Lawrence Berkeley National Laboratory
Recent Work

Title
Chromatin proteins are determinants of centromere function

Permalink
https://escholarship.org/uc/item/5cq1f61r

Author
Kaufman, Paul D.

Publication Date
2002

eScholarship.org Powered by the California Digital Library
University of California

Chromatin proteins are determinants of centromere function

Judith A. Sharp and Paul D. Kaufman*

***Lawrence Berkeley National Laboratory and**
University of California, Berkeley
Stanley Hall, Mail Code 3206
Berkeley, California 94720
510-486-6488 (FAX)
510-486-5846 (TEL)
pdkaufman@lbl.gov

Chromatin proteins are determinants of centromere function

Table of contents

1. Introduction
2. Centromere structure among eukaryotes is divergent
   2.1 Centromere sequence elements in *Saccharomyces cerevisiae*
   2.2 Centromeric repeats in *Schizosaccharomyces pombe*
   2.3 Centromeric repeats in higher eukaryotes
3. Chromatin deposition and centromere function in budding yeast
   3.1 Overview of chromatin subdomains at centromeric loci in budding yeast
   3.2 Building nucleosomal arrays: a role for chromatin assembly factors at silent and centromeric chromatin
      3.2.1 Nucleosome assembly proteins in budding yeast
      3.2.2 Nucleosome assembly proteins at centromeric chromatin
      3.2.3 Are nucleosome remodeling factors acting at the arrays?
4. Transcriptional silencing of centric heterochromatin domains in *S. pombe* and *Drosophila*
   4.1 Conserved heterochromatin proteins at centromeres in higher eukaryotes
   4.2 Marking pericentric nucleosomes: histone H3 lysine 9 methylation and the propagation of centromeric heterochromatin
   4.3 Factors downstream of HP1 heterochromatin association
      4.3.1 Recruitment of cohesin to centromeres: a requirement for specialized chromatin
      4.3.2 HP1 and the large subunit of CAF-I
5. Assembly of the kinetochore: the formation of nucleosomes containing an H3 isoform at centromeric chromatin
6. Concluding remarks
7. Acknowledgements
8. References

1. Introduction

Recent advances in the identification of molecular components of centromeres have demonstrated a crucial role for chromatin proteins in determining both centromere identity and the stability of kinetochore-microtubule attachments. Although we are far from a complete understanding of the establishment and propagation of centromeres, this review seeks to highlight the contribution of histones, histone deposition factors, histone modifying enzymes, and heterochromatin proteins to the assembly of this sophisticated, highly specialized chromatin structure. First, an overview of DNA sequence elements at centromeric regions will be presented. We will then discuss the contribution of chromatin to kinetochore function in budding yeast, and pericentric heterochromatin

domains in other eukaryotic systems. We will conclude with discussion of specialized nucleosomes that direct kinetochore assembly and propagation of centromere-defining chromatin domains.

2. Centromere structure among eukaryotes is divergent

During cell division, mitotic spindles attach to chromosomes and act to partition sister chromatids to daughter cells. Centromeres are defined as the discrete loci that direct spindle attachment to chromosomes through the action of the kinetochore, a microtubule-binding, chromatin-associated protein complex (Van Hooser and Heald 2001). DNA sequence analysis of centromeric regions has revealed that although the primary DNA sequence and structure may be conserved among centromeres within an organism, there is certainly no one centromere-defining sequence common to all eukaryotes.

2.1 Centromere sequence elements in *Saccharomyces cerevisiae*

The centromeres of the budding yeast *Saccharomyces cerevisiae* are the simplest among eukaryotic model organisms (Figure 1). A DNA sequence of approximately 125 bp from centromeric regions is sufficient to confer mitotic stability to recombinant plasmids (Fitzgerald-Hayes et al. 1982; Hieter et al. 1985; Clarke and Carbon 1980). Comparison of centromere DNA sequences among the sixteen chromosomes in budding yeast has revealed three common elements, CDEI, CDEII, and CDEIII (Panzieri et al. 1985; Cumberledge and Carbon 1987; Gaudet and Fitzgerald-Hayes 1987). The 8 bp CDEI element is not essential for centromere activity, but deletion of CDEI does cause a 10- to 30- fold increase in mitotic chromosome loss. The CDEII element is 78-86 bp long and has ~90% AT content. Deletion of the entire CDEII region abolishes centromere function; however, small deletions or insertions have only minor effects on chromosome segregation. The CDEIII element is a 26 bp imperfect palindrome with seven positions at the core invariant among all sixteen chromosomes. Single point mutations of the invariant nucleotides of CDEIII destroy centromere activity (McGrew et al. 1986).

2.2 Centromeric repeats in *Schizosaccharomyces pombe*

In contrast to the simple *S. cerevisiae* centromere DNA, centromeres in the fission

yeast *Schizosaccharomyces pombe* are much more extended in length and bear a higher degree of resemblance to centromeres in higher eukaryotes (Figure 2). Centromere size among the three *S. pombe* chromosomes ranges from 35-110 kb, and is inversely correlated to total chromosome length (Chikashige et al. 1989; Murakami et al. 1991; Clarke and Baum 1990; Wood 2002). Despite the differences in size, all three centromeres share both overall organization and conserved sequence elements. The core regions – *cnt1*, *cnt2*, and *cnt3* – are 48% identical over a 1.4 kb region and are flanked by two large imperfect, inverted repeat sequences termed *imrL* and *imrR*. These inner repeats are flanked by variable numbers of outer repeat sequences. These outer repeats, *otrL* and *otrR*, consist of *dg* and *dh* repeats separated by another small repeat sequence. While the *dh* repeats share 48% identity over a 1.8 kb region, *dg* repeats are 97% identical over a 1.8 kb region and thus represent the largest conserved element common to all *S. pombe* centromeres. Although the *S. pombe* centromere regions are devoid of protein coding genes, a number of tRNA genes are present in single or tandem clusters in the inner and outer repeats, and have been proposed to mark boundaries between protein domains (Takahashi et al. 1991; Partridge et al. 2000). None of the sequence elements present in fission yeast centromeres bear any resemblance to those of budding yeast.

When naked DNA templates containing partial centromere sequences are introduced into *S. pombe*, two distinct phenotypes are observed (Steiner and Clarke, 1994; Ngan and Clarke 1997). Plasmids containing the both the core region and the *dg* element are competent to acquire centromere activation rapidly after transformation (Hahnenberger et al. 1991; Baum et al. 1994; Ngan and Clarke 1997). Plasmids containing the core region alone can acquire mitotic stability, albeit in a manner that requires selection over several generations, suggesting that establishment of centromere function is stimulated by the dg elements. Thus, there are primary DNA sequence requirements for centromere formation in *S. pombe*.

2.3 Centromeric repeats in higher eukaryotes

Centromeres of higher eukaryotes have been exceedingly challenging to analyze structurally, because they are comprised of simple sequence repeats. Natural human centromeres lie within arrays of 171 bp α-satellite DNA that extend from 1-4 Mb (Choo 2001). Although α-satellite DNA is a major component of human centromeres, other satellite and repeat elements are also present. Indeed, repetitive DNA appears to be the one element common to the natural centromeres of higher eukaryotes. These repeats can range in size from 12 bp repeats in *Drosophila* to 340 bp repeats found in porcine

centromeres (Abad and Villasante 2000; Miller et al. 1993).

Is α-satellite DNA sufficient for centromere formation? Studies of abnormal human chromosomes suggest that alphoid DNA is not required at all once a locus has acquired centromere activity. Occasionally, centromeres form at ectopic sites on the chromosome by an unknown mechanism. These novel, centromere-forming sites are called neocentromeres, and have been observed at loci devoid of alphoid DNA (du Sart et al. 1997). Thus, α-satellite DNA is not required for maintenance of centromere function after establishment. Furthermore, not all α-satellite DNA directs formation of centromeres. In individuals with a Robertsonian translocation, end to end fusion of two distinct chromosomes results in a large, mitotically stable pseudodicentric chromosome. Despite the presence of two α-satellite-rich loci that formed centromeres in the parent chromosomes, only one locus is associated with the cytological marks of an active centromere (Warburton et al. 1997).

In contrast, the requirements for establishing centromere function on naked DNA appear to differ from those for inheritance of a functional centromere. For example, investigations addressing the structural requirements for *de novo* centromere formation on naked DNA templates found that reintroduction of cloned α-satellite arrays into human cells resulted in the formation of 1-10 Mb minichromosomes with bona fide centromeres (Harrington et al. 1997; Ikeno et al. 1998; Schueler et al. 2001). When a non-alphoid DNA template is reintroduced into cells, it is not competent for centromere activation, even if the DNA is derived from a known neocentromere locus (Saffery et al. 2001). These data suggest there may be some requirement for alphoid DNA sequence in centromere formation. How non-alphoid neocentromeric loci acquire centromere identity or whether this process involves alphoid DNA supplying a function *in trans* is not known.

Because of the disparities in correlating centromeric sequence with function in higher eukaryotes, centromere inheritance has been proposed to be mediated by a chromatin-based epigenetic mechanism (Karpen and Allshire 1997). Important questions remain regarding the mechanism of how α-satellite establishes centromere function on naked DNA. Because it is clear that chromatin proteins are integral to centromere structure and function, the following discussion addresses the roles of such proteins in various experimental systems.

3. Chromatin deposition and centromere function in budding yeast

3.1 Overview of chromatin subdomains at centromeric loci in budding yeast

The simple primary sequence of centromeres in budding yeast belies their complex protein composition (Figure 3). The 125 bp conserved core sequence is contained within a 250 bp nuclease-resistant region (Bloom and Carbon 1982). Genetic and biochemical experiments designed to isolate protein components of budding yeast kinetochores have identified a number of proteins required for the integrity of the core structure reviewed in (Cheeseman et al. 2002). A subset of these proteins bind specifically to the CDE elements present in centromeric DNA. For example, CDEI recruits the Cbf1 protein to mark the left boundary of the protected core domain (Saunders et al. 1988; Bram and Kornberg 1987; Baker et al. 1989; Cai and Davis 1989; Jiang and Philippsen 1989). Chromatin digestion of *cbf1*Δ cells results in a slightly reduced area of nuclease protection of the core centromere, and both CDEIΔ and *cbf1* Δ cells display only minor defects in chromosome segregation (Cai and Davis 1990). Therefore, it is thought that these components make a nonessential contribution to the overall integrity of the kinetochore structure. More notably, the 4-subunit CBF3 complex (Ndc10, Cep3, Ctf13, Skp1) binds the CDEIII sequence (Lechner and Carbon 1991; Connelly and Hieter 1996). Mutations in CDEIII which disrupt CBF3-binding *in vitro* also cause a profound disruption of the nuclease-resistant core and a high frequency of chromosome missegregation *in vivo* (Saunders et al. 1988). All genes encoding subunits of the CBF3 complex are essential for viability. Therefore, the recruitment of CBF3 to the CDEIII element is absolutely required for accurate chromosome segregation. The CDEII spacer element is occupied by a specialized nucleosome containing Cse4, an isoform of histone H3 (Section 5; Stoler et al. 1995; Keith and Fitzgerald-Hayes 2000). High resolution chromatin immunoprecipitation analysis has determined that Cbf1, Ndc10, and Cse4 are all restricted to the core centromeric domain (Meluh and Koshland 1997; Meluh et al. 1998).

Flanking both sides of the core region are highly phased nucleosome arrays that can extend for 2 or more kilobases along the chromosome arms (Bloom and Carbon 1982). The integrity of these pericentric nucleosomal arrays is dependent on core histones: the appearance of novel nuclease digestion sites within the array is observed in cells bearing point mutations in histone H2A, as well as for cells depleted for histones H2B or H4 (Pinto and Winston 2000; Saunders et al. 1990). In both cases, cells with altered histones are largely incapable of segregating chromosomes, thus correlating the integrity of the pericentric nucleosomal arrays with proper mitotic chromosome transmission.

Some evidence suggests that the core centromere and pericentric nucleosomal arrays are structurally interdependent. Depletion of histones H2B or H4 causes an increase in the accessibility of the core centromeric region, suggesting that the pericentric array might function to stabilize protein-protein contacts within the core kinetochore complex (Saunders et al. 1990). Conversely, recruitment of CBF3 to CDEIII appears to be critical for building the pericentric chromatin, because point mutations within CDEIII result in greatly increased nuclease sensitivity of not just the core, but also the flanking regions (Saunders et al. 1988).

The elegant little centromeres of budding yeast are sometimes dismissed as a poor model for understanding centromere structure in higher eukaryotic organisms. Because budding yeast centromeres replicate early in S phase (Raghuraman et al. 2001), lack cytologically dense chromatin staining, and lack an HP1 homolog (Section 4.1), budding yeast centromeric regions are not likened to the centric heterochromatin found in higher eukaryotes. However, as discussed in the next section, recent links between chromatin deposition proteins and centromere function in budding yeast are likely to provide insights into conserved molecular components of chromatin-mediated centromere inheritance.

3.2 Building nucleosomal arrays: a role for chromatin assembly factors at silent and centromeric chromatin

3.2.1 Nucleosome assembly proteins in budding yeast

The *de novo* formation of nucleosomes is coupled temporally to the passage of replication forks: a core tetramer of histones H3 and H4 is deposited first, followed by the incorporation of two dimers of histones H2A and H2B to form the nucleosome particle (Camerini-Otero et al. 1976; Worcel et al. 1978). This process is mediated by histone chaperones that prevent the formation of disordered histone-DNA complexes (Ruiz-Carrillo et al. 1979; Ellison and Pulleyblank 1983). A DNA replication-coupled chromatin assembly activity was first identified in HeLa cell nuclear extracts (Stillman 1986). Purification of this activity identified a three-subunit complex, named chromatin assembly factor-I (CAF-I) (Smith and Stillman 1989). The replication-dependent nucleosome assembly activity of CAF-I *in vitro* resembles the two-step mechanism observed *in vivo* (Smith and Stillman 1991); CAF-I performs the first step of nucleosome formation, deposition of histones H3 and H4. Subsequent addition of histones H2A and H2B completes nucleosome formation.

Genes encoding the three subunits of CAF-I are conserved throughout the eukaryotes (Kaufman et al. 1995; Kaufman et al. 1997; Tyler et al. 1996; Tyler et al. 2001; Kaya et al. 2001; Quivy et al. 2001). Consistent with its activity *in vitro*, CAF-I subunits colocalize with replication foci during S-phase in human cells (Krude 1995). The recruitment of CAF-I to replication forks occurs via an interaction with PCNA (proliferating cell nuclear antigen), the processivity factor for eukaryotic DNA polymerases (Shibahara and Stillman 1999). Taken together, these results evoke a simple model for replication-coupled nucleosome assembly: PCNA recruits CAF-I to sites of DNA synthesis, CAF-I then mediates nucleosome assembly on nascent DNA strands.

However, genetic studies in budding yeast indicate that cells require the action of multiple chromatin assembly factors. The three subunits of CAF-I are encoded by the *CAC1*, *CAC2*, and *CAC3* genes (Kaufman et al. 1997). Yeast cells with deletions of single or multiple *CAC* genes display viability and growth phenotypes that are indistinguishable from those of wild-type cells under normal growth conditions (Kaufman et al. 1997; Enomoto et al. 1997; Kaufman et al. 1998; Sharp et al. 2002). Furthermore, digestion of bulk chromatin in yeast *cac* mutants with micrococcal nuclease results in nucleosomal ladders that are nearly identical to those of wild-type cells (Sharp et al. 2002). These findings are inconsistent with the hypothesis that CAF-I acts alone to assemble chromatin across the genome.

The search for additional nucleosome assembly factors has identified two principal candidates that are themselves functionally related. The first set, the Hir (histone regulatory) proteins, were originally identified as factors critical for the transcriptional repression of histone genes (Osley and Lycan 1987; Xu et al. 1992). Homology searches indicated that Hir1 is significantly similar to the Cac2 subunit of CAF-I, suggesting conserved gene function (Kaufman et al. 1998). *CAC2* and *HIR1* orthologs are present in all eukaryotes, and together define a distinct subfamily of WD-repeat containing proteins. Further data linking *CAC* and *HIR* gene function came from a synthetic viability screen: mutations in the four known HIR genes (*HIR1*, *HIR2*, *HIR3*, and *HPC2*) all cause synthetic growth defects in *cac1*Δ cells (Kaufman et al. 1998; Qian et al. 1998; Sharp et al. 2001). These growth defects hold true for any *cac hir* double mutant gene combination, suggesting that Hir proteins function together as a complex.

A second protein related to CAF-I function is Asf1. Asf1 was originally described as a factor that abolished heterochromatic gene silencing in budding yeast when overexpressed (Le et al. 1997; Singer et al. 1998). *Drosophila* Asf1 protein was then isolated as a component of the RCAF complex that stimulated the histone deposition activity of CAF-I (Tyler et al. 1999). Like CAF-I, Asf1 binds to histones H3 and H4 and

possesses histone deposition activity (Tyler et al. 1999; Sharp et al. 2001). The gene encoding Asf1 is conserved in budding yeast; *ASF1* is not essential, but like *cac hir* yeast cells, *cac asf1* double mutants display synergistic growth defects (Tyler et al. 1999; Sharp et al. 2001). Epistasis analysis has demonstrated that Asf1 and Hir proteins function in the same genetic pathway for heterochromatin function (see below), and physical interactions between Asf1 and three of the Hir proteins suggest they function together in the context of a larger complex to contribute to nucleosome formation (Sharp et al. 2001; Sutton et al. 2001; Gavin et al. 2002).

CAF-I, Hir proteins, and Asf1 may act to assemble chromatin in a locus-specific manner in budding yeast. CAF-I contributes to position-dependent transcriptional silencing at all the known heterochromatic loci in budding yeast – telomeres, the cryptic *HMR* and *HML* mating-type cassettes, and rDNA (Kaufman et al. 1997; Enomoto et al. 1997) (Monson et al. 1997; Enomoto and Berman 1998; Smith et al. 1999). A synergistic loss of gene silencing at heterochromatic loci is observed in both *cac hir* and *cac asf1* double mutants (Tyler et al. 1999; Kaufman et al. 1998; Qian et al. 1998). Therefore, CAF-I and Hir/Asf1 proteins are thought to comprise two pathways for heterochromatin formation (Sharp et al. 2001; Sutton et al. 2001). In addition to performing histone deposition, chromatin assembly factors may recruit other complexes that have locus-specific silencing functions, a hypothesis supported by the physical interactions of Cac1 and Asf1 with the putative acetyltransferase Sas2 (Meijsing and Ehrenhofer-Murray 2001; Osada et al. 2001).

Although the silencing functions of chromatin assembly factors are linked to replication proteins, these roles may not be restricted to S phase. The ability of CAF-I, Hir proteins, and Asf1 to contribute to telomeric silencing requires the *POL30*-encoded PCNA, encoded by the *POL30* gene in *S. cerevisiae* (Zhang et al. 2000; Sharp et al. 2001). Because the association of Cac1 with chromatin requires wild-type *POL30* gene function, PCNA is thought to provide a recruitment activity in targeting the CAF-I complex (Zhang et al. 2000). However, CAF-I can function outside S phase to maintain silencing at *HML*, suggesting the requirement for PCNA could be independent of its role in DNA replication during S phase (Enomoto and Berman 1998).

3.2.2 Nucleosome assembly proteins at centromeric chromatin

The discovery that CAF-I and Hir proteins contribute to the integrity of centromeric chromatin in *S. cerevisiae* came from experiments characterizing the slow-growth phenotype of *cac hir* cells (Sharp et al. 2002). This slow-growth phenotype

results from a delay in traversing the metaphase-to-anaphase transition during mitosis. The mitotic delay in *cac hir* cells is mediated in part by the spindle assembly checkpoint. Increased rates of minichromosome missegregation and the presence of cytologically visible lagging chromosomes suggested that *cac hir* cells possessed a structural defect at centromeres that weakened the stability of microtubule-kinetochore attachments. Consistent with this hypothesis, the nucleosomal array flanking the core centromere is dramatically perturbed in *cac hir* cells; significant increases in nuclease accessibility to the core region are also detected specifically in the double-mutant cells. Thus, CAF-I and Hir proteins have an overlapping role in building or maintaining centromeric chromatin structure in budding yeast.

Localization experiments strengthened the argument that CAF-I and Hir proteins act at centromeric chromatin (Sharp et al. 2002). The chromatin-associated pool of CAF-I and Hir proteins can be visualized in chromosome spread preparations: subunits of both complexes display a punctate localization pattern with a discrete number of foci. A subset of CAF-I and Hir1 foci colocalize with kinetochore proteins. Chromatin immunoprecipitation of the large subunit of CAF-I demonstrated that it is present at centromeric loci, and is distributed across a 10 kb region centered on the core of *CEN3*. The failure to observe Cac1 association with a number of euchromatic loci in these experiments demonstrates that CAF-I displays selective association with discrete chromatin domains.

Future studies that address the full impact of chromatin deposition proteins at centromeric and silent chromatin will be informative. The nucleosomal arrays present at these loci might form a scaffold that is required for the recruitment of other factors that contribute to the functionally active chromatin state. Do chromatin deposition complexes simply act to build or maintain the scaffold, or could they also be more directly involved in recruiting other factors? Little is known about what features, if any, are common to the centromeric nucleosomal arrays and those of the heterochromatic, transcriptionally silent mating-type loci and telomeres. For example, there are no reports which test whether the centromeric nucleosomal arrays exert a silencing effect on neighboring genes. More importantly, it will be crucial to test whether the role for chromatin assembly factors at centromeric loci is conserved in higher organisms.

3.2.3 Are nucleosome remodeling factors acting at the arrays?

The RSC nucleosome remodeling factor is a large multisubunit protein complex that is broadly conserved and is both biochemically and structurally related to the

SWI/SNF nucleosome remodeling complex (Cairns et al. 1996). Both RSC and SWI/SNF complexes display a nucleosome-stimulated ATPase activity. In the presence of ATP, both complexes can disrupt nucleosome positioning on mono- or polynucleosomal substrates. RSC also catalyzes the transfer of a histone octamer to naked DNA *in trans* (Lorch et al. 1999). Such activities are proposed to "open" chromatin and therefore facilitate transcription of target promoters *in vivo*. Disruption of genes encoding RSC or SWI/SNF subunits indicates that these complexes have distinct biological functions -- SWI/SNF subunits are dispensable for viability in budding yeast, but RSC subunits are not (Cairns et al. 1996). While the essential function of RSC is not known, it is reasonable to hypothesize that RSC activity could be required for either the transcription of an essential gene, or the remodeling of a chromatin domain required for viability.

Analysis of conditional mutants in RSC subunits reveal a function for RSC during mitosis (Cao et al. 1997; Tsuchiya et al. 1998; Angus-Hill et al. 2001). Budding yeast cells carrying temperature sensitive alleles of *NPS1/STH1* arrest in G2/M in a manner that depends on the spindle checkpoint; removal of spindle checkpoint gene function in these cells causes a drastic reduction of cell viability (Tsuchiya et al. 1998). Therefore, cells lacking RSC activity possess a defect that is linked to centromere function. Nuclease accessibility studies performed on chromatin from cells lacking RSC function resulted in increased accessibility of the both core region and the flanking nucleosomal array (Tsuchiya et al. 1998). The subnuclear distribution of RSC that is associated with chromatin is currently not known in budding yeast. However, immunolocalization of a human RSC protein on mitotic chromosomes showed a highly localized kinetochore staining pattern (Xue et al. 2000). Together, these data suggest a centromere-linked remodeling function of RSC may be conserved.

4. Transcriptional silencing of centric heterochromatin domains in *S. pombe* and *Drosophila*

4.1 Conserved heterochromatin proteins at centromeres in higher eukaryotes

Transcriptional repression of genes placed within pericentric heterochromatin has been described in *Drosophila* and *S. pombe* (Spofford 1967; Allshire et al. 1994); a number of mutations that alleviate this phenomenon of position-dependent transcriptional silencing have been identified. Strikingly, multiple genes required for heterochromatic centromeric silencing in both *Drosophila* and *S. pombe* display significant molecular homology (Su(var)2-5/HP1 and *swi6*$^{+}$; Su(var)3-9 and *clr4*$^{+}$, respectively). HP1 and

Su(var)3-9 homologs are present in all eukaryotes examined with the exception of budding yeast and plants (Eissenberg and Elgin 2000).

In multiple cases, loss of centromeric transcriptional silencing is linked to defects in chromosome segregation. Figure 4 shows proteins that are associated with heterochromatin and core centromeric regions in *S. pombe*. In *S. pombe*, while null alleles of *swi6*, *clr4*, *rik1*, and *chp1* are all viable, all cause the derepression of a reporter gene embedded within centromeric regions and result in elevated loss rates of a minichromosome (Allshire et al. 1995; Ekwall et al. 1996; Doe et al. 1998). *Drosophila* HP1 null mutants are lethal and display abnormal chromosome segregation (Eissenberg et al. 1992; Kellum and Alberts 1995); less severe alleles suppress position effect variegation of a chromosomal translocation of the white gene, as do alleles of Su(var)3-9 (Eissenberg et al. 1990; Reuter and Spierer 1992). Mouse cells deficient in the two loci encoding Suvar39h show greatly increased frequencies of chromosome instability, and ~ 1/3rd of double null adult mice develop tumors (Peters et al. 2001).

An alignment of Su(var)3-9 with the Polycomb-group protein E(Z) and trithorax-group protein TRX revealed a shared motif dubbed the SET domain (Tschiersch et al. 1994). Subsequent homology searches revealed that the SET domain was present in all eukaryotes, and also that six related plant sequences recovered had been assigned methyltransferase activity (Rea et al. 2000). When histones were tested as potential substrates, purified Su(var)3-9 family members catalyzed the transfer of a methyl group specifically on Lys 9 of histone H3 (Rea et al. 2000; Nakayama et al. 2001). Further, the activity of Su(var)3-9 is required *in vivo*, because cells lacking Su(var)3-9 gene function display little or no H3 Lys 9 methylation in *Drosophila* and *S. pombe* (Schotta et al. 2002; Nakayama et al. 2001).

Common to both HP1 and Su(var)3-9 family members is a conserved stretch of ~50 amino acids termed the chromodomain. Originally identified as a region of similarity between HP1 and Polycomb (Paro and Hogness 1991), chromodomains are present in a number of proteins, all of which are involved in some aspect of chromosome metabolism (Eissenberg 2001). The chromodomains of HP1 and Su(var)3-9 are essential for centromeric silencing and fidelity of chromosome segregation (Platero et al. 1995; Ivanova et al. 1998; Wang et al. 2000; Nakayama et al. 2001).

4.2 Marking pericentric nucleosomes: histone H3 lysine 9 methylation and the propagation of centromeric heterochromatin

Peptide binding studies were used to define the specificity of interaction between chromodomain of HP1 and the N-terminal tail of histone H3 (Bannister et al. 2001; Nakayama et al. 2001; Jacobs et al. 2001). The HP1 chromodomain specifically binds histone H3 peptides methylated at Lys 9, but not methylated at Lys 4. Further, the interaction is specific to the chromodomain of HP1, because other chromodomain containing proteins did not bind histone H3 peptides methylated at Lys 9. This suggests a mechanism for recruiting HP1 specifically to sites of heterochromatin. Indeed, histone H3 Lys 9 methylation is greatly enriched at all *S. pombe* heterochromatic regions tested, including the *mat* locus and the *imr* and *otr* repeats of *cen1* (Nakayama et al. 2001; Noma et al. 2001). Epistasis experiments have determined that histone H3 Lys 9 methylation occurs prior to HP1 recruitment: mutations in *clr4* which abolish H3 Lys 9 methylation *in vivo* fail to target Swi6 to heterochromatin, and yet H3 Lys 9 methylation is unperturbed in *swi6* null cells (Ekwall et al. 1996; Noma et al. 2001; Nakayama et al. 2001; Bannister et al. 2001). Together, these data suggest a "histone code" resides within the various combinations of covalent histone modifications to specify locus-specific activity within the context of chromatin (Strahl and Allis 2000; Jenuwein and Allis 2001).

Based on these studies, a model for mitotically stable, self-propagating heterochromatin has been proposed. After DNA replication, nucleosomes are distributed randomly to sister chromatids (Jackson 1988; Jackson 1990). Heterochromatic loci would retain the histone H3 Lys 9 methylation on the inherited parental nucleosomes, and therefore be "marked" for the reassembly of heterochromatin. Su(var)3-9 would then be recruited to these sites to catalyze the methylation reaction on newly incorporated nucleosomes. The resulting array of nucleosomes bearing histone H3 Lys 9 methylation would provide a strong binding site for the recruitment of HP1 onto chromatin. Subsequent oligomerization of HP1 along the "marked" domain and recruitment of other proteins would form functional heterochromatin.

However, despite the attractiveness of this model, several questions remain. For example, it is not clear how Su(var)3-9 itself is recruited to heterochromatin. A simple solution might have involved the targeting of Su(var)3-9 via methylated H3 Lys 9. However, although Su(var)3-9 does possess a chromodomain, Su(var)3-9 protein fails to interact with a H3 N-terminal tail peptide methylated on Lys 9 *in vitro* (Bannister et al. 2001). Furthermore, Su(var)3-9 retains full activity in the presence of an H3 tail peptide that is either unmodified or methylated on Lys 4 (Rea et al. 2000; Nakayama et al. 2001). Together these data suggest that Su(var)3-9 loses affinity for the H3 N-terminus after Lys 9 methylation.

In contrast, histone modifying enzymes that act upstream of Su(var)3-9 recruitment to centric heterochromatin act to modulate the activity of Su(var)3-9. A mutation in a histone deacetylase was observed to decrease H3 Lys 9 methylation at centric heterochromatin in chromatin immunoprecipitation experiments, indicating that acetyl groups on histone tails inhibit Su(var)3-9 activity (Nakayama et al. 2001). Consistent with these data, the treatment of cells with drugs that inhibit histone deacetylases has long been known to inhibit centromeric silencing in *S. pombe* and *Drosophila* (Ekwall et al. 1997; Reuter et al. 1982). Further, evidence for a biochemical interaction between *Drosophila* Su(var)3-9 and HDAC1 indicates that histone deacetylases could act in a direct manner to recruit Su(var)3-9 (Czermin et al. 2001). Other proteins acting at heterochromatin could act similarly to recruit Su(var)3-9. For example, a role for HP1 in recruiting Su(var)3-9 has been proposed, because HP1 interacts with Su(var)3-9 biochemically (Aagaard et al. 1999).

Taken together, these data suggest a reaction cycle that is an extension of the model described above (Nakayama et al. 2001; Bannister et al. 2001). Histone deacetylases acting at newly incorporated nucleosomes embedded within "marked" heterochromatin would facilitate the recruitment of Su(var)3-9 to the H3 tail. After catalyzing the H3 Lys 9 methylation reaction, Su(var)3-9 would then rapidly dissociate from chromatin, exposing a high-affinity binding site for free HP1 molecules. Chromatin-associated HP1 would then recruit free Su(var)3-9 to modify neighboring nucleosomes, enabling the H3 Lys 9 methylation reaction to spread along chromatin.

The structural requirements that determine the spreading of HP1 along chromatin have not yet been fully elucidated. In an elegant study of the distribution of Swi6 at the *mat* locus (Noma et al. 2001), it was discovered that two boundary elements effectively block the spreading of Swi6 and H3 Lys 9 methylation into adjacent regions containing H3 methylated at Lys 4, a signature of transcriptionally active chromatin (Strahl et al. 1999; Noma et al. 2001). Whether such boundary elements exist in pericentric heterochromatin remains to be determined, as does the identification of factors that act together with H3 Lys4 methylation to block HP1 spreading. Further, only limited information is available regarding the protein domains within the HP1 molecule that serve to facilitate spreading *in vivo*. The chromoshadow domain mediates dimerization of HP1 in solution (Smothers and Henikoff 2000; Brasher et al. 2000), making it tempting to speculate that this domain will be important for the oligomeric state of chromatin-bound HP1. Finally, it will be important to determine whether other factors downstream of HP1 recruitment and H3 Lys 9 methylation are required for the formation of functional heterochromatin.

4.3 Factors downstream of HP1 heterochromatin association

4.3.1 Recruitment of cohesin to centromeres: a requirement for specialized chromatin

A complex of cohesin proteins provides the molecular "glue" to hold sister chromatids together after replication (Guacci et al. 1997; Michaelis et al. 1997; Jallepalli et al. 2001; for review, see Cohen-Fix 2001). At the time of mitosis, cohesin proteins are concentrated at centromeres and at discrete sites along chromosome arms in budding yeast (Blat and Kleckner 1999; Megee et al. 1999; Tanaka et al. 1999) and human cells (Waizenegger et al. 2000; Hauf et al. 2001; Sonoda et al. 2001). Cohesin proteins are thought to assist formation of stable microtubule-kinetochore attachments by properly orienting kinetochores toward the spindle poles.

Two recent studies report the requirement for heterochromatin to recruit cohesin to centromeric regions in *S. pombe*. Mutations in *swi6* abolish the association of cohesin subunits with centromeric regions but not arm sites (Bernard et al. 2001; Nonaka et al. 2002). Physical interactions between Swi6 protein and the Psc3 cohesin subunit suggest that Swi6 functions directly to recruit cohesin (Nonaka et al. 2002).

There is an epigenetic component to cohesin recruitment to centromeres in budding yeast (Tanaka et al. 1999), although in this organism there is no clear link between heterochromatin proteins and cohesin recruitment. Core components of the kinetochore (Ndc10, Mif2, Cse4) are required to recruit cohesin to an ectoptically placed centromere. However, only subtle changes in cohesin recruitment are observed at a natural *CEN* locus upon inactivation of essential kinetochore proteins. These results suggest that natural, established centromeres, but not neocentromeres, bear a "mark" that is sufficient to maintain localization of the cohesin complex when core centromere function is lacking. However, this mark appears to be linked to the core region itself, because excision of the natural *CEN* core sequence is sufficient to abolish cohesin association (Megee et al. 1999).

Cohesin in budding yeast spreads along chromatin for approximately 5 kb on either side of centromeres, overlapping the phased nucleosome arrays there (Blat and Kleckner 1999; Megee et al. 1999; Tanaka et al. 1999). However, it is not known whether nucleosome depletion has any impact on cohesin association. Cells lacking both CAF-I and Hir protein function have altered centromeric nucleosomeal arrays, but display proper loading of the Mcd1 cohesin subunit onto centromeres (Sharp et al. 2002). These data suggest that cohesin recruitment to *S. cerevisiae* centromeres is

at least partially independent of pericentric chromatin structure.

It is not known whether cohesin association with centromeres is required for the silencing function of heterochromatin. Some evidence of a silencing role for cohesins has been reported in budding yeast: alleles of the *SMC1* and *SMC3* cohesin components disrupted the silencing activity of a boundary element at *HMR* (Donze et al. 1999), and at least one cohesin subunit, Mcd1, is associated with this boundary element (Laloraya et al. 2000). However, in *S. pombe*, alleles of cohesin subunits have not been tested for centromeric silencing defects, nor is it known whether the silencing and cohesin recruiting functions of Swi6 are genetically separable.

4.3.2 HP1 and the large subunit of CAF-I

The p150 subunit of mammalian CAF-I interacts with HP1 in a manner that requires a conserved pentapeptide HP1-binding motif (Murzina et al. 1999). *In vitro*, this motif is dispensable for the activity of CAF-I (Kaufman et al. 1995), and HP1 does not inhibit CAF-I activity *in vitro* (Murzina et al. 1999). However, this motif is important for the localization of p150 to HP1-containing heterochromatin during G2 in mouse cells. This suggests that CAF-I can be targeted to HP1-containing regions in a manner that is independent of ongoing DNA replication, and that CAF-I could act to replenish nucleosomes within heterochromatin in mammals. This activity for CAF-I has been suggested previously for CAF-I in budding yeast (Sharp et al., 2002) Exploring HP1-CAF-I association in a more genetically tractable organism, such as *S. pombe*, may help to define how this interaction may impact heterochromatin assembly or function.

5. Assembly of the kinetochore: the formation of nucleosomes containing an H3 isoform at centromeric chromatin

Centromeric chromatin in all eukaryotes is uniquely marked by the presence of a histone H3 isoform. The founding member of this protein family, CENP-A, was discovered in humans as a major autoantigen of sera isolated from patients diagnosed with CREST syndrome (Earnshaw and Rothfield 1985; Valdivia and Brinkley 1985). CENP-A homologs have since been shown to be universal in eukaryotes, with homologs identified in mouse, *Drosophila* (Cid), *S. pombe* (Cnp1), *C. elegans* (HCP-3), and *S. cerevisiae* (Cse4) (Kalitsis et al. 1998; Henikoff et al. 2000; Takahashi et al. 2000; Buchwitz et al. 1999; Stoler et al. 1995). CENP-A is essential for high-fidelity chromosome segregation, because removing CENP-A by targeted gene disruption, antibody microinjection, or RNA-mediated interference confers a lethal phenotype in

every organism tested to date. Comparison of amino acid sequences among CENP-A family members and histone H3 has shown the highest degree of conservation occurs at the predominantly α-helical C-terminal histone fold domain, which defines the core region in the nucleosome structure (Luger et al. 1997). In contrast, the N-terminal tails are highly divergent among organisms. Mutagenesis of CENP-A reveals several sites in the histone fold domain are required for centromere-specific targeting in human cells (Shelby et al. 1997) and viability in budding yeast (Keith et al. 1999). The N-terminal tail domain is instead thought to mediate the recruitment and stable association of other kinetochore proteins (Chen et al. 2000).

CENP-A localizes to the inner plate of complex mammalian kinetochores (Warburton et al. 1997), and is intimately associated with α-satellite DNA, a major component of centromeric loci (Vafa and Sullivan 1997). Early studies demonstrated histone-like biochemical properties of CENP-A and cosedimentation of the CENP-A protein with mononucleosome particles (Palmer et al. 1987). More recent work with purified components has established that CENP-A can indeed replace histone H3 in reconstituted nucleosomes (Yoda et al. 2000). Together, these data indicate that the CENP-A protein forms a specialized nucleosome particle associated with centromeric chromatin domains.

Localization studies in a host of organisms have indicated deposition of CENP-A onto centromeric DNA is an early event in kinetochore assembly. The recruitment of the inner kinetochore component CENP-C requires CENP-A in mouse, *C. elegans*, and human cells (Kalitsis et al. 1998; Oegema et al. 2001; Van Hooser et al. 2001); CENP-A is required to mediate the centromeric localization of the ROD motor protein, POLO kinase, spindle checkpoint protein BUB1, and cohesin subunit MEI-S332 in *Drosophila* (Blower and Karpen 2001), and the Ctf19 kinetochore protein in *S. cerevisiae* (Measday et al. 2002). However, when the converse localization dependency was tested in these studies, CENP-A showed proper centromere localization in every case. These experiments suggest that CENP-A is a primary determinant of kinetochore assembly.

However, CENP-A deposition is not sufficient to direct kinetochore assembly. Altering expression levels or expression timing of CENP-A in human cells results in the association of CENP-A with non-centromeric regions (Shelby et al. 1997; Van Hooser et al. 2001). CENP-A can recruit at least a subset of other kinetochore proteins to these ectopic sites, and yet neocentromere activity is not observed. In *S. cerevisiae*, loss of CAF-I and Hir protein function in budding yeast causes the deposition of Cse4 to non-centromeric loci (Sharp et al. 2002). Because these sites exclude the CBF3 component Ndc10, it is unlikely that these Cse4-containing sites form competent neocentromeres.

The kinetochore-forming activity of CENP-A therefore occurs in a more complex chromatin context in multiple organisms examined.

Very little is known about the putative factors that act *in vivo* to assemble CENP-A nucleosomes at newly replicated centromeric DNA. In budding yeast, the CBF3 component Ndc10 is required to maintain Cse4 association with the core centromere (Ortiz et al. 1999). However, the apparent lack of conservation of the Ndc10 protein or other CBF3 subunits among other eukaryotes makes them poor candidates for a CENP-A assembly factor. Additionally, because CBF3 is crucial not only for structural integrity but also for the subnuclear localization and clustering of centromeric domains, it is possible that Ndc10 could be acting in a way that is one or more steps removed from the actual Cse4 deposition event. In *S. pombe*, Mis6 is required for the localization of Cnp1 to centromeres by the criterion of indirect immunofluorescence (Takahashi et al. 2000). However, this function of Mis6 is not conserved in budding yeast (Measday et al. 2002), and no definitive candidates for Cse4 deposition factors have emerged.

The assembly of CENP-A containing nucleosomes to nacent chromatin is uncoupled from replication. The timing of newly synthesized CENP-A protein is in G2, which is a period distinct from replication timing of centromeres (Shelby et al. 2000). Therefore, the putative deposition factor must act to incorporate new CENP-A nucleosomes at sites that may contain both old CENP-A nucleosomes and new H3-containing nucleosomes after replication. A role for the histone octamer transfer activity of the RSC nucleosome remodeling factor in catalyzing this reaction has been proposed (Sullivan 2001). Whatever the assembly mechanism turns out to be, it will be exciting to watch this developing story.

6. Concluding remarks

Chromatin proteins act at many levels to contribute to centromere function. In budding yeast, pericentric chromatin domains are built with the assistance of chromatin deposition proteins; these domains appear to be part of a higher-order chromatin structure important for stable microtubule-kinetochore attachments. In *S. pombe* and other eukaryotes, protein recruitment to centromeric regions is modulated by histone modifying enzymes that mark centromeric chromatin domains for subsequent heterochromatin assembly. In turn, heterochromatic regions then serve to recruit other factors required for chromosome segregation. Finally, in all eukaryotes, assembly of the microtubule-bunding kinetochore structure requires an isoform of histone H3. How different organisms coordinate chromatin elements to ensure high-fidelity chromosome

segregation is a major question in biology today.

7. Acknowledgements

We apologize to those whose work we have not discussed due to space limitations. The authors would like to thank Iain Cheeseman and members of the Kaufman laboratory for comments on this manuscript. This work was supported by Department of Energy funds administered through the Lawrence Berkeley National Laboratory, National Science Foundation Grant MCB-9982909, and by National Institutes of Health grants 1 R01 GM55712.

8. References

Aagaard L, Laible G, Selenko P, Schmid M, Dorn R, Schotta G, Kuhfittig S, Wolf A, Lebersorger A, Singh PB, Reuter G, Jenuwein T (1999) Functional mammalian homologues of the *Drosophila* PEV-modifier Su(var)3-9 encode centromere-associated proteins which complex with the heterochromatin component M31. EMBO J. 18: 1923-1938.

Abad JP, Villasante A (2000) Searching for a common centromeric structural motif: *Drosophila* centromeric satellite DNAs show propensity to form telomeric-like unusual DNA structures. Genetica 109: 71-75.

Allshire RC, Javerzat JP, Redhead NJ, Cranston G. (1994) Position effect variegation at fission yeast centromeres. Cell 76: 157-169.

Allshire RC, Nimmo, ER, Ekwall, K, Javerzat, JP, Cranston G. (1995) Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. Genes Dev. 9: 218-233.

Angus-Hill ML, Schlichter A, Roberts D, Erdjument-Bromage H, Tempst P, Cairns BR (2001) A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the chromatin remodeler RSC in gene expression and cell cycle control. Mol. Cell 7: 741-751.

Baker RE, Fitzgerald-Hayes M, O'Brien TC (1989) Purification of the yeast centromere binding protein CP1 and a mutational analysis of its binding site. J. Biol. Chem. 264: 10843-10850.

Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410: 120-124.

Baum M, Ngan VK, Clarke L (1994) The centromeric K-type repeat and the central core are together sufficient to establish a functional *Schizosaccharomyces pombe*

centromere. Mol. Biol. Cell. 5: 747-761.

Bernard P, Maure JF, Partridge JF, Genier S, Javerzat JP, Allshire RC (2001) Requirement of heterochromatin for cohesion at centromeres. Science 294: 2359-2342.

Blat Y, Kleckner N (1999) Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. Cell 98: 249-259.

Bloom KS, Carbon J (1982) Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. Cell 29: 305-317.

Blower MD, Karpen GH (2001) The role of *Drosophila* CID in kinetochore formation, cell-cycle progression and heterochromatin interactions. Nat. Cell Biol. 3: 730-739.

Bram RJ, Kornberg RD (1987) Isolation of a *Saccharomyces cerevisiae* centromere DNA-binding protein, its human homolog, and its possible role as a transcription factor. Mol. Cell. Biol. 7: 403-409.

Brasher SV, Smith BO, Fogh RH, Nietlispach D, Thiru A, Nielsen PR, Broadhurst RW, Ball LJ, Murzina NV, Laue ED (2000) The structure of mouse HP1 suggests a unique mode of single peptide recognition by the shadow chromo domain dimer. EMBO J. 19: 1587-1597.

Buchwitz BJ, Ahmad K, Moore LL, Roth MB, Henikoff SA (1999) A histone-H3-like protein in *C. elegans*. Nature 401: 547-548.

Cai M, Davis RW (1990) Yeast centromere binding protein CBF1, of the helix-loop-helix protein family, is required for chromosome stability and methionine prototrophy. Cell 61: 437-446.

Cai MJ, Davis RW (1989) Purification of a yeast centromere-binding protein that is able to distinguish single base-pair mutations in its recognition site. Mol. Cell. Biol. 9: 2544-2550.

Cairns BR, Lorch Y, Li Y, Zhang M, Lacomis L, Erdjument-Bromage H, Tempst P, Du J, Laurent B, Kornberg RD (1996) RSC, an essential, abundant chromatin-remodeling complex. Cell 87: 1249-1260.

Camerini-Otero R, Sollner-Webb B, Felsenfeld G (1976) The organization of histones and DNA in chromatin: evidence for an arginine-rich histone kernel. Cell *8*: 333-347.

Cao Y, Cairns BR, Kornberg RD, Laurent BC (1997) Sfh1p, a component of a novel

chromatin-remodeling complex, is required for cell cycle progression. Mol. Cell. Biol. 17: 3323-3334.

Cheeseman IM, Drubin DG, Barnes G (2002) Simple centromere, complex kinetochore:linking spindle microtubules and centromeric DNA in budding yeast. J. Cell. Biol. In press.

Chen Y, Baker RE, Keith KC, Harris K, Stoler S, Fitzgerald-Hayes M (2000) The N terminus of the centromere H3-like protein Cse4p performs an essential function distinct from that of the histone fold domain. Mol Cell Biol 20: 7037-7048.

Chikashige Y, Kinoshita N, Nakaseko Y, Matsumoto T, Murakami S, Niwa O, Yanagida M (1989) Composite motifs and repeat symmetry in S. pombe centromeres: direct analysis by integration of NotI restriction sites. Cell 57: 739-751.

Choo KH (2001) Domain organization at the centromere and neocentromere. Dev. Cell 1: 165-177.

Clarke L, Baum MP (1990) Functional analysis of a centromere from fission yeast: a role for centromere-specific repeated DNA sequences. Mol. Cell. Biol. 10: 1863-1872.

Clarke L, Carbon J (1980) Isolation of a yeast centromere and construction of functional small circular chromosomes. Nature 287: 504-509.

Cohen-Fix O (2001) The making and breaking of sister chromatid cohesion. Cell 106: 137-140.

Connelly C, Hieter P (1996) Budding yeast SKP1 encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell 86: 275-285.

Cumberledge S, Carbon J (1987) Mutational analysis of meiotic and mitotic centromere function in *Saccharomyces cerevisiae*. Genetics 117: 203-212.

Czermin B, Schotta G, Hulsmann BB, Brehm A, Becker PB, Reuter G, Imhof A (2001) Physical and functional association of SU(VAR)3-9 and HDAC1 in *Drosophila*. EMBO Rep. 2: 915-919.

Doe CL, Wang G, Chow C, Fricker MD, Singh PB, Mellor EJ (1998) The fission yeast chromo domain encoding gene chp1(+) is required for chromosome segregation and shows a genetic interaction with alpha-tubulin. Nucleic Acids Res. 26: 4222-4229.

Donze D, Adams CR, Rine J, Kamakaka RT (1999) The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. Genes Dev. 13: 698-708.

du Sart D, Cancilla MR, Earle E, Mao JI, Saffery R, Tainton KM, Kalitsis P, Martyn J,

Barry AE, Choo KH (1997) A functional neo-centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. Nat. Genet. 16: 144-153.

Earnshaw WC, Rothfield N (1985) Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma. Chromosoma 91: 313-321.

Eissenberg JC (2001) Molecular biology of the chromo domain: an ancient chromatin module comes of age. Genes Cells 275: 19-29.

Eissenberg JC, Elgin SC (2000) The HP1 protein family: getting a grip on chromatin. Curr. Opin. Genet. Dev. 10: 204-210.

Eissenberg JC, James TC, Foster-Hartnett DM, Hartnett T, Ngan V, Elgin SC (1990) Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U S A 87: 9923-9927.

Eissenberg JC, Morris GD, Reuter G, Hartnett T (1992) The heterochromatin-associated protein HP-1 is an essential protein in *Drosophila* with dosage-dependent effects on position-effect variegation. Genetics 131: 345-352.

Ekwall K, Nimmo ER, Javerzat JP, Borgstrom B, Egel R, Cranston G, Allshire R (1996) Mutations in the fission yeast silencing factors clr4+ and rik1+ disrupt the localisation of the chromo domain protein Swi6p and impair centromere function. J. Cell Sci. 109: 2637-3648.

Ekwall K, Olsson T, Turner BM, Cranston G, Allshire RC (1997) Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. Cell 91: 1021-1032.

Ellison MJ, Pulleyblank DE (1983) Internal structure of discrete nucleohistone complexes which form in vitro under conditions of physiological ionic strength. J. Biol. Chem. 258: 13314-13320.

Enomoto S, Berman J (1998) Chromatin assembly factor I contributes to the maintenance, but not the reestablishment, of silencing at the yeast silent mating loci. Genes Dev. 12: 219-232.

Enomoto S, McCune-Zierath PD, Gerami-Nejad M, Sanders M, Berman J (1997) *RLF2*, a subunit of yeast chromatin assembly factor I, is required for telomeric chromatin function *in vivo*. Genes Dev. 11: 358-370.

Fitzgerald-Hayes M, Clarke L, Carbon J (1982) Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. Cell 29: 235-244.

Gaudet A, Fitzgerald-Hayes M (1987) Alterations in the adenine-plus-thymine-rich region of CEN3 affect centromere function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 7: 68-75.

Gavin A-C et al (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415: 141-147.

Guacci V, Koshland D, Strunnikov A (1997) A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae*. Cell 91: 47-57.

Hahnenberger KM, Carbon J, Clarke L (1991) Identification of DNA regions required for mitotic and meiotic functions within the centromere of *Schizosaccharomyces pombe* chromosome I. Mol. Cell. Biol. 11: 2206-2215.

Harrington JJ, Van Bokkelen G, Mays RW, Gustashaw K, Willard HF (1997) Formation of de novo centromeres and construction of first-generation human artificial microchromosomes. Nat. Genet. 15: 345-355.

Hauf S, Waizenegger IC, Peters JM (2001) Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. Science 293: 1320-1323.

Hegemann JH, Fleig UN (1993) The centromere of budding yeast. Bioessays 15:451-460.

Henikoff S, Ahmad K, Platero JS, van Steensel B (2000) Heterochromatic deposition of centromeric histone H3-like proteins. Proc. Natl. Acad. Sci. U S A 97: 716-721.

Hieter P, Pridmore D, Hegemann JH, Thomas M, Davis RW, Philippsen P (1985) Functional selection and analysis of yeast centromeric DNA. Cell 42: 913-921.

Ikeno M, Grimes B, Okazaki T, Nakano M, Saitoh K, Hoshino H, McGill NI, Cooke HMasumoto H (1998) Construction of YAC-based mammalian artificial chromosomes. Nat. Biotechnol. 16: 431-439.

Ivanova AV, Bonaduce MJ, Ivanov SV, Klar AJ (1998) The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. Nat. Genet. 19: 192-195.

Jackson V (1988) Deposition of newly synthesized histones: hybrid nucleosomes are not tandemly arranged on daughter DNA strands. Biochemistry 27: 2109-2120.

Jackson V (1990) In vivo studies on the dynamics of histone-DNA interaction: evidence for nucleosome dissolution during replication and transcription and a low level of dissolution independent of both. Biochemistry 29: 719-731.

Jacobs SA, Taverna SD, Zhang Y, Briggs SD, Li J, Eissenberg JC, Allis CD, Khorasanizadeh S (2001) Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. EMBO J. 20: 5232-5241.

Jallepalli PV, Waizenegger IC, Bunz F, Langer S, Speicher MR, Peters JM, Kinzler KW, Vogelstein B, Lengauer C (2001) Securin is required for chromosomal stability in human cells. Cell 105: 445-457.

Jenuwein T, Allis CD (2001) Translating the histone code. Science 293: 1074-1080.

Jiang WD, Philippsen P (1989) Purification of a protein binding to the CDEI subregion of *Saccharomyces cerevisiae centromere* DNA. Mol. Cell. Biol. 9: 5585-5593.

Kalitsis P, MacDonald AC, Newson AJ, Hudson DF, Choo KH (1998) Gene structure and sequence analysis of mouse centromere proteins A and C. Genomics 47: 108-114.

Kamakaka RT, Bulger M, Kaufman PD, Stillman B, Kadonaga JT (1996) Post-replicative chromatin assembly by *Drosophila* and human Chromatin Assembly Factor-I. Mol. Cell. Biol. 16: 810-817.

Karpen GH, Allshire RC (1997) The case for epigenetic effects on centromere identity and function. Trends Genet. 13: 489-496.

Kaufman PD, Cohen JL, Osley MA (1998) Hir proteins are required for position-dependent gene silencing in *Saccharomyces cerevisiae* in the absence of Chromatin Assembly Factor I. Mol. Cell. Biol. 18: 4793-4806.

Kaufman PD, Kobayashi R, Kessler N, Stillman B (1995) The p150 and p60 subunits of chromatin assembly factor 1: a molecular link between newly synthesized histones and DNA replication. Cell 81: 1105-1114.

Kaufman PD, Kobayashi R, Stillman B (1997) Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cell lacking chromatin assembly factor-I. Genes Dev. 11: 345-357.

Kaya H, Shibahara KI, Taoka KI, Iwabuchi M, Stillman B, Araki T (2001) FASCIATA genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. Cell 104: 131-142.

Keith KC, Baker RE, Chen Y, Harris K, Stoler S, Fitzgerald-Hayes M (1999) Analysis of primary structural determinants that distinguish the centromere-specific function of histone variant Cse4p from histone H3. Mol Cell Biol 19: 6130-6139.

Keith KC, Fitzgerald-Hayes M (2000) CSE4 genetically interacts with the

Saccharomyces cerevisiae centromere DNA elements CDE I and CDE II but not CDE III. Implications for the path of the centromere DNA around a Cse4p variant nucleosome. Genetics 156: 973-981.

Kellum R, Alberts BM (1995) Heterochromatin protein 1 is required for correct chromosome segregation in *Drosophila* embryos. J. Cell Sci. 108: 1419-1431.

Krude T (1995) Chromatin assembly factor 1 (CAF-1) colocalizes with replication foci in HeLa cell nuclei. Exp. Cell Res. 220: 304-311.

Laloraya S, Guacci V, Koshland D (2000) Chromosomal addresses of the cohesin component Mcd1p. J. Cell Biol. 151: 1047-1056.

Le S, Davis C, Konopka JB, Sternglanz R (1997) Two new S-phase-specific genes from *Saccharomyces cerevisiae*. Yeast 13: 1029-1042.

Lechner J, Carbon J (1991) A 240 kd multisubunit protein complex, CBF3, is a major component of the budding yeast centromere. Cell 64: 717-725.

Lorch Y, Zhang M, Kornberg RD (1999) Histone octamer transfer by a chromatin-remodeling complex. Cell 96: 389-392.

Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8Å resolution. Nature 389: 251-260.

McGrew J, Diehl B, Fitzgerald-Hayes M (1986) Single base-pair mutations in centromere element III cause aberrant chromosome segregation in Saccharomyces cerevisiae. Mol. Cell. Biol. 6: 530-538.

Measday V, Hailey DW, Pot I, Givan SA, Hyland KM, Cagney G, Fields S, Davis TN, Hieter P (2002) Ctf3p, the Mis6 budding yeast homolog, interacts with Mcm22p and Mcm16p at the yeast outer kinetochore. Genes Dev. 16: 101-113.

Megee PC, Mistrot C, Guacci V, Koshland D (1999) The centromeric sister chromatid cohesion site directs Mcd1p binding to adjacent sequences. Mol Cell 4: 445-450.

Meijsing SH, Ehrenhofer-Murray AE (2001) The silencing complex SAS-I links histone acetylation to the assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae*. Genes Dev. 15: 3169-3182.

Meluh PB, Koshland D (1997) Budding yeast centromere composition and assembly as revealed by in vivo cross-linking. Genes Dev 11: 3401-3412.

Meluh PB, Yang P, Glowczewski L, Koshland D, Smith MM (1998) Cse4p is a component of the core centromere of *Saccharomyces cerevisiae*. Cell 94: 607-613.

Michaelis C, Ciosk R, Nasmyth K (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91: 35-45.

Miller JR, Hindkjaer J, Thomsen PD (1993) A chromosomal basis for the differential organization of a porcine centromere-specific repeat. Cytogenet. Cell Genet. 62: 37-41.

Monson EK, de Bruin D, Zakian VA (1997) The yeast Cac1 protein is required for the stable inheritance of transcriptionally repressed chromatin at telomeres. Proc. Natl. Acad. Sci. USA 94: 13081-13086.

Murakami S, Matsumoto T, Niwa O, Yanagida M (1991) Structure of the fission yeast centromere cen3: direct analysis of the reiterated inverted region. Chromosoma 101: 214-221.

Murzina N, Verreault A, Laue E, Stillman B (1999) Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. Mol. Cell 4: 529-540.

Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292: 110-113.

Ngan VK, Clarke L (1997) The centromere enhancer mediates centromere activation in *Schizosaccharomyces pombe*. Mol. Cell. Biol. 17: 3305-3314.

Noma K, Allis CD, Grewal SI (2001) Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Nature 293: 1150-1155.

Nonaka N, Kitajima T, Yokobayashi S, Xiao G, Yamamoto M, Grewal S, Watanabe Y (2002) Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast. Nat. Cell Biol. 4: 89-93.

Oegema K, Desai A, Rybina S, Kirkham M, Hyman AA (2001) Functional analysis of kinetochore assembly in *Caenorhabditis elegans*. J. Cell Biol. 153: 1209-1206.

Ortiz J, Stemmann O, Rank S, Lechner J (1999) A putative protein complex consisting of Ctf19, Mcm21, and Okp1 represents a missing link in the budding yeast kinetochore. Genes Dev. 13: 1140-1155.

Osada S, Sutton A, Muster N, Brown CE, Yates JR, Sternglanz R, Workman JL (2001) The yeast SAS (something about silencing) protein complex contains a MYST-type putative acetyltransferase and functions with chromatin assembly factor ASF1. Genes Dev. 15: 3155-3168.

Osley MA, Lycan D (1987) Trans-acting regulatory mutations that alter transcription of *Saccharomyces cerevisiae* histone genes. Mol. Cell. Biol. 7: 4204-4210.

Palmer DK, O'Day K, Wener MH, Andrews BS, Margolis RL (1987) A 17-kD centromere protein (CENP-A) copurifies with nucleosome core particles and with histones. J. Cell Biol. 104: 805-815.

Panzieri L, Landonio L, Stotz A, Philippsen P (1985) Role of conserved sequence elements in yeast centromere DNA. EMBO J. 4: 1867-1874.

Paro R, Hogness DS (1991) The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. Proc. Natl. Acad. Sci. U S A 88: 263-267.

Partridge JF, Borgstrom B, Allshire RC (2000) Distinct protein interaction domains and protein spreading in a complex centromere. Genes Dev. 14: 783-91.

Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C, Weipoltshammer K, Pagani M, Lachner M, Kohlmaier A, Opravil S, Doyle M, Sibilia M, Jenuwein T (2001) Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 107: 323-337.

Pinto I, Winston F (2000) Histone H2A is required for normal centromere function in *Saccharomyces cerevisiae*. EMBO J. 19: 1598-1612.

Platero JS, Hartnett T, Eissenberg JC (1995) Functional analysis of the chromo domain of HP1. EMBO J. 14: 3977-3986.

Qian Z, Huang H, Hong JY, Burck CL, Johnston SD, Berman J, Carol A, Liebman SW (1998) Yeast Ty1 retrotransposition is stimulated by a synergistic interaction between mutations in Chromatin Assembly Factor-I and histone regulatory (Hir) proteins. Mol. Cell. Biol. 18: 4783-4792.

Quivy JP, Grandi P, Almouzni G (2001) Dimerization of the largest subunit of chromatin assembly factor 1: importance in vitro and during *Xenopus* early development. EMBO J 20: 2015-2027.

Raghuraman MK, Winzeler EA, Collingwood D, Hunt S, Wodicka L, Conway A, Lockhart DJ, Davis RW, Brewer BJ, Fangman WL (2001) Replication dynamics of the yeast genome. Science 294:115-121.

Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406: 593-599.

Reuter G, Dorn R, Hoffmann HJ (1982) Butyrate sensitive suppressor of position-effect

variegation mutations in *Drosophila melanogaster*. Mol. Gen. Genet. 188: 480-485.

Reuter G, Spierer P (1992) Position effect variegation and chromatin proteins. Bioessays 14: 605-612.

Ruiz-Carrillo A, Jorcano JL, Eder G, Lurz R (1979) In vitro core particle and nucleosome assembly at physiological ionic strength. Proc. Natl. Acad. Sci. USA 76: 3284-3288.

Saffery R, Wong LH, Irvine DV, Bateman MA, Griffiths B, Cutts SM, Cancilla MR, Cendron AC, Stafford AJ, Choo KH (2001) Construction of neocentromere-based human minichromosomes by telomere-associated chromosomal truncation. Proc. Natl. Acad. Sci. U S A 98: 5705-5710.

Saunders M, Fitzgerald-Hayes M, Bloom K (1988) Chromatin structure of altered yeast centromeres. Proc. Natl. Acad. Sci. U S A 85: 175-179.

Saunders MJ, Yeh E, Grunstein M, Bloom K (1990) Nucleosome depletion alters the chromatin structure of *Saccharomyces cerevisiae* centromeres. Mol. Cell. Biol. 10: 5721-5727.

Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G (2002) Central role of Drosophila SU(VAR)3-9 in histone H3-K9 methylation and heterochromatic gene silencing. EMBO J. 21: 1121-1131.

Schueler MG, Higgins AW, Rudd MK, Gustashaw K, Willard HF (2001) Genomic and genetic definition of a functional human centromere. Science 294: 109-115.

Sharp JS, Fouts ET, Krawitz DC, Kaufman PD (2001) Yeast Histone Deposition Protein Asf1p Requires Hir Proteins and PCNA for Heterochromatic Silencing. Current Biology 11: 463-473.

Sharp JS, Franco AA, Osley MA, Kaufman PD (2002) Chromatin assembly factor I and Hir proteins contribute to building functional kinetochores in *S. cerevisiae*. Genes Dev. 16: 85-100.

Shelby RD, Monier K, Sullivan KF (2000) Chromatin assembly at kinetochores is uncoupled from DNA replication. J. Cell Biol. 151: 1113-1118.

Shelby RD, Vafa O, Sullivan KF (1997) Assembly of CENP-A into centromeric chromatin requires a cooperative array of nucleosomal DNA contact sites. J. Cell Biol. 136: 501-513.

Shibahara K, Stillman B (1999) Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. Cell 96: 575-85.

Singer MS, Kahana A, Wolf AJ, Meisinger LL, Peterson SE, Goggin C, Mahowald M, Gottschling DE (1998) Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. Genetics 150: 613-632.

Smith J, Caputo E, Boeke J (1999) A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. Mol. Cell. Biol. 19: 3184-3197.

Smith S, Stillman B (1989) Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication. Cell 58: 15-25.

Smith S, Stillman B (1991) Stepwise assembly of chromatin during DNA replication *in vitro*. EMBO J. 10: 971-980.

Smothers JF, Henikoff S (2000) The HP1 chromo shadow domain binds a consensus peptide pentamer. Curr. Biol. 10: 27-30.

Sonoda E, Matsusaka T, Morrison C, Vagnarelli P, Hoshi O, Ushiki T, Nojima K, Fukagawa T, Waizenegger IC, Peters JM, Earnshaw WC, Takeda S (2001) Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. Dev. Cell 1: 759-770.

Spofford JB (1967) Single-locus modification of position-effect variegation in *Drosophila* melanogaster. I. White variegation. Genetics 57: 751-766.

Steiner NC, Clarke L (1994) A novel epigenetic effect can alter centromere function in fission yeast. Cell 79: 865-874.

Stillman B (1986) Chromatin assembly during SV40 DNA replication *in vitro*. Cell 45: 555-565.

Stoler S, Keith KC, Curnick KE, Fitzgerald-Hayes M (1995) A mutation in CSE4, an essential gene encoding a novel chromatin-associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. Genes Dev. 9: 573-586.

Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403: 41-45.

Strahl BD, Ohba R, Cook RG, Allis CD (1999) Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in *Tetrahymena*. Proc. Natl. Acad. Sci. U S A 96: 14967-14972.

Sullivan KF (2001) A solid foundation: functional specialization of centromeric chromatin. Curr. Opin. Genet. Dev. 11: 182-188.

Sutton A, Bucaria J, Osley MA, Sternglanz R (2001) Yeast ASF1 Protein is Required for Cell-Cycle Regulation of Histone Gene Transcription. Genetics 158: 587-596.

Takahashi K, Chen ES, Yanagida M (2000) Requirement of Mis6 centromere connector for localizing a CENP-A-like protein in fission yeast. Science 288: 2215-2219.

Takahashi K, Murakami S, Chikashige Y, Niwa O, Yanagida M (1991) A large number of tRNA genes are symmetrically located in fission yeast centromeres. J. Mol. Biol. 218: 13-17.

Tanaka T, Cosma MP, Wirth K, Nasmyth K (1999) Identification of cohesin association sites at centromeres and along chromosome arms. Cell 98: 847-858.

Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, Reuter G (1994) The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3-9 combines domains of antagonistic regulators of homeotic gene complexes. EMBO J. 13: 3822-3831.

Tsuchiya E, Hosotani T, Miyakawa T (1998) A mutation in NPS1/STH1, an essential gene encoding a component of a novel chromatin-remodeling complex RSC, alters the chromatin structure of Saccharomyces cerevisiae centromeres. Nucleic Acids Res. 26: 3286-3292.

Tyler JK, Adams CR, Chen SR, Kobayashi R, Kamakaka RT, Kadonaga JT (1999) The RCAF complex mediates chromatin assembly during DNA replication and repair. Nature 402: 555-560.

Tyler JK, Bulger M, Kamakaka RT, Kobayashi R, Kadonaga JT (1996) The p55 subunit of *Drosophila* Chromatin Assembly Factor-I is homologous to a histone deacetylase-associated protein. Mol. Cell. Biol. 16: 6149-6159.

Tyler JK, Collins KA, Prasad-Sinha J, Amiott E, Bulger M, Harte PJ, Kobayashi R, Kadonaga JT (2001) Interaction between the Drosophila CAF-1 and ASF1 chromatin assembly factors. Mol Cell Biol 21: 6574-84.

Vafa O, Sullivan KF (1997) Chromatin containing CENP-A and alpha-satellite DNA is a major component of the inner kinetochore plate. Curr. Biol. 7: 897-900.

Valdivia MM, Brinkley BR (1985) Fractionation and initial characterization of the kinetochore from mammalian metaphase chromosomes. J. Cell Biol. 101: 1124-1134.

Van Hooser AA, Heald R (2001) Kinetochore function: the complications of becoming attached. Curr. Biol. 11: R855-R877.

Van Hooser AA, Ouspenski II, Gregson HC, Starr DA, Yen TJ, Goldberg ML, Yokomori K, Earnshaw WC, Sullivan KF, Brinkley BR (2001) Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A. J. Cell Sci. 114: 3529-3542.

Waizenegger IC, Hauf S, Meinke A, Peters JM (2000) Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell 103: 399-410.

Wang G, Ma A, Chow CM, Horsley D, Brown NR, Cowell IG, Singh PB (2000) Conservation of heterochromatin protein 1 function. Mol. Cell. Biol. 20: 6970-6983.

Warburton PE, Cooke CA, Bourassa S, Vafa O, Sullivan BA, Stetten G, Gimelli G, Warburton D, Tyler-Smith C, Sullivan KF, Poirier GG, Earnshaw WC (1997) Immunolocalization of CENP-A suggests a distinct nucleosome structure at the inner kinetochore plate of active centromeres. Curr. Biol. 7: 901-904.

Wood V, et al (2002) The genome sequence of *Schizosaccharomyces pombe*. Nature 415: 871-880.

Worcel A, Han S, Wong ML (1978) Assembly of newly replicated chromatin. Cell 15: 969-977.

Xu H, Kim UJ, Schuster T, Grunstein M (1992) Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 12: 5249-5259.

Xue Y, Canman JC, Lee CS, Nie Z, Yang D, Moreno GT, Young MK, Salmon ED, Wang W (2000) The human SWI/SNF-B chromatin-remodeling complex is related to yeast RSC and localizes at kinetochores of mitotic chromosomes. Proc. Natl. Acad. Sci. U S A 97: 13015-13020.

Yoda K, Ando S, Morishita S, Houmura K, Hashimoto K, Takeyasu K, Okazaki T (2000) Human centromere protein A (CENP-A) can replace histone H3 in nucleosome reconstitution in vitro. Proc. Natl. Acad. Sci. U S A 97: 7266-7271.

Zhang Z, Shibahara K, Stillman B (2000) PCNA connects DNA replication to epigenetic inhertance in yeast. Nature 408: 221-225.

Figure legends

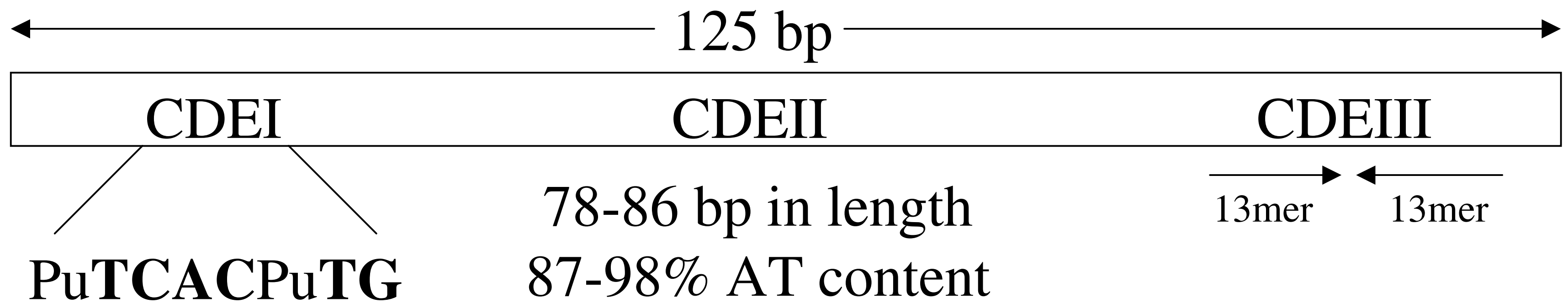
Figure 1. Consensus centromere in *S. cerevisiae* (after Hegeman and Fleig, 1993). Sequence elements present at all sixteen chromosomes are denoted CDEI, CDEII, and CDEIII. Invariant residues of the CDEI and CDEIII elements are marked in bold.

Figure 2. Consensus centromere in *S. pombe*. The core centromere is flanked by imr and otr repeat sequence elements. See text for details.

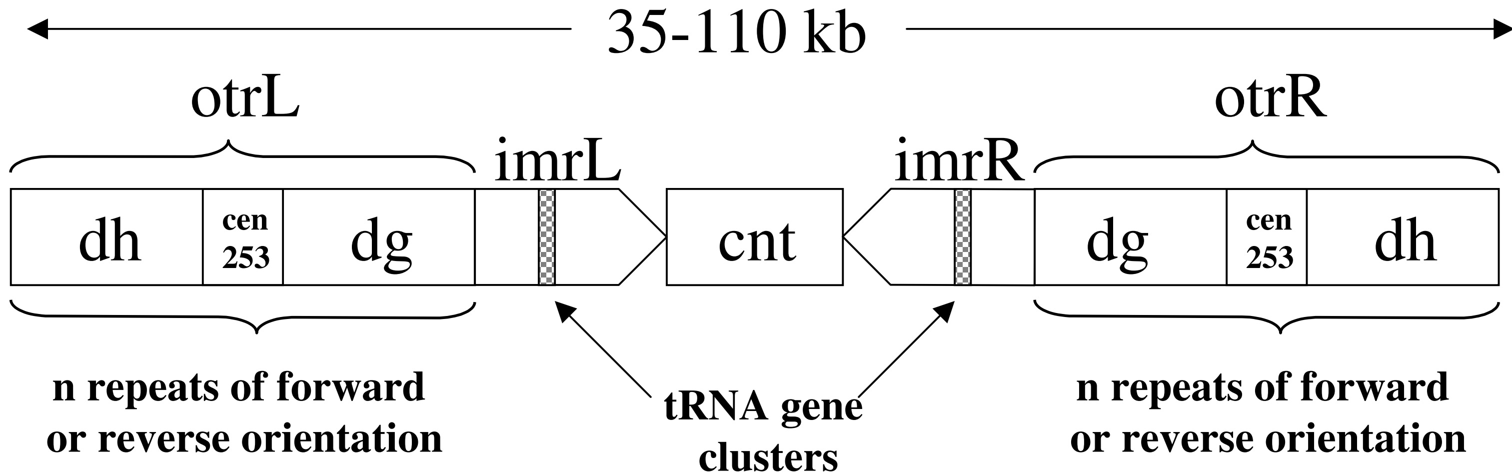
Figure 3. Chromatin domains at *S. cerevisiae* centromeres. Kinetochore proteins localize to the conserved core seqence, some have been omitted for clarity. Kinetochore regions are flanked by highly positioned nucleosomes that extend outward along chromosome arms. The CAF-I and cohesin protein complexes pictured above the diagram indicate widespread association across the entire *CEN* region (Blat and Kleckner 1999; Megee et al. 1999; Tanaka et al. 1999; Sharp et al. 2002). The Hir1 protein has been localized to centromeres by indirect immunofluorescence (Sharp et al., 2002).

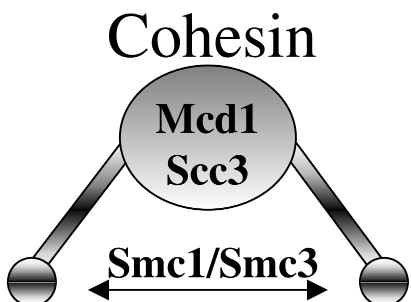
Figure 4. Chromatin domains at *S. pombe* centromeres. The core centromere is occupied by specialized Cnp-1 (CENP-A homolog) containing nucleosomes and kinetochore proteins. A tRNA gene (stippled boxes) marks a transition zone betweeen the core-associated proteins and the heterochromatic outer repeats. Swi6 (HP1 homolog) and Chp1-associated regions of centric heterochromatin define a zone of strong transcriptional silencing and nuclease-resistant chromatin. Clr4 (Su(var)3-9 homolog) and Rik1 are required for histone H3 Lys 9 methylation (Nakayama et al. 2001).

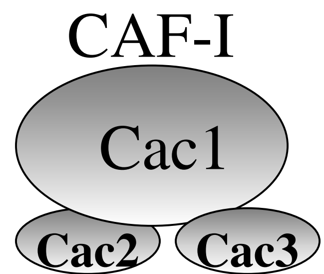
Figure 1.

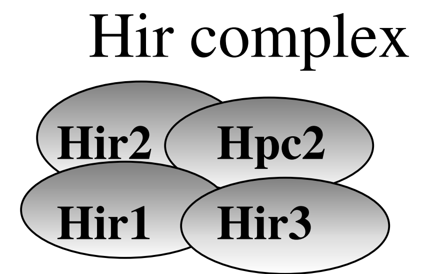


Schizosaccharomyces pombe









> 2 kb
phased nucleosomes

Mif2
Cbf1
Cse4
Skp1
Ctf13
Ndc10
Cep3
Okp1 Ctf19
Mcm21
Ctf3 Mcm16
Mcm22

> 2 kb
phased nucleosomes

CBF3

CORE CENTROMERE

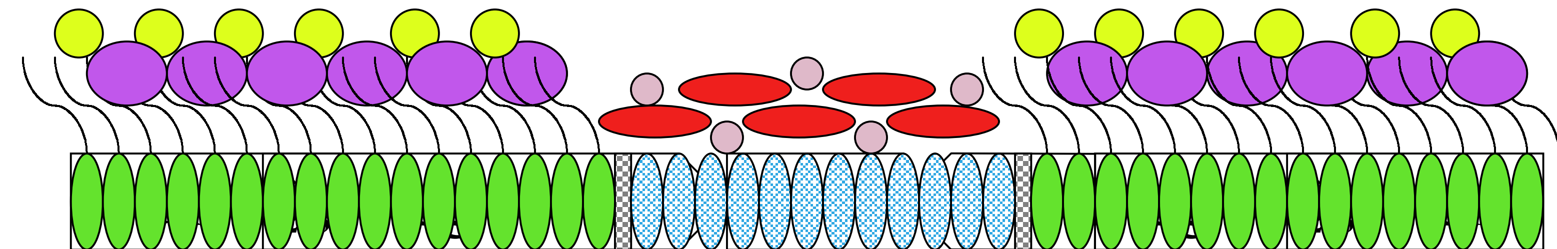
weak silencing,
nuclease sensitive

HETEROCHROMATIN

strong silencing,
nuclease resistant

Cnp1-containing
nucleosomes

HETEROCHROMATIN



Swi6

histone H3 Lys 9$^{\text{Methyl}}$

Chp1

Mis6

Mis12

Clr4
Rik1