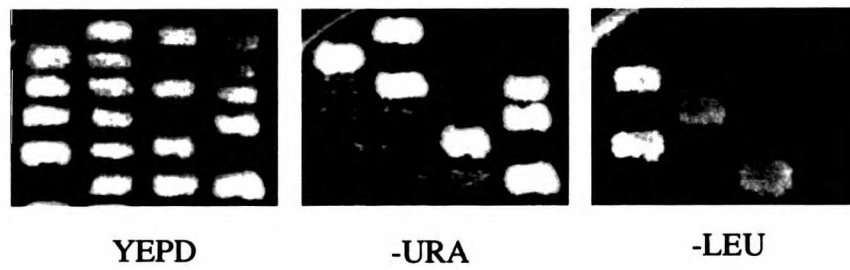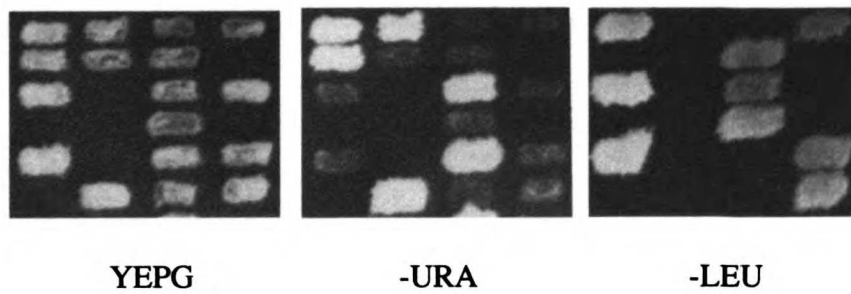
Figure 3-3

A

Wild type

30⁰ C   chd1Δ chd1Δ crp1Δ

crp1Δ

Wild type

37⁰ C   chd1Δ chd1Δ crp1Δ

crp1Δ

B

crp1Δ::LEU2/+
ynl047cΔ::URA3/+

-LEU   -URA

C

pGal::CRP1::URA3/+
ynl047cΔ::LEU2/+
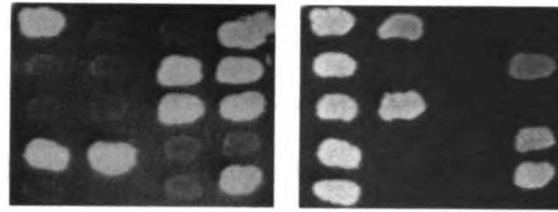

YEPD   -URA   -LEU
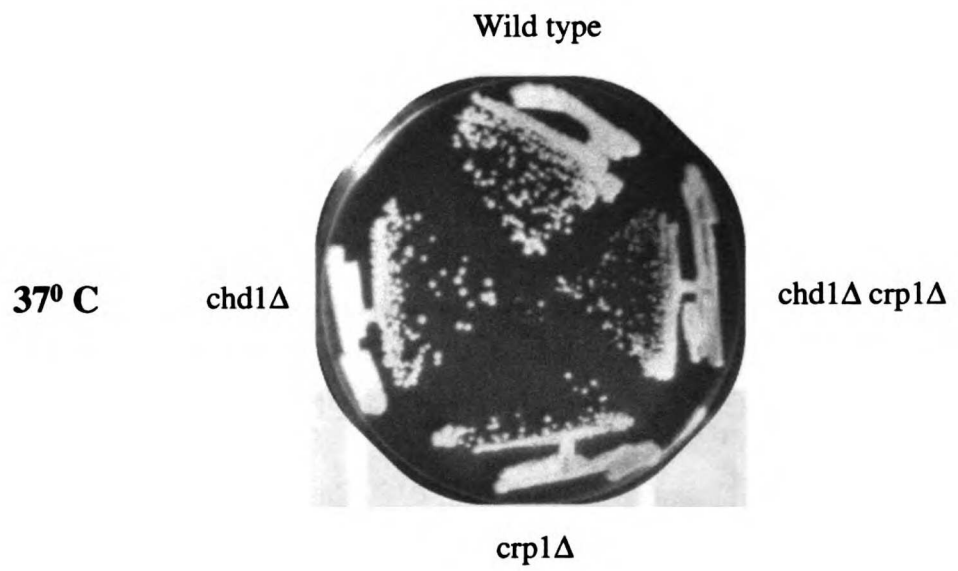
D

pGal::CRP1::URA3/+
ynl047cΔ::LEU2/+

YEPG   -URA   -LEU

E

ynl047cΔ ::URA3/+
chd1Δ::HIS3/+

-URA   -HIS

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SUMMARY AND PERSPECTIVES

Nucleosome modifying proteins are found in all eukaryotes and are important regulators of chromatin structure. For many of these factors, such as the CHD proteins, the role of their nucleosome-altering activities *in vivo* is not clear. To better understand the cellular function of CHD proteins, we utilized biochemical, genetic, and genomic approaches to characterize the *S. cerevisiae* Chd1p protein.

Since *CHD1* was observed to be nonessential, and because its role in yeast was not known, a synthetic lethal screen was implemented to identify genes that share similar functions with *CHD1*. Two *swi2* mutants were determined to have synthetic interactions with *chd1*. Further genetic analyses indicated that *CHD1* also interacts with other genes encoding components of the SWI/SNF complex. These results suggested that Chd1p may function as a nucleosome remodeling factor and as a transcriptional regulator.

To determine if Chd1p possesses chromatin remodeling activity, we sought to obtain purified Chd1p in order to perform *in vitro* nucleosome disruption assays. To this end, Chd1p was purified from yeast to near homogeneity, with no other proteins associating in stoichiometric amounts. *In vitro* analyses demonstrated that purified Chd1p perturbed the structure of nucleosomes in an ATP-dependent manner. Unlike SWI/SNF activity, which greatly increases the sensitivity of nucleosomal DNA to digestion by DNase I, Chd1p protected certain regions from DNase I attack while enhancing cleavage at other sites. These findings show that Chd1p remodeling activity is distinct from SWI/SNF.

To assess the role of Chd1p in transcription, we carried out genome-wide expression profiling using micro arrays consisting of ~6000 yeast genes. In a *chd1*

deletion, the expression of 2-4% of genes were affected, both positively and negatively. Northern blot analysis of a subset of genes confirmed the results seen in the micro array experiment. Therefore, these findings are consistent with the idea that Chd1p is involved in transcription control.

From the group of genes whose expression were affected by the absence of Chd1p, we attempted to identify genes directly regulated by Chd1p. Chromatin immunoprecipitations (Ch-IP) were performed to examine if Chd1p is associated at the promoters of affected genes. Relative to the *ACT1* control, Chd1p was not found to be enriched at a subset of genes examined. However, because we now know that Chd1p is selectively cross linked to the coding sequences of genes, these results are not unexpected.

As an alternative approach to identify genes directly regulated by Chd1p, Ch-IP/micro array analysis was performed. Using this method, we determined that Chd1p is selectively associated with genes involved in protein synthesis, most of which encode ribosomal proteins. Chd1p recruitment is dependent on transcriptional activity, as Chd1p is not enriched at RP genes under conditions of minimal expression. This finding indicates that Chd1p is a positive regulator of RP genes. Of particular interest, we observed that Chd1p was preferentially enriched along the entire coding region of genes and not at the promoter sequences. This result, along with previous observations, strongly suggests that Chd1p has a role in transcription elongation.

To better understand how Chd1p is activity and localization is regulated, we carried out an affinity chromatography purification of proteins that interact with a chromo domain fragment of Chd1p. Three proteins were identified by this approach: Hsl7p,

Ask10p, and Yil105 (Crp1p). The interaction between Chd1p and Crp1p has been verified by co-immunoprecipitation experiments. These results designate Crp1p as only the second protein believed to be a chromo domain-interacting factor (the other being histone H3). Genetic analyses indicate that either CRP1 or its homologue, *YNL047C*, is required for viability. Preliminary genome-wide expression profiling of *CHD1* and *CRP1* have uncovered a subset of genes that may be regulated by both Chd1p and Crp1p. Because Crp1 has similarity to a transcription factor, it is possible that Crp1 recruits Chd1p to specific chromosomal sites.

In summary, the collective results of our multiple methods of analyses indicate that Chd1p is a chromatin remodeling factor and a transcriptional regulator in yeast. Chd1p has overlapping functions with the SWI/SNF complex and can alter the structure of nucleosomes *in vitro*, suggesting that Chd1p regulates transcription by modulating chromatin architecture. Our genomic analyses has revealed a subset of genes whose expression are affected by the absence of Chd1p. In addition, we examined the distribution of Chd1p on chromosomes and have detected a specific association between Chd1p and a class of genes involved in protein synthesis. The Ch-IP analysis also supports a role for Chd1p in transcription elongation. Finally, we have identified a protein (Crp1) that interacts with Chd1p via the chromo domain. Crp1p may act to recruit Chd1p to specific genes.

Future directions

There are several aspects of Chd1p function that require further analyses. One important issue relates to the role of Chd1p in transcription elongation. It is unclear how Chd1p interacts functionally with the transcription machinery to regulate elongation.
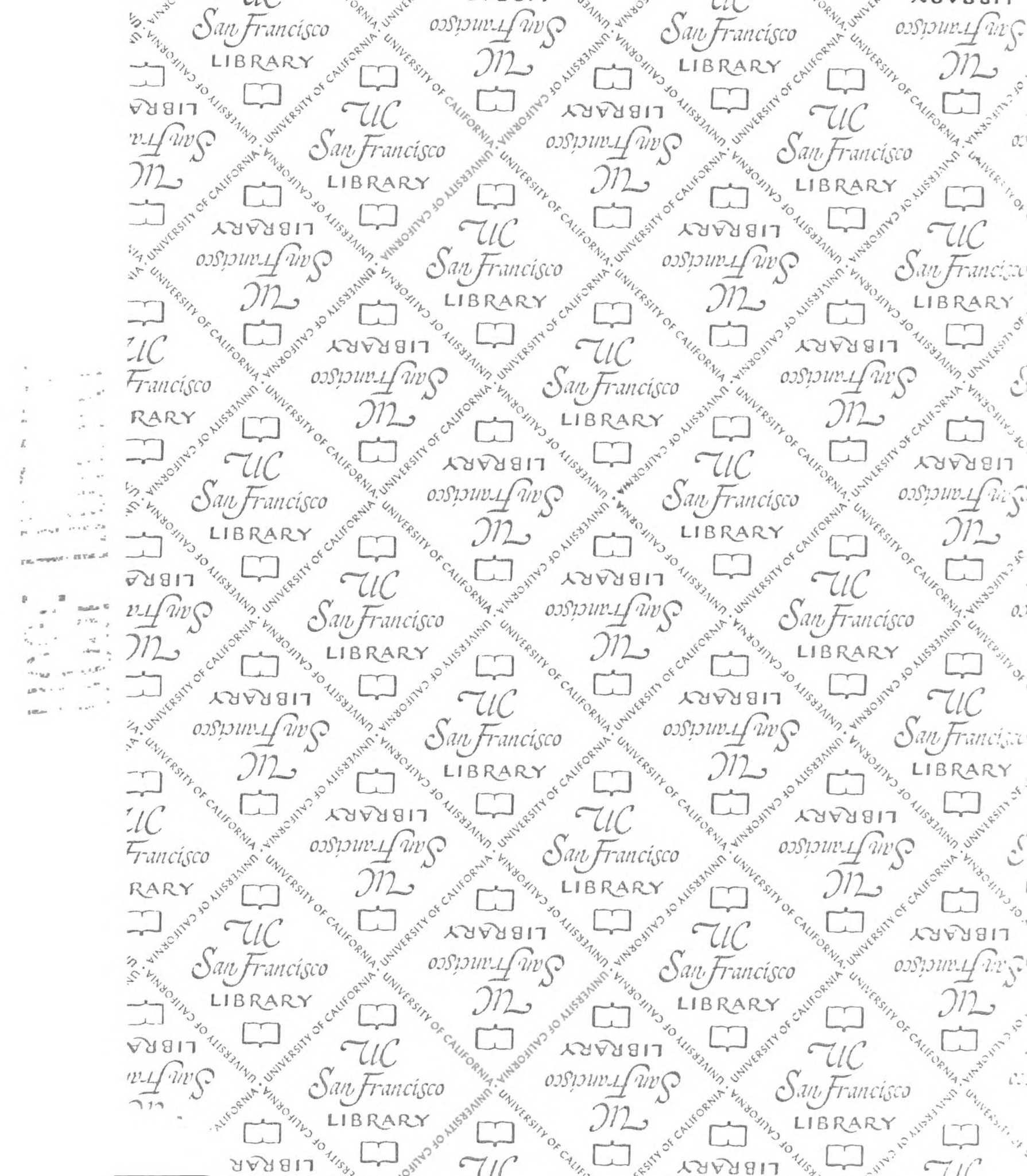
Chd1p may physically interact with the yeast elongation complex FACT, or directly with the RNA polymerase apparatus. Because both yFACT and the holoenzyme have been purified, it will be possible to assay for a direct interaction between Chd1p and these two complexes. In addition, because vitro transcription systems using chromatin templates have been developed, the effect of Chd1p activity on transcription can be examined, and it may be possible to uncover the molecular mechanism of action for Chd1p.

It is not known how Chd1p is specifically recruited to RP genes. Rap1p or Esa1p may localize Chd1p to the RP loci. Since Rap1 and Esa1p regulate other classes of genes, further Ch-IP/PCR analysis can be carried out to examine if Chd1p is also enriched at these genes. Also, the Ch-IP/microarray profiles of Rap1p and Esa1p can be compared to Chd1p in order to determine if there is a significant overlap in localization between Chd1p and Raps1p or Esa1p. Alternatively, Chd1p may be recruited by a transcription elongation factor.

Crp1p is not required for Chd1p localization at RP genes. However, Crp1p may recruit Chd1p to other loci or may act to modulate Chd1p activity. Ch-IP/PCR analysis of the genes affected by the absence of either Chd1p or Crp1p (as identified by our expression profiling) may reveal the set of genes regulated by both proteins. Additional Ch-IP/microarray analyses for both Chd1p and Crp1p may also clarify the functional relationship between the two proteins. Furthermore, purified Crp1p can be added to the remodeling assay to access if it alters the manner in which Chd1p disrupts the structure of nucleosomes.

Base on the synthetic lethal phenotype of a *chd1 swi/snf* double mutant, Chd1p and SWI/SNF may have similar or overlapping functions. Additional comparisons of the

expression or Ch-IP profiling of Chd1p and Swi2p may identify common targets or processes regulated by both factors. However, there are differences between the remodeling activities of Chd1p and SWI/SNF. Examining Chd1p and SWI/SNF activities using other types of remodeling assays will assist in understanding the significance of these differences.