REVIEW OF LITERATURE

Most eukaryotic chromosomes contain a specialized domain, the centromere, that mediates critical mitotic and meiotic functions, including kinetochore nucleation, spindle attachment, and sister chromatid cohesion. These processes ensure faithful chromosome partitioning during cell division. Intense scrutiny of the DNA and proteins that comprise revealed their fundamental roles in chromosome mechanics and, in some cases, enabled the creation of artificial chromosomes. The centromeric DNA of several lower eukaryotes has been completely defined; much less is known about the centromeric DNA of higher eukaryotes, including plants.

The centromeres from many lower eukaryotes such as fungal organisms were mapped by tetrad analysis, a powerful genetic tool used to map sites of genetic exchange relative to the centromere (Clarke, 1998). Tetrad analysis is possible when meiotically-related daughter cells can be recovered and analyzed: yeasts such as Saccharomyces cerevisiae and Schizosaccharomyces pombe package their meiotic products into ascis that can be readily separated and characterized. Using tetrad analysis and chromosome walking (Clarke and Carbon, 1980) identified the centromere of S. cerevisiae chromosome III, mapping the chromosomal regions that attach to the spindle and migrate to opposite poles in meiosis I, thus providing a functional definition of the centromere. Chromosome walking delineated a small region of ~ 20 kb, and subsequently, artificial chromosomes were used to show that only 125 bp were needed for centromere function (Cottarel et al., 1989). A highly conserved copy of this sequence resides at the centromere of every S. cerevisiae chromosome. This minimal region meets two stringent criteria: first, artificial chromosomes containing the 125 bp region segregate efficiently through mitosis, with loss rates approaching those of normal chromosomes (>99% transmission in the absence of selection); and second, these constructs partition efficiently through meiosis, as assayed by tetrad analysis (Carbon and Clarke, 1990). Similar genetic analyses led to the elucidation of the S. pombe centromere (Baum, et al., 1994): Chromosome walking on all three chromosomes pinpointed regions ranging from 40 to 100 kb. Unlike S. cerevisiae centromeres, each S. pombe centromere contains a divergent core region surrounded by repetitive elements. S. pombe artificial
chromosomes define a minimal centromere region that confers high-fidelity transmission: this ~7 kb fragment consists of a portion of the core and a single flanking repeat element.

In the centromeres from higher eukaryotes tetrads analysis has not been used to map the centromeres of most higher eukaryotes because the products of individual meioses are relatively difficult to obtain and analyze. This limitation has hampered progress toward genetically defining these centromeres in their native contexts. As an alternative, the DNA sequences that comprise higher eukaryotic centromeres are typically localized genetically by analyzing the assortment of chromosome fragments and rearrangements. These techniques have limited resolution: breakpoints close to the centromeres cannot always be obtained, and the activity of centromeres can be altered when they are removed from their natural context. Nonetheless, such methods have localized centromere functions to regions ranging from a few hundred kb to several megabases in maize, tomato, rice, Drosophila and humans (Sun et al., 1999; Farr et al., 1995; Heller et al., 1996; Harushima, et al., 1998; Kaszas and Birchler, 1996; Frary et al., 1996]. The relatively large chromosome size typical of these organisms facilitates cytological analysis of centromeres, and the genetically defined regions typically correlate with the centromeric constriction (Jiang et al., 1996; Fransz et al., 1998). These studies have been the subject of several recent reviews (Birchler, 1997; Richards, 1998; Willard, 1998; Murphey, et al., 1998; Dong, et al., 1998). The emerging trend is that the large regions that encompass higher eukaryotic centromeres resemble the centromeres defined in S. pombe. All higher organisms examined thus far, including plants, contain distinctive and numerous repetitive elements at each centromere; however, other than these repeats, a conserved sequence that resides at the centromere of each chromosome has not been found (Dong, et al., 1998; Round, et al., 1997).

Genomic sequencing is underway in several multicellular organisms, promising to yield a dramatically improved understanding of centromeric DNA in the coming years. The first complete sequence of a higher eukaryote, that of the nematode C. elegans, unfortunately does not clarify the understanding of centromeres: C. elegans has holocentric chromosomes that attach to spindle fibers along their entire length. In contrast, the complete sequence of Arabidopsis thaliana, an organism with typical centromeres, will be obtained within the next two years (Meinke, et al., 1998).
*Arabidopsis* is particularly suited for elucidating centromere functions — among all multicellular model organisms, it is uniquely amenable to tetrad analysis. The *Arabidopsis* quartet mutation causes the four products of male meiosis to remain attached, yielding tetrads of pollen grains (Preuss, et al., 1994). Recently, tetrad analysis was used to genetically map all five centromeres in *Arabidopsis* (Copenhaver, 1998) high resolution genetic mapping will allow a precise definition of the DNA sequences that confer centromere function.

The role of DNA sequence in centromere formation:

Eukaryotic centromeres, which are located in large blocks of highly repetitive DNA, have been notoriously difficult to sequence (Lamb and Birchler, 2003). Centromeres are a paradox in that their basic function is highly conserved across eukaryotes but their sequences are divergent, even between closely related species (Henikoff et al., 2002). Suggested that the DNA sequence may not be essential in centromere formation (Sullivan et al., 2001). It has been difficult to address this issue because of a lack of complete sequence for any higher eukaryotic centromere. Deletion of large regions of the human Y chromosome has shown that centromere activity is associated with a block of tandemly repeated 171 base-pair (bp) units, termed α-satellite DNA (Heller et al., 1996). Every human centromere is associated with arrays of this α-satellite DNA that can be several megabases (Mb) in size. Designer PCR markers used for constructing a 500 kilobase (kb) containing of bacterial artificial chromosomes (BACs) that covers a region that is immediately adjacent to, and including part of a 3 Mb array of α-satellite located at the centromere of the human X chromosome. *Arabidopsis* centromeres include a 178 bp α-satellite repeats, which is organized in tandem arrays that range in size from 0.4 Mb to 1.4 Mb on different chromosomes and are located between regions enriched for various α-satellites and other repetitive elements (Copenhaver et al., 1999; Haupt et al., 2001). The clusters of α-satellite DNA in human and the 178 bp centromeric element in Arabidopsis are organized in similar ways, although their primary sequence are completely unrelated. Interestingly, centromeres of other plants have also been shown to contain DNA elements of similar length, and this reflects a common requirement for centromere function. In *Drosophila* there are no DNA sequences that are located at every centromere, suggesting that primary centromeric sequence alone is
neither sufficient nor necessary for centromere formation. *Drosophila* centromeres are unusual in being composed of sequences that are abundant elsewhere in the genome whereas implants and mammals this is not the case under normal circumstances. Robertsonian translocations, which are whole-arm rearrangements between acrocentric chromosome can link two centromeres and yet the resulting chromosome stably transmitted through mitosis and meiosis. Further more, in *in situ* analysis using antibodies against essential kinetochore proteins, such as CENP-C, the centromere-specific variant of histone H3 in human, has shown that only one of the two centromeric locations remains function (Warburton et al., 1977). Neocentromere has the usual hallmarks of a centromere-it form bound (Warburton et al., 1997; Saffery et a., 1997). The importance of chromatin structure for centromere function is supported by the presence of specific-specific variants of histone H3 found in the centromeric chromatin of all eukaryotes. The variants interact with the other core histone proteins, H2a, H2b, and H4 to form a type of nucleosome that is present only at functional centromeres. It has been suggested that nucleosomes containing centromeric histone H3 are indispensable for centromere function and likely to serve as anchors for kinetochore formation. A model proposing that correct spacing of centromeric and normal nucleosomes if required for centromere function is supported by recent data from *Drosophila* and in human cells showing that stretched chromatin form centromeres if organized into blocks of centromeric nucleosomes interspersed between blocks of nucleosomes containing the normal core histone H3 (Blower et al., 2002). This spacing may be facilitated by the satellites present at centromeres. Analysis of centromeric histone H3 in related species of mammals, flies, and plants has shown that the variants are highly similar to core histone H3 proteins in the regions that interact with the other histone proteins (Malik et al., 2002; Henikoff and Malik 2002; Yoda et al., 2000). Meiotic drive (a distortion of chromosome segregation) resulting from preferential positioning of ‘stronger’ centromeres to the egg during female meiosis might be the mechanism for this coevolution (Malik et al., 2002; Henikoff and Malik 2002). Centromere function is independent of the underlying sequence. Such models are formulated to explain how nucleosomes containing centromeric histone H3 are maintained at all functional centromeres regardless of the DNA sequence with which they are associated. Another model predicts that extant nucleosomes containing
centromeric histone H3 are distributed to each strand during replication and subsequently used in post replication recruitment of additional centromeric nucleosomes. Models for centromere formation that do not rely on sequence must account for certain elements, such as the human α-satellite DNA and the *Arabidopsis* 178bp repeat, that are present at every centromere in normal karyotype within a given species. Possibility that secondary structure or even higher order DNA structure could be a factor in determining centromere position and function. Similarly, epigenetic models of centromere formation, proposing regulation at the chromatin level, would not exclude fine-tuning of primary sequence. In either model, formation of a centromere with a new sequence would be allowed as long as the region permitted the proper higher-order DNA organization.

**The Centromere paradox: stable inheritance with rapidly evolving DNA:**

Henikoff et al., (2001) identifies the stable inheritance of rapidly evolving centromere DNA sequences as a ‘centromere paradox’. DNA methylation can mediate epigenetic inheritance during development and even between generations of complex organisms (Martien, 2001). Chromation-based mechanisms are thought to maintain developmental state (Lyko, 1999). Several authors have argued that centromeres, the sites of spindle attachment at mitosis and meiosis, can also be maintained epigenetically (Willard, 1998). These point to a novel chromatin-based mechanism for the maintenance of centromere location during multiple rounds of cell division. This mechanism may be responsible for the enigmatic organization of centromeric DNA and for the rapid onset of reproductive isolation as species emerge.

In animals and plants, centromeres are contained within regions of highly repetitive satellite DNA, which confounds even the most powerful mapping methods. Chromosomes in *Saccharomyces cerevisiae* are exceptional in that they lack satellite sequences and their centromeres have been precisely localized. Each of these “point” centromeres specifies spindle attachment with only ~ 125 base pairs (bp) of DNA. However, this simplicity is evolutionarily derived, as centromeres from other fungal lineages include arrays of repeats (Carnbareri et al., 1998), much like what is found in animals and plants. The idea that specific repeated sequence elements might specify centromere location for largest chromosomes when present in a sufficient number of
copies (Willard, 1990), has fallen out of favor for reasons that have been extensively reviewed (Willard, 1998). Most compelling is the lack of any common repetitive elements in many "neocentromeres" that occasionally are found in humans. Even fine-structure mapping of two human neocentromeres has failed to detect \(\alpha\)-satellite or other tandem repeats found near centromeres (Lo et al., 2001).

Speculations that non-DNA sequence determinants maintain centromeres (Willard, 1998). Such determinants might account for examples of reversible centromere inactivation (Aguda et al., 2000). There are proteins found only at centromeres, and these are present at centromeres only at mitosis (Suffery et al., 2000). Some of these form the kinetochore, a proteinaceous structure that assembles on centromeric chromatin and connects the centromere to spindle microtubules. The kinetochore appears at prophase and disappears at telophase (Caig et al., 1999). Other protein complexes, such as cohesions, are not part of the kinetochore but also disappear from centromeric regions at mitosis.

CENP-A is present at native centromeres and at neocentromeres (Suffery et al., 2000) but is absent from centromeres that are mutated (Tyler-Smith et al., 2000) or inactivated (Sullivan and Willard, 1998). In vivo, each centromeric nucleosome consists of a histone octamer with CENP-A instead of H3 (Shelby et al., 1997). CENP-A has counterparts in other eukaryotes. Cse4p in \(S.\ cerevisiae\) (Meluh et al., 1998), HCP-3 in \(Caenorhabditis\ elegans\) (Buchwitz et al., 1999), Cid (for centromere identifier) in \(D.\ melanogaster\) (Henikoff et al., 2000), and SpCENP-A in \(Schizosaccharomyces\ pombe\) (Takahashi et al., 2000) are exclusively centromeric. Even in \(C.\ elegans\), where the holokinetochore extends from one end of the chromosome to the other, HCP-3 is found at the underlying holocentromere. At the other end of the spectrum, the point centromeres of \(S.\ cerevisiae\) have what may be a single Cse4p-containing centromeric nucleosome (Meluh et al., 1998). Centromeric histones contain sequence features that distinguish them from histone H3. Although histone H3 is evolutionarily constrained, centromeric histones are strikingly divergent. We attribute this difference to the necessity of H3 to interact with the entire genome.
Nucleosomes are distributed between daughter strands at replication and current evidence favors a similar distributive segregation of centromeric nucleosomes. New centromeric nucleosomes must be assembled, and H3-containing nucleosomes must be excluded to prevent a gradual degradation of centromeric identity. Two general models have been proposed for this selective assembly. In one, old centromeric nucleosomes specifically direct the deposition of new centromeric nucleosomes (Willard, 1998). This process could be mediated by an interaction between centromeric nucleosomes or facilitated by a nucleosome loading factor (Takahashi et al., 2000). A second model supposes that centromeric histones are available only at a restricted time or nuclear location during the cell cycle (Shelby et al., 1997). Genetic evidence that heterochromatin is required for centromere function.

Centromeric repeats comprise the most rapidly evolving DNA sequences in eukaryotic genomes, differing even between closely related species. These satellite changes are brought about by a variety of mutational processes, including replication slippage, unequal exchange, transposition, and excision. Such rapid change is paradoxical: Why has not a single optimal sequence been fixed at centromeres? A clue comes from examination of centromeric histones. These are expected to maintain favorable interactions with centromeric satellite (Shelby et al., 1999). Comparison of Cid from closely related Drosophila species reveals that both the NH2-terminal tail and the histone core domain contain regions that have undergone frequent episodes of adaptive evolution (Malik and Henikof, 2001). This is unexpected for a histone molecule, as histones are among the most evolutionarily constrained eukaryotic proteins. Understanding the basis of these adaptive changes could resolve the paradox of rapidly evolving centromeres.

Asymmetry at female meiosis may be the key. Of the four products of meiosis, three are lost and only one becomes the oocyte nucleus. There is evidence that the asymmetry of the meiotic tetrad provides an opportunity for chromosomes to compete for inclusion into the oocyte nucleus by attaining a preferable orientation at meiosis. Centromeres that can exploit this opportunity at meiosis I will “win”, and even a slight advantage at each female meiosis will be enough to rapidly drive a centromere to
fixation. Additional recruitment of centromeric nucleosomes, for example, by the expansion of centromeric satellite, would confer this advantage. Genetic evidence that some animal and plant centromeres are "stronger" at meiosis dates back nearly half a century (Nowitski, 1955). In maize, centromere strength is characteristic of heterochromatic "knobs". Which display poleward movement and meiotic drive during female meiosis (Aguda et al., 2000), and a similar drive process might contribute to the success of selfish B chromosomes (Rhoades, 1942). In humans, a variety of Robertsonian translocations, with two adjacent centromeres, consistently display a higher than expected transmission ratio (Villena et al., 2001). In females, the winning centromeres simply exploit the inherently destructive process of forming the egg and thus might not reduce fecundity. However in Drosophila males, heterochromatic differences between paired chromosomes at meiosis I can cause nondisjunction. Three recent papers in Journal of Biology – Talbert et al., (2004) – reiterate that a 'centromere drive' is responsible for assignment of a favoured genome to the meiotic product of female meiosis in mammals and plants and eventually forms the egg. In an excellent study that explains the functional conservation in spite of DNA sequence variation in centromeres, Talbert et al., (2004) show that the DNA binding domains of an essential protein component of the functional centromere, CENPC, shows a wide range of variation which is under positive selection. This variation is particularly evident in the CENPC domain which actually binds the centromeric DNA sequences. This has prompted the authors to propose a centromere drive model (on the lines of meiotic drive) that organisms could build centromeres of differential strength and the genome with a stronger centromeres wins the place in the egg while others with weaker centromeres loose out.

Copenhaver and Preuss (2002) have also provided further insights regarding centromere paradoxes: Neither the DNA sequences that comprise higher eukaryotic centromeres, nor the structure of their centromeric chromatin are fully understood; consequently, only conjectural models for centromere function are available. Appropriate explanations must account for several puzzling observations, including the diversity of centromere DNA sequences, the evolutionary conservation of some centromere binding proteins, and the accumulation of repetitive DNA in the centromeric regions.
In many higher eukaryotes arrays of repetitive DNA measuring approximately 170 bp have been identified at the centromere, yet the sequence of these repeats is typically species specific. The centromeres from the budding yeasts *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Candida glabrata*, and *S. cerevisiae* are more similar in size and sequence composition, yet none function efficiently when introduced into *S. cerevisiae* or *S. pombe* artificial chromosomes (Clarke, 1998). By contrast, the proteins that bind to centromeres display remarkable conservation between species. For example, homologs of the human centromere binding proteins CENP-A, CENP-B, and CENP-C have been identified in *S. cerevisiae* or *S. pombe*. CENP-A encodes a modified histone H3 protein, and CENP-B, which binds to repetitive arrays, shares sequence similarity with transposase (Clarke, 1998). The diverse nature of centromeric DNA sequences in higher eukaryotes together with the observed homology among centromere binding proteins support the idea that the conservation of centromere functions derives from a higher order structure, rather than a particular DNA sequence. However, the lack of a complete DNA sequence from any higher eukaryotic centromere leaves the possibility open that a conserved sequence nucleates centromere formation in multicellular organisms.

The ubiquity of repetitive elements at the centromeres of higher eukaryotes led to the suggestion that repeats are required for centromere function. These repetitive arrays have been postulated to form essential higher order structures, bind key centromeric proteins, or serve as targets for critical DNA modification. Support for the role of repetitive centromeric DNA has come from the construction of human artificial chromosomes (Ikeno, et al., 1998; Harrington, et al., 1997). Constructs containing long arrays of human satellite DNA together with telomeres acquire modest stability in cell lines. However, rearrangements of these arrays were frequently observed; consequently, it remains possible that incorporation of essential centromeric sequences was selected in vivo. Importantly, the role of repetitive DNA has been called into question by the recent characterization of highly stable human neocentromeres — centromeric constrictions that assemble at an atypical site on the chromosome. Many of these neocentromeres contain neither alpha satellite DNA nor display associated CENP-B protein (du Sart, et al., 1997). These recent findings raise the possibility that centromeric repeats are not required for
centromere functions, but rather that the intrinsic nature of the centromeric regions, particularly the relatively low level of meiotic recombination, may lead to the accumulation of repetitive DNA.

There are several models for centromere function in higher eukaryotes: In multicellular organisms, the similarities between centromere functions and the paradoxes still remaining have lead to the generation of several competing models that aim to explain centromere activity. These models can be grouped into two categories: i) sequence-based models that claim higher centromeres, like those from *S. cerevisiae*, depend on a specific sequence, but that this sequence may be dispersed or appear in multiple copies along the centromeric region, and ii) sequence-independent models that invoke epigenetic modification of centromeric DNA, higher order DNA structures, or an entirely passive role for sequences that comprise the centromere.

Although the paradigms established in lower eukaryotes are appealing, they have yet to be demonstrated in more complex systems. Currently, the most complete understanding of higher centromeres comes from extensive characterization of *Drosophila* mini-chromosomes. These studies argue that *Drosophila* centromeres may be composed entirely of repetitive DNA. Final proof of this possibility will be achieved after complete DNA analysis rules out the presence of small islands of unique sequence. In addition, because these minichromosomes have undergone significant rearrangements, they may reflect neocentromeric rather than native centromeric activity. Nonetheless, this body of work demonstrates strong distinctions between higher and lower eukaryotic centromeres context.

If higher eukaryotes do rely on particular DNA sequences for centromere function, then those sequences may span a relatively large portion of the chromosome. The constricted regions of higher chromosomes clearly encompass megabases of DNA, with multiple spindle fiber attachments during cell division. In some cases, for example the maize B chromosomes, it is possible to split a centromere, yielding two chromosome fragments that segregate normally [10]. These observations suggest that critical DNA sequences may be spread throughout the centromeric region, with the space between such elements filled by apparently nonfunctional DNA.
Because a unique centromere sequence has not been demonstrated in higher eukaryotes, several recent models focus on non-sequence based mechanisms. Such models are appealing and could explain why centromeres moved from their native host into another eukaryotic species are stable. Yet centromeres transferred first through E. coli can rarely function in a heterologous species. Two strong examples of epigenetic regulation have been demonstrated in Drosophila and S. pombe. Fragments of Drosophila chromosomes that are normally telomeric can acquire modest segregation stability after residing near a centromere (Williams, et al., 1998). Control fragments that have never resided near a centromere do not share this property. These observations led to the proposal that centromere activity can spread to adjacent DNA through an unknown epigenetic mechanism. While these results are intriguing, the relatively low transmission rates (14 to 24%) indicate that complete stability is not acquired by these fragments. Epigenetic control of centromere function in S. pombe has been proposed following the quantitative analysis of artificial chromosome transmission (Steiner and Clarke, 1994). Curiously, a construct bearing a partial centromere was transmitted with a high fidelity in some colonies and a low fidelity in others. This difference in transmission frequency was not due to mutation, and rapid switching between states, through an unknown mechanism, was observed. These findings are quite unusual for yeast artificial chromosomes and reveal a potentially exciting level of epigenetic control; however, only partial centromeres display this activity, and inclusion of the complete centromeric sequence always resulted in highly stable transmission (5 X 10^4). Thus, for both Drosophila and S. pombe, the proposed epigenetic regulation may be most critical in cases where only a partial centromere is present.

Determining centromere identity is a complicated task (Sullivan et al., 2001). Eukaryotic centromeres are encoded by non-conserved but contain highly conserved proteins, such as CENPA. Furthermore, centromeric DNAs that lack centromere function and non-centromeric DNAs can gain centromere function. Despite a lack of conservation in centromeric DNA sequence, (A-T) rich or repetitive DNA might be a primary substrate for kinetochore assembly. CENPA is an essential kinetochore histone that might epigenetically mark sites for kinetochore formation and is required for the proper localization of kinetochore and sister-chromatid cohesion proteins. It is also required for
mitotic and cell-cycle progression. Its incorporation into centromeres is independent of: Centromeric DNA replication, because replication studies of human and *Drosophila* centromeres indicate that centromeric DNAs are replicated asynchronously in S phase, and in humans are replicated before CENPA is loaded onto chromatin.

Eukaryotic centromere regions are composed of several spatial and functional domains. Epigenetic propagation of centromere identity might be achieved through the activity of centromere-specific chromatin-assembly factors, which load CENPA into centromeric nucleosomes. Despite recent advances in our understanding of centromere function, many mechanistic details remain to be understood.

Tyler-Smith and Floridia (2000) have shown that there are diverse evolutionary solutions to centromere structure. Centromeres remain enigmatic and poorly understood. A complete genomic sequence must contain the centromeric DNA and genes for all the centromeric proteins. Delineation of a region by tetrad analysis is yeast and *Arabidopsis*. Centromere function is conserved among eukaryotes, it has been clear for decades that centromere morphology varies in an astonishing way. In *S. cerevisiae*, no specific structure is visible; in *C. elegans* the chromosomes are holocentric and microtubules attach it at many positions along the chromosomes. In most plants and animals, the centromere forms a visible primary constriction at metaphase and the kinetochore is a distinct structure resolved into subregions. Hopes initially focused on the centromeric DNA. A "magic sequence" would be common to all centromeres. The finding in the last few years by clinical cytogeneticists of neocentromere (noncentromeric sequences that acquire centromeric function) in humans. By chance in 1980 of anticentromere antibodies in human patients with an autoimmune disorder best known by the acronym CREST. This led to the identification of a set of centromeric proteins designated CENPs including CENP-A CENP-B and CENP-C. Proteins such as the *S.cerevisiae* centromere binding factor 3 (CBF3) complex and the involvement of *Drosophila* zeste-white 10 (ZW10) were identified in this way. Genomic sequencing projects should provide plentiful new information on centromeric DNA sequence. *Arabidopsis* centromere thus conform to the pattern of organization seen in most species: abundant tandem and other repeated sequences, but no specific sequence conservation. The sequence information does not give direct information about centromere function. "The absence of evidence" becomes
The centromere/kinetochore complex interacts with chromatin on one side and microtubules on the other. Chromatin proteins such as histones, and microtubules constitutes such as tubulins are very highly conserved. Centromeric-specific histone H3 variants, CENP-A homologs, have been identified in all species. Thus morphologically diverse centromeres, containing unrelated DNA sequences, used some orthologous proteins, and it is possible that a few, such as the CENP-A homologs, are universal. However, each centromere also appears to contain some specific proteins. In most species, centromere activation and inactivation seem to be regulated by epigenetic events, heritable changes without a corresponding change in DNA sequence (Karpen and Allshire 1997). The nature of the epigenetic changes is a crucial question and the basic mechanisms are still not understood. 19 of 20 centromeric proteins were found to be present at each of the two neocentromeres tested (Saffery et al., 2000); the one exception was CENP-B, a satellite binding protein that remains present at inactive centromeres and can be knocked out in the mouse genomes without affecting centromere function. An epigenetic mechanism into a sequence-specific one for S.cerevisiae. The evolutionary distribution of homocentric centromeres, which are found in organisms as diverse as plants and insects as well as Caenorhabditis, suggests that changes from one mode to the other have occurred on several occasions. There are similarities between the protein components of homocentric and monocentric centromeres: Caenorhabditis HCP-3 (holocentric protein-3) is homologous to CENP-A while two other holocentric proteins, HCP1 and 2, are both homologous to mammalian centromeric protein CENP-F (Pidoux and Allshire 2000).

Mythreye and Bloom (2003) have studied differential kinetochore protein requirements for establishment versus propagation of centromere activity in Saccharomyces cerevisiae. Dicentric chromosomes undergo beakage-fusion-bridge cycle as a consequence of having two centromeres on the same chromatid attach to opposite spindle poles in mitosis. Suppression of dicentric chromosome breakage reflects loss of kinetochore function at the kinetochore-microtubule or the kinetochore-DNA interface. Using a conditionally functional dicentric chromosome in vivo, they demonstrated that kinetochore mutants exhibit quantitative differences in their degree of chromosome breakage. Mutations in ch14/mcm17/ctf17 segregate dicentric chromosomes through
successive cell divisions without breakage, indicating that only one of the two centromeres is functional. Centromere DNA introduced into the cell is unable to promote kinetochore assembly in the absence of CHL4. In contrast, established centromeres retain their segregation capacity for greater than 25 generations after depletion of Chl4p. The persistent mitotic stability of established centromeres reveals the presence of an epigenetic component in kinetochore segregation.

Accurate chromosome segregation during mitosis requires the assembly of centromeric DNA and proteins to form the kinetochore, which couples chromosome movement to dynamic spindle microtubules. In *Saccharomyces cerevisiae*, the core centromere is defined by a distinctive nuclease-protected chromatin domain encompassing 106-200 bp of DNA (Bloom and Carbon, 1982; Funk et al., 1989). This protected chromatin domain is required for proper centromere function, as evidenced by the requirement of histone genes (Smith et al., 1996; Pinto and Winston, 2000) and chromatin remodeling enzymes (Tsuchiya et al., 1998), mutations which disrupt the structure and decrease the fidelity of chromosome segregation. The CEN DNA-histone complex together with CBF3 comprises the inner kinetochore, which the outer kinetochore includes Okp1p, Ctf19p, Mcm21p, Ctf3p, and Ndc80p (Ortiz et al., 1999; Measday et al., 2002). Spindle outer domain proteins that bind microtubules and kinetochores include components of the Dam1p-Duo1p complex.

That higher-order chromatin structure contributes to function is evidenced by the decreased segregation fidelity of deletion derivative chromosomes (10-100 kb in size) (Newlon, 1988). Mutations that show a marked size-dependent segregation defect. Whereas the presence of a single centromere on each chromosome is essential for accurate chromosome segregation, multiple centromeres on a single chromosome are deleterious. After bipolar attachment during cell division, dicentric chromosomes rearrange, giving rise to stable monocentric derivative chromosomes. Mutations in kinetochore genes that decrease the fidelity of chromosome segregation display decreased dicentric chromosome breakage as well. The presence of two weakened kinetochores on a dicentric chromosome may decrease the chance that both centromeres are attached to opposite spindle poles at a given time, thereby suppressing chromosome breakage.
Alternatively, dicentric chromosomes with one functional and one dysfunctional centromere might behave like a monocentric.

Differential centromeric states have been reported in a number of organisms (Ault and Lyttle, 1988; Steiner and Clarke, 1994). Minichromosomes carrying only a fraction of the Schizosaccharomyces pombe centromere adopt a mitotically stable or mitotically unstable state. A mitotically unstable centromere switches to the stable state at a frequency of 0.6-0.7% (Steiner and Clarke, 1994). This was the first demonstration of the presence of two heritable states within a population in the absence of DNA. Coexistence of different centromeric states within a cell has also been observed upon application of drugs that block histone deacetylation. The mitotic stability of a modified histone state indicates an epigenetic component in the transmission of centromeric proteins in the process of kinetochore replication. Although a number of epigenetic mechanisms have been identified to promote the inheritance of stable state, the mechanisms required to initiate or specify which heritable states are to be propagated have not been determined.

In this study, we have identified ch14/mcm17/ctf17 as a mutant that fails to assemble kinetochores on naked centromere DNA. In contrast, established centromeres are faithfully transmitted for over 25 generations in the absence of Ch14p. Thus there are distinct heritable states of centromeric chromatin in budding yeast. Ch14p is required for the specification of the mitotically stable state.

Established centromeres seem to be capable of recruiting kinetochore components in the absence of Ch14p. Lack of Ch14p, however, abolishes recruitment of the same kinetochore components to newly activated centromeres. Consistent with the idea that established centromeres are propagated in an epigenetic manner in contrast to new centromeres that require de novo assembly. Understanding the mechanism that specifies kinetochore protein loading on to naked DNA.

Molecular structure of a functional centromere:

Sun et al., (1997) have reviewed the molecular structure of a functional Drosophila centromere. Centromeres play a critical role in chromosome inheritance but are among the most difficult genomic components to analyze in multicellular eukaryotes. The centromeric DNA is associated with the kinetochore, a structure that attaches to
microtubules and helps direct chromosome movements along the spindle (Pluta et al., 1995). The centromere also plays a role in sister chromatid cohesion and separation (Miyazaki and Orr-Weaver 1994). In *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* has centromeres of two yeast species differ greatly in size and do not share any significant sequence identity (Clarke and Carbon 1985; Schulman and Bloom 1991). Centromeres of multicellular organisms are usually embedded in large blocks of heterochromatin (White 1973). Heterochromatin contains many repetitive sequences that have hindered molecular-genetic studies of higher eukaryotic centromeres. The detailed mapping of a higher eukaryotic centromere is challenging task because of the prevalence or repetitive sequences. A single 420 kb region of the minichromosome centric heterochromatin is both necessary and sufficient for fully stable chromosome inheritance and is primarily composed of AATAT and AAGAG satellites interspersed with transposable elements. Transposable elements comprise about 10% of this region of the centric heterochromatin. Single elements are interspersed in the AATAT but not the AAGAG satellite and are also clustered in *Maupiti*. The conservation of the centromeric transposable elements is very high. The sequence similarity suggests that these centric elements are recent insertions and are likely to be active, or they are ancient insertions conserved due to selective/functional constraints. Derivatives with deletions in *Bora Bora* (e.g. J21A) display a high rates of sister chromatid nondisjunction and low rates of loss, suggesting that this region may be primarily responsible for sister chromatid cohesion, rather that kinetochore formation (Murphy and Karpen 1995a). Do multicellular eukaryotes such as Drosophila utilize a similar mechanism to determine which region will act as a centromere and how it will function? Our sensitive FISH analyses corroborate previous results (Bonaccorsi et al., 1990; Lohe et al., 1993) and demonstrated that the AAATAT and AAGAG satellites are present at multiple places in the genome, predominantly in regions that never act as functional centromeres. The distribution of AAATAT and AAGAG satellites with the genome indicates that these satellites are neither necessary nor sufficient for centromere function. Transposable elements in *Drosophila* are known to be present in multiple heterochromatic and euchromatic locations throughout the genome; none have been found to be centromere-specific or present at all centromeres (Gatti and Pimpinelli 1992; Carmena and Gonzalez 1995; Pimpinelli et al.,
However, among the centromeric transposable elements identified here, the distribution of Doc, F, and G were analyzed. It is unclear if these analyses were sensitive enough to identify single elements (Gatti and Pimpinelli 1992; Carmona and Gonzalez 1995; Pimpinelli et al., 1995). Demonstrated that at least the fourth chromosome centromere does not contain even one copy of a 412 element. Maupti, which appears to be essential for normal chromosome transmission displays unusual structural features. In addition to cluster of transposable elements, it contains an A+T-rich sequence. The A+T-rich sequence could play a special role in centromere function, since, many sequences unique to centromeres in other organisms are A+T-rich; these include CDEII in S.cerevisiae centromeres, central core in centromeres (Clarke and Carbon 1985; Schulman and Bloom 1991; Tyler-Smith et al., 1993; Brown et al., 1994). However, the A+T rich sequence is only present in one small region in the normal Drosophila genome. None of the sequences identified to date in the Dp1187 centromere fulfill the criteria for a centromere “magic sequence” consistent with the S.cerevisiae model, a specific primary DNA sequence that is both necessary and sufficient for centromere function on all chromosomes. If specific small sequences are involved in centromere function, they most likely function as nucleating sites to recruit other nonspecific sequences during centromere assembly, since there is a clear requirement for a larger (420kb) region of heterochromatin. It is also possible that different Drosophila centromeres contain different essential primary sequences. Alternatively, particular combinations of DNAs, and nucleotide composition, could determine centromere identity and function, rather than the primary sequences. For example, the juxtaposition of the two satellites and/or the interspersed of the transposons in the AATAT repeats may be specific to centromeres and required for function as “Combinatorial” model. The satellites/transposon structure is a common pattern in the Dp1187. Centromere function may be achieved by formation of a specific higher order structure (HOS) (Zinkowski et al., 1991; Vig., 1994; Sunkel and Coelho 1995). An overall three-dimensional organization that results from special DNA architectures (e.g. DNA binding) and/or DNA-protein interactions in the centromere. Different DNA sequences could function as centromeric components in different organisms, even in chromosomes of the same organism (Clarke et al., 1993), as long as they facilitate the formation of the appropriate HOS. Repetitive, A+T rich DNA
seems to be a common feature of centromeric DNAs in different organisms (e.g., Rattner 1991; Alfenito and Birchler 1993; Clarke et al. 1993). The AATAT and AAGAG satellites found in the Dp1187. The transposable elements may contribute to formation of this HOS; alternatively, the transposable elements simply could be tolerated by the centromere if they do not disrupt the HOS. Formation and propagation of a centromere-specific HOS may be epigenetically regulated. Epigenetic mechanisms have been proposed to account for heritable changes in gene function that cannot be explained by changes in DNA sequence (Russi et al., 1996). Epigenetics and the concept of higher order structure emphasize the importance of centromere structure beyond the primary sequence level, which can account for the numerous cases of centromere plasticity reported for mammals, flies, and pombe. First, centromeric DNA is not always sufficient for centromere function. In fission yeast, it has been shown that centromeric DNA can be associated with two functionally different states (Steiner and Clarke 1994). Similarly, the global genomic distribution of the AATAT and AAAGAG satellites and the inactivation of none of the centromeres instable dicentric chromosome in humans and flies (Hsu et al., 1995; Earnshaw and Migeon 1985; Ault and Lyttle 1988; Page et al., 1985; Sullivan and Schwartz 1995) demonstrated that the same type of sequences at different places in the genome may or may not be associated with centromere function. Secondly, centromeric DNA is not always necessary for centromere function; centromere activity can be associated with normally noncentromeric DNAs. “Neocentromere activity” of normally noncentomeric DNA has also been demonstrated to account for the transmission of structurally acentric derivatives. Formation of a centromere-specific higher order structure by nonspecific sequences may be the underlying mechanism for the observed neocentromere activation. Once a functional centromere is present, it is stably propagated at that site through multiple DNA replications and cell divisions obey a mechanism that seems to be independent of the underlying DNA sequence. Epigenetic regulation provides a plausible explanation for these observations. Centromere identity and function may be self-propagating and may be inherited by DNA and/or protein marking (DNA methylation and histones) (Ekwall et al., 1997). In other words, centromere identity may be determined by the fact that a region functioned as centromere in the previous cell division. We also need to determine if proteins that modify chromatin structure or HOS
(histone acetylases) affect centromere function, as has been suggested for \textit{S. pombe} (Ekwell et al., 1997). Epigenetic mechanisms account for both centromere plasticity and stability.

Ventura, et al., (2001) provides an evolutionary perspective on centromere emergence. The centromere repositioning paradox, discovered while studying the cytogenetic conservation of phylogenetic chromosome IX in primates (Montefalcone et al., 1999). In some species the centromere shows an evolutionary history independent from the surrounding markers. Its position can be reconciled with these markers only by assuming additional, peculiar pericentric inversion (which we named flip-flop inversions) or by hypothesizing evolutionary emergence of centromeres. Distinction between these two alternatives is critical to our understanding of the processes of chromosomal evolution. The flip-flop hypothesis does not imply any new biological mechanism and would just add further support to the opinion that intrachromosomal rearrangements are very frequent in evolution (Muller et al., 2000). Evolutionary appearance of centromeres, on the contrary, would represent a novel, unpredicated biological property.

Centromere repositioning not mediated by chromosome rearrangement can be hypothesized to occur either through a transposition event that would insert centromeric sequences into an euchromatic region or through neocentromere emergence. As far as we know, however, no examples are available in mammals of neocentromere seeding caused by insertional transposition of a centromeric block into a noncentromeric region. On the contrary, since the first convincing report of neocentromere occurrence in humans (duSart et al. 1997), several additional cases have been described.

Two hypotheses were proposed to explain the centromere repositioning paradox. Evolutionary inversions occurred in chromosome IX leaving the order of the markers unchanged. The evolutionary emergence of neocentromeres (new centromeres): The degradation of the ancestral centromeres toward simple DNA has been very rapid The centromere position in EMA was found to be telomeric in all the studied markers. Their FISH signals clearly show that their orientation is maintained in both HAS and LCA, thus proving that the difference in centromere position was not caused by a pericentric inversion.
Proteins found only at the centromeres are the main candidates for maintaining the location of the centromere. In mammals, this protein is CENP-A and it also functions as the centromeric histone. It is found at native centromeres and neocentromeres but not at mutated or inactivated centromeres. The interaction between the centromeric histone and the centromeric DNA results in the approximately nucleosomal repeat lengths found for satellites.

Using the *Arabidopsis* tetrads mutants Preuss (1994) has established where the centromeric region starts and stops on each of the five chromosomes. Now, by building "minichromosomes", she and her colleagues are on their way to pinpointing where and how on that region the proteins attach in meiosis. Kaelly Dawe of the University of Georgia developed such minichromosomes in maize (see Mlot, 2000). Cytologists captured the first image of the centromere in the late 1800s. For decades both cell biologists and geneticists have attacked the problem of how chromosomes segregate and the role the centromere plays in this process. Thus, centromere became the baseline for measuring distance to markers on the chromosome arms. In 1980, using tetrad analysis, researchers narrowed the location of the centromere to about a 4000-base stretch of DNA in budding yeast, the first organism to achieve the landmark at that level of resolution. Eventually, as molecular technologies improved, yeast’s functional centromere—the precise bases involved in hitching chromosomes to the proteins—was whittled down to a 125-base stretch. Eukaryotes have devised many ways to segregate their chromosomes—in other words one centromere did not fill all. The chromosomes are jungles of difficult loops and repetitions, or heterochromatin, in contrast to the smoothing runs of readable DNA, or euchromatin, on the chromosome arms. Central regions of the chromosomes, stretches of nothing but repeated bases. Among the five chromosomes, these genetically defined centromeric region vary in length from 11.4 megabases to 1.9 megabases, says Preuss. That’s about 7% of the entire genome. Inside the region were found more repetitive sequences, most notably, recognizable sequences of 180 base pairs repeated hundred of times on all five chromosomes. 200 genes in *Arabidopsis* centromeric regions, at least 50 of which are expressed. About 40 of these genes appear only once in the genome sequence. Given the diversity of centromeres in different organisms, there isn’t a universal code to look for. Experimental minichromosome that are stripped-down version
of an Arabidopsis chromosomes. They contain all the essential parts: the centromere; telomere DNA from the chromosome ends; genes of interest or indicator genes, such as green fluorescent proteins, and elements to ferry the packed into cells. For other organisms, including humans. Apart from their use as a tool to explore chromosomal functioning, they have an applied side in genetic engineering as well. Researchers believe they will provide a controlled means of “stacking” large numbers of genes—say, for pathogen resistance—into an organism that could also be engineered to be eliminated when necessary.

Different cell types have adopted similar strategies to maximize the chance of error-free chromosome segregation. Centromere, is required on both prokaryote and eukaryotic chromosomes to facilitate this process. The centromere must provide a robust scaffold to support such forces and must ensure that, after chromosome replication, a kinetochore is nucleated on both sister chromatids, allowing the propagation of the selected centromeric site from one cell generation to the next. The process that ensures the propagation of kinetochore assembly at a site is particularly as it provides a way for the cell to conserve what was previously successfully achieved and to reuse it for future episodes of segregation.

Inspection of a variety of eukaryotic cell with diverse categories of centromeres reveals that there is not conservation of the primary DNA sequence (reviewed by Mellone and Allshire, 2003). The possibility that specific DNA sequences operate in cis to recruit centromere proteins and to mediate kinetochore assembly has been the subject of constant debate (Karpen and Allshire 1997; Wiens and Sorger 1998; Sullivan et al., 2001; Sullivan 2001; Malik and Henikoff 2002). In Fission yeast, Drosophila and humans, however, larger parts of centromeric region can be deleted without dramatically acting chromosome segregation. Even when supposedly fundamental core sequences are eliminated, these chromosomes still acquire centromere function and propagate their activity by what appears to be an epigenetic mechanism. The existence of neocentromere-chromosomal sites that on rare occasions become associated with centromere activity (Karpen and Allshire 1997; Wiens and Sorger 1998; Sullivan et al., 2001 Sullivan 2001; Choo 2001; Lo et al., 2001) supports the idea that centromere assembly is regulated by epigenetic forces. The fact that a kinetochore drifts along the
alpha-satellite array in response to the presence of a nearby telomere again indicates that kinetochores are malleable structure with no fixed abode. Despite the divergence of centromeric DNA there are clearly several highly conserved centromere proteins. The most fundamentally important is perceived to be CENP-A which (Malik and Henikoff 2002) only associates with active centromeres. As CENP-A is a histone H3 variant, it is attractive as a potential instrument through which the establishment, propagation and marking of the chromosomal site of kinetochore assembly may occur. In different Drosophila species, a region within loop 1 of CENP-A has been found to be critical for targeting to centromeres (Vermaak et al., 2002). CENP-A is incorporated in centromeric nucleosomes in a mechanism that appears to exclude histone H3 (Lo et al., 2001; Blower et al., 2002). The mechanism by which CENP-A is located at only one site per chromosome (holocentrics expected), apparently with no strict requirement, is the subject of much conjecture. CENP-A is required for the location of another essential centromere component, CENP-C (Howman et al., 2000; Oegema et al., 2001; Moore and Roth 2001; Hooser et al., 2001). It is also clear that the mere presence of CENP-A is not sufficient to trigger kinetochore assembly. Sites along chromosome arms including inactivated centromeres would not support kinetochore assembly, even after forced incorporation of CENP-A/CENP-C, because they lack the mark that can only be provided by tension/microtubule attachment. The incorporation of CENP-A into centromeric chromatin ultimately leads to the assembly of the kinetochore, which is composed of several sub complexes. The number of identified centromere-associated proteins has increased enormously over past two years. Approximately ten constitutive vertebrate centromere proteins (CENPs) have been described, and dependency relationships have been established. Despite the relatively simple organization of centromeres at the DNA level, an unexpectedly large number of proteins contribute to budding yeast centromere function (Hooser and Heald 2001; Kitagawa and Hieter 2001). In total 28 proteins were assigned to these complexes, five of which represent previously uncharacterized kinetochore proteins. The microtubules-associated outer kinetochore component Dam1 was found to be a crucial downstream target of the Aurora B kinase homologue, Ipl1. Phosphorylation of Dam1 by Ipl1 is proposed to be required in order to destabilize aberrant kinetochore-microtubule association (Cheeseman et al., 2002). Therefore,
heterochromatin and cohesion may combine to form a rigid structure that holds sister kinetochores in tight back-to-back conformation, thus maximizing interaction so the two kinetochores with opposite poles and ensuring that each one only associates with microtubules form one pole.

The role that DNA sequence plays in controlling the formation of this centromeric chromatin varies from organism to organism reviewed by Wiens and Sorger (1998). Kinetochores assemble only on centromeric sequences present once on each chromosomes. In rare cases as in Drosophila and humans however, kinetochore can assemble at positions lacking detectable centromeric DNA. In these organisms, the location of centromeric chromatin can vary among genetically identical cells. The formation of one and only one kinetochore per chromosome could be explained by the selective binding of kinetochore proteins to DNA elements found only at centromere. This appears to be true in the budding yeast S.cerevisiae A 125 bp sequence, present once on each. Centromeres in eukaryotes other than budding yeast range from 40-100 kb in the fission yeast S. pombe to several megabases in humans. The analysis of abnormal human chromosomes has shown that the presence of an apparently intact centromere is not sufficient to drive kinetochore formation. A Robertsonian translocation, is formed by linking together two short arms from different chromosomes to form a large dicentric that is mitotically stable. How does an apparently dicentric chromosome escape the destructive breakage and rejoining cycles that are the usual fate of dicentrics? Although Robertsonian translocations appear to have two centromeres, they contain only one active kinetochore (Warburton et al., 1997). Both centromeres in Robertsonian translocations usually contain alpha satellite DNA and bind CENP-B and in any cases there is no evidence for gross deletions or rearrangements at the centromeres. However, there is usually only one primary constriction (the visible manifestation of a kinetochore in the light microscope) located at one of the two parental centromeres. When kinetochores are visualized by immunofluorescence with antibodies against CENP-A, CENP-C and CENP-E, staining is observed only at the site of the active centromere, with the inactive centromere showing little if any staining. How are the active and inactive centromeres on Robertsonian translocation selected? Two finding suggest that the process is random. First, when Robertsonian translocations from different individuals but involving the same
two chromosomes are compared, whether of the two centromeres can form the active kinetochore, second, in rare cases, the tissues of patients with Robertsonian translocations are mosaic with respect to the active centromere. Rare cases of mosaicism may arise either because the newly formed dicentric retains both active kinetochores for a few divisions in embryogenesis, or because the site of the active kinetochore occasionally switches in somatic cells. The existence of mosaicism shows that either centromere can form functional kinetochore. However, the general rule in individuals Robertsonian translocations is that the same centromere is active in all cells implying that, once established, the position of the active kinetochore can propagate accurately from mother to daughter. Take together, these data argue that Robertsonian translocation contain two potentially functional centromeres only on active kinetochore that is accurately propagate during cell division. Assembly of human kinetochores may not strictly require the presence of centromeric DNA. A recent examination of neocentromere formation in *Drosophila* confirms that functional kinetochores can established at normally noncentromeric DNA in organisms other that humans (Williams et al., 1998). It seems likely that the location of kinetochores is determined by the position of centromeric chromatin. One interesting consequence of having centromere position specified by specialized chromatin structures is that kinetochore activity can be subjected to epigenetic effects. The connection between epigenetic regulation and chromatin is not unexpected. Epigenetic control of centromere specification may exist because fusion chromosomes play a role in evolution and speciation. The formation of centromeric chromatin can be divided into distinct, but related steps of nucleation and propagation. Nucleation may be controlled by the DNA sequence. DNA must play a role because kinetochores on normal chromosomes are never found at neocentromere-compete locations. Second, nucleation may be controlled by strictly limiting the amounts or activity of key *trans*-acting factors. Third nucleation could be spatially restricted along an entire chromosome by signals that emanate from particular chromosomes structures such as kinetochores or telomeres (Williams et al., 1998). Centromeric chromatin must differ from other chromatin in the very tight control that is exerted over its formation and propagation.
Chromatin assembly and the kinetochore:

Krude (2002) has discussed chromatin assembly in relation to kinetochore formation. During replication of genomic DNA, bulk chromatin is assembled from histone proteins on the two nascent DNA daughter strands. This reaction is mediated by chromatin assembly factors which target histones to their site of assembly into nucleosomes (Mello and Almouzzi, 2001).

The centromeric DNA is bound by a functional protein complex known as the kinetochore, which physically links the centromeric DNA to the spindle apparatus [3]. The requirements for assembly of functional kinetochores are far from fully understood at present. A recent paper (Sharp et al., 2002) has reported that two protein complexes involved in regulating chromatin assembly are also required for building functional kinetochores Two key processes of chromosome maintenance during cell division have thus been brought into one common molecular focus.

What defines functional kinetochores at centromeres? The relatively simple centromeres of the budding yeast *Saccharomyces cerevisiae* contain only 125 base pairs of centromeric DNA (CEN). CENs are subdivided into three centromere DNA elements, CDE are subdivided into three centromere DNA elements, CDE I-II, which are present on each centromere. The core of a yeast kinetochore contains a protein complex termed CBF3 bound to CDE III.

In yeast, a distinct chromatin structure at centromeres extends for several thousand base pairs beyond the CEN. The centromere-specific histone H3 variant Cse4p. (CENP-A in higher eukaryotes) is incorporated into centromeric nucleosome particles. Recent research on kinetochore function has consequently focused on centromeric chromatin and the epigenetic information that it contains (Sullivan, 2001).

Kinetochore-Microtubule interactions:

He et al. (2001) have made a molecular analysis of kinetochore-microtubule attachment in budding yeast. Kinetochores have both DNA and microtubule binding activities. *S. cerevisiae* centromeres include CBF3 and the specialized H3 histone Cse4p (Stoler et al., 1995). The assembly of kinetochores in *S. cerevisiae* appears to begin with
the binding of CBF3, a four-protein complex to the essential CDEIII region of centromeric DNA. Cells carrying temperature sensitive mutations in CBF3 subunits (Ndc10p, Cep3p, Ctf13p, or Skp1p) experience greatly elevated chromosome loss under semipermissive conditions.

Historically, an important question about chromosome-microtubule attachment has been the identity of the kinetochore-associated motors. In animal cells, the kinesin-related motor proteins (KRP) CENP-E and MCAK have been shown to function in kinetochore-dependent chromosome movement, as has dynein in yeast, it is not known which among the six KRP and dynein motors are kinetochore bound. Both the ATP-dependent sliding of motor proteins along microtubules and the GTP-dependent depolymerization of microtubule fibres are capable of generating sufficient force to move chromosome (Hunter and Wordeman, 2000). Thus nonmotor microtubule associated proteins (MAPs) may function to link kinetochores and microtubule plus ends during periods of polymer growth and shrinkage.

Among these kinetochore proteins are several that bind to microtubules, or have animal cell homologs that are microtubule binding. The proteins are involved directly in the formation of microtubule attachment sites.

The mitotic spindle is a large multicomponent machine with long range physical interactions among kinetochores, microtubules, and spindle poles. It is therefore important to inquire into the physical basis by which proteins might become associated with CEN DNA. We can imagine three possibilities. First, centromere association might be highly indirect, involving distant interactions mediated by microtubules. This seems unlikely because neither α-tubulin, γ-tubulin, nor the spindle pole component Spc42p detectably coprecipitated with CEN DNA by ChIP, and the available evidence suggests that ChIP is a reliable and highly selective cross-linking method (Meluh and Broach, 1999). Second, centromere association might involve binding rectly to DNA, as in the case of CBF3, or binding to CBF3 in a multiplayer kinetochore structure (Ortiz et al., 1999). In this case, a protein should be found associated with kinetochores independent of whether the kinetochores are linked to microtubules. Third, a protein that binds to
microtubules might show centromere association through the attachment of microtubule plus ends to kinetochores.

Among the eleven proteins discussed in this paper, four-Stu2p, Bik1p, Dam1p, and Cin8p-have been shown previously to bind microtubules and mutations. Multiple proteins appear to be involved in the attachment of kinetochores to microtubules, including both motor proteins and MAPs, and these proteins play at least partially overlapping roles. Second, the formation of kinetochores with at least some microtubule binding activity, however aberrant, is required for mitotic checkpoint function. Third, mutations in different proteins give rise to different defects in chromosome movement, including a complete loss of attachment (in ndc10, ndc80, and nuf2), slow movement of chromatids that have apparently achieved bipolar attachment (in stu2), and close association with a single pole, presumably reflecting monopolar microtubule attachment (dam1 and ipl1). Fourth, many proteins involved in kinetochore-microtubule attachment also localize to other microtubule-based structures and appear to have more than one.

Biggins and Walczak (2003) provide additional insights as to how microtubules attach to kinetochores. Kinetochore, a proteinaceous structure that assembles on the centromeric DNA. First, it is the site of attachment of microtubules to the chromosome to allow the chromosomes to properly align and segregate on the spindle. Second, it contains molecular motor proteins that orchestrate the complex movements of chromosomes during mitosis. Finally, the kinetochore serves as the site of assembly for the checkpoint machinery—a set of proteins that assures that the chromosomes are properly attached to and aligned on the spindle prior to the initiation of anaphase.

C. elegans, has chromosomes with multiple kinetochores, holocentric chromosomes. Although the fidelity of segregation depends on kinetochores assembling on one and only one region of a chromosome, it is not yet clear what specifies the site of assembly. The region of DNA to which kinetochore proteins bind is the centromere. α-satellite repeats do not have a strict DNA sequence and, therefore, organisms must also rely on an epigenetic component to direct kinetochore assembly. In general, all eukaryotic organisms employ both sequence based and epigenetic components to varying degrees to propagate kinetochore assembly.
The epigenetic mark for kinetochore assembly is unknown, but one feature of all kinetochores is a histone H3 variant called CENP-A in vertebrates of Cse4p in budding yeast (for reviews, see Sullivan, 2001). Active neocentromeres the histone H3 variant is always present. This raises the intriguing possibility that the centromeric H3 epigenetically marks the centromere. However, since CENP-A is not sufficient to initiate kinetochore assembly (Van Hooser et al., 2001), the minimal requirements for kinetochore formation still need to be elucidated.

The underlying centromeric sequences and the epigenetic components direct formation of the proteinaceous kinetochore structure. The two sister kinetochores assemble during early mitosis on each side of the primary constriction of the sister chromatids, orienting them toward the opposing spindle poles, to which they will become attached. The mature kinetochore forms during late prometaphase to metaphase in vertebrate cells, but prekinetochores exist throughout the entire cell cycle.

Extending from the trilaminar plate is a region termed the fibrous corona, which is only clearly visible when MTs are not attached to the kinetochore. When MTs attach to the kinetochore, they terminate in the outer plate (Ris and Wit, 1981). Recent analysis by an alternative EM technique thought to provide better preservation of the ultrastructure has produced a different view of the kinetochore (McEven et al., 1998). The vertebrate kinetochore appears as a mat of lightly stained material that abuts the centromeric heterochromatin. The MTs end in this mat, and it is, therefore, thought to correspond to the outer plate of conventionally fixed samples. Intriguingly, the holocentric chromosomes of C. elegans have a similar appearance when prepared by this technique (Howe et al., 2001), suggesting that the higher order structure of the kinetochore is similar despite the different numbers of MT attachment sites along the chromosomes. The general structure will be conserved throughout eukaryotes (Ding et al., 1993).

The vertebrate 'search-and-capture' model of mitosis serves as the textbook paradigm for how chromosomes become properly attached to and aligned on the spindle. The growing MT end distal from the spindle pole (centrosome), called the plus end, probes three-dimensional space until the MT is captured by a kinetochore. Proper bi-oriented attachment generates tension on the sister kinetochores.
The mitotic or spindle checkpoint is a signal transduction cascade that mediates cell cycle arrest if there are errors in the attachment of MTs to kinetochores. Signal originating at the kinetochore. Elegant micromanipulation and genetic studies have demonstrated that MT attachment and/or tension defects can activate the checkpoint (Li and Nicklas, 1995). MT attachment is required to generate tension at the kinetochore, but tension contributes to stabilizing attachments. Defects in spindle function, kinetochore assembly and spindle pole duplication all activate the checkpoint, which is consistent with the checkpoint sensing kinetochore attachment or tension.

Spindles can also self-organize around DNA. This mechanism of spindle assembly occurs in the acentriolar spindles of female meiosis (McKim and Hawley, 1995) and in vertebrate cells in which centrosomes have been removed by laser ablation.

A major goal in the field is to determine how the complex kinetochore assembles faithfully during every cell cycle. More than 40 budding yeast kinetochore proteins have been identified due to various technological advances. In addition, the purification of yeast spindle poles fortuitously identified many outer kinetochore proteins that co-purified with MTs (Wigge and Kilmartin, 2001). Several of the yeast proteins have mammalian homologs, suggesting strong conservation of kinetochore components and function. Kinetochore can be broken down into the following subcomplexes – CBF3, Ctf19, Ndc80, Dam1 and lpl1. Essential proteins includes the budding yeast H3 variant Cse4p, the CBF3 complex, and CENP-C homolog Mif2. It is unclear which proteins(s) initiate (s) kinetochore assembly. CBF3 may be the scaffold for yeast kinetochore assembly, because it is essential for the maintenance of every kinetochore protein that has been tested.

The assembly process can be roughly broken down into the DNA-binding proteins directing assembly of Ctf9, followed by the Ndc80, lpl1 and Dam1 complexes. Many kinetochore proteins, such as motor proteins and MT-associated proteins (Maps) be added to the picture of yeast kinetochore assembly. In other organisms, the histone variant CENP-A and the inner kinetochore protein CENP-C are required for the assembly of many proteins. When CENP-A is mistargeted to non-centromeric DNA, it is able to recruit CENP-C and other inner kinetochore proteins (Van Hooser et al., 2001).
Therefore, kinetochore assembly is not simply hierarchical, but is instead driven by multiple pathways. Given the large number of kinetochore proteins, the current challenge is to understand how their assembly into a functional kinetochore is regulated. The introduction of siRNA technology in cell culture will advance studies in this area.

In addition to motor proteins, there are a number of plus-end MT binding proteins that play important roles in both kinetochore-MT interactions and regulation of kinetochore-MT dynamics. In addition to the proteins that directly mediate kinetochore MT interactions, there are also proteins that regulate these interactions, such as the Aurora B/lpl1 protein kinase. Aurora B/lpl1 is an important regulator of kinetochore function, and understanding its activity will be a key area of future research. The emerging picture of MT attachment to the kinetochore in multicellular eukaryotes is that a number of players are required to set up the initial attachment, and inhibition of these proteins causes defects in chromosome alignment.

Cheeseman et al. (2002) have provided key data regarding linking spindle microtubules and centromeric DNA in budding yeast. Chromosome segregation during mitosis requires a physical connection between spindle microtubules and chromosomes. This attachment occurs at proteinaceous structures called kinetochores that assemble on centromeric DNA. Identification of >30 yeast kinetochore proteins, many of which are conserved among eukaryotes.

Subsets of kinetochore proteins interact physically in discrete complexes or function together in signaling modules. Here we propose a classification of individual kinetochore proteins and these complexes according to whether they function at the interface with centromeric DNA (inner kinetochore proteins), at the interface with spindle microtubules (outer kinetochore proteins), or at the interface between the inner and outer kinetochore proteins (central kinetochore proteins).

Correct chromosome segregation requires that one and only one kinetochore assembles on each chromosome. To achieve this, a subset of kinetochore proteins functions to specifically recognize and bind to centromeric DNA. In budding yeast, centromeric DNA is 125 bp long and is conserved among the different chromosomes.
In contrast, metazoan centromeric DNA can be megabases in length and does not contain easily identifiable DNA consensus sequences (for review see Choo, 1997). Despite the differences between yeast and metazoan centromeric DNA, the kinetochores that assemble on this DNA in both cases are organized around centromeric nucleosomes that contain specialized histone H3 like proteins (yeast Cserp or its metazoan homologue CENP-A (Meluh et al., 1993). Since Cserp/CENP-A-containing nucleosomes are found only at centromeres, there must be a mechanism to target these nucleosomes specifically to centromeric DNA. Yeasts have solved this problem in part through the activities of additional DNA binding kinetochore proteins. The yeast centromeric nucleosome binds to an 80-bp sequence termed CDEII that spans the middle of the centromere.

The DNA sequences on either side of CDEII (termed CDEI and CDEII) also serve as binding sites for distinct proteins. The most important of these is the CBF3 complex (Ndc10p, Cep3p, Ctf13p, and Skp1p), which binds at CDEII (Lechner and Carbon, 1991). In the absence of CBF3, kinetochore function is abolished in vivo and in vitro (Goh and Kilmartin, 1993; Sorger et al., 1994), and the association of all known kinetochore proteins with the centromere, including Cse4p (Ortiz et al., 1999), is disrupted. In contrast, the association of CBF3 with centromere DNA in vivo does not require Cserp (Measday et al., 2002). Therefore, the specific binding of CBF3 to CDEII helps define the position of the yeast kinetochore.

The yeast inner kinetochore also contains two additional DNA-binding proteins. CDEI serves as a binding site for a homodimer of Cbf1p (Mellor et al., 1990). Although Cbf1p is not essential for kinetochore function, it induces the bending of DNA (Niedenthal et al., 1993) and may therefore contribute to the higher order structure of the kinetochore. Cbf1p has structural similarity and limited sequence identity to CENP-B, which binds to metazoan centromeric DNA and also induces DNA bending (Tanaka et al., 2001).

Inner kinetochore proteins do not associate directly with microtubules or the microtubule-binding components of the outer kinetochore. Therefore, we propose that "central kinetochore" proteins mediate the linkage between the inner and outer kinetochore proteins. One important central kinetochore component appears to be the
Ctf19 complex (Ctf19p, Mcm21p, and Okp1p), which binds to each of the inner kinetochore components described above (Oritz et al., 1999). By virtue of its two-hybrid interaction, the Ctf19 complex also appears well positioned to link together the Ctf3 complex (Ctf3p, Mcm16p, and the Ndc80 complex (Ndc80p, Spc24p, Spc25p. The Ndc80 complex is especially important for kinetochore function, since mutants in this complex are completely defective for chromosome segregation, similar to CBF3 mutants. There are also a variety of kinetochore proteins, which are less defined in terms of their physical interactions.

The most critical function of a kinetochore is to connect chromosomes to microtubules. However, until recently it was unclear how yeast kinetochores attach to spindle microtubules. In metazoans, microtubule-associated motors, including CENP-E, dynein, and XKCM1, play roles in mediating kinetochore-microtubule attachments and in subsequent chromosome movements during congression and anaphase A (for review see Heald, 2000). Yeast lack CENP-E, and yeast dynein appears restricted to cytoplasmic microtubules. However yeast Kip3p may be an XKCM1 homologue (Severin et al., 2001), suggesting that it may function at kinetochores.

Nonmotor microtubule-associated proteins (MAPs) also may mediate kinetochore-spindle attachments. The first kinetochore-associated MAP to be identified was Dam1p, a component of the Dam1p complex (Dam1p, Duo1p, Dad1p, Spc19p, Spc34p, Dad2p, Ask1p, Dad3p, and Dad4p (Cheeseman et al., 2001a; Janke et al., 2002; Li et al., 2002; unpublished data). This complex localizes to kinetochores in an Ndc10- and Ndc80-dependent manner (Enquist-Newman et al., 2001; Jones et al., 2001; Janke et al., 2002) and is essential for chromosome segregation. Electron microscope studies of vertebrate kinetochores revealed that microtubule plus ends make end-on attachments with the kinetochore. This observation suggests a role for plus-end-binding MAPs in the microtubule kinetochore linkage. Although there is no direct evidence for such an end-on attachment in yeast, Biklp, a member of the Clip-170 family of plus-end tracking MAPs, does localize to kinetochores independent of its microtubule-binding activity (He et al., 2001; Lin et al., 2001).
To ensure that the kinetochore proteins described above function properly, the conserved mitotic checkpoint monitors the formation of bipolar kinetochore-microtubule attachments and, in the event of an error, arrests a cell in metaphase (for review see Amon, 1999). In budding yeast, the primary signal for this checkpoint is the absence of an attachment between the centromere and the spindle, implicating kinetochore proteins as the source of this signal. In addition to sensing attachment defects, the yeast mitotic checkpoint also appears to monitor tension on the kinetochore. Bipolar attachments exert sufficient force on the kinetochore to pull centromeric regions apart before anaphase (Goshima and Yanagida, 2000; He et al., 2000). It additionally requires Ip11p (Biggins and Murray, 2001), an aurora protein kinase.

It is less clear how yeast kinetochores change through the cell cycle. Centromeres are positioned near the spindle poles throughout the cell cycle (Jin et al., 2000). This localization requires microtubules and functional kinetochores, suggesting that active attachments between the spindle and the kinetochore exist during the majority of the cell cycle. However, changes in kinetochore function may occur during events such as the assembly of a new kinetochore, during spindle assembly to facilitate the formation of bipolar attachments, and during anaphase when kinetochores move to the spindle poles.

Several proteins have been identified that may regulate these and other aspects of kinetochore function. The best characterized of these regulatory factors is the Ip11p protein kinase, which is essential for chromosome segregation. Ip11p can associate with kinetochores (Biggins and Murray, 2001; Kang et al., 2001) and directly with microtubules (Kang et al., 2001). Therefore, it is well positioned to regulate kinetochore-microtubule attachments. Ip11p associates closely in vivo with Sli15p, an INCENP homologue that plays a role in activating Ip11p’s kinase activity and possibly in substrate recognition (Kang et al., 2001).

The kinetochore-microtubule linkage is very dynamic and chromosomes move continuously during mitosis reviewed by Grancell and Sorger, (1998). Chromosome movement is powered by microtubule-based molecular motors, which use the energy of ATP hydrolysis to generate force, and by microtubule depolymerization, which liberate energy stored in the microtubule lattice by GTP hydrolysis during assembly (Inoue and
Salmon 1995). Which kinetochore proteins are responsible for which aspects of chromosome movement, and to determine the relative importance of motors and microtubule dynamics in generating force. Spindle microtubules can be divided into four classes on the basis of the structure they contact. The first, class, astral microtubules, contact the cell cortex, where they play a role in orienting the spindle pole-to-pole microtubules. Motor proteins found in the overlap region cause pole-to-pole microtubules to slide relative to each other by controlling the length of the spindle. The third class of microtubules interact with chromatin via chromokinesins, motor proteins on the chromosome arms, which generate a polar ejection force that pushes chromosomes towards the middle of the spindle. The fourth class of microtubules bind to kinetochores to link chromosomes to the spindle. Just after nuclear envelope breakdown most CENP-E is associated with microtubules. Subsequently, CENP-E becomes concentrated at kinetochores, presumably by traveling along microtubules by plus-end-motor activity (Yao et al., 1997). CENP-E localizes to the interface between chromatin and microtubules even early in mitosis, when partially assembled kinetochores have yet to assume mature electron-dense structure. A motor's polarity is usually defined as the direction it moves on non-dynamic (usually taxol-stabilized) microtubules in the presence of ATP. Classical kinesin is a plus-end-directed motor, but some kinesin-like motors move towards the minus ends of microtubules dynein is also a minus-end directed motors. Kinetochore-mediated chromosome movement is dependent on a large number of different proteins, most of which have not yet been identified, that must act in concert to attach chromosomes to microtubules, control the oscillatory movements of prometaphase, maintain chromosomes position at the metaphase plate, and move chromosome polewards at anaphase. And move chromosome at anaphase. CENP-E demonstrates that the identification and characterization of kinetochore-associated microtubules motor proteins is a good first step in the search for mechanistic understanding chromosome segregation.

Attachment of chromosomes to the microtubules is the product of chance encounters. The following is a concise account of the events involved in chromosome segregation during mitosis and meiosis extracted from Nicklas (1997) and the references therein. Microtubules grow outward from each pole of the spindle If a microtubule
happens, by chance, to encounter a kinetochore, it may be captured by the kinetochore and consequently the chromosome becomes attached to the spindle. Microtubules contribute a self-assembly process with the remarkably, perhaps unique property that energy is used to purchase instability rather than stable bonds. Partner kinetochores lie back-to-back, that is the capture surfaces of partner kinetochores face in opposite directions. A kinetochore tends to capture microtubules from the pole its capture surface faces. Chances makes errors in initial attachment inevitable. Chance encounters often result in the attachment of both partner chromosomes to the same pole, a "monopolar" attachment. Unless the error is corrected, one daughter cell will receive two copies of the chromosome and the other daughter cell will receive none. Alternative to chance is a precision biochemical machine. Such a machine corrects errors in DNA replication: a protein complex detects the errors with great sensitivity and then corrects them with great precision. However, errors in chromosome attachment are not corrected by a protein machine, probably because of the scale and nature of the error. Correction of faulty attachments required both the capture of new microtubules and release from the old. That kinetochore must also be free to move—it must have been released from the initial connection that tethered one to the lower pole. Otherwise, the mitotic motors will be unable to move the kinetochore very fast, and error capture and release are improbable events. In fact, capture is only possible at all because microtubules that touch the kinetochore at any angle can captured. Tension is the source of order. Tension is absent in the improper attachment. A particularly interesting possibility is a microtubule motor protein as the link between each microtubule and the pole. Microtubule motors grasp microtubules with a dynamic, not a static, grip: the grip is dependent on conditions (such as the presence or absence of tension, perhaps) rather than permanent. Some candidates for polar motor-linkers have been identified. Selection extracts order from disorder, music from noise. Evolution by natural selection begins with random genetic changes, mutations, selection among mutants result a in a nonrandom outcome, improved adaptation. In addition to monopolar attachments, another error commonly occurs—one attachment is missing. Diverse cells have a quality-control checkpoint that detects such errors. The checkpoint delays the onset of anaphase and the completion of cell division, allowing time for the tardy kinetochore to attach to the spindle. The checkpoint is an
adaptation to living with chance, an invention that buys time for the capture of microtubules by unfavorably placed kinetochores. Mantid spermatocytes have three sex chromosomes, a Y chromosome and two genetically distinct X chromosomes. Genetic balance requires sperm that carry either the Y chromosome or the two X chromosome. This desirable state allows if spermatocytes, the Y attached to one pole and the two X’s to the opposite pole. Often, however, the three chromosomes fail to remain connected and one X chromosome is free, not connected to the other two sex chromosomes. Monopolar attachment is common in meiosis and uncommon in somatic cell mitosis. Conversely, missing attachments are common in mitosis but rare in meiosis. These disparities arise from an important difference in the construction of mitotic versus meiotic chromosomes. The kinetochores of chromosomes in mitosis lie in a pit. Chromosomes in meiosis, however, have relatively exposed kinetochore which can capture microtubules over a large angle. Consequently, a kinetochore can easily attach to either pole, and partner kinetochores often attach to the same pole. Hence, monopolar attachment is frequent. Pairing and recombination result in chromosomes in which the partner kinetochores are linked by long, sometimes convoluted stretches of rather flexible chromatin. Hence, the partner kinetochores are not rigidly constrained to face in opposite directions, as they are in mitosis, and both may capture microtubules from the same pole. Kinetochores were processed as they are in somatic mitosis, encounters with microtubules from the opposite pole would be very rare and most errors would go uncorrected. Kinetochores, in meiosis and in somatic cell mitosis illustrate the compromise imposed by reliance on chance encounters for chromosome attachment to the spindle. In meiosis coping with chance favors a moderately protruberant kinetochore, which facilitates the correction of monopolar attachment errors. Tension controls the checkpoint with tension-sensitive protein phosphorylation. Certain kinetochore proteins in mammalian and insect cells are phosphorylated before the chromosomes attach to the spindle, and there become dephosphorylated after chromosomes attach properly. For checkpoint control, the kinetochores proteins of misattached chromosomes remain phosphorylated. Chromosomes with both kinetochores attached to the same pole are relaxed, not under tension, whereas properly attached chromosomes are under tension from opposed mitotic forces. The kinetochores of misattached, relaxed chromosomes have highly
phosphorylated proteins. When immunostained with an antibody specific for the phosphorylated state. We do not know how tension produces kinetochore protein dephosphorylation. One possibility is a direct effect on protein conformation, just like an allosteric response to binding a ligand. Tension, whether from mitotic forces or from experimenter’s tugging, invariably has two effects: kinetochore dephosphorylation and a “go ahead” signal to the checkpoint. Conversely, the absence of tension, whether from natural causes. A particularly convincing correlation between phosphorylation and the checkpoint response comes from evolutionary differences in sex chromosomes. Two yeast checkpoint proteins have relatives that are kinetochore components in other cells. The common theme is the transduction of mechanical force into chemical change, just the opposite of the transduction of chemical change into mechanical force seen in muscle and other motile systems.

The epigenetic mark for kinetochore assembly is unknown, but one feature of all kinetochores is a histone H3 variant called CENP-A in vertebrates or Cse4p in budding yeast (Biggins and Walczak, 2003). The active neocentromeres the histone H3 variant is always present. This raises the intriguing possibility that the centromeric H3 epigenetically marks the centromere. However, since CENP-A is not sufficient to initiate kinetochore assembly, the minimal requirements for kinetochore formation still need to be elucidated.

The underlying centromeric sequences and the epigenetic components direct formation of the proteinaceous kinetochore structure. The two sister kinetochores assemble during early mitosis on each side of the primary constriction of the sister chromatids, orienting them toward the opposing spindle poles, to which they will become attached. The mature kinetochore forms during late prometaphase to metaphase in vertebrate cells, but prekinetochores exist throughout the entire cell cycle.

Extending from the trilaminar plate is a region termed the fibrous corona, which is only clearly visible when MTs are not attached to the kinetochore. When MTs attach to the kinetochore, they terminate in the outer plate. Recent analysis by an alternative EM technique thought to provide better preservation of the ultrastructure has produced a different view of the kinetochore. The vertebrate kinetochore appears as a mat of lightly stained material that abuts the centromeric heterochromatin. The MTs end in this mat,
and it is, therefore, thought to correspond to the outer plate of conventionally fixed samples. Intriguingly, the holocentric chromosomes of *C. elegans* have a similar appearance when prepared by this technique, suggesting that the higher order structure of the kinetochore is similar despite the different numbers of MT attachment sites along the chromosomes, the general structure will be conserved throughout eukaryotes.

The vertebrate 'search and capture' model of mitosis serves as the textbook paradigm for how chromosomes become properly attached to and aligned on the spindle. The growing MT end distal from the spindle pole (centrosome called the plus end, probes three-dimensional space until the MT is captured by a kinetochore. Proper bi-oriented attachment generates tension on the sister kinetochores. The mitotic or spindle checkpoint is a signal transduction cascade that mediates cell cycle arrest if there are errors in the attachment of MTs to kinetochores. Elegant micromanipulation and genetic studies have demonstrated that MT attachment and/or tension defects can activate the checkpoint. MT attachment is required to generate tension at the kinetochore, but tension contributes to stabilizing attachments. Defects in spindle function, kinetochore assembly and spindle pole duplication all activate the checkpoint, which is consistent with the checkpoint sensing kinetochore attachment or tension.

This mechanism of spindle assembly occurs in the acentriolar spindles of female meiosis and in vertebrate cells in which centrosomes have been removed by laser ablation. A major goal in the field is to determine how the complex kinetochore assembles faithfully during every cell cycle. More than 40 budding yeast kinetochore proteins have been identified due to various technological advances. In addition, the purification of yeast spindle poles fortuitously identified many outer kinetochore proteins that co-purified with MTs. Several of the yeast proteins have mammalian homologs, suggesting strong conservation of kinetochore components and function. Kinetochore can be broken down into the following subcomplexes. Essential proteins includes the budding yeast H3 variant Cse4p, the CBF3 complex, and the CENP-C homolog Mif2. It is unclear which protein(s) initiate(s) kinetochore assembly. CBF3 may be the scaffold for yeast kinetochore assembly, because it is essential for the maintenance of every kinetochore protein that has been tested.
The assembly process can be roughly broken down into the DNA-binding proteins directing assembly of Ctf19, followed by the Ndc80, Ipl1 and Dam1 complexes. Many kinetochore proteins, such as motor proteins and MT-associated proteins (MAPs) to be added to the picture of yeast kinetochore assembly. In other organisms, the histone variant CENP-A and the inner kinetochore protein CENP-C are required for the assembly of many proteins. When CENP-A is mistargeted to non-centromeric DNA, it is able to recruit CENP-C and other inner kinetochore proteins.

Therefore, kinetochore assembly is not simply hierarchical, but is instead driven by multiple pathways. Given the large number of kinetochore proteins, the current challenge is to understand how their assembly into a functional kinetochore is regulated. The introduction of siRNA technology in cell culture will advance studies in this area. Once kinetochores assemble, they must capture dynamic MTs. The types of kinetochore defects that result in chromosome mis-segregation include a complete lack of MT attachments, partial or weak MT attachments, or a defect in making bi-oriented MT attachments.

In the first class of yeast mutants, MT attachments are completely absent and all of the DNA remains in the mother cell when the spindle elongates. Because the cell cycle continues in the absence of attachments the spindle checkpoint is not activated. Members of the CBF3 and Ndc80 complexes attachments or instead abolish kinetochore assembly. CENP-E, a plus-end directed kinesin-related motor that localizes to kinetochores, is the best studied protein whose inactivation causes a congression defect.

Vertebrate MT-kinetochore attachment also requires the conserved Ndc80 complex, which is required for congression, but has various effects on the spindle. Another important class of kinetochore motor proteins are the Kin I kinesins (MCAK/XKCM1/klp5/6). In addition to motor proteins, there are a number of plus-end MT binding proteins that play important roles in both kinetochore-MT interactions and regulation of kinetochore-MT dynamics.

In addition to the proteins that directly mediate kineto-chore-MT interactions, there are also proteins that regulate these interactions, such as the Aurora B/Ipl1 protein kinase. Aurora B/Ipl1 is an important regulator of kinetochore function and understanding its activity will be a key area of future research. The emerging picture of MT attachment
to the kinetochore in multicellular eukaryotes is that a number of players are required to set up the initial attachment, and inhibition of these proteins causes defects in chromosome alignment.

Dernburg (2001) has provided some insights into the function of kinetochore in holocentric chromosomes. Holocentric chromosomes bind to microtubules along their entire length and move broadside to the pole from the metaphase plate. Holocentric chromosomes are scattered throughout the plant and animal kingdoms, and may be products of convergent evolution. The development of the holocentric nematode worm Caenorhabditis elegans as a robust molecular genetic system, the availability of extensive genome sequences for both C. elegans and monocentric species and the harnessing of RNA interference (RNAi) as an experimental technique (Fire et al. 1998). As these tools are enabling holocentric behavior to be studied at a molecular level, that many components and mechanisms underlying kinetochore function are highly conserved between holocentric and monocentric chromosomes, including ZW10, CENP-C (HCP-4).

Each of these C. elegans centromere or kinetochore proteins thus shares similarity with centromere-associated factors from monocentric organisms, technique of RNA interference in C. elegans. This reverse genetic approach makes it technically straightforward to examine the consequences of depleting a specific gene product even when a mutant allele is unavailable, have reshaped the way we might think about some of the longstanding riddles surrounding holocentric chromosome behavior.

What sequence determinants underlie the positioning of holocentric centromeres? How does mitotic chromosome segregation work when the kinetocore is distributed along each chromatid? In a holocentric organism, what stabilizes the karyotype? How do holocentric chromosomes accomplish meiosis? Work from Moore and Roth 2001 and Oegema et al. 2001 underscores the power of RNAi by demonstrating that construction of a mitotic kinetochore involves a hierarchical assembly of protein factors, which would have been more difficult to establish by traditional mutant analyses.

Diverse molecular mechanisms regulate spindle function (Walczak, 2000). The cell has an elegant macromolecular machine, the mitotic spindle, made up of microtubules and associated proteins, to segregate its chromosomes. Microtubules are dynamic, polar polymers composed of α- tubulin heterodimers that are nucleated from
the centrosomes and extend to the spindle equator where they interact with the chromosomes. The chromosomes are attached to the spindle via kinetochores, specialized proteinaceous structures at the centromere that interact with microtubules.

Several themes emerge, first microtubule nucleation is necessary, either from centrosomes or in the region of chromatin. Second, microtubule dynamics must be regulated for a spindle to be assembled. Third, motor proteins are key players, both in the assembly of the spindle and in the segregation of chromosomes. Finally, accurate movement of chromosomes requires a complex integration of motor-protein action and microtubule dynamics. Microtubule nucleation is one of the key early events during spindle assembly. α-tubulin is required to nucleate microtubules from the centrosome that are assembled into the spindle.

Microtubule dynamics must be precisely controlled to allow formation of a spindle. In the self-assembly pathway of spindle formation, microtubule dynamics must be regulated in the region around chromatin to allow for the local nucleation of microtubules that will be sorted and arranged into a bipolar spindle. The proper assembly and function of the mitotic spindle would not be possible without the presence of motor proteins, which couple the energy of ATP hydrolysis to force production. Once a bipolar spindle is assembled, the chromosomes must be aligned at the metaphase plate and then segregated during anaphase to the two daughter cells. Depolymerization at poles due to poleward flux may be the main driving force for chromosome segregation in embryonic systems.
Somatic chromosomes from the root tip cells of *Dendrobium* Queen-Sonia and *Paphiopedilum insigne* were examined under transmission electron microscope for ultrastructural details of somatic metaphase chromosomes.

Structure of the kinetochore in these two taxa (Figs. 1,2,7,8,9,10,11,12) of ball-and-cup type comparable to the kinetochores seen in other higher plants. The kinetochore shows a convex shaped structure when attached to the microtubules (MTs). About 8-10 MTs are attached to the kinetochore per section which is comparable to the high number of KMTs observed in other plants examined. In the present study the number of MTs attached are estimated at about 100 to 200 per Kinetochore depending on the number of sequential sections examined (Data computed while observation).

An unusual feature observed in *Dendrobium* Queen-Sonia (Figs. 3 & 4) was the presence of membraneous structures interspersed with the metaphase as well as anaphase chromosomes. However these membraneous structures are not seen in the present studies on *Paphiopedilum insigne*.

Fig. 4 also show a number of mitochondria's in the vicinity of the mitotic spindle along with membraneous structures described above.
Fig. 1 & 2: Dendrobium Queen-Sonia

Fig. 1: Electronmicrograph showing somatic Anaphase chromosomes (sectional view). About 8-10 Microtubules (KMTs) are seen attached to the Kinetochore (K). A number of microtubules (MTs) unattached to any Kinetochore are also seen (X14,603).

Fig. 2: Electronmicrograph showing somatic metaphase chromosomes (sectional view). About 8-10 Microtubules (KMTs) are seen attached to the Kinetochore (K) (X20,890).
Fig. 3 & 4: Dendrobium Queen-Sonia

Fig. 3: Electron micrograph showing membranous structure intermingled with the late metaphase chromosomes (sectional view). Note the abundance of membranous structures (MS) along with highly condensed late metaphase chromosomes (X 11,800).

Fig. 4: Electron micrograph showing a somatic cell at mid-anaphase (sectional view). Note the presence of membranous structure (MS) associated not only chromosomes but also the spindle MTs. A few mitochondria (Mi) also seen in the vicinity of mitotic apparatus (X9,900).
Figs. 5 & 6: Paphiopedilum insigne

Fig. 5: Electronmicrograph of a somatic cell nucleus at the interphase. A conspicuous nucleolus (Nu) and chromatin (Ch) evenly distributed within the nucleus are prominent (X10,000).

Fig. 6: Electronmicrograph of a somatic cell showing sectional view of a few metaphase chromosomes. The alignment of metaphase chromosomes at the cell equatorial zone is evident in preparation for anaphase (X12,000).
**Figs. 7 & 8: Paphiopedilum insigne**

**Fig. 7:** Electronmicrograph of a somatic cell nucleus at the metaphase chromosomes (sectional view) showing kinetochore (K) and kinetochore microtubules (KMTs). Note the number of KMTs attached to the higher plant kinetochore - a distinctive feature (X16,000).

**Fig. 8:** Electronmicrograph showing details as above (sectional view) (X15,000).
Fig. 9 & 10: Paphiopedilum insigne

Fig. 9: (Sectional view) The details in 7 & 8 are evident in this electronmicrograph also (X15,000).

Fig. 10: Kinetochore (K) and kinetochore microtubules (KMTs) are prominent in the electronmicrograph of the somatic cell metaphase chromosomes (X21,000). Note parts of the chromosomes are visible due to superthin sections (700Å°) observed.
Figs. 11: Paphiopedilum insigne

Fig. 11: The electronmicrograph of X12,000 shows kinetochore details from somatic metaphase chromosomes. Note the prominent Kinetochore (K) attached to about 15-20 kinetochore microtubules (KMTs) - a characteristic feature of large plant chromosomes (high CEN values).
Figs. 12 & 13: Paphiopedilum insigne

Electronmicrographs showing late Anaphase/Telophase configurations. Fig. 13 (X16,000) shows formation of a phragmoplast (PP) while Fig. 14 (X17,000) shows a late stage of cell plate (CP) formation. Fig. 5 and Figs. 13 & 14 are provided to emphasise that kinetochore studies are possible only at the right stage of metaphase chromosomes when they are highly compact and attached to KMTs.