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The Centromere Frontier: Kinetochore Components, Microtubule-Based Motility, and the CEN-Value Paradox

Minireview

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The kinetochore, an integrated nucleic acid and protein complex, directs chromosome movement during mitosis and meiosis. Chromosome segregation depends upon the coordinated assembly of kinetochore components at the centromere DNA and attachment to the bipolar array of spindle fibers emanating from microtubule-organizing centers. Both kinetochores and spindles have been the focus of recent efforts aimed at elucidating a mechanism that accounts for the exquisite fidelity of reactions involving chance encounters.

While activities of a single protein (such as protein phosphorylation, DNA binding, and transcriptional activation) may be highly conserved, individual components of complex structures may not be as forgiving of evolutionary alterations. The phylogenetic distance between yeast, the first source of centromere DNA, and humans, from which kinetochore proteins were initially isolated, has precluded the development of an in vitro kinetochore assembly system. Thus, the recent isolation of kinetochore proteins and centromere DNA from homologous systems, allowing biochemical as well as genetic analyses, will contribute to understanding the multifunctional nature of the kinetochore and how it is integrated into the cell cycle circuitry.

Kinetochore Proteins

Human patients with the variant of scleroderma known as CREST (calcinosis, Raynaud phenomenon, esophageal dismotility, sclerodactly, and telangiectasia) produce autoantibodies that react with a number of distinct mammalian proteins (centromere proteins [CENPs] and inner centromere proteins [INCENPs]; Table 1). These antibodies have been instrumental in delineating domains within the tripartite kinetochore structure (Earnshaw and Rattner, 1989; Earnshaw and Bernat, 1991).

In yeast, proteins have been purified that specifically bind centromere DNA elements (CDEs). In a tour de force of protein biochemistry, limiting centromere-binding factors (CBF3A, CBF3B, and CBF3C) have been identified that form a 240 kd tertiary complex, CBF3 (Lechner and Carbon, 1991). These proteins, which recognize yeast CDEIII with high specificity in vitro, are essential kinetochore components.

Table 1. Mammalian Centromere-Binding Proteins

Protein	Motifs	Phenotype[a]
CENP-A	Histone-like	
CENP-B	Acidic serine-rich region	G2–M arrest
CENP-C	Hydrophilic and highly basic	
CENP-D	GTP-binding (RCC1 homolog)	
CENP-E	Kinesin-like microtubule-based motor	Metaphase arrest
INCENPA	Coiled-coil domain	
INCENPB	Coiled-coil domain	

[a] Effects resulting from antibody injection.

Genes encoding yeast centromere-binding factors have been identified by mutations affecting chromosome segregation, by cell cycle–dependent spindle defects, and by partial sequence of purified proteins (Table 2). For example, the gene encoding CBF3A, one of the centromere-binding factors in the 240 kd tertiary complex, has been cloned by three independent routes. The *ctf14* mutation in this gene has been isolated in a genetic screen for chromosome transmission fidelity (*ctf*) mutants (Spencer et al., 1990), followed by secondary screens based on transcription through the centromere and dicentric chromosome breakage (Doheny et al., 1993 [this issue of *Cell*]). Another mutation in the same gene (*ndc10* for nuclear division cycle) has been identified by screening a library of temperature-sensitive mutants for spindle defects (Goh and Kilmartin, 1993). The mutant *ndc10-1* accumulates polyploid and aploid cells because of a defect in chromosome segregation: chromosomes remain at only one pole of the anaphase spindle. Finally, the gene has been cloned using partial amino acid sequence of purified CBF3A (*CBF2*; Jiang et al., 1993).

The gene encoding CBF3C (Lechner and Carbon, 1991) has been isolated as another chromosome transmission fidelity mutant, *ctf13* (Doheny et al., 1993). Interestingly, its product contains a short stretch of serines, similar to that found in mammalian CENP-B.

The first indication that centromere protein function is subject to posttranslational controls came from the isolation of a mutant encoding a putative protein kinase that could suppress centromere DNA mutations (meiosis and centromere regulatory kinase, *MCK1*; Shero and Hieter,

Table 2. Budding Yeast Genes Encoding Centromere-Binding Proteins

Gene	Motifs	Centromere Specificity	Protein	Mutant Phenotype
CBF1/CPF1/CEP1	Helix-loop-helix	CDEI	CP1	Methionine auxotrophy
CBF2/NDC10/CTF14	Nucleotide-binding domain	CDEIII	CBF3A	Accumulation of aploid and polyploid cells
CTF13	Acidic serine-rich region[a]	CDEIII	CBF3C	G2–M arrest

References for *CBF1*: Baker and Masison, 1990; Cai and Davis, 1990; Mellor et al., 1990.
For *CBF2*, see Jiang et al., 1993; Goh and Kilmartin, 1993; Doheny et al., 1993.
[a] Similar to CENP-B.

1991). A mutation in the same gene was independently isolated as a meiotic activator (Neigeborn and Mitchell, 1991) whose product is serine and tyrosine phosphorylated in vivo (*YPK1*; Dailey et al., 1990). Considering the diversity of *MCK1* function, it may not phosphorylate a centromere protein per se.

However, phosphorylation is likely to be directly involved in CBF3 function. Phosphatase treatment of the CBF3 protein complex abolishes its DNA binding activity, even in the presence of soluble assembly factors. A yeast centromeric complex isolated from nocodazole-treated cells binds more efficiently to microtubules assembled in vitro than do centromeres isolated from an asynchronous population (Kingsbury and Koshland, 1991). Thus, both DNA and microtubule binding are likely to be cell cycle–regulated events. The emerging view is an ordered pathway of kinetochore assembly, whose regulation includes posttranslational modifications of bound components.

A striking result in the discovery of centromere proteins is their diversity, which probably exceeds the variety of components directly involved in generating chromosome movements. Coordinating centromere function with cell cycle progression not only requires regulation of kinetochore proteins, but a signaling apparatus to relay the status of chromosome attachment to the cell cycle regulators.

Centromere DNA and Kinetochore Assembly

The ability to manipulate endogenous chromosomes and to construct artificial minichromosomes has led to the identification of centromere DNA from the budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe. The DNA sequence required for chromosome segregation in S. cerevisiae is 125 bp, which is .006%–.06% of the chromosome. In contrast, the S. pombe centromeres constitute 1%–2% of the chromosome (Clarke, 1990), reminiscent of mammalian cells in which DNA localized to the primary constriction can account for a few percent of the chromosome.

In primates, hierarchical arrays of a 171 bp α-satellite sequence can apparently provide at least partial centromere function (Haaf et al., 1992). α-Satellite DNA is an AT-rich sequence containing the DNA motif recognized by CENP-B in vitro (Table 1; Matsumoto et al., 1989). In African green monkey cells transfected with human α-satellite DNA, the localization of CENP antigens is indistinguishable from patterns of in situ hybridization to human α-satellite DNA. Thus, human α-satellite DNA directs the binding of simian CENP antigens in vivo. Moreover, depending upon the site of integration, autonomous chromosome segregation or dicentric chromosome behavior has been observed. These data indicate that a simple repeated sequence may direct the binding of CENP antigens, and in a concerted way may act as structural determinants for chromosome segregation. However, because the transfected cell lines underwent selection, it will be important to determine the primary sequence organization at the sites of α-satellite DNA integration.

How does DNA sequence direct the assembly of kinetochore components? Certainly the binding of CENP-B is not sufficient for centromere function, as CENP-B has been localized to the inactive centromere of dicentric chromosomes (Earnshaw et al., 1989). African green monkey α-satellite DNA is organized into ordered arrays of nucleosomal subunits. The histone-like nature of CENP-A (Palmer et al., 1991) is indicative of a nucleosomal component in the mammalian centromere. The 125 bp S. cerevisiae centromere is organized into a chromatin structure spanning 160–220 bp, which is essential for function. Like CENP-B, the binding of the CBF3 complex is also insufficient for yeast centromere function. CDEIII itself, while capable of binding the CBF3 complex, is segregationally impaired (Lechner and Carbon, 1991; Schulman and Bloom, 1993). Histone proteins are required for the formation of centromeric chromatin structure observed in vivo (Saunders et al., 1990) and may provide a structural framework for the binding of CBF3. The requirement of factors (in the form of Escherichia coli extract, yeast extract, or casein) for CBF3 binding to centromere DNA in vitro reflects the complexity in kinetochore assembly.

Kinetochores as Motile Organelles

Chromosomes are dynamic entities exhibiting a range of motions, including initial microtubule attachments and rapid movements toward the pole (Rieder and Alexander, 1990), oscillations to and away from the pole, congression toward the spindle equator, and, finally, segregation to the spindle pole in anaphase (reviewed by Salmon, 1989). The ability of yeast extract containing CBF3 proteins to translocate centromere DNA–coated beads along microtubules establishes a powerful physiological assay for kinetochore function and emphasizes the fundamental role of mechanochemical enzymes in yeast chromosome movement (Hyman et al., 1992). Chromosome movement is apparently elicited by mechanochemical enzymes that bind the kinetochore region and translocate along the microtubule. Since microtubules are highly conserved, the basic mechanism by which chromosomes are transported along microtubules may also be highly conserved. However, no yeast centromere-binding proteins exhibit homology to dynein or kinesin, the known microtubule-based motor proteins. It is unknown whether such proteins have yet to be discovered, a new class of microtubule-based motors resides at the kinetochore, or the mechanistic features of mitosis have changed substantially in evolution.

Kinetochore movements have recently been described in fission yeast (Funakabi et al., 1993). Fission yeast centromeres, which consist of highly repetitive elements that can be visualized with fluorescent probes (Uzawa and Yanagida, 1992), colocalize with the spindle pole body during G1, S, and early G2 phases. Once the bipolar microtubule array is established, centromeres migrate to the central spindle. At a critical microtubule length, the sister centromeres separate and migrate back to the spindle poles. There are indications that similar centromere movements occur in budding yeast (Goh and Kilmartin, 1993). CBF3A is juxtaposed to the spindle pole body for most of the interphase stage of the cell cycle. In a number of cells with short mitotic spindles, CBF3A is visualized along the spindle matrix. Thus, in both fission and budding yeasts, centromeres seem to move from their subnuclear position in interphase to the spindle in metaphase and back to the poles at anaphase.

Kinetochore Signaling

The mode of chromosome attachment to the spindle may be more robust in mammals and higher plants, in which chromosomes are dispersed in interphase and form a well-defined metaphase plate during mitosis. The ability of detached chromosomes to delay mitotic progression indicates that the cell can monitor chromosome attachment to the bipolar spindle (Nicklas and Arana, 1992). Recent work reveals that the intracellular signal for this delay may emanate from the kinetochore itself.

A single aberrant kinetochore is sufficient to induce a cell cycle delay in yeast (Spencer and Hieter, 1992). The delay does not reflect defects in chromosome segregation, as 50-fold more cells pause than undergo chromosome loss. Temperature-sensitive mutations in the gene encoding CBF3C also result in a cell cycle delay at restrictive temperature, with a phenotype similar to that observed for centromere DNA mutations (Doheny et al., 1993). Wild-type cells can, therefore, delay cell cycle progression in response to chromosome abnormalities. Yeast cells grown under selective pressure for excess centromeres also exhibit a mitotic delay and increased loss of endogenous chromosomes (Runge et al., 1991, and references therein). The introduction of centromeres that are segregationally impaired but competent to bind the CBF3 complex negatively affects chromosome segregation as well (Schulman and Bloom, 1993). Thus, the state of kinetochore assembly and/or microtubule attachment may be subject to cell cycle surveillance. Unassembled or partially defective centromeres may fail to signal the transition from metaphase to anaphase or may activate a checkpoint response that delays cell cycle progression (Weinert and Hartwell, 1988).

Microinjection of CREST autoimmune antisera blocks normal chromosome movements in vivo (Bernat et al., 1990; Simerly et al., 1990), demonstrating an essential role for the autoantigens in kinetochore function. However, the precise role of a particular antigen remained elusive until the corresponding structural analysis (Bernat et al., 1991). Injection of autoimmune antisera prior to G2 results in the inability to assemble a kinetochore. Injection of antibodies in G2 (within a few hours of mitosis) results in a metaphase arrest. Surprisingly, these chromosomes also lack kinetochores. To address whether the kinetochore is actually absent from the chromosome or perturbed to an extent that its appearance is compromised upon microtubule binding, cells were injected with antisera and colcemid was added a few hours later. Colcemid disrupts microtubules, but kinetochores will form in a cell cycle–dependent fashion. Late G2–injected cells contain full kinetochores. Thus, kinetochores are present but structurally unstable upon microtubule binding. These experiments define two execution points in the pathway of kinetochore assembly. Prior to G2, antibodies block the assembly of the kinetochore. Following assembly, a maturation step is required for kinetochore endurance throughout mitosis.

What features of kinetochores do cell cycle regulators monitor? Possible signals include kinetochore assembly, maturation, and/or attachment to spindle microtubules. The isolation of mutants whose functions are required for cell cycle delay in response to the microtubule-depolymerizing drug benomyl indicates that microtubules play an integral role in cell cycle surveillance as well (Li and Murray, 1991; Hoyt et al., 1991).

Chromosomal Passengers

In mammalian condensed mitotic chromosomes, INCENPs are in the centromeric domain. However, upon chromosome to pole movement in anaphase, these proteins detach from the chromosomes and remain at the metaphase plate. By telophase, they are localized at the midbody (Earnshaw and Bernat, 1991). This behavior has led to the suggestion that kinetochores may transport INCENPs to the metaphase plate. Since the position of the spindle is intimately involved in specifying the location of the contractile ring for cell division (Strome, 1993), the INCENPs may in turn establish the plane of cell division. The microtubule-based motor protein CENP-E (Table 1) also exhibits a transition from centromere to midbody (Yen et al., 1992).

Evolution and Centromere Size: The CEN-Value Paradox

The apparent sequence divergence as well as disparity in the size of centromeric domains throughout evolution remains enigmatic. The amount of DNA per microtubule is fairly constant throughout phylogeny (50-fold range; Table 3). In contrast, centromeric DNA spans 3–4 orders of magnitude over a 300-fold difference in genome size. Thus, even if the budding yeast centromere represents a primitive kinetochore, the elaboration of this region throughout evolution must have a significant advantage that cannot be accounted for simply by the increased number of microtubule-binding sites.

What is responsible for the increased complexity of centromere size (CEN value) in evolution? The diversity of mi-

Table 3. Average Number of DNA Base Pairs per Kinetochore Microtubule

Type of Organism	Species	DNA Content[a] (bp)	Chromosome Number[a]	MTs per Chromosome	DNA (bp) per MT
Protozoan	Chlamydomonas reinhardtii	1.09×10^8	19	1	5.7×10^6
Budding yeast	S. cerevisiae	1.4×10^7	16	1	0.87×10^6
Budding yeast	Kluyveromyces lactis	1.4×10^7	6	1	2.3×10^6
Fission yeast	S. pombe	1.4×10^7	3	2–4	1.5×10^6
Fruit fly	Drosophila melanogaster	1.65×10^8	4	6–21	4.1×10^6
Grasshopper	L. migratoria	6.5×10^9	11	18–23	2.8×10^7
Human	Homo sapiens	3.9×10^9	23	20–30	6.7×10^6
Plant	Haemanthus katharinae	1.06×10^{11}	18	120	4.9×10^7

MT, microtubule.
[a] Haploid.

totic strategies defies a simple unifying hypothesis. However, certain mechanistic features have clearly evolved. In primitive mitoses, differential growth of membranes of the nuclear envelope may result in segregation of daughter spindle poles and consequently chromosome segregation (Raikov, 1982, and references therein). This may represent an early mechanism, since membranous features of segregation are utilized in prokaryotes. Upon nuclear envelope breakdown in some protists, fungi, and higher eukaryotes, spindle microtubules grow from the poles and only attach secondarily to the centromere. The advent of microtubule capture represents a significant step in kinetochore evolution.

A single microtubule-binding site per chromosome may be sufficient to generate force for chromosome movement (see Rieder and Alexander, 1990), but may not be large enough to ensure efficient microtubule capture. With increasing cell volume, capture time increases. Thus, increasing CEN value might enhance the efficiency of microtubule capture, and the evolution of mitosis from membranous mechanisms to open capturing systems might select for organisms with larger CEN values.

Increasing the number of microtubule-binding sites presents an additional structural problem. Multiple binding sites are viable only if they are interspersed with the correct periodicity to ensure the proper orientation of the microtubules. Improper orientation could, in principle, have the same disastrous consequences as multiple centromeres: chromosome aberrations such as breakage, deletions, and rearrangements (Hill and Bloom, 1989, and references therein).

The kinetochore differentiation seen in higher eukaryotic centromeres may well represent reiteration of individual microtubule-binding sites (see Zinkowski et al., 1991) that enhance the fidelity of chromosome segregation. Increasing size and multiplicity of binding requires a mechanism that detects when a sufficient number of microtubules are bound. The proposal that particular features of the kinetochore are monitored prior to the onset of anaphase increases the diversity of kinetochore components. Finally, commensal partners (such as the INCENPs) may further contribute to kinetochore complexity and size.

The number of kinetochore components will likely continue to abound. The development of physiological assays for kinetochore function will prove invaluable in dissecting the variety of functions provided by this complex structure. We look forward to additional puzzles and solutions in the molecular nature of this structure.

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