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Centromere domain organization and histone modifications

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Abstract

Centromere function requires the proper coordination of several subfunctions, such as kinetochore assembly, sister chromatid cohesion, binding of kinetochore microtubules, orientation of sister kinetochores to opposite spindle poles, and their movement towards the spindle poles. Centromere structure appears to be organized in different, separable domains in order to accomplish these functions. Despite the conserved nature of centromere functions, the molecular genetic definition of the DNA sequences that form a centromere in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, in the fruit fly *Drosophila melanogaster*, and in humans has revealed little conservation at the level of centromere DNA sequences. Also at the protein level few centromere proteins are conserved in all of these four organisms and many are unique to the different organisms. The recent analysis of the centromere structure in the yeast *S. pombe* by electron microscopy and detailed immunofluorescence microscopy of *Drosophila* centromeres have brought to light striking similarities at the overall structural level between these centromeres and the human centromere. The structural organization of the centromere is generally multilayered with a heterochromatin domain and a central core/inner plate region, which harbors the outer plate structures of the kinetochore. It is becoming increasingly clear that the key factors for assembly and function of the centromere structure are the specialized histones and modified histones which are present in the centromeric heterochromatin and in the chromatin of the central core. Thus, despite the differences in the DNA sequences and the proteins that define a centromere, there is an overall structural similarity between centromeres in evolutionarily diverse eukaryotes.

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Introduction

The centromere is the region of the chromosome that is responsible for its segregation at mitosis and meiosis. Centromeres are therefore essential for genetic stability and there are several examples where defects in centromere function are associated with aneuploidy (aberrant chromosome number) causing birth defects or with cancer. Centromeres have been molecularly defined in several genetic model organisms and several centromere proteins have been characterized (for reviews, see Refs. 1,2). In this review we present a survey of centromere DNA sequences and centromere proteins from yeasts, *Drosophila* and humans. We focus on the comparison of centromere domain structure in the light of recent cytological

characterization of *Drosophila* and *Schizosaccharomyces pombe* centromeres (3,4). We also address the exciting mechanistic insights regarding the involvement of histone modifications in centromere assembly and function.

Centromeric DNA sequences

A survey of centromere DNA sequences from yeast, *Drosophila* and humans is presented in Table 1. In human cells the centromeric DNA consists of AT-rich α-satellite repeats (5). However, this DNA sequence is not absolutely required for centromere assembly and function since so-called 'neocentromeres' can sometimes be formed in regions of the chromosome which lack α-satellite DNA (6,7). In *Drosophila*, the centromere has been assigned on a Dp1187 minichromosome to a 420-kb region rich in repetitive DNA sequences (8). Also in *Drosophila* neocentromeres can in some cases be formed on other centromere sequences (9). Thus, both in human and *Drosophila* cells there is plasticity with respect to the DNA sequences that can form a centromere. In fission yeast (*S. pombe*) the centromere DNA encompasses 40- to 100-kb regions of the chromosome and has a symmetric organization. The minimal DNA sequence requirement for an *S. pombe* centromere is a central core sequence (*CC/cnt*) flanked by a portion of repeated arrays that consist of inner (*imr/B*) and outer (*otr/K + L*) sequences (10-12). In budding yeast, centromeres are relatively small and 120 bp of DNA is sufficient for a fully functional centromere (13). Thus, at least in humans and *Drosophila* the exact DNA sequences are not important for the formation of a functional centromere and in general there is no conservation between species with regard to the DNA sequences that can form a centromere.

Centromere proteins

A survey of centromere proteins from two yeasts, *Drosophila* and humans is presented in Table 1. Several centromere proteins have been defined in humans through the binding of autoantibodies from human patients with scleroderma. These immunoglobulins react with several distinct centromere proteins (CENP) (14,15). In *Drosophila* many centromere proteins have been defined: MEI-S332, POLO, PROD, ROD, ZW10, BUB1, BUB3, CENP-meta, HP1 and CID (DmCENP-A) (16-24). These proteins are part of different subdomains of the *Drosophila* centromere: the pericentric heterochromatin, the inner plate and the outer kinetochore (3). In *S. pombe* several centromere proteins have also been identified: Swi6, Chp1, Cnp1, Mis6, Mis12, Ndc80, Nuf2, Spc24, Alp14/Dis1 and Mtc1 (25-34). Chromatin immunoprecipitation crosslinking experiments have demonstrated that these proteins are also part of different centromeric subdomains. For example, Cnp1 (*S. pombe* CENP-A) and Mis6 proteins both bind to the central core region but not to the flanking regions (27,29,30). Conversely, the chromodomain proteins Swi6 and Chp1 bind to the flanking repeats but not to the central core region (35). This indicates that there are at least two distinct structural and functional domains in *S. pombe* centromeres. In budding yeast, centromere proteins have been defined and extensively studied. Recently, budding yeast kinetochore proteins were redefined using cytological live analysis and dynamic criteria (36). A total of 11 proteins including the *S. cerevisiae* counterparts of Nuf2, Ndc80, Spc24 and Spc25 were defined as kinetochore proteins in this study. The kinetochore proteins are likely to be anchored to the centromere via a DNA-binding protein complex CBF3 (37) and Cse4, which is the budding yeast CENP-A homologue, forming a specialized nucleosome which in turn forms the centromeric chromatin (38). Notably the pericentric heterochromatin is lacking in budding yeast centromeres and the corresponding heterochromatin pro-

teins are missing. The *S. cerevisiae* centromeres are thus smaller in size, indicating that the heterochromatin component may have been lost during the evolution of these 'trimmed down' kinetochores. To summarize, several centromere proteins have now been defined in the various organisms and although many factors appear to be organism specific it is possible to discern some degree of conservation (underlined proteins in Table 1). Interestingly, the variant histone CENP-A at the inner plate/central core seems

Table 1. A survey of centromere DNA sequences and proteins in humans, *Drosophila,* fission yeast and budding yeast.

Centromere	Human	*Drosophila*	Fission yeast	Budding yeast
DNA sequences (references - see text)	Tandem arrays of 171 bp Monomer α-satellite repeats	Simple satellites and single complete transposable elements	A 15-kb central core with unique sequences (*cnt* and *imr*) flanked by 20-100 kb or (*otr*/*K*+*L*) repeats	Three conserved regions CDE-I TCACATGAT, CDE-II 80-90 bp >90% A+T, and CDE-III TGATTTCCGAA
Minimal DNA sequence requirement for function (references - see text)	<500 kb of DXZ1 α-satellites are sufficient for centromere function	420 kb is sufficient for centromere function on a Dp1187 minichromosome	7 kb of central core + 2 kb of the *otr*/*K* repeat sufficient for function	120 bp of DNA sufficient for function (9). Deletion of CDE-I or CDE-II reduces centromere function but point mutations in the central CCG in CDE-III completely inactivate the centromere
Centromere proteins (references - see text)				
Heterochromatin	CENP-B INCENP M31 (mouse) SUV39H1 (mouse)	 HP1 Su(var)3-9* PROD MEI-S322	Cph1 (CENP-B homologue 1) Swi6, Chp1 Clr4*	
Inner plate/ central core	CENP-A CENP-C	CID (DmCENP-A)	Cnp1 (SpCENP-A) Mis6 Cph2 (CENP-B homologue 2)	Cse4 (ScCENP-A)
Outer kinetochore	HEC, Nuf2R CENP-E CENP-F hBUB1 XMAP215* CLIP170* BimC kinesins* Aurora B	 CENP-meta BUB1 BUB3 ZW10 POLO ROD	Ndc80, Nuf2, Spc24 Bub1 Alp14/Dis1 Mtc1	Ndc80, Nuf2, Spc24, Spc25 Dam1 Spc19 Spc34 Stu2 Bik1 Cin8 Ipl1

Homologous proteins are underlined and placed on the same horizontal line.
*Localization at centromere not determined.

to be completely conserved and parts of the Ndc80 protein complex (although hitherto not described in *Drosophila*) seem to be a conserved component of most eukaryotic kinetochores.

Centromere domain structure

Recent studies have revealed that the positions of some centromere proteins are conserved within the multilayered kinetochore structures from *S. pombe* to humans (Figure 1). In *S. pombe* the centromere proteins Mis6 and Cnp1 (SpCENP-A) associate exclusively with central core DNA, while the Swi6 protein binds to the surrounding repeats, suggesting that distinct protein interaction domains exist within the *S. pombe* centromeres (35). Electron microscopy and immunofluorescence light microscopy of the precisely positioned centromeres in interphase cells have revealed that the central core and flanking regions indeed occupy cytologically distinct positions within a heterochromatic domain. In addition, an 'anchor' structure containing the Ndc80 putative kinetochore protein was found between this heterochromatic domain and the spindle pole body (4). As seen by electron microscopy, the human metaphase centromere is multilayered, containing several substructures: a fibrous corona, an outer and an inner plate, as well as the space between them (39). Beneath the inner plates is the heterochromatic region. Each of these substructures appears to comprise a distinct protein composition. The fibrous corona contains CENP-E, dynein and dynactin, the outer plate contains CENP-F, the inner plate contains CENP-C and CENP-A, and the underlying heterochromatin contains CENP-B, INCENP, HP1 and SUV39H1 (40-46). Live analysis of cells expressing CENP-A and CENP-B green fluorescent protein fusion proteins indicates that, although the human kinetochore unfolds and refolds during interphase, the human pre-kinetochore structure remains ordered in interphase. CENP-A localization is limited to the edge of a larger CENP-B heterochromatin domain even before the typical double dot structure appears in G2 (47,48). At metaphase, CENP-A is a component of the inner plate in the human centromere (44). The human homologue of Ndc80, HEC, is localized to the outer part of HeLa cell centromeres (31). There are also human homologues of additional components of the

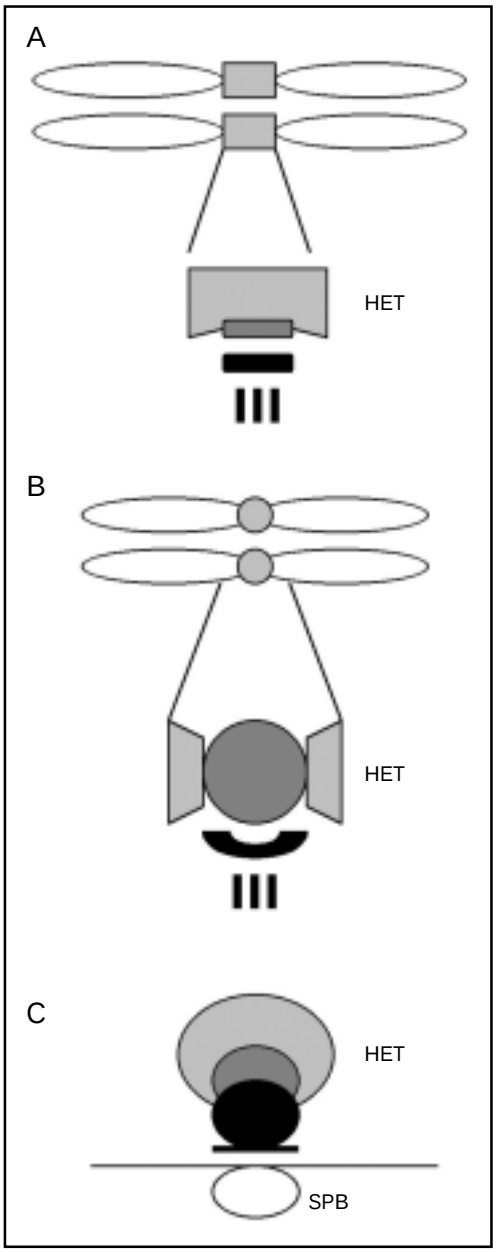

Figure 1. A schematic representation of the centromere domain organization at metaphase in humans (A), at metaphase in *Drosophila* (B), and the centromere cluster near the SPB at interphase in *Schizosaccharomyces pombe* (C). The heterochromatic (HET) domains (light gray), the inner kinetochore/inner plate/central core structures (dark gray) and the outer plate/outer kinetochore/anchor structures (black) are indicated. The vertical lines from the outer plate/kinetochore in A and B represent kinetochore microtubules. SPB = spindle pole body.

budding yeast kinetochore, i.e., XMAP215, CLIP170 and BimC kinesins but their localization in the human centromere has not been determined (36).

Histone modifications

Several different covalent modifications of the histone proteins have been reported to affect centromeric chromatin. These are acetylation, methylation and phosphorylation. Acetylation of histones is carried out by histone acetyl transferases at several conserved lysine residues of the N-terminal tails of histone H3 and H4. In general, histones in heterochromatin are relatively underacetylated when compared to those present in euchromatin (49,50). The opposite reaction, the deacetylation of histone tails, is performed by histone deacetylases (51). For *S. pombe*, *Drosophila* and human cells it has been shown that the centromeres are underacetylated (50,52). Treatment of *S. pombe* and human cells with the histone deacetylase inhibitor trichostatin A increases acetylation in centromeric heterochromatin and disrupts centromere function (50,53). The underacetylated state of the flanking repeats is necessary for binding of Swi6 to *S. pombe* centromeres and for binding of HP1 to human pericentric heterochromatin (50,53). Swi6 has been shown to associate with centromeric heterochromatin in fission yeast, and this association has been shown to require Clr4 (25,54). Recently, Clr4 belonging to a conserved chromo- and SET-domain family of proteins was shown to modify the N-terminal tail of histone H3 by methylation (55,56). Indeed H3 methylation was shown to be required for Swi6 binding to centromeric *otr/K* chromatin which is methylated at lysine 9 of histone H3 (57,58). It has been shown that deacetylation of histone H3 by histone deacetylases is required to allow for the subsequent methylation of this residue by the Clr4 histone H3 methyltransferase in a stepwise mechanism (57). The mouse SUV39H1 is a homologue of Clr4 with the same enzymatic function. It has been shown to be required for chromosome segregation and interacts with M31 (mouse HP1 homologue) in pericentric heterochromatin (46). SUV39H1 specifically accumulates in the centromere during prometaphase but dissociates from centromeric positions in anaphase (59). Su(var)3-9 in *Drosophila* was originally defined as a gene required for position effect variegation, i.e., silencing of the centromeric marker gene but it remains to be tested whether the protein itself is associated with centromeres. Thus, underacetylation of histones H3 and H4 and methylation of lysine 9 of histone H3 are required for heterochromatin formation and/or proper chromosome segregation in several different organisms. What is then the exact function of centromeric heterochromatin?

It is known from *S. pombe* that trichostatin A-induced acetylated centromeres display lagging chromosomes in anaphase cells. This phenotype was recently shown to be the consequence of a defective centromeric cohesion function since the cohesin Rad21 is dependent on Swi6 for its association with this region (60). Thus, although this remains to be demonstrated directly, it is conceivable that underacetylation of centromeric outer domain at least in *S. pombe* is a prerequisite for the centromeric cohesion function. Yet additional modifications are known to occur in centromeric histones. Phosphorylation occurs both of "normal histone H3" and of the histone H3 variant, CENP-A, localized to the central core of the centromere (61). In human cells this phosphorylation has been shown to occur in three phases of the cell cycle. Pericentric regions first accumulate H3 phosphorylation, followed by general phosphorylation of all chromatin and lastly by phosphorylation of CENP-A in the centromere (61). Interestingly, the Aurora B kinase colocalizes with INCENP at metaphase chromosomes and is responsible for phosphorylation of histone H3 as well as

being required for metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation (62,63). Notably the budding yeast homologue of Aurora kinase, Ipl1, is present in the kinetochore of this organism (36). The exact targets of the Ipl1 kinase at the budding yeast kinetochores are currently not known but Ipl1 is required for segregation of sister chromatids at anaphase (64).

Kinetochore assembly

The domain organization of the centromere described in *Drosophila* (3) is similar to the one described in *S. pombe* and humans. These findings now allow us to address the function of the different centromere domains in the genetic model organisms *Drosophila* and *S. pombe*. To this end, the study of Blower and Karpen indicated that CENP-A (CID) has a very central role in several centromere functions. Injection of antibodies against CID and/or RNAi inhibition of CID expression resulted in a multitude of centromeric defects including prophase arrest, indicative of an assembly defect, and lagging chromosomes which is indicative of cohesion defect. Inhibition of CID led to delocalization of several centromere proteins (POLO, ROD, BUB1, CENP-meta) indicating that the incorporation of all these factors is normally dependent on CID. In budding yeast Cse4 is necessary for proper chromosome segregation. In a *cse4* conditional mutant, the core centromere chromatin structure is disrupted at the restrictive temperature (38,65). Similarly, in fission yeast a mutation in the *mis6*$^{+}$ (*mis*-segregation) gene causes a failure of the Cnp1 (SpCENP-A) protein to bind the central core chromatin leading to massive aneuploidy (29,30). Although it remains to be investigated whether other kinetochore components are lost from centromeres in *mis6-1* cells, the strong defects indicate that CENP-A may be required for kinetochore assembly also in *S. pombe*. What targets CENP-A to centromeres? Replication timing might be important to form a functional centromere and has been suggested as an important determinant for CENP-A targeting (66). In *Drosophila* it has been shown that the centromeres replicate in the early S-phase while surrounding heterochromatin replicates later (67). This replication pattern might be crucial since a human neocentromere 10q25.3 causes the surrounding DNA to replicate later in the S-phase as compared to a wild-type chromosome 10 (68). However, other investigations show that replication timing does not matter for the incorporation of CENP-A and formation of a functional centromere (69). In fission yeast *otr/K* repeats in the centromeres replicate early (70) and CENP-A is expressed early in the S-phase (30). However, there is no evidence for an early replication requirement for CENP-A incorporation into *S. pombe* centromeres.

Conclusions and perspectives

Centromere DNA sequences were recently defined molecularly in humans and can be compared with those of genetic model organisms, i.e., two evolutionarily distant yeasts (*S. pombe* and *S. cerevisiae*) and *Drosophila*. Perhaps surprisingly, given the crucial function of centromeres in maintaining genomic stability, there is no obvious conservation of the DNA sequences which form centromeres in the different eukaryotes. Even the centromere proteins are in many cases species-specific but comparative approaches have revealed some key components such as the histone H3 variant CENP-A which is required for centromere function in all systems. Despite the differences in DNA sequences and protein composition of centromeres, the structural organization at the electron microscope level reveals a conserved multilayered domain organization between *S. pombe*, *Drosophila* and humans (Figure 1). These centromeres are based on a

heterochromatic part of the chromosome that contains a special chromatin structure with underacetylated histones. It is conceivable that this structural similarity is conserved to accommodate the common subfunctions of a centromere: kinetochore assembly, sister chromatid cohesion, binding of kinetochore microtubules, orientation of sister kinetochores to opposite spindle poles, and their movement towards the spindle poles.

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