

Kinetochores in Mitosis and Meiosis

Review

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Summary

Kinetochores are the major point of contact between spindle microtubules and chromosomes. They are assemblies of more than 50 different proteins and take part in regulating and controlling their own interaction with the spindle. We review recent advance in understanding how kinetochores are properly placed onto the chromosome, and how their interaction with the microtubules of the spindle is regulated. Kinetochores orientation in meiosis I shows some particular features, and we also discuss similarities and differences between mitosis and meiosis I.

Introduction

Kinetochores

Kinetochores are complex proteinaceous structures, which are assembled on chromosomes and represent the major point of contact between microtubules of the mitotic spindle and chromosomes. The presence of kinetochores is essential for proper chromosome segregation, since chromosome fragments that lack a kinetochore are not inherited faithfully and are quickly lost (reviewed in Mazia, 1961). Kinetochores are not mere passive attachment points for microtubules, but contribute directly to regulating the interaction with the spindle. Three different types of kinetochores have been found to date (for reviews see Cleveland et al., 2003; Dernburg, 2001). Budding yeast has small kinetochores, which are not visible by standard light or electron microscopy, and which bind a single microtubule. Despite their small size, these kinetochores are highly complex, consisting of around 60 subunits, which are organized in a number of subcomplexes (for an extensive review, see McAinsh et al., 2003). In many other organisms, kinetochores are around 100 to 500 nm in diameter, well visible by microscopy and bind several microtubules (around 3 to 40 depending on the organism). Some plants, *C. elegans*, and other animals have so-called holocentric chromosomes, where the kinetochores cover the whole length of the chromosome. Many kinetochore proteins are conserved from yeast to humans and also in species with holocentric chromosomes (Kitagawa and Hieter,

2001), but whether larger kinetochores are in fact assemblies of multiple budding yeast-type kinetochores is not known (for an outline of a “repeat subunit model” see Zinkowski et al., 1991).

In organisms with monocentric chromosomes (chromosomes with a localized kinetochore), the kinetochores assemble on a specialized genomic region, the centromere. In budding yeast, the centromere is defined by its 125 bp DNA sequence. In fission yeast and higher eukaryotes, the centromere is not defined by sequence, it is much larger (around 1 Mbp in humans), consists mainly of repeated elements, and is largely heterochromatic (for review, see Amor et al., 2004; Biggins and Walczak, 2003) (Figure 1B). One feature that all kinetochore-assembling regions have in common is the presence of specialized nucleosomes, in which histone H3 is replaced by a variant, CENP-A.

The Importance of Proper Kinetochore Orientation

In order to faithfully hand down the genetic information to the daughter cells, the two DNA copies that are generated in S phase need to be segregated to opposite poles during mitosis. After DNA replication the two copies of each chromosome are held together, which allows their identification as entities that have to be distributed to opposite poles in mitosis. Cohesion between the two copies is mediated by a protein complex called “cohesin”. Cohesin subunits form a ring-like structure, and it is possible that cohesin molecules encircle sister DNA strands thereby providing a physical link (for reviews, see Nasmyth, 2002; Uhlmann, 2004). In mitosis, chromosomes condense and the two copies become visible as “sister chromatids”. One kinetochore is assembled on each of the two sister chromatids of a chromosome, and both sister kinetochores become attached to opposite spindle poles by metaphase. In anaphase, cohesin is cleaved by the protease separase allowing the sister chromatids to move to opposite poles. Defects in the kinetochore, in cohesion, or in any of the factors that promote biorientation lead to chromosome missegregation and hence aneuploidy (an abnormal number of chromosomes) in the daughter cells. Aneuploidy is deleterious for cells, not only because it can cause essential genetic information to get lost, but also because imbalances in chromosome number severely perturb gene dosage and thereby cellular function. Prominent examples are the clinical syndromes caused by excess copies of chromosome 21 (Down syndrome), chromosome 13 (Patau syndrome), 18 (Edward syndrome) or the sex chromosomes (Klinefelter and Turner syndrome). The excess number of chromosomes results from chromosome missegregation during meiosis, and the aneuploidy is passed on from the resulting germ cell to the embryo. Aneuploidy is also a hallmark of many cancers, and might contribute to tumorigenesis (Draviam et al., 2004), for example by increasing the number of oncogenes or eliminating tumor suppressors.

Different Aspects of Kinetochore Orientation

In this review, we will discuss recent advances in the molecular biology of kinetochore orientation. We will treat two aspects of kinetochore orientation separately,

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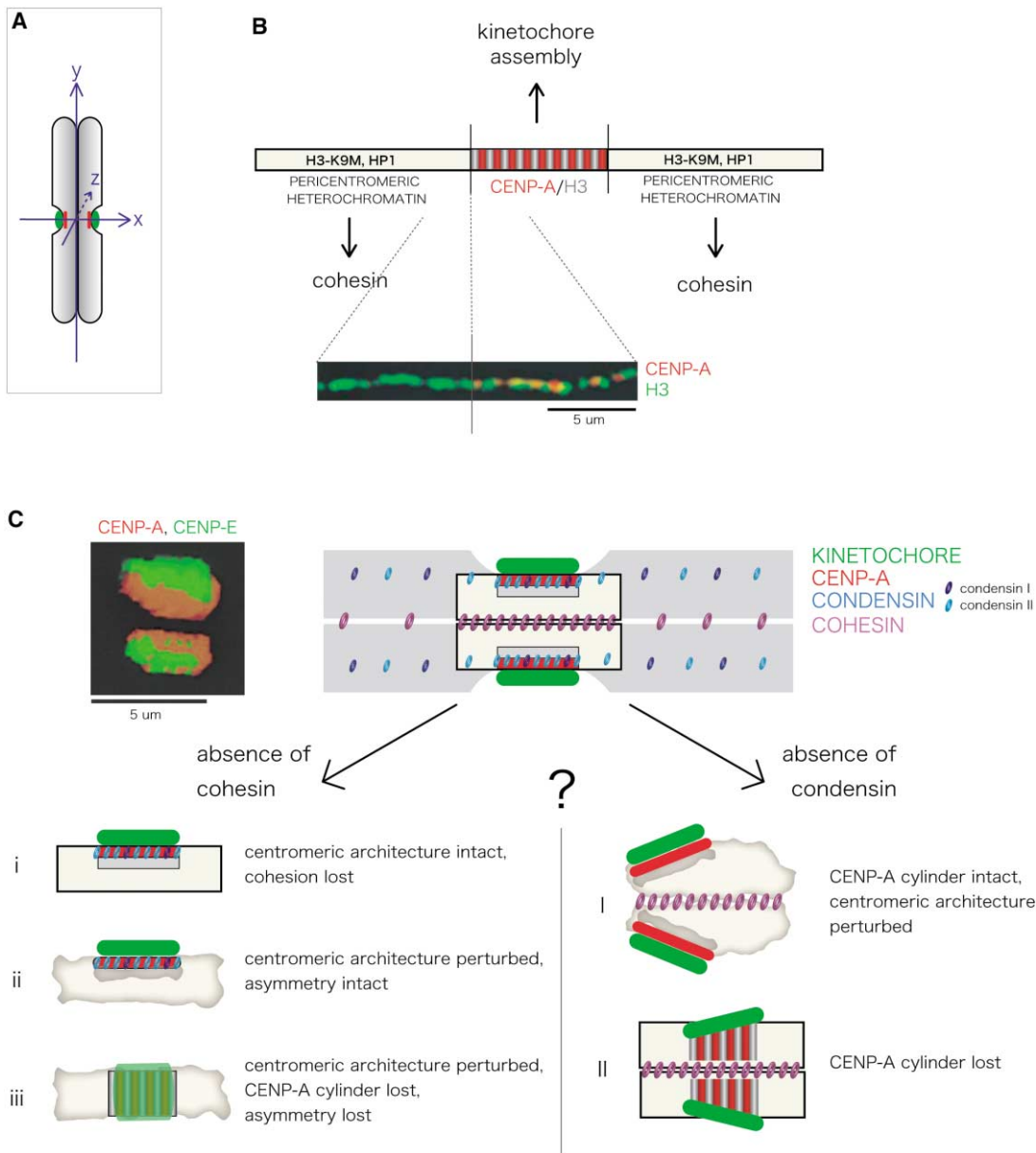


Figure 1. Back-to-Back Orientation of Sister Kinetochores on Mitotic Chromosomes

(A) Axes of a mitotic chromosome.

(B) Linear model of the centromere. Note that the budding yeast centromere only consists of a 125 bp region, with possibly only one CENP-A nucleosome. Fission yeast presumably only has one CENP-A region flanked by heterochromatin. Higher eukaryotes have the alternating CENP-A/H3 pattern depicted here, flanked by heterochromatin (also see Figure 4). For more detailed models see Amor et al. (2004). (H3-K9M = histone H3 methylated at lysine 9, HP1 = heterochromatin protein 1). The micrograph shows CENP-A and histone H3 along a chromatin fiber. Photos in (B) and (C) are reprinted with permission from the author, Gary H. Karpen.

(C) Three-dimensional model of centromere and kinetochores. CENP-A is found in two cylinder-like structures on both sides of the long (y) axis of the chromosome (red). The spatial relationship between H3-containing inner regions (gray) and H3-containing pericentromeric heterochromatin (light yellow) is in fact unclear. Lower image, possible scenarios in the absence of cohesin or condensin: (i) Only cohesion between sister chromatids is lost, centromeric structure is unaffected. (ii) The centromere is affected by cohesin loss; the overall structure remains intact. (iii) Aurora-B does not localize properly to the centromere in the absence of cohesin (Morishita et al., 2001; Sonoda et al., 2001). Along with loss of Aurora-B, condensin and centromeric architecture is lost, the kinetochore is not localized correctly. (I) Condensin is not required for formation of the CENP-A cylinder, but centromeric architecture is looser, so that kinetochores do not strictly face opposite sides. (II) Condensin is required for formation of the CENP-A cylinder, the kinetochores do not localize properly to opposite sides.

the geometric/morphological aspect and the dynamic aspect. By morphology/geometry we mean how the kinetochores are arranged on the chromosome, e.g., the back-to-back orientation of sister kinetochores on mi-

totic chromosomes. Proper kinetochore “geometry” is not sufficient for correct chromosome attachment, since, for example, transient malorientation of chromosomes on the mitotic spindle is frequent during prometa-

phase even if sister kinetochores are properly assembled on opposite sides of the chromosome. The cell needs additional mechanisms that ensure correct orientation with respect to the spindle poles. We will discuss such dynamic aspects of spindle attachment separately—although of course geometry of kinetochores and spindle attachment are interdependent.

Kinetochores in Mitosis ***Back-to-Back Orientation of Sister Kinetochores in Mitosis***

The observation that each chromosome has two kinetochores in mitosis, and that they face in opposite direction was made more than 50 years ago. Such an arrangement of the kinetochores is thought to facilitate biorientation, since attachment of one kinetochore to one pole orients the other kinetochore toward the other pole (discussed in Bajer and Molè-Bajer, 1972). Surprisingly little is known about the molecular mechanisms underlying the back-to-back orientation of kinetochores. Presumably, the structure of the underlying chromatin is crucial. Nucleosomes containing the histone H3-variant CENP-A are only found in two cylinder-like structures on either side of the long axis (*y* axis, Figure 1A and 1C) of human or *Drosophila* chromosomes, and kinetochore proteins either overlay with or cover these structures on their outside. However, if single chromatin fibers are inspected, CENP-A and histone H3-containing nucleosomes alternate (Figure 1B), suggesting that some internal structure restricts CENP-A nucleosomes to the outer face of the chromatid, whereas histone H3 nucleosomes are buried toward the interface of the two sister chromatids (Blower et al., 2002) (Figure 1C). The CENP-A containing chromatin fiber constituting the kinetochore binding site is flanked on both sides by pericentromeric heterochromatin (Figure 1B). On the condensed chromosome, the pericentromeric heterochromatin seems to underlie the CENP-A cylinder and maybe exceed it in the longitudinal (*y*-) axis of the chromosome (Sugimoto et al., 2001, Figure 1C, reviewed by Amor et al. 2004). It is conceivable that the pericentromeric heterochromatin contributes to determining the kinetochore assembly site by providing the structural basis for assembly of the CENP-A cylinder, and/or by shielding the inner and lateral parts of the CENP-A cylinder from access of kinetochore proteins. Since the CENP-A containing chromatin is placed asymmetrically to one side of each chromatid's long axis, molecular mechanisms must exist that determine this asymmetry. Cohesin is presumably located at the interface of the two sister chromatids (Figure 1C), thereby "marking" the internal surface of each sister chromatid. Cohesin might therefore also be required to determine that CENP-A is located on the opposite side, but this notion has not been tested. Pericentromeric heterochromatin is particularly rich in cohesin compared to chromosome arms (Tomonaga et al., 2000; Watanabe et al., 2001), and even in budding yeast, where centromeric heterochromatin is lacking, cohesin is enriched around centromeres (Blat and Kleckner, 1999; Megee et al., 1999; Tanaka et al., 1999). In the absence of heterochromatin proteins, which are required to recruit cohesin (Bernard et al., 2001b; Nonaka et al., 2002), chromosome segregation

is defective. Close cohesion between sisters at the centromere might be required to resist the pulling forces of the spindle exerted on the kinetochore, but it might also be necessary to orient the two centromeric domains in opposite direction or to establish centromeric architecture (Sonoda et al., 2001; Tanaka et al., 2000) (Figure 1C).

Recent work has implicated condensin in the back-to-back orientation of sister kinetochores (Hagstrom et al., 2002; Ono et al., 2004) (Figure 1C). Condensin is a protein complex similar to cohesin in its overall structure and type of subunits. It localizes to the core of sister chromatids and is involved in compacting DNA (Hirano, 2002). Human cells contain two forms of condensin, condensin I and II, which differ in some of their subunits (Ono et al., 2003). Whereas both condensins alternate on chromosome arms, condensin II is enriched in the centromere/kinetochore region, where it is found to largely overlap with CENP-A (Figure 1C). Interestingly, depletion of condensin by RNAi disturbs kinetochore orientation so that sister kinetochores move closer together and do not face opposite sides anymore (Ono et al., 2004). *C. elegans* which has holocentric chromosomes only possesses a condensin complex similar to condensin II, which colocalizes with CENP-A all along the length of the sister chromatids. Depletion of *C. elegans* condensin by RNAi also leads to seemingly disorganized centromeres (Hagstrom et al., 2002). In which way centromere architecture is perturbed is presently unclear (Figure 1C). Also, it cannot be excluded that the distorted appearance is a secondary effect of abnormal kinetochore-spindle interactions.

The Primary Constriction

On monocentric chromosomes, the centromeric region is smaller in diameter than the remainder of the chromosome (Figure 1A), which led to its cytological description as "primary constriction". Kinetochores therefore appear embedded in a "pit" on the chromosome, and it has been proposed (e.g., Nicklas, 1997) that this configuration facilitates biorientation, since the kinetochore is shielded from microtubules, which do not reach it at the right (orthogonal) angle. It has recently been found that two mitotic kinases, Aurora-B and Polo-like kinase 1 (Plk1), are required to form the primary constriction (Giménez-Abian et al., 2004; Ono et al., 2004). In the absence of either kinase, the centromeric region shows the same width as the chromosome arms, and sister kinetochores are found further apart from each other. Whether the bipolar attachment of chromosomes is indeed negatively influenced when kinetochores are thus exposed is unclear. Both Aurora-B and Plk1 depleted cells do show a high frequency of chromosomes that do not achieve biorientation. However, the phenotypes are distinct and other functions of each kinase probably contribute to this defect. Although Aurora-B is required for the enrichment of condensin II at the centromere, depletion of condensin by RNAi does not affect the primary constriction (Ono et al., 2004), indicating that condensin II is not directly responsible for the structure. A possible candidate is cohesin, whose enrichment at the centromere might cause tighter cohesion between sisters resulting in the primary constriction (Giménez-Abian et al., 2004). Indeed, chicken cells lacking the cohesin subunit Scc1 also lack a strong centromeric constriction (Sonoda et al., 2001). Both Aurora-B and Plk1 have been

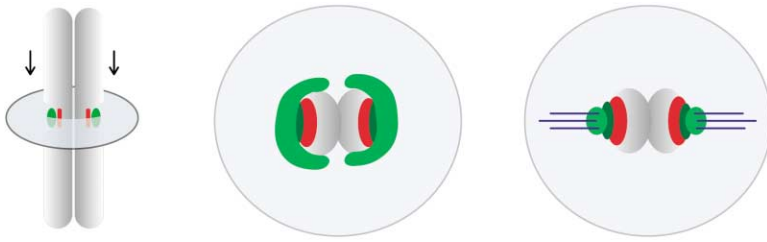


Figure 2. Changes in Kinetochore Morphology upon Attachment
View along the long (y-) axis of the chromosome onto the centromeric region as depicted on the left side. Middle image: Unattached kinetochores show a crescent shape. The effect is pronounced when cells have been treated for a prolonged time with microtubule-destabilizing drugs. Right image: Kinetochore attached to microtubules have a very localized appearance. Outer kinetochore proteins (as CENP-E) are in light green. Whether kinetochore proteins more close to the centromere (inner kinetochore proteins, dark green) also change shape is unknown. CENP-A (red) and the centromere seem to be unaffected by the changes (see Cooke et al., 1990; Hoffman et al., 2001).

implicated in removing cohesin from chromosome arms in early mitosis (Giménez-Abian et al., 2004; Losada et al., 2002; Sumara et al., 2002), but it is unknown whether and how these kinases influence cohesin at the centromere.

Changes in Kinetochore Morphology upon Attachment

Whereas kinetochores of metaphase chromosomes have a very localized appearance on opposite sides of the chromosome's long axis, it has been noted that outer regions of the kinetochore can become drastically more expanded when cells are treated with spindle poisons (Cooke et al., 1990; Hoffman et al., 2001; Rieder, 1982, and references therein). Under such circumstances, when kinetochores do not interact with microtubules, some kinetochore proteins seem to almost encircle the chromosome at the primary constriction (Figure 2). In contrast, centromeric proteins beneath the kinetochore do not seem to be affected by this change (Cooke et al., 1990; Hoffman et al., 2001). To a lesser extent, such wider distribution of the outer kinetochore is also seen during unperturbed prometaphase, when some kinetochores have not yet captured microtubules (Rieder, 1982). It is possible that the increase in kinetochore surface maximizes the chance of microtubule attachment, but the significance and molecular details of this process have not been studied.

Dynamic Aspects of Kinetochore Orientation in Mitosis

Establishing Biorientation: Pulling and Resisting

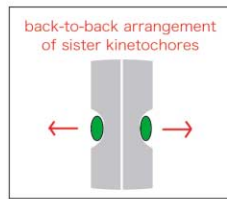
Mitotic spindles can be established by at least two different pathways (for a recent review, see Gadde and Heald, 2004). In the absence of centrosomes, microtubules nucleate from the kinetochores, and are then ordered into a bipolar spindle. When centrosomes are present, microtubules are usually nucleated from the centrosomes and attach to kinetochores in a process that has been termed "search and capture" (Kirschner and Mitchison, 1986). How the biorientation of sister kinetochores is achieved has been studied almost exclusively in cell types where the second mechanism prevails (reviewed in Rieder and Salmon, 1998). In somatic cells of higher eukaryotes, kinetochores assemble on each sister chromatid during prophase, and become accessible for the microtubules of the spindle upon nuclear envelope breakdown. Dynamically unstable microtubules probe the space ("search"), and get stabilized once they contact a kinetochore ("capture"). Some chromosomes, which happen to be oriented favorably, become attached with

the two sister kinetochores to opposite spindle poles almost immediately. In contrast, many chromosomes first become attached by one of their kinetochores to one pole, and only later capture microtubules from the other pole with the other kinetochore. Since the encounter of kinetochores and microtubules is a stochastic event, misattachment (monotelic, syntelic, merotelic; see Figure 3) is common during prometaphase. However, only bipolar kinetochore-microtubule attachments are stable (i.e., do not convert into other attachments). In contrast to monooriented chromosomes, bioriented chromosomes are under tension from spindle forces acting in opposite direction. Micromanipulation experiments by Koch and Nicklas in meiotic cells have demonstrated that the physical appliance of tension can lead to a stabilization of attachment (Nicklas and Koch, 1969). Together, this suggests that biorientation is favored over malorientation, because cells are able to convert the presence of tension at the kinetochore/centromere into a biochemical signal which leads to a stabilization of microtubule-kinetochore attachment (Note: chromosomes attached in a merotelic fashion might also be under tension, in particular when one kinetochore is attached to one pole, and the other to both poles. Indeed, merotelic errors are not always corrected before anaphase [Cimini et al., 2001, 2003]).

The generation of tension not only requires pulling forces exerted by the microtubules but also a counteracting force. The latter is provided by cohesion between sister chromatids. As expected, cohesin is essential for biorientation (Sonoda et al., 2001; Tanaka et al., 2000). It is interesting to note, that it is indeed cohesion (and not specifically cohesin), which is required for biorientation, since in the absence of cohesin the inhibition of topoisomerase II (which causes sisters to stay closely connected by catenation of DNA strands) is sufficient to allow biorientation (Dewar et al., 2004; Vagnarelli et al., 2004).

Back-to-back orientation of sister kinetochores is thought to facilitate their attachment to opposite poles. Dewar, Tanaka, and colleagues recently tested whether back-to-back orientation is required by engineering a circular minichromosome in budding yeast (Dewar et al., 2004). This minichromosome harbors two centromeres, of which one can be inactivated by transcription across the centromere, and the replication origin can be eliminated by site-directed recombination. This allows them to propagate the minichromosome in budding yeast cells (origin present, one kinetochore), and to study the requirements for biorientation (origin absent,

MITOSIS



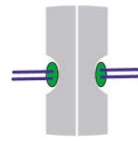
attachment:

kinetochores in relation to spindle poles

orientation:

chromosome in relation to spindle poles

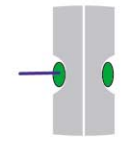
sister kinetochores attached to opposite poles



amphitelic

bi-orientation

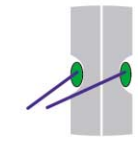
only one of the sister kinetochores attached to one pole



monotelic

(monotelic)
mono-orientation

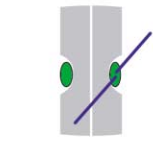
both sister kinetochores attached to the same pole



syntelic

(syntelic)
mono-orientation

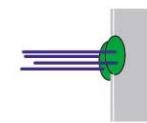
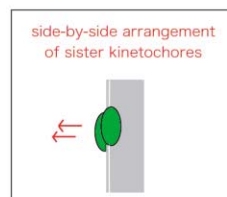
one of the sister kinetochores attached to both poles



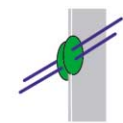
merotelic

(merotelic)
bi-orientation

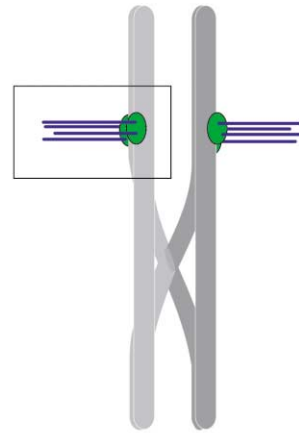
MEIOSIS I



mono-orientation of sister kinetochores



bi-orientation of sister kinetochores
(side-by-side arrangement can be preserved or not)



bi-orientation of bivalent



mono-orientation of bivalent

Figure 3. Types of Chromosome Attachment to the Spindle

Note that in meiosis I, attachment/orientation of sister chromatids and attachment/orientation of homologs have to be distinguished.

two kinetochores). Even when the distance between the two centromeres was 10 kilobases, unreplicated minichromosomes with two kinetochores oriented faithfully, indicating that back-to-back orientation is not strictly required for biorientation at least in budding yeast.

The Role of the Aurora-B Kinase in Promoting Biorientation

A key molecule in promoting biorientation is the Aurora-B kinase (Ipl1 in budding yeast), which localizes to the centromeric region of chromosomes from prophase until metaphase. In the absence of Ipl1, sister chromatids frequently segregate to the same pole, and so do the two kinetochores of the engineered minichromosome (Biggins et al., 1999; Dewar et al., 2004; Tanaka et al., 2002). The primary defect seems to be the inability of *ipl1* mutant cells to adequately respond to an absence of tension at the kinetochore (Biggins and Murray, 2001; Tanaka et al., 2002). When budding yeast cells are made to enter mitosis without previously undergoing DNA replication, tension and biorientation cannot be established because each unreplicated chromosome only has a sin-

gle kinetochore. In this situation, chromatids segregate to the poles randomly. In *ipl1* mutant cells, in contrast, almost all chromatids segregate to the old of the two spindle pole bodies (Tanaka et al., 2002). Since in budding yeast, kinetochores stay attached to spindle poles throughout interphase, this result implies that Ipl1 is required to release microtubule-kinetochore attachments, which fail to establish tension.

In metazoans, Aurora-B is required for the alignment of chromosomes on the metaphase plate and proper chromosome segregation (Carmena and Earnshaw, 2003). Immunofluorescence microscopy has demonstrated the frequent presence of syntelic chromosomes in human cells, in which Aurora-B has been inhibited (Hauf et al., 2003). In *C. elegans*, interfering with Aurora-B function leads to a phenotype, which is consistent with an increase in merotelic attachment (Kaitna et al., 2002). These observations suggest that Aurora-B is also required to eliminate defective microtubule-kinetochore attachments in organisms other than budding yeast.

How does Aurora-B correct malorientation? Lampson

and colleagues (Lampson et al., 2004) have followed the fate of syntelic chromosomes in living mammalian cells in which Aurora-B had first been inactivated by the chemical inhibitor Hesperadin and then been reactivated by washing out Hesperadin. Somewhat surprisingly, syntelic chromosomes first move to the pole they are attached to, keeping both sister kinetochores attached to microtubules. Later they acquire amphitelic attachment, but how reorientation is achieved could not be observed in these experiments. It is possible that one or both kinetochores detach from microtubules to make bipolar attachment possible. Reattachment might occur via capture by spindle microtubules, or by nucleating microtubules from the kinetochore, which are then integrated into the existing spindle (Khodjakov et al., 2003). Since there are multiple microtubule binding sites on one kinetochore, it is also possible that the kinetochores do never get completely detached, but instead one of the kinetochores also acquires microtubules from the opposite pole (i.e., it becomes merotelic, Figure 3). Such a transient merotelic attachment would twist the chromosome, orienting the kinetochores more toward opposite poles, and thereby eventually facilitate biorientation. Another possibility is that twisting is achieved by generating force at the microtubule-kinetochore interface. Kinetochores can contact microtubules at their side (lateral attachment) or at their end (end-on attachment) (Rieder and Salmon, 1998). It is possible that a change from lateral to end-on attachment on one of the kinetochores twists the chromosome and forces the other kinetochore into the direction of the other spindle pole. Although an attractive hypothesis, it is currently unclear whether such a mechanism actually exists.

Some of the targets of Aurora-B/Ipl1 are beginning to be revealed. In budding yeast, a number of kinetochore proteins is phosphorylated by Ipl1 (Cheeseman et al., 2002). A particular interesting substrate is Dam1, since mutation of the phosphorylation sites leads to a phenotype that mimics the *ipl1* mutant phenotype. In human cells, the kinesin MCAK (mitotic centromere associated kinesin) has recently been found to be a substrate of Aurora-B (Andrews et al., 2004; Lan et al., 2004; Ohi et al., 2004). MCAK is a member of the KinI/Kinesin-13 (Lawrence et al., 2004) family of kinesins, which promote the depolymerization of microtubules. Phosphorylation by Aurora-B inhibits the depolymerization activity of MCAK in vitro. Both phosphorylation and dephosphorylation might be necessary for biorientation in vivo, since both phospho-mimicking and nonphosphorylatable mutants of MCAK cause misattachment of chromosomes when overexpressed in cells (Andrews et al., 2004). Whereas our knowledge about the downstream events of Aurora-B/Ipl1 kinase activity is increasing, it remains unclear, how the kinase is regulated by the absence or presence of tension.

Particularities of Kinetochore Orientation in Meiosis

In meiosis, haploid gametes are generated from diploid cells. This is accomplished by two rounds of cell division following a single round of DNA replication. In the first meiotic division (also called reductional), maternal and paternal chromosomes are separated, whereas in the

second meiotic division sister chromatids are segregated to opposite poles similar to a mitotic division (equational division). Elegant micromanipulation experiments by Paliulis and Nicklas (2000) have shown that the properties that allow the specialized chromosome separation at meiosis I are determined by the chromosome, not by the mitotic spindle or other cytoplasmic factors. When meiosis I chromosomes are moved to a meiosis II spindle, they behave as in meiosis I (and vice versa). What are the features of chromosomes that make the specialized division of meiosis I possible?

Firstly, movement of both sister chromatids to one spindle pole during meiosis I is facilitated by putting the two kinetochores in very close proximity in a side-by-side arrangement (Figure 3). Secondly, recombination during meiotic prophase results in chiasmata which together with the cohesion between sister chromatids keep homologous chromosomes connected. Like cohesion between sister chromatids in mitosis, this physical connection is essential for ensuring bi-polar attachment of homologs. Thirdly, in order to segregate homologs during meiosis I, arm cohesin has to be removed, but cohesin in the centromeric region is preserved, which is necessary for the biorientation of sisters in meiosis II (reviewed in (Miyazaki and Orr-Weaver, 1994; Petronczki et al., 2003)).

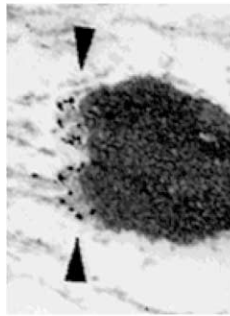
Geometry of Kinetochores in Meiosis I

Side-by-Side Arrangement of Sister Kinetochores

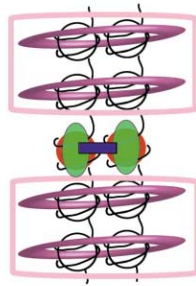
After crossover recombination during meiotic prophase, homologous chromosomes are connected by chiasmata and form what is called a bivalent (Figure 3). Bivalents possess four kinetochores, and in contrast to mitosis, the two sister kinetochores of each chromosome have to be segregated to the same pole in anaphase I. Electron microscopic analysis has shown that in striking contrast to mitosis, sister kinetochores orient side-by-side in meiosis I, which probably facilitates their orientation to the same pole (Goldstein, 1981; Lee et al., 2000; Parra et al., 2004) (Figure 4). (We call this orientation to the same pole “monopolar” or “monoorientation” [see Figure 3, Toth et al., 2000] rather than “coorientation” [e.g., Lee and Amon, 2003], a confusing term that was originally meant to describe the mechanism by which homologous chromosomes become bioriented in meiosis [Östergren, 1951]).

Studies in yeast have recently begun to elucidate the molecular factors required for the monopolar orientation of sister kinetochores. In fission yeast, monoorientation of sister kinetochores requires the meiotic cohesin subunit Rec8 (Watanabe and Nurse, 1999). When Rec8 is replaced by its mitotic counterpart Rad21, sister chromatids stay closely connected until anaphase I, but monopolar attachment of sister kinetochores is not established, and sister chromatids therefore segregate to opposite poles (Yokobayashi et al., 2003). Chromatin immunoprecipitation demonstrated that Rad21 ectopically expressed during meiosis localizes to the chromosome arms and pericentromeric heterochromatin, but not to the central region of the centromere, on which the kinetochore assembles (Figure 4, the central region is in red). This is similar to its localization in mitosis. In contrast, Rec8 localizes to arms, pericentromeric heterochroma-

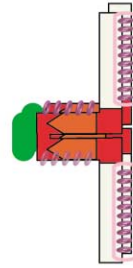
MEIOSIS I



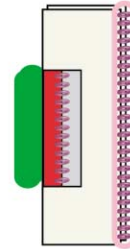
1 μm



S. cerevisiae

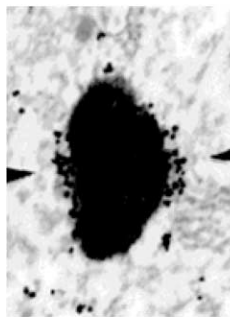


S. pombe

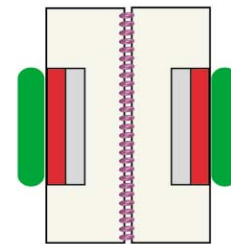
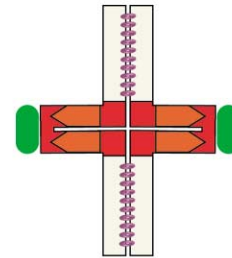
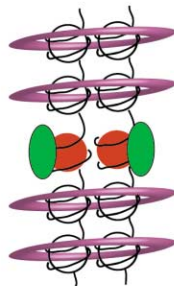


metazoans

MEIOSIS II



1 μm





- | | | | |
|---|------------------------------------|--|--|
|  | CENP-A containing nucleosomes |  | monopolin |
|  | highly homologous inverted repeats |  | meiotic cohesin (Rec8) |
|  | pericentromeric heterochromatin |  | protection of pericentromeric cohesin (Shugoshin proteins) |
|  | kinetochore | | |

Figure 4. Models for the Formation and Resolution of the Side-by-Side Arrangement of Sister Kinetochores in Meiosis I

In *S. cerevisiae*, monopolin together with other factors (e.g., Spo13, which does not localize to the kinetochore) is required for monopolar orientation of sister kinetochores in meiosis I. Monopolin might act by “clamping” together the two sister kinetochores (Rabitsch et al., 2003). Resolution of monopolar orientation probably depends on the removal of monopolin. In *S. pombe*, meiotic cohesin (with the subunit Rec8) is required for monopolar orientation (aided by other factors, as for example Moa1). Side-by-side orientation might be resolved by removing cohesin from CENP-A containing chromatin. We speculate that a similar mechanism acts in metazoans (model on the right side). Cohesin at pericentromeric heterochromatin is protected from removal during meiosis I by Shugoshin proteins (Kitajima et al., 2004), which is necessary to keep sister chromatids connected up to metaphase II. The electron micrographs show sister kinetochores of pig oocytes in meiosis I and II (arrowheads). The kinetochore protein CENP-E is labeled by gold particles. Electron microscopic images reproduced from Molecular Reproduction and Development. Vol. 56, 51–62, March 2000.

tin, and to the central region of the centromere (Figure 4). In order to establish cohesion, cohesin has to be present on chromatin during DNA replication (Uhlmann and Nasmyth, 1998). Similarly, Rec8 has to be present during premeiotic S phase to establish monoorientation of sister kinetochores (Watanabe et al., 2001). This raises the possibility that the inner centromeric regions of sisters get connected during premeiotic S phase, which is necessary for their monoorientation. In fission yeast, inverted repeats highly identical to each other are present in the inner centromeric region (orange arrows in Figure 4), and it has therefore been proposed that the central region of the centromere loops out from the pericentromeric heterochromatin (Takahashi et al., 1992). Bringing together these two loops by cohesion might orient the kinetochores assembling on the inner

region (Watanabe et al., 2001) (Figure 4). Cytological evidence for such a phenomenon is as yet missing.

Rec8 is required to establish monoorientation, but it is not sufficient. Ectopic expression of Rec8 in mitosis does not induce monoorientation although Rec8 is found at the inner centromere by chromatin immunoprecipitation (Watanabe and Nurse, 1999; Yokobayashi et al., 2003). Other meiosis-specific factors that aid Rec8 in establishing monoorientation must therefore exist. Genetic screening in fission yeast has revealed one such factor, Moa1 (monopolar attachment) (S. Yokobayashi and Y.W., unpublished data). Moa1 localizes specifically to the inner centromeric region from prophase to metaphase of meiosis I, but disappears in anaphase I. In the absence of Moa1, sister chromatids frequently move to opposite poles in anaphase I. Yet other factors, however,

remain to be discovered, since overexpression of *Moa1* and *Rec8* in mitosis is still not sufficient for monoorientation.

To allow the biorientation of sisters in meiosis II, the side-by-side arrangement of sister kinetochores has to be resolved. Cytological observation has shown that sister kinetochores start changing to a back-to-back orientation during anaphase I (e.g., Parra et al., 2004). Consistently, the micromanipulation experiments by Paliulis and Nicklas (2000) demonstrated that chromosomes can be easily bioriented on a meiosis II spindle, as soon as they have reached late anaphase I. An attractive hypothesis is that side-by-side can be converted to a back-to-back orientation by removing cohesin complexes in the inner centromeric region during anaphase I (Figure 4). Whether cohesin is indeed lost from the inner centromere during anaphase I remains to be tested. Alternatively or additionally, the removal of *Moa1* or other factors required for monoorientation might allow biorientation.

Rec8 is conserved in all eukaryotes. Is the function of *Rec8* in monoorientation of sister kinetochores a conserved feature? We do not know about the mechanisms of monoorientation in animals. However, in the absence of maize *Rec8*, sister chromatids apparently establish bipolar attachment, suggesting that maize *Rec8* is also required for monoorientation (Z. Cande Lab, personal communication). In contrast, studies in budding yeast show a different picture. When *Rec8* is absent and replaced by its mitotic counterpart *Scs1* during meiosis, monoorientation of sister kinetochores is still established (Toth et al., 2000), and *Rec8* therefore cannot be obligatory. *Mam1*, *Csm1*, and *Lrs4* are proteins required for monoorientation in *S. cerevisiae* (Rabitsch et al., 2003; Toth et al., 2000). Together, they form the so-called "monopolin" complex. *Mam1* (monopolar microtubule attachment during meiosis I) is a meiosis-specific protein, which similar to *Moa1* localizes to kinetochores from prophase to metaphase I and is not present thereafter. *Csm1* and *Lrs4* are not meiosis-specific, but dramatically change their localization from the nucleolus to the kinetochore during meiosis I (Figure 4). The localization to the kinetochore requires the Polo-like kinase *Cdc5* (Clyne et al., 2003; Lee and Amon, 2003). In the absence of the monopolin complex, sisters biorient in meiosis I, but fail to segregate since cohesin in the centromeric region is preserved, as it should be. Maintenance of the monopolin complex at kinetochores also requires *Spo13* (B. Lee, B. Kiburz and A. Amon, personal communication). *Spo13* has a double role in meiosis I, since it is not only required for monopolin maintenance and therefore monoorientation, but also for the protection of centromeric cohesion up to metaphase II (Lee et al., 2002; Shonn et al., 2002).

Orthologs of the monopolin subunits have so far not been found in metazoans. It is conceivable that budding yeast employs different mechanisms from metazoans for establishing side-by-side kinetochores, due to the smaller size of its centromeric region (Figure 4). One of the monopolin subunits, *Csm1*, shows homology to a fission yeast protein, *Pcs1*. In the absence of *Pcs1* however, chromosome segregation goes awry in mitosis and meiosis II, but to a lesser extent in meiosis I (Rabitsch et al., 2003), indicating that the function of *Csm1* in

monoorientation of sister kinetochores is also not conserved in fission yeast.

In both yeasts, monoorientation is regulated by factors at the kinetochore/centromere. Consistently, monoorientation of sisters does not require chiasmata formation (Kitajima et al., 2003; Klein et al., 1999). However, association between homologous chromosomes can enhance the fidelity of sister kinetochore monoorientation (Shonn et al., 2002; Yamamoto and Hiraoka, 2003). In the absence of chiasmata, biorientation of sisters is the only way to stabilize microtubule-kinetochore interactions. In contrast, in the presence of chiasmata, syntelic attachment of sister kinetochores can also create tension at the kinetochore and stabilize attachment, which might favor orientation of sisters to one pole even if they are not arranged side-by-side.

Dynamic Aspects of Kinetochore Orientation in Meiosis I

Biorientation of Homologs

In contrast to the monoorientation of sister chromatids, the biorientation of homologs in meiosis I relies on chiasmata (Klein et al., 1999; Molnar et al., 2001b). Chiasmata together with cohesion between chromosome arms provide the connection between homologs which is essential to establish tension, and therefore to stabilize the biorientation of bivalents (see Figure 3). The recent demonstration by the Tanaka lab (Dewar et al., 2004) that *Ipl1* can promote biorientation even if kinetochores are loosely connected by long DNA strands suggests that *Ipl1* and its orthologs also promote the biorientation of bivalents (on which the two pairs of kinetochores are distantly connected by chiasmata). Support comes from the fact that fission yeast *Ark1* and mouse *Aurora-B* localize to the centromeric regions adjacent to the kinetochores in meiosis I (Parra et al., 2003; Petersen et al., 2001), and therefore are at the right place at the right time. Experimental evidence for an involvement of *Aurora-B* in the biorientation of bivalents is as yet missing. Similar to the situation in mitosis, it is imaginable that the biorientation of bivalents would additionally be facilitated by a direct back-to-back interaction of sister kinetochore pairs. In *Drosophila*, the centromeres of homologs can associate dependent on centromeric heterochromatin even without connection along arm regions (Dernburg et al., 1996; Karpen et al., 1996). Likewise in budding yeast, homologous (or even nonhomologous) centromere regions can initially pair in the absence of chiasmata, although the interaction is disrupted during metaphase of meiosis I, presumably by spindle forces (Kemp et al., 2004). Since segregation of homologs is often faithful (i.e., to opposite sides) in these cases, chiasmata-independent homolog interaction via the centromere can contribute to the biorientation of bivalents.

In mitosis, timing of anaphase is controlled by a network of proteins called the "spindle checkpoint". Unattached kinetochores or the lack of tension at kinetochores keep the checkpoint active, which inhibits anaphase (reviewed by Musacchio and Hardwick, 2002). In the absence of checkpoint components, anaphase happens precociously (i.e., without 'waiting' for un- or malattached chromosomes to become bioriented), which leads to errors in chromosome segregation. Ana-

phase of meiosis I also seems to be controlled by the spindle checkpoint, since in yeast the absence of attachment or the absence of chiasmata (which leads to a failure to produce tension) cause a delay in meiosis I (Molnar et al., 2001a; Shonn et al., 2000). Consequently, components of the spindle checkpoint are required for the faithful segregation of homologs to opposite poles in meiosis I (Bernard et al., 2001a; Shonn et al., 2000, 2003). As expected delaying anaphase by different means gives the necessary time and decreases the rate of nondisjunction (Shonn et al., 2000). However, cytological analysis suggests that cells deficient in a particular component of the checkpoint (Mad2) are slower in acquiring biorientation than wild-type cells or cells deficient in Mad3 (another checkpoint component) (Shonn et al., 2003). This suggests that Mad2 has a role in promoting biorientation apart from its role in delaying anaphase. How Mad2 exerts this role is mysterious at present.

Concluding Remarks

During the last century, generations of cytologists have produced a huge amount of data on the morphology of chromosomes and their kinetic behavior throughout mitosis and meiosis. They quickly came to the conclusion that kinetochores are of crucial importance for chromosome inheritance (Schrader, 1953). Today, we know that kinetochores are highly complex structures. But we are still missing crucial insight into how kinetochores are arranged on the chromosome, and how they interact with the spindle. However, one picture that already emerges is that the mechanisms of kinetochore assembly and orientation seem highly conserved among species—as one might expect—but at the same time the process is astoundingly versatile. For example, the kinetochore-assembling chromatin always contains CENP-A, but the underlying chromatin can be anything from large stretches of repetitive repeats over euchromatin to the specific 125 bp sequence of budding yeast. Another example is the fact, that at least some organisms possessing holocentric chromosomes assemble localized kinetochores during meiosis (Dernburg, 2001), which probably is brought about by only a few molecular changes. We also anticipate that biorientation of homologs in meiosis I is governed by the same mechanisms as biorientation of chromosomes in mitosis, and only a few key changes (chiasmata and side-by-side orientation of kinetochores) are needed to adapt the process. The task for the future will be to understand how this versatility is reconciled with the high degree of conservation.

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