The Centromere: Hub of Chromosomal Activities

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Centromeres are the structures that direct eukaryotic chromosome segregation in mitosis and meiosis. There are two major classes of centromeres. Point centromeres, found in the budding yeasts, are compact loci whose constituent proteins are now beginning to yield to biochemical analysis. Regional centromeres, best described in the fission yeast *Schizosaccharomyces pombe*, encompass many kilobases of DNA and are packaged into heterochromatin. Their associated proteins are as yet poorly understood. In addition to providing the site for microtubule attachment, centromeres also have an important role in checkpoint regulation during mitosis.

Centromeres have multiple roles during mitosis and may also have essential functions in interphase. (i) The centromere is the site of formation of the kinetochore, the buttonlike structure at the chromosomal surface that binds spindle microtubules and regulates chromosome movements in mitosis. The DNA sequence that specifies centromere location on the chromosome is referred to as the *CEN* locus. (ii) The centromere is the final locus of sister chromatid pairing in mitosis and, therefore, must receive the signal that triggers the release of sister chromatids at the metaphase-anaphase transition. Centromeres presumably contain the machinery necessary to effect this separation. (iii) The centromere is involved in cell cycle checkpoint control. In most cells, centromeres of mitotic chromosomes that have not yet achieved a stable bipolar orientation on the spindle send a signal that delays the onset of the metaphase-anaphase transition. (iv) The centromere acts as a marshaling area for the chromosomal passenger proteins, proteins that transfer from the chromosomes to the mitotic spindle during metaphase or anaphase (*1*). These are thought to be mitosis-specific cytoskeletal proteins (Fig. 1).

In this review, we discuss several aspects of centromere structure and function that have received particular attention in recent years. For other reviews we refer the reader to references (*2*–*4*).

The Two Classes of Centromeres: Point and Regional

Although centromere functions are conserved in all cells, centromeres exhibit bewildering structural variability between species. This ranges from holocentric chromosomes, where microtubules attach along the entire length of the chromatid, to chromosomes with localized centromeres, where microtubules attach to a single region that often appears constricted relative to the chromosome arms. Although holocentric chromosomes occur in many arthropods and plants as well as in the well-studied genetic model organism *Caenorhabditis elegans* (*5*), their structure remains poorly understood. This review will therefore focus on localized centromeres.

There are two classes of localized centromeres, point and regional centromeres. Point centromeres have been identified in the budding yeasts *Saccharomyces cerevisiae* (*3*–*5*), *Schizosaccharomyces uvarum* (6), and *Kluyveromyces lactii* (*7*). These compact loci encompass three conserved DNA elements (CDEs): CDEI, CDEII, and CDEIII (*8*). [The point centromeres of *Yarrowia lipolytica* have a different sequence organization (*9*).] The 25–base pair (bp) CDEIII is absolutely required for centromere function and is the binding site for an essential protein complex, termed CBF3 (*10*). Mutations in CDEIII or in genes encoding the CBF3 polypeptides disrupt chromosome segregation (*3*–*5*). The 8-bp CDEI sequence also appears in the promoters of a number of genes. Its binding factor, Cpf1p (*11*), is a transcriptional activator when bound to these noncentromeric sequences. This is paradoxical because transcription directed toward the core *CEN* sequences abolishes centromere function in vivo (*12*). CDEI and CDEIII are separated by CDEII, an A:T-rich region whose primary sequence is not well conserved but whose length is important for centromere function.

The point centromere is packaged into a compact nuclease-resistant chromatin structure of ~250 bp (*13*) that binds a single microtubule (*14*). Several findings suggest a model in which this structure may be assembled around a modified nucleosome. A reduction in amounts of core histones H2B or H4 alters centromeric chromatin (*15*), and *CSE4*, a gene required for chromosome segregation, encodes a highly divergent histone H3–related molecule (*16*). The human centromeric polypeptide CENP-A is also a very divergent histone H3 (*17*). Because the preferred length for CDEII, 78 to 86 bp, corresponds to roughly one turn of the DNA around the nucleosomal core, this model predicts that CDEI and CDEIII might be juxtaposed in space (Fig. 2).

Regional centromeres, the other class of

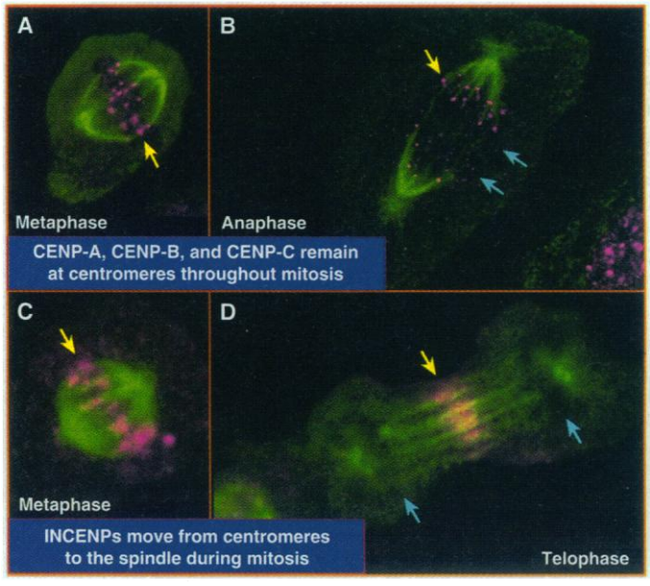

Fig. 1. Different behavior of intrinsic proteins of the centromere and chromosomal passenger proteins during mitosis. Both the CENP antigens [(**A**), labeled red with autoimmune serum] and passenger proteins, the INCENPs [(**B**), labeled red with rabbit serum], concentrate at centromeres during metaphase. The CENP antigens remain at centromeres during anaphase (**C**), whereas the INCENPs transfer to the overlapping microtubules of the central spindle (**D**). The position of the CENP and INCENP antigens in mitosis is indicated by yellow arrows. Microtubules are labeled green, and the blue arrows indicate the position of the chromosomes during anaphase and telophase.

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localized centromeres, encompass kilobases of DNA and include both unique and repeated DNA elements. The organization of the repeated DNA component can exhibit considerable polymorphisms between strains, as it does in *S. pombe* (*18*), or between individuals, as it does in humans (*19*). Whereas a single *CEN* locus accomplishes all aspects of centromere function in the point centromere, in regional centromeres different sequences may have become specialized to regulate kinetochore assembly and sister chromatid pairing.

Centromeres of *S. pombe* are the paradigm for the regional centromere. These loci span 40 to 100 kb (*20*), with the smallest functional centromeres containing ≥19 kb (*21*). The chromatin structure and functional organization of these centromeres are complex. The centromeres of *S. pombe* all contain a 4- to 7-kb central core sequence embedded within a domain containing both inverted and direct repeats. This central core contains several functionally redundant domains (*21*). A 2.1-kb region of the K/dg-type flanking repeat is also required for centromere function (*20*). This region confers a special chromatin structure on the central core sequences (*22*) and has a number of binding sites for proteins that have yet to be identified. It is not known if the bundles of two to four microtubules bound by *S. pombe* kinetochores (*23*) are associated with specific DNA sequences, as is the case in the point centromere, or if the regional centromere adopts a three-dimensional configuration that nucleates assembly of a proteinaceous kinetochore. It cannot be excluded that regional centromeres contain point *CEN* loci that have yet to be identified.

In most organisms, centromeres are composed of heterochromatin, a specialized form of chromatin that remains condensed throughout the cell cycle, replicates late in the S phase, and suppresses the expression of most genes. Like classic heterochromatin, *S. pombe* centromeres are not transcribed and can exert a position effect on reporter genes that are inserted into the centromeric array (*24*). Certain mutants that affect silencing of the mating type loci (*clr4*, *rik1*, and *swi6*) also affect centromeric silencing (*25*). These mutations also compromise the fidelity of chromosome segregation, suggesting that transcriptional suppression and centromere function are mechanistically linked. Recent work on the *swi6*$^+$ gene reveals that Swi6p is enriched in centromeric and telomeric heterochromatin (*26*). Swi6p has a sequence motif, the chromo domain, that has been found in several proteins that are involved in the stable repression of gene expression (*27*). One of these proteins, HP1, is concentrated in heterochromatin (*28*), particularly at centromeres in mammals (*29*). The other protein, polycomb, is thought to stabilize chromatin domains in a transcriptionally inactive state.

In a further parallel with heterochromatin, the establishment of centromeric activity in *S. pombe* is subject to functional variegation. When certain artificial chromosomes are introduced into *S. pombe* cultures, a substantial difference in chromosome stability is seen between different colonies (*30*). Thus, centromere function may require not only a minimal set of sequences, but the acquisition of specific epigenetic modifications by those sequences.

A recent study suggests a fundamental similarity of organization between the *S. pombe* and *Drosophila melanogaster* centromeres. *Drosophila* centromere function requires *Bora Bora*, a 220-kb "island" containing complex sequence DNA (*31*). For full centromere function, *Bora Bora* must be flanked on either the 5′ or 3′ side by another ~200 kb of simple sequence satellite DNA. It has been suggested that *Bora Bora* nucleates kinetochore formation, whereas the simple-sequence DNA regulates sister chromatid pairing.

Several groups are attempting to define functional *CEN* sequences in mammals, with the ultimate goal of constructing stable artificial chromosomes. This approach is hindered by the extraordinary variability of centromeric DNA sequences between species. Only one centromeric sequence element is known to be conserved between primates and rodents. This is the 17-bp binding site for CENP-B (*32*, *33*). A summary of the centromere components of mammals is presented in Fig. 3. The "CENP-B box" is found in human α-satellite DNA, *Mus musculus* minor satellite DNA, and in a minimal functional form (9 of 17 bp conserved) in the 79-bp centromeric satellite DNA of *Mus caroli* (*34*). In African green monkey α-satellite DNA the CENP-B box occurs at a frequency ≤0.1% of that found in human cells (*35*), raising doubts as to whether the binding of CENP-B to this sequence can be a major determinant of centromere structure and function. Such concerns were previously raised by the demonstration that CENP-B protein and CENP-B box DNA are both undetectable on the human Y chromosome (*36*).

Current evidence indicates that α-satellite DNA does have an important role in centromere function. When incorporated into ectopic locations on chromosome arms, transfected α-satellite DNA can perturb the segregation of sister chromatids (*37*). Whether this occurs because of the assembly of extra kinetochores or because of effects on sister chromatid separation remains to be determined. One approach to identifying human centromeric DNA involves the use of telomere-mediated fragmentation of chromosomes within the centromere. This approach has enabled the localization of the Y chromosome centromere to a region of ~70 kb within the α-satellite array (*38*). The most stable of these fragmented chromosomes also retain unique sequences from the p arm of the Y chromosome. The parallel to *S. pombe* and *Drosophila* regional centromeres is noteworthy.

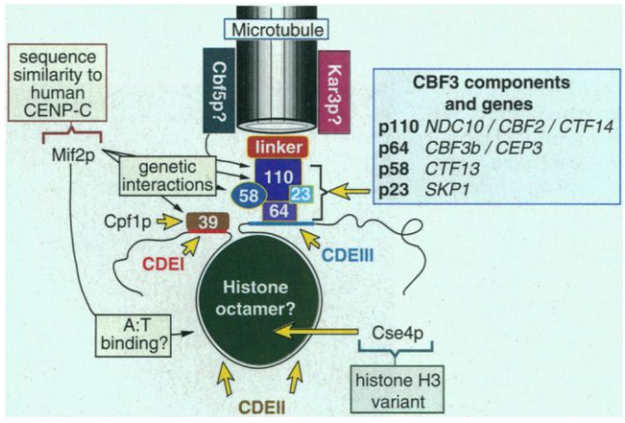

Fig. 2. Hypothetical model for the budding yeast kinetochore. The 125-bp *CEN* DNA is shown wrapped around a nucleosomal core, which may contain the histone H3 variant Cse4p (*16*). Whilst this model is consistent with the results of mutational analysis of the histones and length constraints on the CDEII sequence, direct evidence for such a configuration has yet to be obtained. Conserved DNA element I (CDEI, 8 bp) is complexed with the 39-kD Cpf1p [also known as CP1 and CBF1 (*11*)]. The four-subunit CBF3 complex binds the 25-bp element CDEIII. The CBF3 subunits include p110 [encoded by the *NDC10/CBF2/CTF14* gene (*68*, *69*)], p64 [*CBF3b/CEP3* (*70*)], p58 [*CTF13* (*69*)], and p23 [*SKP1* (*71*)]. The detailed functions of these components are unknown, although p64 contains zinc finger motifs and has therefore been suggested to be the DNA binding subunit. p23 is conserved in species from *Arabadopsis* to mammals (*71*). The hypothetical linker molecule is inserted as a result of experiments demonstrating that CBF3 alone is not sufficient to cause *CEN* DNA to associate with microtubules in vitro (*72*). The kinesin-related protein Kar3p appears to be responsible for centromere movement on microtubules under in vitro conditions (*73*). Mif2p, which is required for chromosome segregation and spindle assembly (*74*, *75*), interacts genetically with p39, p64, and p110. Cbf5p, which can bind to microtubules, also interacts genetically with p110 (*56*). Mif2p shares several short regions of amino acid similarity with the human kinetochore protein CENP-C (*75*, *76*).

Centromeres May Function During Interphase

Whereas the functions of mitotic centromeres are well documented, the role of centromeres during interphase remains enigmatic. The persistence of condensed centromeres during interphase was first revealed by the staining of discrete foci in interphase nuclei with serum from autoimmune patients (*39*). These foci correspond to specialized heterochromatin domains (*40*). The demonstration that these foci of antibody staining colocalize with α-satellite DNA suggested a tight association of at least some "intrinsic" proteins with the centromere throughout the cell cycle (*41*). Association of these proteins with centromeres during interphase is apparently required for assembly of functional kinetochores. Microinjection of centromere antibodies during interphase disrupts kinetochore assembly and blocks progression through mitosis (*42*, *43*). Thus, centromeres are not quiescent during interphase but require specific interactions with other proteins to prepare for their subsequent roles in chromosome segregation during mitosis.

Mammalian centromeres occupy distinct, nonrandom positions in the interphase nucleus. However, these centromeres are not entirely clustered or polarized as has been described in fission yeast (*44*) and *Drosophila* (*45*). Instead, mammalian centromeres tend to congregate near the nuclear periphery and around nucleoli, although they can also be found throughout the nuclear volume. These interphase centromere positions are not static, nor does their arrangement appear to conform to one pattern for all cell types. Rather, within a cell type, centromere distribution with regard to these two nuclear landmarks reproducibly changes during cell cycle progression (*46*) and in response to the functional (*47*) and transcriptional states of the cell (*48*, *49*). This has led to the thinking that highly repeated sequences such as those at centromeres may organize the interphase nucleus in ways that influence gene expression (*50*).

The association of interphase centromeres with nucleoli in human cells is true not only for the acrocentric chromosomes in which centromeres and nucleolus organizer regions (NOR) are in relatively close proximity, but also for centromeres of non–NOR-bearing chromosomes (*49*, *50*). Centromere proteins are detectable both ultrastructurally and biochemically in isolated human nucleoli (*51*). Furthermore, preliminary evidence has been obtained for a direct biochemical interaction between human kinetochore protein CENP-C (*52*) and the nucleolar transcription factor UBF (also known as NOR90) (*53*). Whereas the functional significance of these associations remains unclear, studies of human autoantibodies suggest that centromere associations with nucleoli may have important consequences. In particular, it has been postulated that a complex between nucleoli and centromeres is the dominant autoantigen in scleroderma spectrum disease (*54*).

A further link between centromeres and nucleoli involves Nap57p, a nucleolar protein from rat liver, which is thought to function as a chaperone for nucleolar proteins (*55*). Nap57p closely resembles Cbf5p, a *S. cerevisiae* protein of unknown function (*55*, *56*) that interacts genetically with CBF3 (Fig. 2) and is also localized to the nucleolus (*56*). The detection of biochemical associations between centromeres and nucleolar proteins suggests that centromeres may have as yet undiscovered functions during interphase.

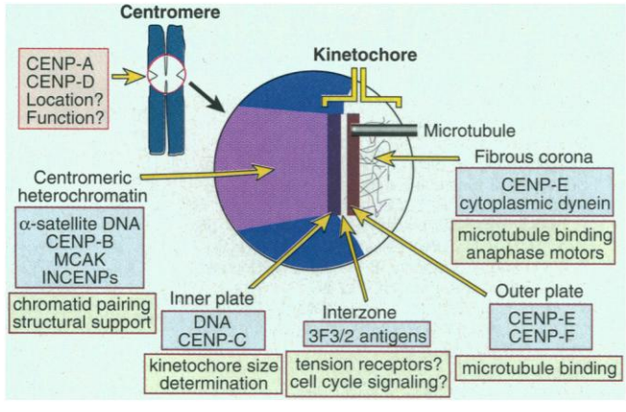

Fig. 3. Components of the mammalian centromere. The condensed centromeric heterochromatin, which may function as a structural foundation for the kinetochore, is rich in α-satellite DNA and its binding protein CENP-B (*33*, *40*) but also contains the kinesin-related MCAK (*77*) and the INCENP chromosomal passengers (*78*). At the surface of the heterochromatin is the inner kinetochore plate. This structure contains both DNA (*79*) and CENP-C (*52*), with the latter being essential for kinetochore assembly (*43*). The outer kinetochore plate, which contains the kinesin-related CENP-E (*80*) and CENP-F (also termed mitosin) (*81*) is involved in microtubule binding. The interzone between the two plates contains the 3F3/2 antigens (*65*) and may be involved in tension sensing and cell cycle signaling. The fibrous corona contains microtubule motor proteins, both of the kinesin [CENP-E (*80*)] and cytoplasmic dynein (*82*) families. The detailed localizations of CENP-A [a divergent histone H3 that resembles yeast Cse4p but is not functionally interchangeable with it (*16*, *17*)] and CENP-D [which appears to correspond to the RCC1 protein, a regulator of nuclear transport (*83*)] are not known.

Centromeres Signal the "Wait Anaphase" Checkpoint

When vertebrate cells enter mitosis, the nuclear envelope breaks down and chromosomes randomly encounter microtubules that emanate from opposing centrosomes. The ensuing processes of microtubule capture by kinetochores and the gradual establishment of a balance of forces between opposing kinetochores and centrosomes ultimately result in the establishment of the metaphase configuration with all chromosomes lined up at the midzone of a bipolar spindle and awaiting the onset of anaphase. If aneuploidy is to be avoided, it is crucial that the cell delay anaphase onset until all chromosomes have achieved this balanced alignment. In recent years, significant advances have been made in the characterization of this checkpoint.

In 1970, Zirkle proposed that a signal emanating from the chromosomes or spindle could prolong metaphase until both kinetochores of every chromosome had been attached to microtubules and brought to the metaphase plate (*57*). Dietz (*58*) had originally proposed and Nicklas (*59*) demonstrated that meiotic kinetochores could somehow sense tension. This led to the proposal that in the absence of tension produced by a stable bipolar attachment, kinetochores transmit an inhibitory signal that delays the metaphase-anaphase transition (*60*). Results consistent with this view have been obtained in *S. cerevisiae*, where cells with mutant centromeres exhibit a mitotic delay (*61*), and in humans, where microinjection of antibodies to centromeres also causes a mitotic delay (*42*).

A recent study provides direct evidence for the involvement of kinetochore tension in releasing cells from the "wait anaphase" checkpoint. In mantid spermatocytes, the presence of a single mono-oriented X chromosome results in a permanent block to anaphase entry, leading to eventual cell death. However, if such a chromosome is placed under gentle tension by pulling it away from the pole with a microneedle, the cells overcome their metaphase block and proceed through anaphase (*62*). Presumably the external application of tension switches off the "wait anaphase" signal-generating mechanism at the kinetochore.

In cultured vertebrate cells, this "wait anaphase" signal can be transmitted by unattached kinetochores. In the rat kangaroo cell line Ptk_1, attachment of the last free kinetochore to the spindle precedes anaphase onset by about 20 min, and the presence of one or more mono-oriented chromosomes blocks the metaphase-anaphase transition (*63*). However, if the unattached kinetochore of such a mono-oriented chromosome is destroyed with a laser, the cell then enters anaphase ~20 min later (*63*). This was interpreted to show that

destruction of the unattached kinetochore abolished transmission of an inhibitory "wait anaphase" signal.

A fortuitous observation provided the first evidence for a link between the attachment status of a kinetochore and its biochemical makeup. In addition to staining other cellular structures, the phosphoepitope-specific monoclonal antibody 3F3/2 preferentially stains kinetochores that are not under tension (*64*). Injection of this antibody into cells delays the metaphase-anaphase transition without affecting chromosome movements (*65*). The kinase-phosphatase balance responsible for expression of this phosphoepitope may either be indicative of, or actually contribute to, the transmission of the "wait anaphase" signal (*64*, *65*).

Recently, a direct experimental link was shown between the expression of the 3F3/2 epitope and kinetochore tension in grasshopper spermatocytes. When bivalents were detached from the metaphase spindle, their kinetochores acquired strong 3F3/2 reactivity. If these bivalents were then directed to reattach to a single spindle pole (no kinetochore tension), 3F3/2 reactivity remained high. However, if such mono-oriented bivalents were then stretched with a microneedle so as to bring one kinetochore under tension, the amount of 3F3/2 epitope at the tense kinetochore underwent a marked decrease (*66*).

Whereas recent studies have made progress toward proving that kinetochores can send signals and that spindle tension status affects kinetochore biochemistry, fundamental questions remain. On the one hand, studies with 3F3/2 and micromanipulation point to tension as a key factor in the "wait anaphase" signal. On the other hand, the laser ablation studies suggest that kinetochore attachment rather than tension is important. When the unattached kinetochore of a mono-oriented chromosome is ablated, this does not put the attached kinetochore under tension, yet it does turn off the "wait anaphase" signal (*63*). These inconsistencies may be due to intrinsic differences between meiosis and mitosis (*67*), or more subtle factors may be involved.

Clearly, no single structure or mechanism can yet be used to define all centromeres. This variability reflects not only the complexity of the functions attributed to the centromere, but also, undoubtedly, the regulatory circuits involved in cell cycle checkpoints that are influenced by the centromere. Continued progress in studying both point and regional centromeres at all stages of the cell cycle should produce a unified view of this important chromosomal structure.

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