Centromeres, checkpoints and chromatid cohesion

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An emerging view is that the formation of active centromeres is modulated in an epigenetic manner reflecting the association of centromeres with heterochromatin. Support for this comes from studies on fission yeast centromeres, the properties of human neocentromeres and dicentric chromosomes, and analyses of *Drosophila* minichromosome deletion derivatives. A link has been established between tension across kinetochores and the phosphorylation status of kinetochore components. Vertebrate homologues of yeast MAD2 have recently been isolated and localized to kinetochores, indicating that components of the spindle integrity checkpoint are conserved. The linkage between sister chromatids is only dissolved at anaphase during mitotic and meiotic divisions. Phenotypic and localization data combined with their pattern of rapid degradation at anaphase have implicated several yeast and *Drosophila* proteins in aspects of sister chromatid cohesion.

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Abbreviations

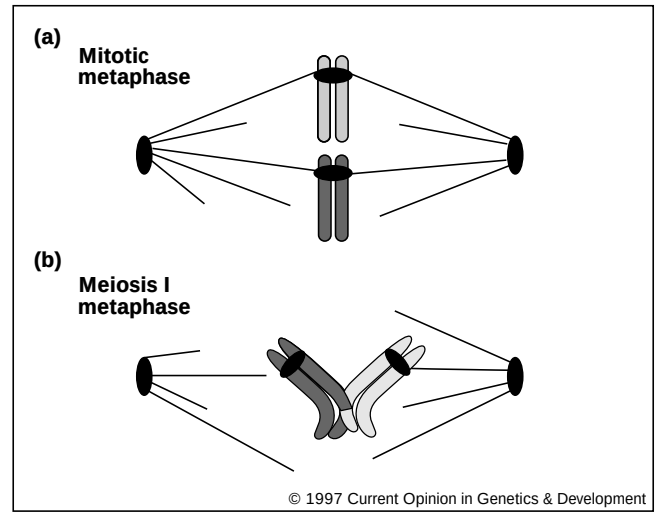
APC	anaphase-promoting complex
BAC	bacterial artificial chromosome
bw^D	*brown^{Dominant}*
GFP	green fluorescent protein
YAC	yeast artificial chromosome

Introduction

Aneuploidy in the form of trisomies is extraordinarily common in human embryos, occurring in a quarter of spontaneously aborted foetuses and 0.3% of newborns. Consequently, abnormal chromosome segregation plays a major role in human health problems [1•]. The process of chromosome segregation on mitotic and meiotic spindles occurs in all eukaryotes. Understanding how various components contribute to accurate chromosome segregation in other organisms should facilitate the analysis of defects which lead to aneuploidy in humans. This review focuses on three aspects of chromosome segregation: the role of heterochromatin in the formation and function of active centromeres; components of checkpoints which act at kinetochores; and the proteins which contribute to sister chromatid cohesion and its regulation.

Centromeres and their associated kinetochores are responsible for the bipolar attachment of chromosomes to the developing mitotic spindle, congression of chromosomes to the metaphase plate, the simultaneous release of sister chromatids and their movement to opposite spindle poles during anaphase [2•]. During meiosis, there is the added complication of having to cope with paired sets of homologous sister chromatids (bivalents) which must remain attached at their centromeres during the first meiotic division and only separate in the second meiotic division (Fig. 1).

Figure 1



The configuration of chromosomes on **(a)** mitotic and **(b)** meiosis I metaphase spindles. In mitosis, homologous chromosomes act independently with their sister kinetochores attaching and segregating to opposite spindle poles. The pairing of homologous chromosomes to form bivalents (four chromatids) during the early stages of meiosis is required to allow homologues to attach to opposite poles of the meiosis I spindle via one of their sister kinetochores. In many systems, pairing results in recombination between the homologues which develop into visible chiasmata (cross-overs). It is these chiasmata that hold the bivalents in place with their kinetochores oriented to opposite poles [1•]. The fourth chromosome in *Drosophila* females segregates without exchange. Pairing of achiasmatic X and fourth chromosomes is mediated by centric heterochromatin [54••,55••]. No recombination occurs in *Drosophila* male meiosis, instead, homologues are held together by pairing mediated by specific euchromatic sequences, not heterochromatin [65].

DNA sequences that act in *cis* to nucleate kinetochore assembly and provide full centromere function in multicellular eukaryotes have been extremely difficult to identify. Recent developments in mammalian, fruitfly and fission yeast systems have revealed that centromere activity is associated with centromeric heterochromatin formed on arrays of repetitive DNA sequences. Several new centromere/kinetochore components have been identified in mammals, flies and yeasts, and some of these proteins are evolutionarily conserved.

The kinetochore is subject to a surveillance system that monitors the presence of monopolar attached or unattached kinetochores and halts sister chromatid separation until proper attachment has been attained. The surveillance system appears to sense tension across bilaterally attached kinetochores and is regulated by phosphorylation. Several proteins have been implicated as components of this checkpoint system. Exciting developments have taken place towards understanding the regulation of sister chromatid cohesion and identifying the 'molecular glue' that is regulated at the metaphase/anaphase transition by the cyclosome or anaphase-promoting complex (APC).

Heterochromatin and the epigenetic regulation of centromere formation

The centromeric domain is cytologically discernible as the primary constriction in the body of a metaphase chromosome in many species [2•,3•]. Large arrays of monotonous tandemly repeated satellite sequences are found in this region of mouse and human chromosomes where the kinetochore has been shown to assemble [2•]. *In vivo* fragmentation of human chromosomes suggests that centromere function may be associated with arrays of alphoid repeats [4,5]. When short arrays of naked alphoid DNA in the form of a purified yeast artificial chromosome (YAC) containing ~70 kb of human chromosome 21-I alphoid (H Cooke, H Matsumoto, personal communication) or *in vitro* tandemly oligomerized alphoid repeats cloned in bacterial artificial chromosomes (BACs; H Willard, personal communication) are reintroduced into human cells, they are assembled *de novo* into structures which contain an active kinetochore in some transformants. Thus, although more detailed analyses of these structures is required, it is possible that alphoid satellite arrays may be sufficient for providing a nucleation site for kinetochore assembly.

In the fruitfly *Drosophila melanogaster*, an elegant series of experiments based on radiation-induced minichromosome derivatives of the X chromosome show that the active centromere is confined to a specific 420 kb region of centric heterochromatin, which contains two different simple repetitive sequences interspersed at intervals with various transposable elements ([6••,7•]; X Sun, J Wahlstrom, G Karpen, personal communication). Complete centromere function in the budding yeast *Saccharomyces cerevisiae* is specified by only 125 bp of DNA [2•,8]. In contrast, centromeres of the fission yeast, *Schizosaccharomyces pombe*, occupy 40–100 kbp and, like their counterparts in larger eukaryotes, they are associated with arrays of repetitive DNA [2•,8]. *S. pombe* centromeres are also heterochromatic as genes placed within them display variegated expression (reversible transcriptional silencing) [9,10•]. The *clr4*, *rik1* and s*wi6* mutations alleviate silencing within the centromere [10•]. These strains are also cold-sensitive, hypersensitive to microtubule destabilizing drugs, and display reduced mitotic chromosome stability and disrupted movement of centromeres to the poles of the spindle during anaphase. Interestingly, the Swi6 and Clr4 proteins share domains of similarity with some *Drosophila* heterochromatin proteins. Thus, mutations that inhibit heterochromatin formation also disturb centromere function [10•,11••,12•]. The ability to assemble a functional *S. pombe* centromere on minimal constructs is also subject to epigenetic regulation; a mitotically stable minichromosome is formed in only a proportion of primary transformants. Once assembled, however, this functional centromere is stably propagated over many generations [13]. This phenomenon may reflect the probability of assembling the DNA into heterochromatin to provide the correct template for nucleating a functional kinetochore. The fact that alphoid repeats only form active centromeres in a proportion of human cell transformants is also suggestive of epigenetic events influencing kinetochore assembly (see above).

Other observations also suggest epigenetic regulation of active centromere formation. In humans, several instances of neocentromere formation have been reported in which an active kinetochore is assembled on derivative chromosomes in a region where no alphoid repeats or centromeres are found normally [14,15]. Certain dicentric human chromosomes may be stable because they are functionally monocentric. This is supported by the observation that a constriction is formed at just one centromere and that just one centromere binds known centromere proteins such as CENP-C and -E [3•]. Clearly, centromere activity must be modulated by epigenetic factors. It is possible that a particular chromatin structure needs to be adopted to favour kinetochore assembly. Histone H3 variants such as CENP-A and Cse4p, which are associated with centromere function in mammals and yeast, may be required [16,17•]. In addition, epigenetic modifications of DNA by methylation [18] or chromatin by acetylation (K Ekwall, R Allshire, unpublished data) might regulate centromere activity. Exploring how centromere inactivation occurs and how such centromeres can be reactivated will be a fruitful avenue of research.

Epigenetic regulation of centromere formation is also apparent on certain minichromosome deletion derivatives of the *Drosophila* X chromosome. Deletion derivatives of the original X *Dp1187* minichromosome, which lack all heterochromatic DNA normally associated with the centromere, are transmitted relatively efficiently through male meiosis [6••]. Subtelomeric regions — that do not normally reside close to the centromere — have taken on characteristics of the centromere such as binding the ZW10 protein (see below), allowing the formation of a neocentromere on these *Dp1187* derivatives (B Williams, T Murphy, M Goldberg, G Karpen, personal communication). The proximity of these normally subtelomeric sequences to centromeric heterochromatin on the original *Dp1187* may have allowed them to develop centromeric properties. Therefore, not only can centromeric hete-

rochromatin repress expression of juxtaposed genes by 'spreading' but it may also impose centromeric properties on adjacent sequences (K Maggert, G Karpen, personal communication). Once 'tainted' by association with the centromere, the structure formed may be maintained by self-propagation over many generations. It is apparent that epigenetic phenomena are not only a hallmark of heterochromatin but also of active centromere assembly itself. Clearly, in *S. pombe*, *Drosophila* and mammals there is an association between repetitive DNA, heterochromatin and centromere functions. It is my opinion that the enigmatic phenomena associated with centromeric heterochromatin reflect the assembly and functions of a normal centromere.

Sensing the tension

Cells can respond to the presence of chromosomes which have only managed to capture microtubules from one pole or where the chromosome remains unattached to either pole of a mitotic spindle. The upshot is to delay anaphase onset, providing additional time to achieve bilateral spindle attachment of all chromosomes before proceeding into anaphase [19]. How are unattached or mono-oriented chromosomes recognized by the cell? In mammalian cell lines, a phosphoepitope recognized by a monoclonal antibody (3F3/2) is only present at kinetochores which are not under tension, such as during the early stages of mitosis or the kinetochores of lagging or unattached chromosomes. Once all chromosomes have congressed at the metaphase plate, staining is lost until the next mitosis [20]. Thus, this phosphoepitope could be the basis of a checkpoint resulting in anaphase inhibition in response to misaligned chromosomes. More recent observations bolster this idea. Microinjection of cells with the 3F3/2 antibody in early mitosis protects the phosphoepitope from dephosphorylation and delays anaphase onset until, eventually, the epitope disappears [21•].

Combining staining of the 3F3/2 antibody with manipulation of chromosomes in insect spermatocytes has proven to be very informative. Anaphase I of meiosis in these insects (mantids) is delayed by a single mono-oriented bivalent lurking close to one pole of the spindle. However, applying tension artificially across the bivalent with a microneedle allows anaphase to proceed [22•]. Prior to creating tension on a mono-oriented grasshopper bivalent, both kinetochores stain intensely with 3F3/2 but the application of tension via a microneedle on one homologue results in reduced staining. It appears that tension across kinetochores in mitosis and male meiosis erases a signal — the 3F3/2 phosphoepitope — that acts to inhibit anaphase onset [23••]. The identification of the kinetochore protein(s) recognized by 3F3/2 and the tension-sensing protein that results in dephosphorylation of the 3F3/2 phosphoepitope will be fundamental for dissecting this tension-dependent checkpoint.

The *MAD* and *BUB* genes of *S. cerevisiae* monitor spindle integrity and were identified as being required to halt cell cycle progression in the presence of microtubule-destabilizing drugs [24,25]. One of their counterparts also appears to be a component of the kinetochore tension checkpoint in vertebrates. The *Xenopus* (*XMAD2*) and human (*hsMAD2*) homologs of the *S. cerevisiae MAD2* gene have been isolated recently [26••,27••]. Antibodies raised against XMAD2 inhibit a spindle assembly checkpoint in frog egg extracts [26••]. Electroporation of human cells with anti-hsMAD2 antibodies renders them insensitive to the normal nocodazole-induced mitotic arrest [27••]. Staining of human cells with either antibody in interphase detects protein associated with the nucleus but during prometaphase or in nocodazole-arrested cells, paired dots of fluorescence appear at the sister-kinetochores. In cells progressing normally through metaphase and anaphase, staining with anti-XMAD2 is no longer detected at kinetochores. However, the unattached kinetochores of mono-oriented chromosomes in newt lung cells still stain with anti-XMAD2 when all other kinetochores lack the antigen [26••]. This suggests that vertebrate MAD2p also plays a role in tension sensing at kinetochores; however, as MAD2p is unmodified by phosphorylation, it cannot contain the 3F3/2 epitope and therefore must act in concert with the 3F3/2 protein. It is likely that other MAD/BUB proteins will be conserved and play a role in this checkpoint.

Monitoring chromosome behaviour in yeast

In budding yeast, centromere misbehaviour is also monitored by the products of the *MAD/BUB* genes [28•–30•]. Short linear minichromosomes containing a centromere mis-segregate frequently in mitosis compared to similarly sized circular forms. Pedigree analyses show that these short linear minichromosomes induce mitotic delays but that these delays are abolished in strains with mutations in the *MAD* genes [28•]. Defective kinetochore components or centromeric DNA have been shown to elicit a mitotic delay which is dependent upon some of the products of the *MAD/BUB* genes [29•,30•]. Active dicentric chromosomes in *S. cerevisiae* also induce a delay in progress through mitosis. Time-lapse analyses of live cells has shown that cells harboring these dicentrics pause for a considerable period in mid-anaphase and that this delay is dependent upon the *RAD9* checkpoint [31•]. In mammalian cells, functional dicentrics lag but do not form bridges on mid-anaphase spindles; further analyses are required to determine if they delay progression through anaphase (B Sullivan, personal communication).

Studies of chromosome segregation in yeast are hampered by the small size of chromosomes and consequently the inability to visualize key events. An ingenious *in vivo* assay has been developed for monitoring sister chromatid separation in live yeast cells [32••]. Insertion of a tandem array of 256 copies of the *lac* operator near the centromere of *S. cerevisiae* chromosome III and simultaneous expression of the *lac*-encoded repressor protein fused to green fluorescent protein (GFP) results in the accumulation of

GFP at this point on the chromosome, which can be visualized by fluorescence microscopy. The separation of one spot into two allows the timing of sister chromatid separation to be monitored accurately with respect to other cellular events. This assay confirms, in live cells, that sister chromatid separation is arrested in response to spindle damage but that it proceeds regardless in *mad* mutants [32••]. This approach of monitoring chromosomal events in live cells will be very useful for assessing the contribution of various components to chromosome segregation. The fusion of GFP to centromere proteins themselves will also provide a useful means for charting centromere dynamics in yeast cells, as similar analyses in live human cells expressing GFP fused to the alphoid satellite binding protein CENP-B have demonstrated [33•].

Regulating sister chromatid separation and anaphase onset

During mitosis, the sister centromeres appear — under some conditions — to be the last region of sister chromatids to remain joined and are released only when all sister kinetochores have captured microtubules from opposite poles. It is intriguing that strands of alphoid satellite can be clearly visualized running between sister kinetochores of extracted human chromosomes at metaphase when the arms of the sister chromatids have separated completely [34•]. This region of the chromosome has been observed to undergo microtubule-dependent stretching during metaphase [33•]. At this stage, it is also known that a pool of topoisomerase II α (TopoII) remains specifically associated with the centromere [35,36]. It is possible that the final act which allows anaphase onset is to release these tethers of alphoid DNA by activating this centromere-associated TopoII pool. It is known that the release of sister chromatids is mediated by APC, which also regulates the ubiquitin-dependent degradation of cyclin B, allowing exit from mitosis [37–42]. Degradation of some other factor, but not cyclins, by APC allows sister chromatid separation to proceed, thereby initiating anaphase [37–40]. This factor is referred to as the chromatid 'glue'. The action of APC on the glue could be indirect such that an inhibitor of anaphase onset maintains the glue and when this inhibitor is degraded by APC, sister chromatids separate and anaphase ensues. Recently, two good candidates, Cut2p and Pds1p — which may either be the glue or an inhibitory regulator of the glue — have been identified in *S. pombe* and *S. cerevisiae* respectively [43••,44,45,46••]. The Cut2 protein is associated with the short metaphase spindle but is degraded at anaphase onset whereas Pds1p is associated with the nucleus. The degradation of both proteins at anaphase is dependent on the presence of a specific region which has similarity to cyclin degradation boxes; this degradation is dependent on components of APC. Expression of non-degradable versions of both Cut2p and Pds1p inhibits sister chromatid separation. Thus, degradation of these proteins by APC is required to promote anaphase. The roles of Cut2p and pds1p appear to be very similar but there are no extensive regions of sequence similarity and, unlike *PDS1*, the *cut2*$^+$ gene is essential.

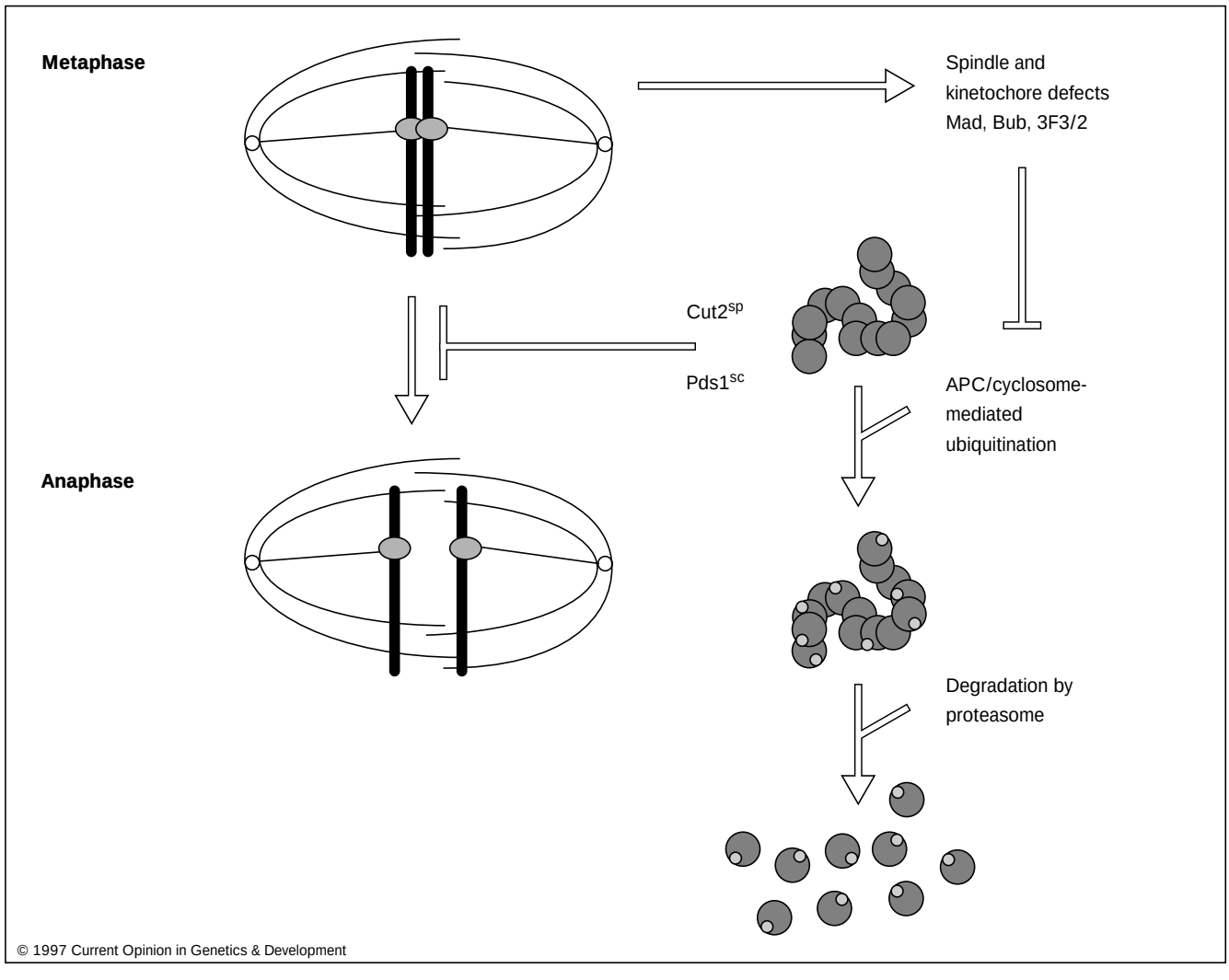
Surprisingly, the spindle integrity checkpoint remains partly intact in *pds1* mutants as, unlike *mad* and *bub* mutants, they do not exit mitosis in the presence of spindle damage [44,45,46••]. The *MAD* and *BUB* products may prevent anaphase by a Pds1p-independent pathway, or they may act via pds1p to inhibit anaphase, but play an additional role in preventing later cell cycle events in response to spindle or chromosome segregation defects. Presumably, the kinetochore tension/spindle integrity checkpoint mediated by the *MAD* and *BUB* products must arrest anaphase by inhibiting the APC-dependent degradation of proteins such as Cut2p or Pds1p at some level, thus preventing sister chromatid separation; how this is achieved is presently unknown.

In *Drosophila*, four genes involved in sister chromatid cohesion have been characterized. The products of the *pimples* and *three rows* genes are required for the separation of sister centromeres during mitosis in embryos [47•]. The Pimples protein is degraded rapidly upon anaphase onset. Neither mutant registers a defective mitotic spindle assembly checkpoint. The MEI-S332 protein is associated with centromeres from late prometaphase of the first meiotic division until anaphase II when sister chromatids normally separate [48••]. In its absence, sister chromatids separate prematurely during anaphase I, suggesting that the MEI-S332 protein is required for tethering sister centromeres during meiosis. The MEI-S332 protein seems to disappear abruptly from chromosomes at the metaphase II/anaphase II transition. The ORD protein plays a complementary role and holds sister chromatids together along their length during the first meiotic division [49]. Lack of ORD results in precocious sister chromatid separation during prophase I. Both MEI-S332 and ORD proteins contain PEST motifs, suggesting that they may be regulated by proteolysis. The *ord* and *mei-S332* mutants display no defects in the separation of sister chromatids during mitosis. From the analysis of these *Drosophila* mutants, it is clear that there are different proteins required to hold sister chromatids together along their length or specifically at the centromere and that these may act exclusively in meiosis or mitosis.

Creating tension in meiosis

In many organisms, bivalents are held together by chiasmata which result from recombination between homologues (Fig. 1) [1•]. As discussed above, in mantid and grasshopper male meiosis I, tension across all bivalents acts as a signal to indicate that all chromosomes have recombined and anaphase can proceed [22•,23••]. In contrast, in *Drosophila* females, the signal of tension across any single bivalent is utilized to halt oocyte development at metaphase I until fertilization [50,51•]. It is of interest to note that, in humans, most trisomies arise from defects in maternal meiosis and many of these are associated with

Figure 2



During the early stages of mitosis, sister kinetochores capture microtubules emanating from both poles of the spindle, resulting in the congression of all chromosomes at the centre. Only when all chromosomes have gathered at the spindle equator can anaphase be triggered by dissolving the tethers between sister chromatids. Proteins, such as Cut2p in *S. pombe* and Pds1p in *S. cerevisiae*, negatively regulate the separation of chromatids [43••,44,45,46••]. These proteins contain destruction boxes and are degraded precisely at anaphase onset, thus allowing chromatid separation. The machinery responsible for the ubiquitination and, consequently, the degradation of proteins such as Cut2p and Pds1p must be negatively regulated by defects in spindle assembly, kinetochore structure, or the presence of an unattached or lagging chromosome. The products of the *MAD* and *BUB* genes are required to monitor spindle integrity and kinetochore structure [26••,27••,28•–30•]. Proteins such as Mad2p and the species recognized by the 3F3/2 antibody signal bilateral attachment of sister kinetochores by sensing the tension that develops across the opposing kinetochores [20,21•,22•,23••,26••]. Mono-oriented chromosomes are not under tension, their kinetochores carry the phosphorylated form of the 3F3/2 protein(s) and the Mad2p, which must signal so as to delay the degradation of proteins that trigger anaphase. Degradation of the Cut2p and Pds1p proteins is mediated by ubiquitination via the APC/cyclosome and proteolysis by the proteasome [43••,46••].

aberrant frequencies and distribution of recombination events on that chromosome [1•]. The importance of recombination in meiosis is emphasized by the finding in male mice that a lack of chiasmata leads to the inhibition of anaphase I [52,53]. Further investigations are required to determine what triggers the coordinate release of chiasmata to induce the onset of anaphase I and whether this is also regulated by APC.

Recombination is not always required to ensure normal segregation of chromosomes in meiosis I. During the first meiotic division in female *Drosophila*, it is known that achiasmatic fourth and X chromosome pairs are segregated normally. Apparently, the centric heterochromatin on each chromosome itself acts as a sorting device, allowing the association of achiasmatic homologous chromosomes in the absence of recombination [54••,55••]; this suggests

that heterochromatin itself is 'sticky' and that each chromosome has a unique heterochromatin structure allowing homologues to find each other in the nuclear milieu. The evidence that pericentric heterochromatin is inherently sticky has been demonstrated persuasively in somatic cells with respect to the behaviour of the *brown*^{Dominant} mutation, *bw*^{D}, in *Drosophila* ([56••,57••]; see Marshall *et al.*, this issue, [pp 259–263]). This type of association of heterochromatic regions in somatic cells is perhaps used in female meiosis for mediating the pairing of achiasmatic homologues.

Novel centromeric proteins

Several other new proteins, in addition to those discussed above, have been found to associate with centromeres. The localization of the Swi6p chromodomain protein at *S. pombe* centromeres is dependent upon the presence of the *clr4*$^{+}$ and *rik1*$^{+}$ products [11••,12•]. The *Drosophila* ZW10 protein localizes with spermatocyte kinetochores in late prometaphase I, stretches along kinetochore microtubules at metaphase I, and reassociates with the kinetochore at anaphase; similar localizations are observed through meiosis II [58•]. Association of ZW10 with kinetochore microtubules correlates with the application of tension across kinetochores at metaphase. Mutations in *zw10* result in a high incidence of lagging chromosomes during mitotic anaphase and nondisjunction of chromosomes during both meiotic divisions. Proteins homologous to ZW10 have been identified in *C. elegans*, *Arabidopsis* and humans. The human ZW10 protein also associates with kinetochores in a cell-cycle regulated manner (M Goldberg, personal communication). Thus, ZW10 represents a conserved centromere/kinetochore component and perhaps plays a role in the recognition of tension across kinetochores. The newly identified CENP-F protein is also dynamic in its association with human kinetochores [59]. It accumulates at kinetochores in late G_2 but remains there only until early anaphase after which it is found at the midzone between the separating chromosome masses and is rapidly degraded. The role of CENP-F at the kinetochore is, as yet, undetermined.

In *S. cerevisiae*, a new kinetochore protein (also known as Cbf3d) was identified by genetic [60••] and physical [61•] interactions with the Ctf13/p58 component of the CBF3 centromere complex and homologs have been identified in *S. pombe* (K Kitagawa, P Hieter, personal communication), *Drosophila* (T Murphy, G Karpen, personal communication), *C. elegans*, *Arabidopsis* and humans [60••,61•]. Skp1p is required for the *in vitro* assembly of the *CEN*–DNA-bound CBF3 complex [61•]. Specific conditional *skp1* alleles display elevated rates of chromosome loss and arrest in mitosis with a similar phenotype to mutations in other components of the centromere complex (*NDC10/CBF2/CTF14*, *CTF13* and *CEP3/CBF3b*) and of APC (*CDC16*, *CDC23* and *CDC27*) [60••]. Human SKP1 was identified as a component of CyclinA/CDK2 complexes and, interestingly, several other *S. cerevisiae skp1* alleles are defective in the G_1/S transition but do not affect mitosis [60••,62••]. Other evidence indicates that the *S. cerevisiae* Skp1 protein is required to direct the ubiquitin-dependent degradation of several key cell cycle regulatory proteins [62••]. It is possible that Skp1p acts to dissolve sister chromatid cohesion at the centromere or to mediate the regulatory ubiquitination of some kinetochore component. It is therefore of interest that the CDC34 ubiquitin-conjugating enzyme — which acts in concert with *SKP1*, *CDC4* and *CDC53* [62••,63•] — ubiquitinates the p110 (*NDC10/CBF2/CTF14*) component of the centromere complex [64]. It will be extremely interesting if the Skp1p homologs present in humans and other organisms are associated with the kinetochore and influence chromosome segregation.

Conclusions

The assembly of active kinetochores is associated with repetitive structures that are heterochromatic in many organisms. The epigenetic phenomena, such as transcriptional repression, associated with heterochromatin may just reflect a complex form of steric hindrance caused by kinetochore assembly. Advances have been made in the identification of proteins that regulate sister chromatid cohesion. How these proteins act in regulating this process is unknown. Several components of the spindle integrity checkpoint are associated with kinetochores and are probably involved in responding to the tension that develops across kinetochores upon bilateral attachment of a chromosome or bivalent. Elucidating the tension-detection mechanism and understanding how this feeds into anaphase onset will be particularly interesting. Identifying the functions of other kinetochore proteins, new and old, and their contribution to chromosome segregation will continue to be a very active and exciting area of research. The development of reagents allowing the dynamics of proteins and chromosomal regions to be tracked in live cells during all stages of mitotic and meiotic cell cycles will provide insights into the mechanism of chromosome segregation.

Note added in proof

Shelby *et al.* [66] have recently described further studies with CENPA. Intriguingly, CENPA must be expressed in late S/G_2 to allow incorporation at centromeres; this again impinges on epigenetic regulation of kinetochore assembly. The work cited as H Willard, personal communication, has now been accepted for publication [67].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Koehler KE, Hawley RS, Sherman S, Hassold T: Recombination and nondisjunction in humans and flies. *Hum Mol Genet* 1996, 5:1495–1504.

A comprehensive review which discusses recombination and nondisjunction in *Drosophila* and humans. Drawing on a wealth of genetic observations in flies, extrapolations are made to explain the high incidence of aneuploidy observed in human neonates and conceptuses.

2. • Pluta AF, Mackay AM, Ainsztein AM, Goldberg IG, Earnshaw WC: The centromere: hub of chromosomal activities. *Science* 1995, 270:1591–1594.

A good recent review of centromere structure and function in various organisms.

3. • Sullivan BA, Schwartz S: Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. *Hum Mol Genet* 1995, 4:2189–2197.

Several dicentric chromosomes are examined in this paper. Centromere activity is generally correlated with the presence of a primary constriction and the two centromere proteins CENP-C and CENP-E. In contrast, the protein CENP-B – which binds directly to repeats of alphoid satellite DNA – is found at both active and inactive centromeres. On some dicentric chromosomes, both centromeres remain active as determined by the presence of these proteins. In addition, activity appears to be modulated in some cells in these dicentric lines as a proportion of the marker chromosomes have a staining pattern consistent with one or other centromere being active.

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6. •• Murphy TD, Karpen GH: Localization of centromere function in a ***Drosophila*** minichromosome. *Cell* 1995, 82:599–610.

This paper represents a real *tour de force*. Irradiation of the 1.3 Mbp *Dp1187 Drosophila* minichromosome resulted in the generation of a series of smaller derivatives, allowing the region critical for mitotic and meiotic transmission to be mapped. Full centromere activity is provided by a 420 kb region embedded within heterochromatin. Transmission of minichromosomes through male and female meiosis requires different amounts of centromeric DNA, perhaps reflecting the significant differences in meiosis I between sexes. Further molecular characterization by pulsed field gel mapping of this centromeric region is presented in [7•]. The centromere is unremarkable, composed of two types of satellite DNAs interspersed with transposable elements.

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See annotation [6••].

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10. • Allshire RC, Nimmo ER, Ekwall K, Javerzat J-P, Cranston G: Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. *Genes Dev* 1995, 9:218–233.

See annotation [12•].

11. •• Ekwall K, Javerzat J-P, Lorentz A, Schmidt H, Cranston G, Allshire RC: The chromodomain protein Swi6: a key component of fission yeast centromeres. *Science* 1995, 269:1429–1431.

See annotation [12•].

12. • Ekwall K, Nimmo ER, Javerzat J-P, Borgstrom B, Egel R, Cranston G, Allshire R: Mutations in the fission yeast silencing factors ***clr4+*** and ***rik+*** disrupt the localisation of the chromodomain protein Swi6p and impair centromere function. *J Cell Sci* 1996, 109:2637–2648.

This represents the latest publication in a series [9,10•,11••] which show that: genes are transcriptionally repressed, but variegate, when placed within *S. pombe* centromeres; this repression at centromeres extends throughout the entire centromeric domain and is dependent on three factors – *clr4+, rik1+* and *swi6+* – previously known to affect silencing at *mat2/mat3* loci; these mutations also display elevated rates of chromosome loss and a high incidence of lagging centromeres on late anaphase spindles; the protein encoded by the *swi6+* gene – which, like *Drosophila* heterochromatin protein 1, contains a chromo domain – is localized at centromeres, telomeres and *mat2/mat3* and localization is dependent on wild-type *clr4+* and *rik1+* genes. These findings suggest that the assembly of a fully functional centromere in *S. pombe* is dependent upon, or results in, a silent heterochromatic structure. There appears to be many parallels between the centromeric domains of *Drosophila* and *S. pombe*.

13. Steiner N, Clarke L: A novel epigenetic effect can alter centromere function in fission yeast. *Cell* 1994, 79:865–874.

14. Voullaire LE, Slater HR, Petrovic V, Choo KH: A functional marker centromere with no detectable α-satellite, satellite III, or CENP-B protein: activation of a latent centromere? *Am J Hum Gen* 1993, 52:1153–1163.

15. Blennow E, Telenius H, De Vos D, Larsson C, Henriksson P, Johansson O, Carter NP, Nordenskjold M: Tetrasomy 15q: two marker chromosomes with no detectable α-satellite DNA. *Am J Hum Gen* 1994, 54:877–883.

16. Sullivan KF, Hechnenberger M, Masri K: Human CENP-A contains a histone H3 related histone fold domain that is required for targetting to the centromere. *J Cell Biol* 1994, 127:581–592.

17. • Stoler S, Keith KC, Curnick KE, Fitzgerald-Hayes M: A mutation in ***CSE4***, an essential gene encoding a novel chromatin associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. *Genes Dev* 1995, 9:573–586.

A conditional mutation in the *S. cerevisiae CSE4* gene causes a 17-fold increase in the mis-segregation of a chromosome bearing a compromised *CEN3* and arrests cells at metaphase/anaphase. *CSE4* is essential, encoding another histone H3 variant (see [16]) showing 64% identity to histone H3 over a 98 residue carboxy-terminal domain and with a novel 138 amino acid amino-terminal extension. Cse4p can be incorporated into yeast chromatin and other data suggest that Cse4p interacts with histone H4 to form a novel type of chromatin. One possibility is that this histone H3 variant plays an important role in centromere structure and function.

18. Mitchell AR, Jeppesen P, Nicol L, Morrison H, Kipling D: Epigentic control of mammalian centromere protein binding: does DNA methylation have a role? *J Cell Sci* 1996, 109:2199–2206.

19. Rieder CL, Schultz A, Cole R, Sluder G: Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J Cell Biol* 1994, 127:1301–1310.

20. Gorbsky GJ, Ricketts WA: Differential expression of a phosphoepitope at the kinetochores of moving chromosomes. *J Cell Biol* 1993, 122:1311–1321.

21. • Campbell MS, Gorbsky GJ: Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase. *J Cell Biol* 1995, 129:1195–1204.

The phosphoepitope detected by the 3F3/2 antibody at kinetochores in prophase and early prometaphase declines at prometaphase except on lagging or unattached chromosomes [20]. Microinjection of this antibody inhibits loss of the epitope upon approach to metaphase and delays anaphase onset. Injected cells displayed normal dynamics of chromosome movement, therefore the antibody does not affect the performance of kinetochore-associated motor proteins. Interfering with normal spindle structure prior to anaphase onset results in the reaccumulation of this phosphoepitope, even at kinetochores which remain attached to the spindle. Electron microscopic studies show that the phophoepitope is located in the middle layer of the trilaminar kinetochore structure. It is proposed that this phosphoepitope is involved in sensing stable attachment of kinetochores to the spindle and regulating the metaphase/anaphase transition.

22. • Li X, Nicklas B: Mitotic forces control a cell-cycle checkpoint. *Nature* 1995, 373:630–632.

In praying mantid spermatocytes, the three sex chromosomes often fail to remain paired prior to metaphase I leaving an unpaired chromosome attached to one spindle pole and delaying anaphase onset for hours. In this elegant paper, the unpaired chromosome is manipulated with a microneedle so that tension is placed across its kinetochore resulting in progression into anaphase.

23. •• Nicklas RB, Ward SC, Gorbsky GJ: Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J Cell Biol* 1995, 130:929–939.
In this paper, the exquisite micromanipulation approach of [22•] is combined with the staining of cells with the 3F3/2 antibody [20,21•] in grasshopper spermatocytes. These experiments show that tension applied by a microneedle alters the phosphorylation of kinetochore protein(s) detected by 3F3/2. Absence of tension across a bivalent is accompanied by intense staining of kinetochores on that chromosome with 3F3/2. Application of tension via a microneedle leads to decreased staining with 3F3/2, presumably because of dephosphorylation of kinetochore protein(s). Intriguingly, the X chromosome, which lacks a pairing partner in grasshopper spermatocytes, always stains dimly and remains unnoticed by the kinetochore-tension checkpoint.

24. Li R, Murray AW: Feedback control of mitosis in budding yeast. *Cell* 1991, 66:519–531.

25. Hoyt MA, Totis L, Roberts BT: ***S. cerevisiae*** genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 1991, 66:507–517

26. •• Chen R-H, Waters JC, Salmon ED, Murray AW: Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. *Science* 1996, 274:242–246.
This paper, together with [27••], demonstrates that the MAD2p component of the budding yeast spindle assembly checkpoint is conserved in vertebrates and that it is localized at kinetochores of unattached chromosomes in prometaphase or cells treated with nocodazole. The pattern of staining is very similar to that described for 3F3/2 [20,21•] allowing one to speculate that the MAD2p and the 3F3/2 protein(s) are in the same pathway. Given the role of MAD2 in spindle assembly and kinetochore checkpoints in vertebrates, it is possible that lesions in *MAD2* will contribute to tumour progression. Intriguingly, a breast tumour cell line fails to arrest mitosis in the presence of nocodazole and expresses reduced amounts of MAD2 [27••].

27. •• Li Y, Benezra R: Identification of a human mitotic checkpoint gene: hsMAD2. *Science* 1996, 274:246–248.
See annotation [26••].

28. • Wells WAE, Murray AW: Aberrantly segregating centromeres activate the spindle assembly checkpoint in budding yeast. *J Cell Biol* 1996, 133:75–84.
Short linear minichromosomes were found to induce mitotic delays. The frequency of delaying cells increases in cells bearing increased copies of even a 'well behaved' circular minichromosome, presumably because the probability of one of the now numerous minichromosomes molecules misbehaving per cell increases. Delays are abolished in *mad* mutants. The *MAD*-dependent delay does not rescue mis-segregation of the offending minichromosome. As most minichromosome mis-segregation events detected are failure to replicate a chromosome or loss of a chromosome (1:0), the cell can clearly not rectify the error but the default response is to delay because a mono-oriented/unattached chromosome is detected.

29. • Wang Y, Burke DJ: Checkpoint genes required to delay cell division in response to nocodazole respond to impaired kinetochore function in the yeast ***Saccharomyces cerevisiae***. *Mol Biol Cell* 1995, 15:6838–6844.
Treatment of *S. cerevisiae* cultures with low nocodazole concentrations does not affect spindle structure but the cell cycle is delayed and cells accumulate with short metaphase-like spindles. The *MAD1-*, *MAD2-*, *BUB1-* and *BUB3-*encoded products are required for this delay. The delay could be caused by defective kinetochore attachment to the spindle as a mutation in the gene encoding the Ctf13/p58 centromere component shows synergistic effects when combined with *mad1*, *mad2*, *bub1*, and *bub3* mutations. Thus, *MAD* and *BUB* genes appear to monitor kinetochore function in budding yeast.

30. • Pangilinan F, Spencer F: Abnormal kinetochore structure activates the spindle assembly checkpoint in yeast. *Mol Biol Cell* 1996, 7:1195–1208.
Previous observations indicated that abnormal kinetochores delay anaphase onset in *S. cerevisiae*; the authors of this paper show that the delay in response to lesions in centromeric DNA (CDEIIΔ31) or kinetochore proteins (*ctf13-30*) is dependent upon functional *MAD2*, *BUB1* and *BUB2* genes. Double mutants of *ctf13-30* with *mad/bub* display a highly elevated incidence of chromosome loss, indicating that these *MAD/BUB* products normally hold anaphase in check, increasing the likelihood of normal chromosome transmission. Abnormal kinetochore structure appears to be recognized by a *MAD2-*, *BUB1-* and *BUB2*-dependent sensor which induces a pre-anaphase delay.

31. • Yang SS, Yeh E, Salmon ED, Bloom K: Identification of a mid-anaphase checkpoint in budding yeast. *J Cell Biol* 1997, 136:345–354.
Activation of a second, conditional, centromere on a chromosome induces a cell cycle delay in *S. cerevisiae*. Cells are delayed in mid-anaphase for 30–120 minutes and this is dependent on the product of the *RAD9* DNA damage checkpoint gene. Thus, the *RAD9*-dependent DNA damage checkpoint remains active from G_2 and through mid-anaphase. It will be interesting to see if this delay is also dependent upon the *PDS1* gene, which is pivotal in preventing sister chromatid separation in response to spindle and DNA damage [44,45,46••].

32. •• Straight AF, Belmont AS, Robinett CC, Murray AW: GFP tagging of budding yeast chromosomes reveals that protein–protein interactions can mediate sister chromatid cohesion. *Curr Biol* 1997, 6:1599–1608.
The expression of a non-tetramerizing form of the Lac repressor protein fused in frame to GFP in yeast cells bearing 256 copies of the *lac* operator adjacent to a centromere provides a clever tool for monitoring sister chromatid separation. A form of the *lac* repressor–GFP fusion, which complexes as tetramers, was found to be sufficient for holding sister chromatids together in *mad* mutants in the presence of nocodazole. Thus, connections between sister chromatids can be mimicked by utilizing a multimerized DNA-binding protein which can simultaneously contact two binding sites on different DNA strands. However, this artificial 'glue' is not sufficient to hold sister chromatids together in the presence of a spindle.

33. • Shelby RD, Hahn KM, Sullivan KF: Dynamic elastic behaviour of α-satellite DNA domains visualized ***in situ*** in living human cells. *J Cell Biol* 1996, 135:545–557.
Expression of the human centromeric alphoid satellite DNA-binding protein CENP-B fused to GFP in human cells was used to follow centromeres throughout the cell cycle. The satellite DNA between kinetochores becomes stretched during mitosis and this is dependent on microtubules. The region expands and contracts during the oscillatory chromosome motions exhibited at metaphase.

34. • Bickmore WA, Oghene K: Visualizing the spatial relationships between defined sequences and the axial region of extracted metaphase chromosomes. *Cell* 1996, 84:95–104.
A visually pleasing demonstration that specific sequences are associated with the axial region of fixed, extracted human metaphase chromosomes and that chromosomal loops extend outwards from this region. Strikingly, alphoid satellite DNA remains in the axial region running between the remnant sister kinetochores.

35. Sumner AT: The distribution of topoisomerase II on mammalian chromosomes. *Chromosome Res* 1996, 4:5–14.

36. Rattner JB, Hendzel MJ, Sommer Furbee C, Muller MT, Bazett-Jones DP: Topoisomerase II α is associated with the mammalian centromere in a cell cycle- and species-specific manner and is required for proper centromere/kinetochore structure. *J Cell Biol* 1996, 134:1097–1107.

37. Holloway SL, Glotzer M, King RW, Murray AW: Anaphase is initiated by proteolysis rather than by the inactivation of maturation promoting factor. *Cell* 1993, 73:1393–1402.

38. Surana U, Amon A, Dowzer C, McGrew J, Byers B, Nasmyth K: Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J* 1993, 12:1969–1978.

39. Irniger S, Piatti S, Michaelis C, Nasmyth K: Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* 1995, 81:269–278.

40. King RW, Peters J-M, Tugendreich S, Rolfe M, Hieter P, Kirschner MW: A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 1995, 81:279–288.

41. Hershko A, Ganoth D, Sudakin V, Dahan A, Cohen LH, Luca FC, Ruderman JV, Eytan E: Components of a system that ligates cyclin to ubiquitin and their regulation by the protein kinase cdc2. *J Biol Chem* 1994, 269:4940–4946.

42. Sudakin V, Ganoth D, Dahan A, Heller H, Hersko J, Luca FC, Rudeman JV, Hersko A: The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol Biol Cell* 1995, 6:185–198.

43. •• Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yanagida M: Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature* 1996, 381:438–441.
At the restrictive temperature, *S. pombe* bearing the *cut2-364* mutation fail to undergo nuclear division but other cell cycle events continue, resulting in the eventual bisection of the nucleus by the septum at cytokinesis. The Cut2p protein is required to allow sister-chromatid separation; it is localized along short metaphase spindles but disappears at anaphase. Degradation at anaphase requires a cyclin-like degradation box located in the amino terminus and components of APC, such as Cut9p. Expression of a version of Cut2p lacking this box inhibits anaphase onset but not cell

cycle progression. Replacement of the Cut2p degradation box with that from cdc13p/cyclin B allows anaphase to occur normally. Surprisingly, cells lacking Cut2p also arrest in metaphase suggesting that Cut2p might be required to set up a structure which holds sister chromatids together and that this structure must pre-exist to enable release to occur at anaphase. The Cut1p protein is known to associate with Cut2p in a complex.

44. Yamamoto A, Guacci V, Koshland D: Pds1p is required for faithful execution of anaphase in the yeast ***Saccharomyces cerevisiae***. *J Cell Biol* 1996, 133:85–97.

45. Yamamoto A, Guacci V, Koshland D: Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). *J Cell Biol* 1996, 133:99–110.

46. •• Cohen-Fix O, Peters J-M, Kirschner M, Koshland D: Anaphase initiation in ***Saccharomyces cerevisiae*** is contolled by the APC-dependent degradation of the anaphase inhibitor, Pds1p. *Genes Dev* 1997, 10:3081–3093.
The *S. cerevisiae* protein Pds1p acts to inhibit anaphase and, hence, is required for sister-chromatid cohesion up until anaphase onset [44,45]. The authors of this paper demonstrate that Pds1p can be ubiquitinated by *Xenopus* APC and is a substrate for APC-dependent degradation in both yeast and *Xenopus* extracts. The Pds1p protein is degraded in yeast at anaphase onset; it contains a cyclin-like degradation box which is necessary for its degradation. Expression of Pds1p lacking this box arrests cells prior to anaphase. Cells lacking Pds1p are hypersensitive to nocodazole and γ-irradiation; thus, Pds1p appears to sit at the apex of spindle and DNA damage checkpoints acting to inhibit anaphase and sister-chromatid separation in response to such insults [45]. The accumulated data of this and [44,45] suggest there must be an additional negative regulator of anaphase in *S. cerevisiae*.

47. • Stratmann R, Lehner CF: Separation of sister chromatids in mitosis requires the ***Drosophila pimples*** product, a protein degraded after the metaphase/anaphase transition. *Cell* 1996, 84:25–35.
The *Drosophila* mutants *pimples* and *three rows* are shown to be defective in sister chromatid separation during mitosis. Cell cycle progression is not blocked, resulting in rereplication of chromosome arms so that four chromatids remain connected at the centromere in mitosis 16 and eight by mitosis 17 in early embryos. Pimples protein is degraded after the metaphase/anaphase transition and contains a region which is similar to cyclin destruction boxes.

48. •• Kerrebrock AW, Moore DP, Wu JS, Orr-Weaver TL: Mei-S332, a ***Drosophila*** protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. *Cell* 1995, 83:247–256.
Mutations in the *Drosophila mei-S332* gene result in precocious sister chromatid separation during late anaphase I in both sexes. Mutations predominantly affecting male or female meiosis map to the amino- and carboxy-terminal regions of the protein respectively. A fully functional MEI-S332 protein fused to GFP localizes to centromeres during meiosis. This fusion protein disappears from chromosomes simultaneously with the onset of anaphase II. This suggests that the Mei-S332 protein is required for sister chromatid cohesion at the centromere prior to anaphase II and that its disappearance regulates sister chromatid separation. No cyclin-like destruction box is apparent in MEI-S332 but it does contain PEST degradation motifs. The evidence indicates that this protein plays no role in mitosis.

49. Bickel SE, Wymann DW, Miyazaki WY, Moore DP, Orr-Weaver TL: Identification of ORD, a ***Drosophila*** protein essential for sister chromatin cohesion. *EMBO J* 1996, 15:1451–1459.

50. McKim KS, Jang JK, Theurkauf WE, Hawley RS: Mechanical basis of meiotic metaphase arrest. *Nature* 1993, 362:364–366.

51. • Jang JK, Messina L, Erdman MB, Arbel T, Hawley RS: Induction of metaphase arrest in ***Drosophila*** oocytes by chiasma-based kinetochore tension. *Science* 1995, 268:1917–1919.
A single crossover event is sufficient to induce metaphase I arrest in female *Drosophila* [50]. This report rules out the possibility that something intrinsic to the chiasmata itself inhibits anaphase I from occurring. Exchange events between the arms of compound chromosomes – where both arms are homologous – did not result in metaphase arrest. The authors conclude that exchanges only induce metaphase I arrest because of the tension created across the kinetochores of the resulting bivalent.

52. Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie D-M, Monell C, Arnheim N, Bradley A *et al.*: Involvement of mouse MLH1 in DNA mismatch repair and meiotic crossing over. *Nat Genet* 1996, 13:336–342.

53. Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R *et al.*: Meiotic pachytene arrest in MLH1-deficient mice. *Cell* 1996, 85:1125–1134.

54. •• Dernberg AF, Sedat JW, Hawley RS: Direct evidence of a role for heterochromatin in meiotic chromosome segregation. *Cell* 1996, 86:135–146.
This paper uses novel cytological methods to investigate the mechanism of segregation of achiasmatic chromosomes during female meiosis I in *Drosophila*. The heterochromatin of homologous chromosomes remains associated from pachytene until metaphase I. The results indicate that, in the absence of chiasmata, homologous heterochromatin interacts to align homologues and orient them to opposite spindle poles.

55. •• Karpen GH, Le M-H, Le H: Centric heterochromatin and the efficiency of achiasmatic disjunction in ***Drosophila*** female meiosis. *Science* 1996, 273:118–122.
The *Dp1187* minichromosome and derivatives described in [6••,7•] are used to demonstrate that centric heterochromatin acts to allow the pairing, alignment and disjunction of these achiasmatic minichromosomes during female meiosis in *Drosophila*. The efficiency of disjunction is dependent on the amount of heterochromatin overlap between the two minichromosomes. Euchromatin and subtelomeric heterochromatin do not contribute to disjunction of *Dp1187*.

56. •• Dernberg AF, Broman KW, Fung JC, Marshall WF, Philips J, Agard DA, Sedat JW: Perturbation of nuclear architecture by long-distance chromosome interactions. *Cell* 1996, 85:745–759.
See annotation [57••].

57. •• Csink AK, Henikoff S: Genetic modification of heterochromatic association and nuclear organization in ***Drosophila***. *Nature* 1996, 381:529–531.
The *brown*^{Dominant} *(bw*^{D}*)* allele is a null mutation created by the insertion of a block of chromosome 2 centromeric satellite DNA into the coding region of the *brown* gene, which is required for normal eye pigmentation. This satellite DNA also causes *trans*-inactivation of the *brown* gene on the normal homologue of chromosome 2. Cytological observations demonstrate that in *bw*^{D}/*bw*$^{+}$ cells, the *brown* locus now associates with the centromeric heterochromatin of chromosome 2 which contains the offending satellite DNA and suggest that silencing of *bw*$^{+}$ on the homologous chromosome in these flies may be dependent on this association. Proteins involved in heterochromatin formation influence the association of *bw*^{D} with centric heterochromatin.

58. • Willams BC, Gatti M, Goldberg ML: Bipolar spindle attachments affect redistributions of ZW10, a ***Drosophila*** centromere/kinetochore component required for accurate chromosome segregation. *J Cell Biol* 1996, 134:1127–1140.
A conserved protein which shows dynamic association with kinetochores and kinetochore microtubules through prophase I and II to anaphase I and II but is left in the midzone at telophase. ZW10 function may be conserved as the human homolog shows similar localization (ML Goldberg, personal communication).

59. Liao H, Winkfein RJ, Mack G, Rattner JB, Yen TJ: CENP-F is a protein of the nuclear matrix that assembles onto kinetochores at late G_2 and is rapidly degraded after mitosis. *J Cell Biol* 1995, 130:507–518.

60. •• Connelly C, Hieter P: Budding yeast ***SKP1*** encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. *Cell* 1996, 86:275–285.
The *S. cerevisiae SKP1* gene was isolated as a high-copy suppressor of a temperature-sensitive mutation (*ctf13-30*) in the p58 component of the CBF3 centromere complex. Mutagenesis of the *SKP1* gene results in alleles which arrest cells in G_1/S or G_2/M and which can intragenically complement. The G_2/M allele loses chromosomes at elevated rates and arrests in pre-anaphase similar to the *ctf13-30* arrest. Overexpression of Ctf13p specifically rescues the temperature sensitivity and missegregation phenotypes of the G_2/M allele. Tagging of the SKP1 protein showed that it is associated with the CBF3 complex. (See also [61•].)

61. • Stemmann O, Lechner J: The ***Saccharomyces cerevisiae*** kinetochore contains a cyclin–CDK complexing homologue, as identified by ***in vitro*** reconstitution. *EMBO J* 1996, 15:3611–3620.
The authors of this paper describe the *in vitro* reconstitution of the *S. cerevisiae* CBF3 centromere complex in association with DNA. The p110 (*NDC10/CBF2/CTF140*) and p64 (*CEP3/CBF3b*) components were produced and purified in heterologous systems. However, p58 (CTF13/CBF3c) had to be purified from *S. cerevisiae* to allow reconstitution. A fourth component, p29 identical to *SKP1*, copurified with p58 and is necessary for CBF3 reconstitution. (See [60••,62••].)

62. •• Bai C, Sen P, Hofman K, Ma L, Goebl M, Harper JW, Elledge SJ: ***SKP1*** connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-Box. *Cell* 1996, 86:263–274.
This paper identified *S. cerevisiae SKP1* as an overexpression suppressor of the *cdc4-1* mutation. *CDC4* acts in concert with *CDC53* and *CDC34*

to degrade Sic1p, an inhibitor of Clb/CDC28 kinases. It is demonstrated that *SKP1* is required for the ubiquitin-mediated proteolysis of Cln2p, Clb5p and Sic1p. These and other proteins which interact with Skp1p contain an F-box motif, implicated in regulation by ubiquitin-mediated proteolysis. Again, alleles of *SKP1* are created which show defects in G_1/S or G_2/M transitions. (See also [61•,64•].)

63. • Willems AR, Lanker S, Patton EE, Craig KL, Nason TF, Mathias N, Kobayashi R, Wittenberg C, Tyers M: **Cdc53 targets phosphorylated G_1 cyclins for degradation by the ubiquitin proteolytic pathway**. *Cell* 1996, 86:453–463.

This paper ties in with [61•,62••] as it demonstrates unequivocally that the G_1 cyclin, Cln2p associates with Cdc53p and that *CDC53* is required for the ubiquitination and degradation of Cln2p. It is also shown that Cdc53 binds to the Cdc34 E2 ubiquitin conjugating enzyme. Cdc4p and Skp1p may be other components of this Cdc53p/Cdc34p ubiquitin–protein ligase complex targeting cyclins and other proteins for cell cycle regulated degradation.

64. Yoon H-J, Carbon J: **Genetic and biochemical interactions between an essential kinetochore protein, Cbf2p/Ndc10p, and the CDC34 ubiquitin-conjugating enzyme**. *Mol Cell Biol* 1995, 15:4835–4842.

65. McKee BD: **The license to pair: identification of meiotic pairing sites in *Drosophila***. *Chromosoma* 1996, 105:135–141.

66. Shelby RD, Hahn KM, Sullivan KF: **Dynamic elastic behavior of alpha-satellite DNA domains visualized *in situ* in living human cells**. *J Cell Biol* 1996, 135:545–557.

67. Harrington JJ, Van Bokkelen G, Mays RW, Gustashaw K, Willard HF: **Formation of de novo centromeres and construction of first-generation human artificial minichromosomes**. *Nat Genet* 1997, in press.