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COMMENTARY

Chromatid cohesion during mitosis: lessons from meiosis

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SUMMARY

The equal distribution of chromosomes during mitosis and meiosis is dependent on the maintenance of sister chromatid cohesion. In this commentary we review the evidence that, during meiosis, the mechanism underlying the cohesion of chromatids along their arms is different from that responsible for cohesion in the centromere region. We then argue that the chromatids on a mitotic chromosome are also tethered along their arms and in the centromere by different mechanisms, and that the functional action of these two mechanisms can be temporally separated under various conditions. Finally, we demonstrate that in the absence of a centromeric tether, arm cohesion is sufficient to maintain chromatid cohesion during prometaphase of mitosis. This finding provides a straightforward explanation for why mutants in proteins responsible for centromeric cohesion in *Drosophila* (e.g. *ord, mei-s332*) disrupt meiosis but not mitosis.

Key words: Sister-chromatid cohesion, Mitosis, Meiosis, Anaphase onset

INTRODUCTION

The equal distribution of chromosomes during mitosis is dependent on the maintenance of chromatid cohesion. This cohesion firmly tethers the sister chromatids of a replicated chromosome to one another until its two kinetochore regions, which lie on opposite sides of the centromere, acquire the proper bipolar attachment to the forming spindle. Then, shortly after the last kinetochore in the cell attaches to the spindle, all of the sister chromatids separate along their length relatively synchronously (over a 1-3 minute period). This 'disjunction' marks the onset of anaphase during which time the sister kinetochores and their associated chromatids move toward the opposing spindle poles. The premature separation of one or more sister chromatids usually leads to the formation of aneuploid daughter cells with unpredictable consequences to the organism (see Fitzgerald et al., 1975; Gabarron et al., 1986).

The molecular mechanism(s) responsible for chromatid cohesion during mitosis remain ambiguous and are an area of active investigation (reviewed by Heck, 1997; Biggins and Murray, 1998). The current view is that unattached kinetochores regulate anaphase onset (and thus chromatid cohesion) by inhibiting the activity of anaphase promoting complexes (APCs). These large macromolecular assemblies target specific proteins for proteolysis by ubiquitinating them (reviewed by Townsley and Ruderman, 1998). In *Saccharomyces cerevisiae* chromatid disjunction involves the APC-mediated degradation of the Pds1 protein (Yamamoto et al., 1996), which exists in a complex with the Esp1 protein (related to the fission yeast Cut1P; Ciosk et al., 1998). When Pds1 is destroyed Esp1 is liberated, and this event somehow induces a class of 'glue' proteins, called cohesins (e.g. Scc1; Michaelis et al., 1997; Guacci et al., 1997), to dissociate from the chromosome. Although this pathway has been suggested to be conserved during mitosis and meiosis in all organisms, its applicability to higher eukaryotes remains to be demonstrated. In *Xenopus* cohesins (including the Scc1 homologue, Xrad21) dissociate from chromosomes at the onset of mitosis, and immunodepleting the cohesin complex (including Xrad21) does not lead to chromatid disjunction (Losada et al., 1998). As noted by Biggins and Murray (1998) these data imply that many vertebrate cohesin proteins are involved in maintaining the linkage of chromatids during interphase and not mitosis, i.e. that the mechanism linking chromatids during interphase involves the cohesin complex, and differs from the mechanism linking chromatids during mitosis (see also Yanagida, 1988).

The separation of sister chromatids at anaphase is a complex process and there are likely other factors involved in regulating the attachment of cohesive molecules including, e.g. transient changes in Ca^{2+} at anaphase onset (Groigno and Whitaker, 1998) and controlled phosphorylation by mitotic kinases (see Hirano et al., 1997; Ciosk et al., 1998). In addition, cytological observations on living and fixed spermatocytes reveal that chromatid disjunction during meiosis is mediated by two mechanistically distinct processes that can be separated temporally: one that leads to the separation of chromatid arms and another that is responsible for disjoining the chromatids in the centromere region. In this article we review and discuss

some of the evidence that supports the view that two similar mechanisms for chromatid cohesion also exist during mitosis.

DIFFERENT MECHANISMS MEDIATE CHROMATID COHESION DURING MEIOSIS I AND II

As the spindle forms in meiosis I the paired homologous chromosomes, commonly referred to as tetrads or bivalents, are attached physically to one another. In most organisms these attachment sites are located at those points along the arms where recombination has occurred between the homologues (i.e. at the chiasmata), but in some cases a physical linkage can be formed and maintained in the absence of crossing over (e.g. *Drosophila* males, see McKee and Karpen, 1990; Ault and Rieder, 1994). Then at the onset of anaphase the cohesive forces holding the homologues together are resolved so that each pair of sister chromatids, now referred to as a dyad, can segregate to the opposing spindle poles. When cohesion between the homologues is broken at anaphase I the forces that hold the sister chromatid arms of each dyad together are similarly destroyed, but the two sister chromatids remain firmly linked by the non-separated centromere region (reviewed by Darlington, 1937; Rhodes, 1961; see also Seto et al., 1969). This centromeric linkage is then maintained until the onset of anaphase II, at which time it too is broken to allow for the proper distribution of the sister chromatids. Thus, during meiosis chromosome disjunction is mediated by two pathways that are activated at different times (reviewed by Bickel and Orr-Weaver, 1996): one leads to the separation of the associated chromosome arms at anaphase I, and another dissolves the centromere linkage between chromatids at anaphase II. As emphasized by Bardhan (1997) the temporal difference seen during meiosis in the separation of chromatids along their arms and at their centromere implies that the molecular basis for cohesion in these two chromosomal regions is different.

The mechanism responsible for the cohesion of paired homologues remains unknown, but it likely involves the same cohesive molecules that hold the constituent replicated chromatids arms together along their length (remember, cohesion between the arms of the sister chromatids is dissolved at the same time that cohesion between the paired homologues is broken; reviewed by Miyasaki and Orr-Weaver, 1994). The mechanism that holds the centromere regions together during meiosis II, after the sister chromatid arms have separated at meiosis I, is similarly vague. When grasshopper spermatocytes in meiosis I and meiosis II are fused the sister chromatids of a meiosis II dyad, which are joined only at the centromere, orient, separate, and segregate normally when micro-manipulated onto a meiosis I spindle (Nicklas, 1977). A common interpretation of this finding is that the machinery for disjoining the centromere region is also functional during meiosis I, but that it is somehow inhibited from working on meiosis I chromosomes. This idea draws considerable support from observations on the behavior of non-natural univalents during meiosis II in grasshopper (Suja et al., 1992; Rebollo and Arana, 1995; Gimenez-Abian et al., 1997; Rebollo et al., 1998) and crane-fly (Janicke and LaFountain, 1984, 1989) spermatocytes. Such univalents are produced when, for various reasons, homologues fail to maintain their association during meiosis I, and each consists only of two sister chromatids (i.e. a dyad). During meiosis I the two chromatids of a non-natural univalent are joined along their arms and at the centromere, and they frequently achieve a stable bipolar orientation on the equator of the metaphase I spindle. However, during anaphase I the arms of the chromatids disjoin but the centromere region usually does not and, as a result, the chromatids rarely segregate normally to the poles. Since chromatid disjunction during meiosis II and mitosis does not require spindle forces (see below) the failure of non-natural univalents to disjoin when bioriented on the meiosis I spindle cannot be ascribed to the possibility that their kinetochore region(s) are not properly connected to the spindle poles (see Janicke and LaFountain, 1989). Instead, the differing behavior during meiosis I, between non-natural univalents and meiosis II dyads manipulated onto a meiosis I spindle, is more consistent with the idea that the centromere region undergoes a change after the onset of anaphase I that 'licenses' it to separate at anaphase II. Unlike a meiosis II dyad, the two dyads comprising a meiosis I tetrad have not been through anaphase I and are thus inhibited from separating in their centromere during anaphase I.

The idea that passing through anaphase I licenses a dyad to disjoin in its centromere region during the subsequent anaphase II is supported by observations on yeast mutants that undergo a single-division meiosis. In these cells some of the chromosomes segregate as during meiosis I (i.e. reductionally) while others segregate as during meiosis II and mitosis (i.e. equationally). Genetic studies reveal that something about the centromere region determines whether a particular chromosome will segregate reductionally or equationally (reviewed by Simchen and Hugerat, 1993). One change known to occur in the centromere between meiosis I and II is that the sister kinetochores on each homologue (dyad), which are normally closely associated and function as a single unit during meiosis I (Goldstein, 1981; Hays and Salmon, 1990), migrate to opposing sides of the primary constriction prior to meiosis II. However, after this migration the kinetochore regions are still seen to be connected in some organisms (e.g. grasshoppers), until the onset of anaphase II, by a prominent strand of material that can be stained with silver (Gimenez-Abian et al., 1997; Solari and Tandler, 1991; Suja et al., 1992). This connecting strand is also present during meiosis I and, although it becomes highly stretched during anaphase, it seldom breaks (see Rebollo and Arana, 1995; Rebollo et al., 1998). As a result, the chromatids comprising a bioriented non-natural univalent on a meiosis I spindle seldom disjoin and usually either remain trapped at the equator during cytokinesis or ultimately migrate into one of the spindle poles.

Together these data support the idea that non-natural univalents that become bioriented on the meiosis I spindle seldom separate at anaphase I because their sister kinetochore regions remain firmly connected by a tether that cannot normally be broken by machinery activated at the onset of anaphase I. On the surface this conclusion appears inconsistent with the observation of Nicklas (1977), discussed above, that meiosis II dyads disjoin normally when placed on a meiosis I spindle. This suggests that the machinery responsible for severing the tether between the kinetochore regions on a meiosis II dyad is also functional during meiosis I. However, since Nicklas's experiments were conducted on primary and secondary spermatocytes that were fused together, the resultant meiosis I/II hybrids may contain the machinery for both arm and centromere separation. Alternatively, since the meiosis II dyad has already

been licensed by passing through anaphase I, it is possible that the centromere linkage holding its chromatids together can now be broken on the meiosis I spindle by the same machinery working to dissolve arm cohesion at the onset of anaphase I.

The composition of the tether that holds the sister kinetochore regions of a dyad (or univalent) together until anaphase II is unknown, but there are several solid candidates. Two proteins, encoded by the *mei-s332* and *ord* genes, have been implicated in centromere cohesion in *Drosophila* (Goldstein, 1980; Kerrebrock et al., 1992, 1995; Bickel et al., 1998). In *mei-s332* mutants the chromatids comprising each dyad prematurely disjoin late in anaphase I, near the time the sister kinetochores separate to opposite sides of the primary constriction (Kerrebrock et al., 1992). Similarly, in *ord* mutants the chromatids comprising each dyad appear to fall apart sometime after the onset of anaphase I but prior to entry into meiosis II, and an analysis of weaker *ord* alleles confirms that *ord* is required for centromeric cohesion after the arms have separated at anaphase I (Bickel et al., 1997). Observations on spermatocytes and oocytes expressing a GFP/mei-s332 fusion protein reveal that mei-s332 is located specifically in the centromere region of meiotic chromosomes until anaphase II (Kerrebrock et al., 1995; Moore et al., 1998). Together these data clearly demonstrate that during meiosis the cohesion of chromatids in the centromere region is mediated by proteins unique to that region. The fact that these centromeric proteins are normally degraded well after those responsible for cohesion between the chromosome arms clearly reveals that different mechanisms are involved in resolving cohesion between chromatids along their arms and within the centromere.

CHROMATID COHESION DURING MITOSIS ALSO APPEARS TO BE MEDIATED BY TWO SEPARATE MECHANISMS

Since it involves the disjunction of sister chromatids, most workers consider the second meiotic division to be analogous to a mitotic division. However, there is a fundamental difference: the chromatids are held together during prometaphase of meiosis II only at the centromere, whereas during prometaphase of mitosis they are joined (at least initially) along their entire length. This raises the interesting possibility that chromosome disjunction during mitosis also requires two different sets of machinery: e.g. one that is normally present during meiosis I that separates the chromatid arms, and another normally found in meiosis II (or during the preceding interphase) that leads to separation in the centromere region.

When somatic cells enter mitosis in the presence of drugs that disrupt microtubule assembly (e.g. colcemid, nocadazole) the chromosomes remain scattered within the cell until it exits mitosis many hours later. During this time the two chromatids comprising each chromosome ultimately become separated, except at the centromere region, to produce the typical 'X' or 'V' shape characteristic of a C-mitosis (mitosis in the presence of colcemid). When the cell finally enters anaphase (C-anaphase) the centromere-based tethers are suddenly and relatively synchronously broken, the chromatids disjoin completely, and the cell exits mitosis shortly thereafter (reviewed by Rieder and Palazzo, 1992). In some cells undergoing a C-mitosis, including e.g. *Haemanthus* endosperm (Mole-Bajer, 1958), newts (Rieder and Palazzo, 1992), and marsupials (PtK_1; Rieder et al., 1994), the chromatids disjoin in the centromere shortly (15-20 minutes) after the arms have become fully resolved. However, in other cases, including e.g. onion root tip (Levan, 1938) and mouse ascites tumor cells (Levan, 1954) division in the centromere is considerably delayed or fails completely after the chromatid arms have separated.

This temporal difference between the separation of chromatid arms and their disjunction in the centromere is not simply a drug-treatment artifact. A similar phenomenon is also

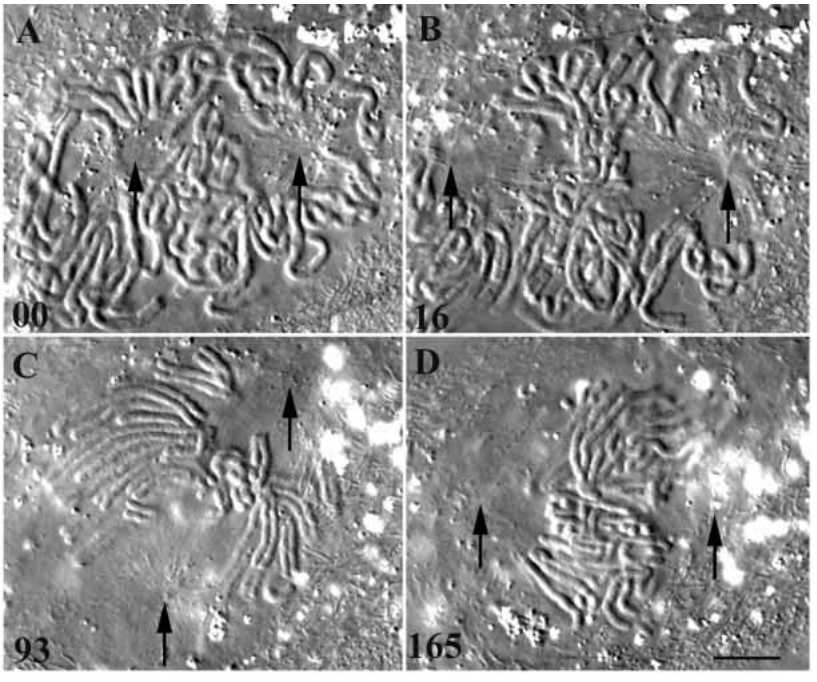

Fig. 1. (A-D) Selected frames from a DIC video recording of a mitotic newt lung cell in which the chromatids become resolved well prior to anaphase onset. (A-B) During the early stages of spindle formation the chromatids comprising each chromosome are not visible. However, when prometaphase is prolonged (e.g. by the presence of one or more monooriented chromosomes) the sister chromatids ultimately become clearly resolved (e.g. C) often well prior to the onset of anaphase (D). Arrows indicate the spindle poles (centrosomes), while the elapsed time (in minutes) is at the bottom left corner of each frame. Bar, 10 μm.

seen in untreated cells, from plants (*Haemanthus*; Mole-Bajer, 1958) to vertebrates (Fig. 1) when the onset of anaphase is delayed, e.g. by persistently unattached kinetochores that delay the metaphase/anaphase transition (reviewed by Rieder and Salmon, 1998). Thus, although the temporal difference during a prolonged mitosis between arm and centromere separation may vary depending on the cell, as during meiosis arm separation can occur prior to separation in the centromere.

Genetic studies in fungi (fission yeast, Ohkura et al., 1989; *Aspergillus*, Doonan and Morris, 1989) reveal that phosphatase activity is required for chromatid separation during mitosis. Indeed, when onion root tip cells are treated with phosphatase inhibitors during mitosis the separation of chromatids at anaphase onset occurs slowly and asynchronously over a 30 minute period (Wolniak and Larsen, 1992). Current evidence indicates that phosphatase activity is not required for arm separation, but instead for chromatid disjunction in the centromere. When HeLa cells are treated during mitosis with okadaic acid (a potent phosphatase inhibitor) the chromosomes fail to disjoin in the centromere regions even though their arms become fully separated (Gosh and Paweletz, 1992; Ghosh et al., 1993). These cells ultimately exit mitosis, and enter the next mitosis with 'diplochromosomes' which, in the presence of colcemid, ultimately become resolved as 4 sister chromatids tethered at a common centromere. Similar diplochromosomes are also found in somatic tissues of some *Drosophila* mitotic mutants after treating larvae with colchicine (e.g. see Gatti and Baker, 1989; Stratman and Lehner, 1996). However, in *Drosophila* it is not clear whether the diplochromosomes result from endoreduplication (repeated rounds of DNA synthesis in the absence of an intervening mitosis), or simply from the failure of sister chromatids to disjoin at the centromere during an earlier mitosis.

Under experimental conditions it is also possible to induce the centromere region of a replicated mitotic chromosome to separate in the absence of arm disjunction. When vertebrate somatic cells are treated with inhibitors of topoisomerase II as they are entering mitosis, spindle formation and chromosome motion to the metaphase plate are not affected. However, when these cells enter anaphase the chromatids separate in the centromere region, but not along their arms. As a result the extent of chromosome poleward motion is severely restricted and a 4N restitution nucleus is ultimately formed (Downes et al., 1991; Gorbsky, 1994). This finding is consistent with previous conclusions that topoisomerase II activity is required during mitosis for chromatid separation. Importantly, however, it also reveals that the activity of topoisomerase II is not required during mitosis for chromatid separation at the centromere.

The separation of chromatids during mitosis is thought to be mediated, as during meiosis, by the sudden activation of APCs (reviewed by Townsley and Ruderman, 1998). The lag observed between arm and centromere separation during a prolonged mitosis, or under experimental conditions that delay anaphase onset, implies either: (a) that arm separation is mediated by a different mechanism not requiring APC activity (and, by analogy, that APC activity may not be required for the separation of bivalents during M1), (b) that the cohesion proteins in the centromere are more resistant to (low levels of?) APC activity than those along the arms, or (c) that the protein(s) responsible for arm and centromere cohesion are targeted for destruction by the APC at different times. However, regardless of the explanation, during meiosis there is clearly something chemically and mechanistically different about the way mitotic chromatids are linked between their arms and in the centromere. In this context it is noteworthy that the centromere region on mitotic chromosomes contains several unique proteins not found along the arms (e.g. Cooke et al., 1987; Rattner et al., 1988, Maney et al., 1998) including the mei-s332 protein (Moore et al., 1998), as well as visible structural elements that link the sister kinetochore regions until anaphase onset (Roos, 1977; Rieder, 1979; Rattner et al., 1988).

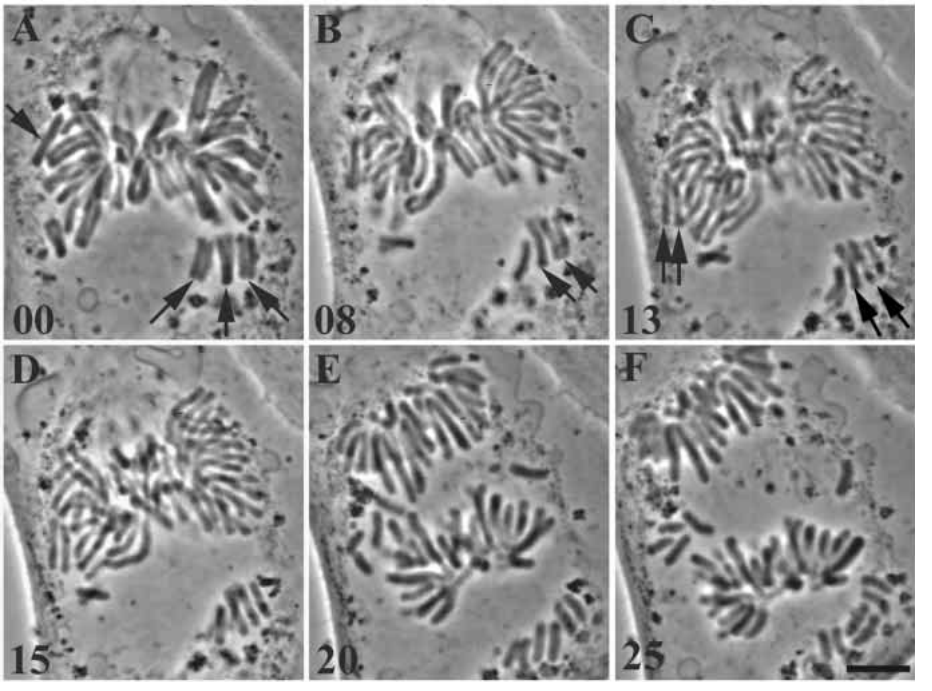

Fig. 2. (A-F) Selected phase-contrast images, from a time-lapse video sequence, showing chromatid disjunction in acentric chromosome fragments created in a newt lung cell by laser microsurgery. During metaphase several fragments are clearly visible near the equator and at the periphery of the spindle (e.g. arrows in A). Over the next 13 minutes, as the chromosome arms become resolved into individual chromatids (B-C), the two chromatids comprising each fragment also become clearly visible and sometimes dissociated from one another (parallel sets of arrows in C). By the time the chromatids have disjoined in the centromere (between C and D) all of the fragments have also separated into two fragments. Time in minutes in lower left corner of each frame. Bar, 10 μm.

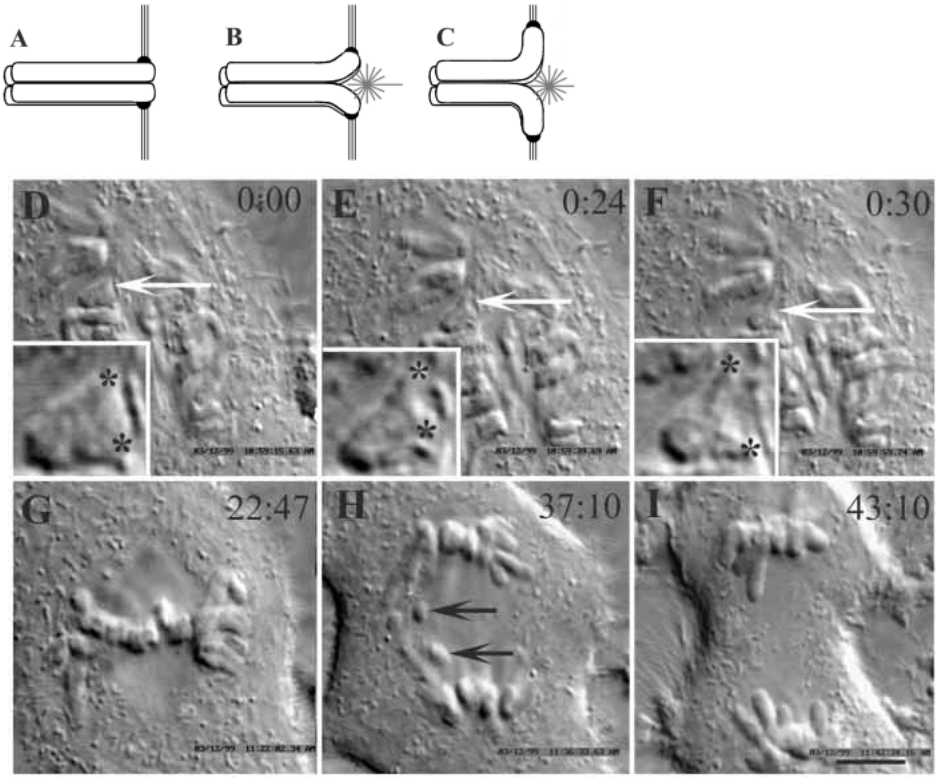

Fig. 3. (A-C) Diagram, modified from Khodjakov et al. (1997b), depicting how the centromere region of a bioriented chromosome can be transected between its sister kinetochores by laser microsurgery. PtK_1 chromosomes are metacentric or sub-metacentric, and when bioriented the arms and kinetochore regions are arranged on the periphery of the hollow spindle as shown in A. At a time when both kinetochores are exhibiting P motion (and thus stretching the centromere) the laser is used to cut the chromosome through the centromere region, between the sister kinetochores (B). This operation produces a bioriented chromosome that is held together only by cohesive forces positioned long the chromosome arms (C). (D-I). Selected DIC images, from a time-lapse video sequence, showing the behavior of a small congressing PtK_1 chromosome after its centromere region is transected between the sister kinetochores. The large white arrow in D-F notes the experimental chromosome prior to D, during E, and after F the operation. This chromosome is also shown at a higher magnification within the insets where the asterisks note the position of the two sister kinetochore regions. As the chromosome was transected through the centromere region (E-F) the distance between the kinetochores increased. When the operation was terminated (F) the centromere region had been severed, but the two chromatids remained tethered along their arms. This chromosome then congressed normally to the metaphase plate (G) where, because of crowded conditions, it became impossible to distinguish from other chromosomes. However, in this particular example it again became visible during anaphase (arrows in H) because the two separated chromatids lag slightly (due to the fact that the centromere region, which was weakened by the operation, is more extensible and becomes stretched poleward). Regardless, as shown in I the force exerted by each kinetochore region remain sufficient to effect normal chromatid segregation. Time in minutes:seconds in upper right hand corner of each frame. Bar, 10 μm.

When progress through mitosis is not impeded, the sister chromatids are usually not resolved by the time of anaphase onset. Under this condition when anaphase occurs the chromatids first separate in the centromere region between the two sister kinetochores. The impression from viewing high temporal and spatial resolution time-lapse records is that the adhering sister chromatid arms are then simply 'peeled' apart as the separating kinetochores pull them towards their opposing poles. However, the fact that acentric chromosome fragments also separate at anaphase onset when mitosis is not prolonged (plants, see Bajer, 1958; Khodjakov et al., 1997a; animals, see Carlson, 1938; Fig. 2), without being acted upon by opposing poleward pulling forces, clearly reveals that they are held together by a 'glue' that is normally dissolved at the metaphase/anaphase transition (but that also can be dissolved, over time, when mitosis is prolonged). Video analyses of vertebrate somatic cells reveals that acentric fragments disjoin into two chromatid fragments when the chromosome arms are finally resolved, which during a prolonged mitosis usually occurs prior to separation in the centromere (Fig. 2).

During meiosis I the glue that holds the associated bivalents together is strong enough to withstand the opposing centromere-based poleward pulling forces, even those that greatly stretch the bivalents during spindle formation (i.e. during the 'prometaphase stretch; reviewed by Schrader, 1944). The fact that the chromatid arms also become resolved at the onset of anaphase I, when the glue holding the homologues together is broken, suggests that this same glue is also responsible for maintaining the cohesion of sister chromatid arms (reviewed by Miyazaki and Orr-Weaver, 1994). Is the glue that holds the chromatid arms together during mitosis similarly strong enough by itself to maintain cohesion in the absence of a centromere tether, or is it weak and easily broken by the kinetochore-based pulling forces? The former possibility is supported by recent work on the temperature sensitive fission yeast *mis6* mutant (Saitoh et al., 1997). The mis6 protein has been shown by GFP-tagging to associate specifically with the centromere. In the absence of a functional *mis6* gene the chromatids in fixed cells appear to remain tethered throughout spindle formation along their arms but not in their centromeres. This suggests that arm cohesion is sufficient, in the absence of centromere cohesion, to support chromatid cohesion until anaphase. To directly test this for vertebrates we used a laser microbeam to sever the centromere on a bioriented chromosome, parallel to the long axis of the chromosome and between its sister kinetochore regions (Fig. 3; see Khodjakov et al., 1997b). As a result of this operation the sister chromatids are no longer tethered in the centromere, but they experience kinetochore-based poleward forces that are attempting to pull them apart. Under this condition when the

centromere is completely severed between the kinetochores during prometaphase, the chromatids remain associated with each other until anaphase onset, at which time they then disjoin and segregate to the opposing poles (Fig. 3). Thus as during meiosis I, arm cohesion alone is sufficient during mitosis to maintain chromatid cohesion in the absence of a centromere linkage.

This is an important conclusion because it provides a ready explanation for why *Drosophila mei-s332* and *ord* mutants, which disjoin their sister chromatids prematurely during or after anaphase I, undergo normal mitoses even though these proteins are found in the centromere regions during both meiosis and mitosis (Kerrebrock et al., 1992; Moore et al., 1998; Bickel et al., 1997, 1998). In the absence of the centromere linkage normally provided by these proteins, the sister chromatids prematurely fall apart during anaphase I of meiosis because at this time the cohesive forces that tether their arms is abrogated. However, even if the centromere linkage provided by these proteins is absent during mitosis, arm cohesion between the sister chromatids is sufficient on its own to support normal bipolar orientation and congression. One prediction of this hypothesis is that, in the presence of nocodazole or colcemid, the sister chromatids in somatic cells of mei-s332 and ord mutants should ultimately disjoin (instead of forming X shaped configurations) once their arms become fully resolved.

SUMMARY AND PERSPECTIVES

Chromosome cohesion during meiosis is mediated by two separate mechanisms. The first holds neighboring chromatid arms together and is resolved at anaphase I. This mechanism may be mediated, in part, by members of the recently identified cohesin complex. The second mechanism holds the chromatids together in the centromere after their arms have separated. This connection can normally be broken only at the onset of anaphase II, and it appears to be mediated by proteins and structural elements unique to the centromere region that involve e.g. the *Drosophila* mei-s332 and ord proteins and their homologues. Chromatid separation during mitosis also appears to be based on two similar mechanisms that normally work concurrently but which can be separated temporally under experimental conditions. In the future it will be important to determine if and how the two mechanisms that operate during meiosis to separate the chromosomes are related to the ones working during mitosis. It will also be important to identify other proteins involved in arm and centromere cohesion, to determine if the dissolution of cohesion in these different regions involves APC activity and, if so, how this activity is differentially regulated between meiosis I, meiosis II, and mitosis.

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