Annu. Rev. Genet. 2001. 35:673–745

DISSEMINATING THE GENOME: Joining, Resolving, and Separating Sister Chromatids During Mitosis and Meiosis

Kim Nasmyth
Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria; e-mail: nasmyth@nt.imp.univie.ac.at

Key Words

■ **Abstract** The separation of sister chromatids at the metaphase to anaphase transition is one of the most dramatic of all cellular events and is a crucial aspect of all sexual and asexual reproduction. The molecular basis for this process has until recently remained obscure. New research has identified proteins that hold sisters together while they are aligned on the metaphase plate. It has also shed insight into the mechanisms that dissolve sister chromatid cohesion during both mitosis and meiosis. These findings promise to provide insights into defects in chromosome segregation that occur in cancer cells and into the pathological pathways by which aneuploidy arises during meiosis.

CONTENTS

THE LOGIC OF MITOSIS AND ITS IMPLICATIONS FOR THE EUKARYOTIC CELL CYCLE 674
THE MOLECULAR BASIS OF SISTER CHROMATID COHESION 681
COHESIN: IS IT THE GLUE? 682
PROPERTIES OF COHESIN SUBUNITS 685
COHESIN IS RELATED TO CONDENSIN 688
ACTIVITIES ASSOCIATED WITH COHESIN AND CONDENSIN 688
LOADING COHESIN ONTO CHROMOSOMES 688
ESTABLISHING COHESION DURING DNA REPLICATION 689
THE SISTER CHROMATID SEPARATING PROCESS 692
SEPARATING SISTERS AT THE METAPHASE TO ANAPHASE TRANSITION 693
IS CLEAVAGE OF Scc1 UNIVERSAL? 695
DO OTHER PROTEINS NEEDED FOR SISTER SEPARATION FUNCTION VIA SEPARASE? 696
DOES SEPARASE HAVE OTHER TARGETS BESIDES Scc1? 697
SEPARASE REGULATION 698
RESOLVING SISTERS DURING PROPHASE 701
COHESIN DISSOCIATION 702

0066-4197/01/1215-0673$14.00

CONDENSIN ASSOCIATION 704
LINKING REREPLICATION WITH CHROMATID SEGREGATION 708
SEPARATING CHROMATIDS DURING MEIOSIS 708
DO MEIOTIC AND MITOTIC CELLS USE THE SAME OR A DIFFERENT COHESION MACHINERY? 709
COHESIN'S ROLE IN RECOMBINATION AND IN BUILDING MEIOTIC AXIAL CORES 710
CHIASMATA 715
ALIGNING HOMOLOGUES ON THE MEIOSIS I SPINDLE 717
RESOLVING CHIASMATA 722
ACHIASMATE CHROMOSOME SEGREGATION 723
RETAINING COHESION AROUND CENTROMERES 724
ANEUPLOIDY IN HUMANS 729
SUMMARY 732

THE LOGIC OF MITOSIS AND ITS IMPLICATIONS FOR THE EUKARYOTIC CELL CYCLE

In all existing living organisms, most cellular constituents, including all structural and enzymatic proteins and nucleic acids, are synthesized under instructions encoded in their genomes. The latter rarely participate directly in cell function and do so largely if not exclusively by encoding the enzymes that make the cell tick. It is hard to imagine that a distinction between "enzymatic" and "hereditary" material existed in our primordial ancestors, many of whose enzymes may have been RNAs duplicated using themselves as templates. Genomes presumably arose to ensure that the progeny of cell division inherited sufficient constituents to duplicate themselves, i.e., to solve the segregation problem. By encoding most instructions in a chemically stable form (DNA) that exists in one or only a few copies and is segregated with high fidelity to opposite poles of the cell prior to cell cleavage, our early ancestors achieved the "continuity of reproduction" that, along with mutation and selection, is an integral part of the Darwinian process. Accurate but not perfect reproduction is the raw material for evolution.

Though the mechanics of genome duplication (i.e., DNA replication) are highly conserved between bacteria and eukaryotic cells, those concerned with genome segregation have little or nothing in common. The mechanics of chromosome segregation during mitotis and meiosis in eukaryotic cells have few if any antecedents in bacteria. Bacteria clearly possess molecules that promote sister chromatid resolution [DNA gyrase and Smc-like proteins (80)], but there is no indication that they possess a cytoskeletal apparatus capable of connecting to chromosomes, let alone any mechanism for holding sister chromatids together for any significant period after their generation during DNA replication. How bacteria segregate their genomes to opposite poles of the cell without either cohesion or cytoskeletal apparatus remains mysterious. A vital clue to this mystery possibly lies in the fact that their chromosomes are replicated in a bi-directional manner from a unique origin. Instead of diverging from each other, the DNA polymerases at both forks remain in a

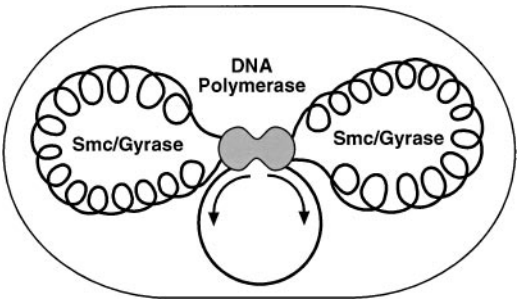
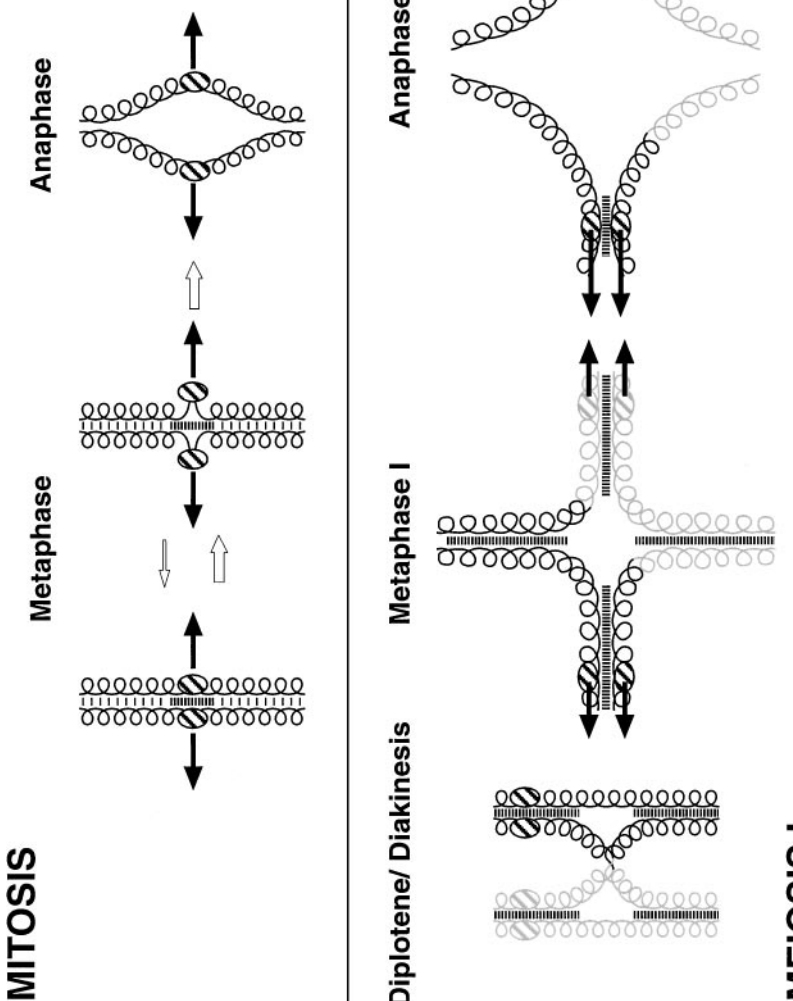


Figure 1 Bacterial chromosome segregation. The DNA polymerases associated with each end of a bi-directional replication fork remain associated and nascent chromatids that emerge are compacted through the action of DNA gyrase and Smc-like proteins.

single location in the middle of the cell (113) while nascent sequences, especially origins, rapidly move toward opposite poles (240). The implication is that bacterial chromosomes are replicated by a stationary "replisome" that uses the energy released by deoxynucleoside triphosphate hydrolysis to push nascent strands toward opposite cell poles (Figure 1).

According to this model, bacterial sister chromatids emerge from opposite sides of the replisome as loops whose distal tips contain the nascent origins. Negative supercoiling produced by DNA gyrase is thought to facilitate the packaging of each nascent chromatid into condensed nucleoid bodies via a process that is facilitated by Smc-like proteins (80). It is unclear how nascent chromatids are encouraged to move in opposite directions as they emerge from the replisome and how this is facilitated by specialized partition proteins (57, 116) that bind near origins in some but not all bacteria. What is abundantly clear, however, is that nascent origins move toward opposite poles of the cell soon after their generation and long before the bulk of the chromosome has been replicated. Thus, the bacterial equivalent of anaphase commences long before the completion of S phase and the phases of the bacterial cell cycle equivalent to the S and M phases of eukaryotic cells coexist. There is therefore no phase equivalent to G2 in the bacterial cell cycle. Though archaea possess DNA replication proteins that resemble eukaryotic ones, there is currently no evidence that their chromosomes are segregated using cohesion/cytoskeletal proteins resembling those used by eukaryotes (17).

Chromosome segregation in eukaryotes is based on a completely different principle. Both mitosis and meiosis rely on five fundamental processes (Figure 2). The first is a tubulin-based cytoskeletal apparatus (the mitotic spindle) capable of moving chromosomes around the cell by virtue of the attachment of microtubules to specialized chromosomal structures, called kinetochores (146). The second is a mechanism capable of holding together the sister chromatids produced by chromosome duplication (sister chromatid cohesion) (147). Without this, cells would not be able to ensure that their kinetochores attach to microtubules from opposite poles (bi-orientation) as opposed to the same poles (mono-orientation) (216). The third is a mechanism that detects whether sister kinetochores have indeed bi-oriented on



the mitotic spindle and destabilizes mono-oriented kinetochore-microtubule connections. The fourth is an apparatus that condenses chromatids (76) and partially resolves sisters from each other before the onset of chromatid separation. The fifth and last is an apparatus capable of severing once and for all the connections that hold sisters together while they are aligned on the metaphase plate, which triggers their poleward segregation during anaphase (154). Less crucial but nevertheless present in most eukaryotic cells is also a surveillance mechanism (checkpoint) (6) that blocks the destruction of sister chromatid cohesion when it detects "lagging" chromosomes, i.e., ones that have not yet bi-orientated on the mitotic spindle.

The complexities of the mitotic and meiotic processes make it easy to lose sight of their underlying logic. By some coincidence, there exists a riddle, whose origins have nothing to do with genetics, that illustrates very nicely the fundamental principle behind mitotic chromosome segregation. Two blind men enter the same department store and each orders five pairs of socks, each pair having a different color. The shop assistant is so confused by this coincidence that he/she places all ten pairs of socks (two red pairs, two blue pairs, etc.) into a single bag and sends one blind man off with all ten pairs and the other with none. By some miracle, the two blind men meet in the street as they leave the department store and discover that one has the other's socks. The question is: How do they sort out the muddle without any outside help? There is a simple solution to their problem. Socks are, of course, sold only as pairs that are joined together. As each pair of socks is removed from the bag, the two socks in each pair are pulled in opposite

Figure 2 Sister chromatid cohesion has a crucial role during mitosis and meiosis. Mitosis: Chromatids (*coils*) are held together by cohesin (*horizontal dashes that connect sister chromatids*), which is enriched in the vicinity of centromeres when sister kinetochores (*hatched ovals*) attach to microtubules of opposite polarity (*arrows*). During metaphase, traction exerted by microtubules on kinetochores tends to split them apart but this is resisted by cohesin concentrated in the surrounding chromatin. Anaphase is initiated by the dissolution of cohesion throughout the chromosome, which takes place due to cleavage of cohesin's Scc1 subunit by separase. Meiosis I: reciprocal crossing over between a maternal (*light gray coil*) and paternal (*black coil*) chromatid links homologous chromosomes together. These crossovers are known as chiasmata. Sister kinetochores attach to microtubules with the same polarity and as a result maternal and paternal sister kinetochore pairs are pulled toward opposite poles at the first meiotic division. During metaphase I, sister chromatid cohesion distal to the chiasmata holds homologous chromosomes together as they are pulled toward opposite poles. Chiasmata are resolved by the dissolution of cohesion along chromosome arms (due to cleavage of cohesin's Rec8 subunit by separase?), which triggers the first meiotic division. Meanwhile, cohesin in the vicinity of centromeres is protected from separase, and sister centromeres therefore remain connected until they are aligned on the meiosis II spindle.

directions by the two blind men, one of whom brandishes a pair of scissors with which he cuts the material connecting each pair. Each blind man deposits the separated socks in his own bag, which will eventually contain two red socks, two blue socks etc... once all ten pairs of socks have been separated. This then is also the logic of mitosis. The blind men pulling on each pair of socks before they are cut apart is analogous to chromosomes being aligned on the mitotic spindle, whereas their severance is analogous to the process that separates sister chromatids at the onset of anaphase. The analogies may extend even deeper, as both blind men and cells are also confronted with the problem of how they ensure that each pair of socks/chromatids are pulled in opposite directions (bi-orientation). Though we do not yet understand how cells ensure that sister kinetochores attach to spindles with opposite polarity, the analogy with the blind men and their socks would suggest that tension generated as a consequence of bi-orientation might stabilize kinetochore-microtubule connections. Indeed, there is good evidence that this is the case (157). This riddle emphasizes that mitosis is a double act that depends as much on the connections that hold sister chromatids together as it does on the cytoskeletal apparatus that actually pulls them to opposite poles of the cell during anaphase.

Sister chromatid cohesion not only makes "mitotic" chromosome segregation possible but also permits it to take place long after chromatids have been generated during S phase. The connections that hold sister chromatids together during G2 and early M phases in eukaryotic cells are, as it were, the marks by which these cells remember which chromatids are merely homologous and which are the "sister" products from the most recent round of DNA replication. Most eukaryotic cells are diploid, and homology would not be an adequate criterion for the disjunction of chromatids to opposite poles. The cohesion apparatus is sufficiently robust that in extreme cases, as in human oocytes, chromatids can still be segregated many decades after they were joined together during premeiotic DNA replication. The temporal separation of chromosome duplication and segregation in eukaryotes and hence the conventional division of their cell cycles (Figure 3) into four discrete phases (G1, S, G2, and M) is unthinkable without sister chromatid cohesion. Its absence in their bacterial cousins is presumably the reason why bacteria have little choice but to link chromosome segregation to duplication.

The temporal separation of S and M phases is in large measure responsible for much of the flexibility of eukaryotic cell cycles. It creates, for example, an opportunity to check whether the chromosome duplication process, during which DNA damage is easily generated, has been completed successfully before embarking on chromosome segregation. As one of the most powerful methods of repairing DNA damage is recombination involving an undamaged sister chromatid, it is important that cells do not separate sisters before damage has been repaired (67). Eukaryotic cells therefore possess numerous surveillance mechanisms (checkpoints) that prevent chromosome segregation if DNA is damaged or if replication is not complete. Such checkpoints either block entry into mitosis, that is, they arrest cells in G2 (211), or they prevent the onset of anaphase, that is, they arrest cells

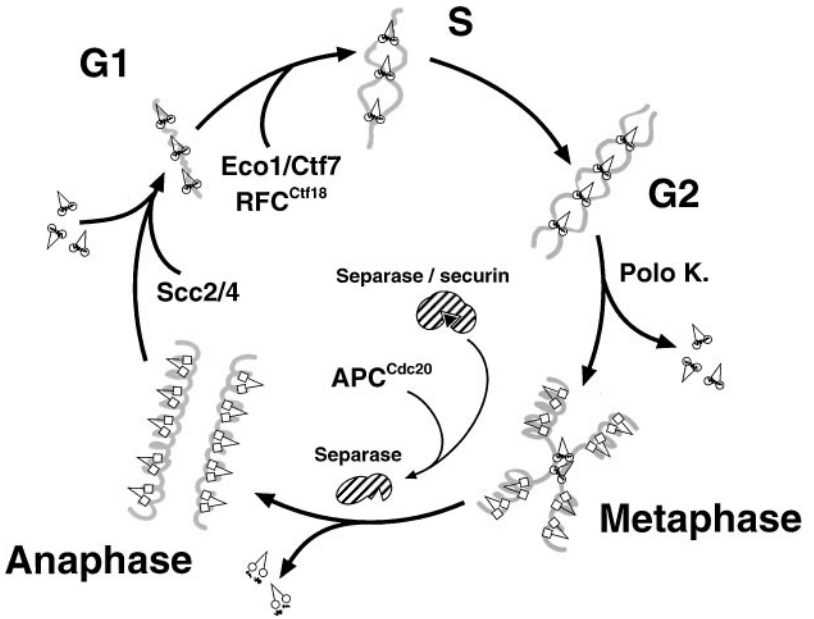


Figure 3 The cohesin/condensin cycle. During telophase, condensin (*Vs with squares at each end*) dissociates from chromatids (*gray lines*) whereas cohesin (*Vs with circles and wavy black line representing Scc1*) associates with them. The binding of cohesin to chromatin depends on a complex composed of Scc2 and Scc4. Bridges between chromatids are built with the help of Eco1/Ctf 7 and RF-C(Ctf18) during passage of replication forks during S phase. Activation of mitotic protein kinases during prophase causes most cohesin to dissociate from chromosomes and triggers the binding and activity of condensin, which resolves sister chromatid arms from each other. Dissociation of cohesin depends on Polo-like kinases, whereas the association of condensin may depend on Aurora B. A small fraction of cohesin persists, largely in the vicinity of centromeres, until all chromosomes have aligned on the mitotic spindle in a bipolar fashion and congressed to the metaphase plate. This inactivates the Mad2-dependent mitotic checkpoint and triggers proteolysis of securin and B-type cyclins by the Anaphase-promoting complex (APC), which activates separase and causes cleavage of Scc1 residing at centromeres.

in metaphase (31). Neither type of checkpoint would be possible without sister chromatid cohesion.

The separation of S and M phases made possible by sister cohesion facilitates another key innovation: the packaging of eukaryotic genomes into a highly condensed and largely inactive state during the chromosome segregation process. The huge genomes of many plants and metazoa could possibly not be segregated into opposite halves of the cell at mitosis were their DNA not highly compacted.

However, the degree of compaction required is largely incompatible with transcription and presumably also DNA replication. Furthermore, the opening up of chromatin associated with transcription and replication would greatly compromise the compaction needed for segregation, except in organisms like yeast with very small genomes. By delaying chromosome segregation until well after duplication has been completed, eukaryotic cells ensure that DNA replication, transcription, and repair proceed while their chromatin is in an open or extended conformation and that mitosis only proceeds after their chromatin has been packaged into a highly condensed state. This fundamental fact has been recognized ever since chromosomes were first detected. Before the discovery of DNA replication, the eukaryotic cell cycle was divided into two phases: mitosis when chromosomes were condensed, and hence visible, and interphase when they were dispersed throughout the nucleus, and hence invisible (130). It is unclear whether the ability to compact chromosomes during mitosis was responsible for the subsequent accumulation of so much junk DNA within our genomes or whether it evolved to deal with this threat to mitosis.

Chromosome duplication and segregation are separated in eukaryotic cells functionally as well as temporarily. Origins of DNA replication and kinetochores have little or no functional connection with each other. Furthermore, kinetochores clearly function without ongoing DNA replication. This has liberated eukaryotic cells from the constraint or tyranny of needing to replicate their chromosomes using a single origin of DNA replication, as occurs in bacteria where chromosome segregation appears to be an integral part of the duplication process. The functional consequences of this emancipation have been wide ranging. Multiple origins per chromosome facilitate duplication of far larger chromosomes/genomes, which has presumably contributed to our ability to carry around a huge surfeit of junk DNA. It also permits a far more rapid execution of genome duplication than is possible in bacteria, without which embryonic cleavage divisions and hence most embryonic development in metazoa would not be possible.

The use of a cytoskeletal/cohesion process to segregate chromosomes is also responsible for another salient feature of the eukaryotic cell cycle: the invariant dependence, at least in the germline, of the reduplication of chromosomes on the segregation of sister chromatids generated during a previous round of duplication. Such linkage is not observed in bacteria, where reinitiation of chromosome duplication often commences before the previous round of duplication has been completed (41). Indeed, their ability to perform this is crucial if their doubling time is to be shorter than the time it takes to complete a single round of duplication (which is 40 min. in *Escherichia coli*). The blind men's game only works if socks are packaged as pairs. Because cohesion between sister chromatids is crucial for the attachment of sister kinetochores to microtubules pointing toward opposite poles, kinetochore reduplication prior to mitosis would create a terrible ambiguity as to which pair of chromatids should be pulled in opposite directions. Though eukaryotic cells frequently reduplicate their genomes without an intervening round of chromosome segregation (a phenomenon known as endo-duplication), they

rarely if ever attempt to undergo mitosis after endo-duplication. Endo-duplication is therefore confined to somatic cells that will never contribute to the germline and have no need to regain a diploid state. The broad outlines of the mechanism by which eukaryotic cells link chromosome reduplication to the segregation process are starting to be understood (see below).

Because sister chromatid cohesion is an integral part of the chromosome segregation process and because this cohesion can only be generated during chromosome duplication, it is vital that eukaryotic cells never attempt to segregate chromosomes without their prior duplication. The dependence of M phase on S phase is therefore partly structural. Surveillance mechanisms may ensure that the attempt is rarely made in normal cells, but it could not be successful even if attempted. There is, of course, one great exception to this rule. Meiotic cells successfully undergo two rounds of chromosome segregation without any intervening round of chromosome duplication and thereby produce haploid progeny from diploid cells. They manage this extraordinary feat by using cohesion along chromosome arms for the first meiotic division and cohesion close to centromeres for the second division (26, 122), a clear case of the exception proving the rule.

THE MOLECULAR BASIS OF SISTER CHROMATID COHESION

A key question has long been whether the connections holding sister chromatids together during G2 and M phase are mediated by special proteins or simply by DNA. It has been suggested in the past, for example, that cohesion could be due either to late replication of centromeric DNA or to the intercatenation of sister DNA molecules arising from the conjunction of adjacent replication forks (152). The first of these two hypotheses was questioned by the finding that centromeres are not particularly late-replicating (33) and the second by the finding that sister chromatids from circular minichromosomes yeast are fully decatenated by Topoisomerase II despite remaining closely cohered in cells arrested in a mitotic state by agents that destabilize microtubules (106).

It is only very recently, however, that specific cohesion proteins have been identified, largely owing to the isolation of yeast mutants unable to maintain sister chromatid cohesion in cells arrested in mitosis (155). These genetic studies have now implicated six distinct classes of proteins in generating or maintaining cohesion (Table 1): (*a*), a four-subunit complex called cohesin, which possibly mediates connections between sisters (63, 118, 144, 222); (*b*) a protein called Pds5, which associates with cohesin on chromosomes (66, 160, 206, 228); (*c*) a separate complex containing at least two subunits, Scc2 and Scc4, which is necessary for cohesin's stable association with chromosomes (28); (*d*) Eco1/Ctf 7, which is neither associated with cohesin nor necessary for its association with chromosomes but is nevertheless essential for generating cohesion during DNA replication (198, 222); (*e*) a large complex related to RF-C (Replication factor C), which though not

TABLE 1 Names of cohesin and securin/separase subunits in various organisms[a]

	Cohesin subunits				Cohesin associated	Securin/separase	
S. cerevisiae	***SMC1***	***SMC3***	***SCC1(MCD1)*** ***REC8***	***SCC3***	***PDS5***	***PDS1***	***ESP1***
S. pombe	***PSM1***	***PSM3***	***RAD21*** ***REC8***	***PSC3*** ***REC11***	***PDS5***	***CUT2***	***CUT1***
A. nidulans		***SUDA***			***BIMD***		***BIMB***
S. macrospora					***SPO76***		
C. elegans	***HIM1***		***COH1,2*** ***REC8***				***SEP1***
D. melanogaster			***RAD21***			***PIMPLES***	***SSE***
A. thaliana			***SYN1, DIF1***				
X. laevis	***XSMC1***	***XSMC3***	***XRAD21***	***SA1, SA2***	***PDS5***	***SECURIN***	***SEPARASE***
H. sapiens	***SMC1*** ***SMC1β***	***SMC3***	***SCC1(RAD21)*** ***REC8***	***SA1, SA2*** ***STAG3***	***PDS5***	***SECURIN***	***SEPARASE***

[a]Where appropriate, meiosis-specific variants are written below their mitotic counterparts. Though there is no need to change actual gene names, it is hoped that future authors will agree to a common nomenclature for the proteins that they encode. Thus, the *ESP1*, *cut1*, *BimB*, *SEP1*, and *SSE* genes all encode separases whereas *SMC1*, *PSM1*, and *HIM1* all encode Smc1 proteins.

essential for cohesion is necessary for its efficient generation (65, 128); and (*f*) DNA polymerase kappa, which is likewise necessary for efficient cohesion (234). Because orthologues of these proteins have been found in all fully sequenced eukaryotic genomes, it is likely that the mechanism by which sister chromatids are bound together is universal and has been inherited from the common ancestor of all eukaryotic cells. With the exception of cohesin's two Smc subunits, none of these proteins have obvious relatives in bacteria or archaea, in which sister chromatid cohesion has yet to be detected.

COHESIN: IS IT THE GLUE?

Cohesin contains four polypeptides: Smc1, Smc3, Scc1 (also known as Rad21 and Mcd1), and Scc3. Somatic cells in vertebrates express two types of Scc3 subunit, which are called SA1 and SA2 (Table 1). All four cohesin subunits form a soluble complex when not bound to chromatin (120, 206, 222) and colocalize in an interdependent manner to discrete sites on chromatin. This raises the possibility, but does not prove, that all four polypeptides always act together. Their necessity for sister chromatid cohesion was first demonstrated in the budding yeast *Saccharomyces cerevisiae*, where most if not all sister DNA sequences remain closely tethered together until metaphase. Yeast cells can be arrested at this stage of the cell cycle by inactivation of a ubiquitin protein ligase called the Anaphase-promoting complex (APC), which mediates the destruction of mitotic cyclins and anaphase inhibitory proteins called securins. APC inactivation prevents their proteolysis, and

the persistence of cyclinB/Cdk1 activity prevents exit from mitosis, while securin blocks the apparatus that destroys cohesion at the metaphase to anaphase transition (see below). Mutational inactivation of any one of cohesin's subunits permits sister chromatid separation, even in the absence of APC activity. An involvement of these proteins in cohesion has since been confirmed in a variety of organisms during meiosis as well as mitosis. Thus, cohesin's depletion from extracts prepared from *Xenopus* oocytes reduces sister chromatid cohesion (118), as do mutations in *SCC1*-like genes in fission yeast (221) and *Arabidopsis thaliana* (12, 20). Furthermore, inactivation of a meiosis-specific version of Scc1 called Rec8 causes loss of sister chromatid cohesion following premeiotic DNA replication in *S. cerevisiae* (104) and *Schizosaccharomyces pombe* (148). Remarkably, inactivation of Rec8's orthologue in *Caenorhabditis elegans* by RNA interference causes the appearance of up to 24 chromatids at diakinesis/metaphase I instead of six bivalents (163).

Unlike some other proteins required for sister chromatid cohesion, cohesin subunits are required both to generate cohesion during DNA replication and to maintain cohesion between chromatids during metaphase (28, 63, 160). By turning off expression of the APC activator protein Cdc20 (see under separase regulation), it is possible to arrest either wild-type or ts *scc1* mutant yeast cells in metaphase with sister chromatid arms closely connected. Subsequently raising the temperature causes sisters to dissociate in mutant but not wild-type cells. This is consistent with the notion that cohesin may actually be part of the bridge that holds sisters together as they come under tension from the mitotic spindle.

It is, however, almost impossible to demonstrate a direct role for a protein in a given process merely by analyzing the phenotypic consequences of its inactivation. For this reason, a crucial breakthrough in this field was the observation that Scc1 is tightly associated with yeast chromatin during metaphase but suddenly disappears at the onset of anaphase (144). Subsequent analysis of this phenomenon led to the discovery that Scc1 is released from yeast chromatin due to proteolytic cleavage by a cysteine endopeptidase called separase. Furthermore, Scc1's cleavage is both necessary and sufficient to trigger anaphase (224, 226). Thus, not only genetics but also physiology points to Scc1 being the real McCoy.

If cohesin does indeed connect sisters, then it should be found at sites where sister chromatids are tightly connected. This does indeed appear to be the case, albeit only at a low level of resolution. Thus, Scc1 is concentrated between chromatids in the vicinity of centromeres in human tissue culture cells during metaphase and is clearly less abundant along chromosome arms, which are less tightly connected (83, 118, 229). Likewise, meiosis-specific forms of Scc1, Smc1, and Scc3 called Rec8, Smc1β, and STAG3, respectively, are all found between chromatids during diakinesis and metaphase I, that is, during the periods of meiosis when chiasmata and chromosome arm cohesion are vital for holding homologous chromosomes together on the metaphase I spindle (C. Heyting, personal communication; 104, 171, 176, 238). Most impressive of all, Rec8 disappears from chromosome arms at the onset of anaphase I, as cohesion is lost from these chromosomal regions but persists in the vicinity of centromeres until the onset of anaphase II, both

in yeast (*S. cerevisiae* and *S. pombe*) and in mammals. Despite these impressive cytological observations linking cohesin's chromosomal distribution with cohesion itself, the direct colocalization of cohesin with bridges connecting sisters at a molecular level remains one of the holy grails in this field.

This issue has also been addressed by the identification, using chromatin immunoprecipitation, of sites along yeast chromosomes to which cohesin subunits are bound. In *S. cerevisiae*, cohesin is found at centromeres and in their vicinity and at specific loci, every 5–10 kb, along chromosome arms (22, 138, 215). Both types of site are sufficient to recruit cohesin to regions of the chromosome that normally lack cohesin, which raises the question whether they can also confer cohesion between sister chromatids. Though cohesin at centromeres and in their immediate vicinity clearly helps to promote kinetochore bi-orientation (216), possibly by providing cohesion, it is surprisingly incapable of resisting the movement of sister kinetochores toward opposite poles during metaphase (60, 72, 216). Centromeres, presumably due to their recruitment of cohesin, are nevertheless capable of conferring cohesion between sisters in the presence of drugs that destabilize microtubules (138). The implication is that the cohesin present at budding yeast centromeres is incapable of preventing the traction of sister sequences toward opposite poles once kinetochores have bi-oriented on the spindle. This could be taken to mean either that cohesin does not in fact confer cohesion between chromatids (37) or that the cohesion conferred by cohesin is insufficient to counteract the splitting force exerted in the immediate vicinity of bi-oriented kinetochores. Time lapse microscopy shows that sister centromeres but not arm sequences separate soon after formation of bipolar spindles in *S. cerevisiae* and that they only occasionally rejoin before the onset of anaphase proper (216). Such "breathing" of sister sequences in the vicinity of centromeres during metaphase is also seen in protozoa, insect cells, and even in mammalian cells and may therefore be a quite general phenomenon (139, 190, 220). In yeast, this precocious sister chromatid separation during metaphase extends for about 10 kb around centromeres and is accompanied by considerable stretching of the chromatin (72, 216), which is presumably unraveled down to nucleosomes (164). Because partial inactivation of Scc1 enlarges the interval that can be separated by bi-oriented kinetochores (T. Tanaka, personal communication), the splitting process is presumably halted by cohesin bound to flanking arm sequences. It is tempting to speculate that tension exerted along the chromsosome increases cohesion within the arms, as would be the case if one would try to separate two intertwined rubber bands. It is otherwise unclear how chromosomes prevent themselves being ripped apart once the first opening has been generated. According to this model, it is cohesion within sequences flanking kinetochores and not at kinetochores themselves that bears the brunt of the load in resisting the complete separation of sister chromatids during metaphase. It is therefore interesting that in *S. pombe*, whose centromeres are larger and more complex than those of *S. cerevisiae*, Scc1 (Rad21) is found not in the inner centromere region to which kinetochore proteins bind and at which microtubules presumably exert their action but rather in the outer centromere regions that flank these (239).

It is possibly because rather extensive regions of cohesion are necessary to oppose the spindle that insertion of individual cohesin association sites only modestly delays centromere splitting, even when present as tandem arrays (215). In conclusion, the study of cohesion association sites, though consistent with the notion that cohesin provides the connections between sister chromatids, has yet to provide a truly conclusive experiment that settles this issue once and for all.

The case for cohesin being the glue that holds sisters together is clearly a strong one: it is the only protein clearly required for cohesion that is at the right places at the right times. Furthermore, proteolytic cleavage of its scissile Scc1 subunit is both necessary and sufficient for triggering chromatid separation. How then might cohesin produce bridges between sister chromatids? What is cohesin's structure and does it alone possess activities consistent with building bridges between chromatids? Studies that address these issues are still in their infancy.

PROPERTIES OF COHESIN SUBUNITS

Cohesin's Smc1 and Smc3 subunits are both members of the SMC (structural maintenance of chromosomes) family of proteins, which are common to bacteria, archaea, and eukaryotes and have roles in chromosome condensation, sister chromatid cohesion, and DNA repair (74). All SMC proteins share five conserved domains: three globular domains separated by two long stretches of coiled-coil interrupted by a hinge region. Both X-ray crystallography (121) and electron microscopy (140) suggest that bacterial and archaeal Smc proteins are homodimers whose coiled coils are antiparallel and bring together the globular N- and C-terminal domains. These contain Walker A and B motifs, respectively, whose appropriate alignment is thought to create an active ATPase of the ABC type frequently found in membrane transporters. The Walker B motif constitutes a nucleotide-binding pocket. SMC proteins therefore form V-shaped molecules that can open or close by virtue of their flexible hinge region. Closure would bring two N- and C-terminal domain pairs together, which by bringing Walker A and B motifs into juxtaposition could modulate ATPase activity as well as create a DNA binding domain (82) (Figure 4*a*, *b*).

The coiled-coils of SMC proteins could either be intramolecular, in which case the N- and C-terminal domains from the same molecule would associate with each other and connections between subunits would be confined to the hinge region, or intermolecular, in which case the N-terminal domain of one Smc molecule would associate with the C-terminal domain of its partner in the complex (Figure 4*a*). According to the first geometry, the two halves of the V-shaped complex would merely be connected by homotypic interactions between the hinge regions of each Smc subunit, whereas according to the second geometry, the two halves would be connected by two continuous polypeptide chains that run in antiparallel manner from one end of the V to the other. Because it contains equal amounts of two different Smc proteins (Smc1 and Smc3), it is suspected that cohesin contains

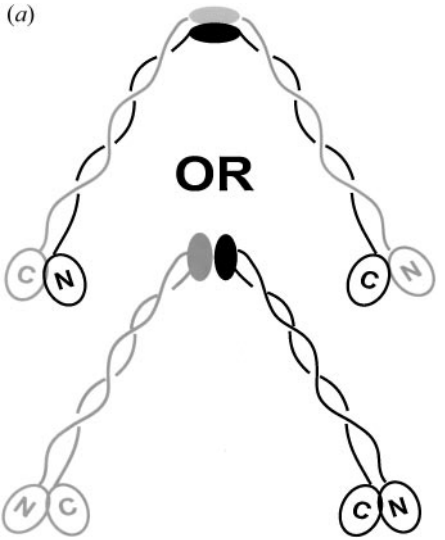


Figure 4 Potential geometries of cohesin's Smc proteins. *A*, cohesin contains an Smc1/Smc3 heterodimer, whose two long stretches of coiled coil are either intermolecular (*above*) or intramolecular (*below*). In the first case, both Smc1 and Smc3 molecules stretch from one end of the V to the other end, whereas in the second case, Smc1 constitutes the left branch and Smc3 the right one. *B*, one possible mechanism by which the Smc1/Smc3 heterodimer might cooperate with Scc1 (*wavy gray lines* containing separase cleavage sites marked by an arrow) to generate bridges between sister chromatids (*thick dark lines*). *Top*: cohesin in an open configuration before it associates with chromatin. *Middle right*: cohesin clasps a DNA duplex or chromatin fiber. The Scc1 subunit, presumably with the aid of Scc3 (SA1, SA2) and Pds5, locks the chromatin's embrace by the Smc1/Smc3 heterodimer. *Bottom*: both sister chromatids are embraced by cohesin after passage of a replication fork through a "closed" cohesin complex. *Middle left*: The cohesin complex can be opened either by phosphorylation of Scc1, Scc3-SA1/SA2, or Pds5 as occurs during prometaphase or as shown by cleavage of the Scc1 subunit. The crystal structures of SMC head domains suggest that left and right Smc1/Smc3 head domains can bind ATP but that hydrolysis might only occur when both head domains are brought together as in the closed configuration. ATP binding and hydrolysis might therefore regulate the opening and closing of cohesin complexes. Closure around a chromatin fiber might also depend on the Scc2/4 complex. Condensin and DNA repair proteins like Rad50 might operate using a similar principle. For example, condensin might close around adjacent coils of the same chromatin fiber.

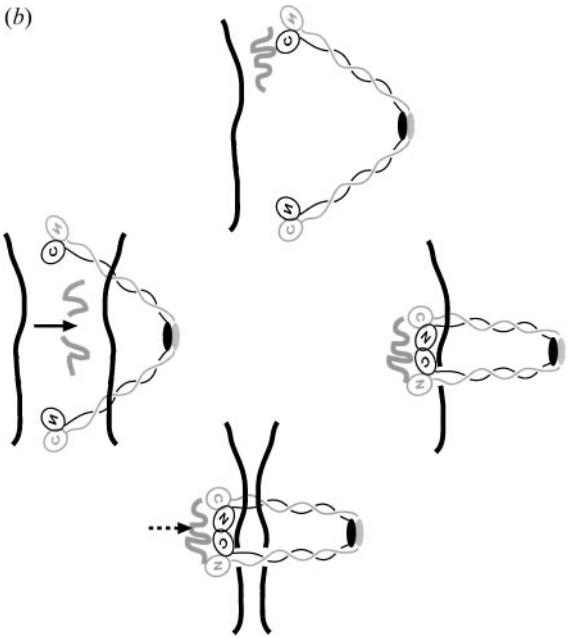


Figure 4 (*Continued*)

an Smc1/Smc3 heterodimer that would be pseudo-symmetrical if its coiled-coils were intermolecular but asymmetric if they were intramolecular. A crucial question is whether cohesion is mediated by Smc1/Smc3 heterodimers in an open or closed configuration. An open heterodimer would bridge the gap between sisters (76, 154), whereas a closed heterodimer could form a ring around them (see Figure 4*b*).

The sequence of cohesin's Scc1 subunit has thus far shed less insight into its structure. Its N- and C-terminal domains are conserved but its central domain, which contains its separase cleavage sites, is much less so and may be rather unstructured. Scc1 must nevertheless be the lynchpin of the cohesin complex because its cleavage by separase causes the sudden dissolution of cohesion at the metaphase to anaphase transition (226).

Though not strictly a subunit, because it is less stably associated with the soluble form of the complex, the Heat repeat containing protein Pds5 (156, 160) clearly has an intimate connection with cohesin. Like Scc1, it is essential for maintaining sister chromatid cohesion during mitosis in *S. cerevisiae* (66, 160) and during meiosis in Sordaria (228). Furthermore, it associates with the same chromosomal sites as cohesin subunits and is released from chromosomes at the metaphase to anaphase transition due to Scc1's proteolytic cleavage. Pds5 presumably interacts directly with cohesin because it can sometimes be coprecipitated with cohesin subunits (206). Pds5 might not be as crucial to cohesion as other cohesin subunits because

it is not an essential gene in the *S. pombe*. Though *pds5* mutants are viable in *S. pombe*, they are defective in maintaining cohesion between sister chromatids during a prolonged G2 arrest (K. Tanaka, personal communication).

COHESIN IS RELATED TO CONDENSIN

Most if not all eukaryotic genomes contain at least two other SMC proteins, Smc2 and Smc4, which are more closely related to Smc1 and Smc3, respectively, than they are to any other members of this family. Remarkably, Smc2 and Smc4 also form a heterodimer that is part of a separate multisubunit complex, called condensin (75), which has an important role in the condensation and resolution of sister chromatids between prophase and metaphase (78). The implication is that unlike bacteria, whose SMC proteins form homodimers, a common ancestor of eukaryotic cells possessed an Smc heterodimer whose duplication led to the evolution of cohesin- and condensin-specific Smc heterodimers. Condensin contains three other subunits: barren/Xcap-H, X-cap-D2, and X-cap-G. It is striking that condensin's D2 and G subunits are, like Scc2 and Pds5, composed of Heat repeats. This raises the possibility that some of these proteins are descended from an ancestral complex containing not only an Smc heterodimer but also Heat repeat–containing proteins.

ACTIVITIES ASSOCIATED WITH COHESIN AND CONDENSIN

Purified condensin and cohesin have both been associated with activities in vitro that might be relevant to their functions in vivo. While condensin is capable of imparting global positive writhe to circular DNA in an ATP-dependent manner (96), cohesin is capable of aggregating DNA molecules in a manner that facilitates intermolecular catenation in the presence of Topo II (119). Unlike the positive writhing induced by condensin, the DNA aggregation produced by cohesin in vitro is ATP-independent, and it is therefore unclear whether it requires Smc1 and Smc3 or is merely a property of its Scc1 subunit. Cohesin's ability to aggregate separate DNA molecules could clearly be relevant to its ability to hold sister chromatids together and is consistent with the notion that this complex does indeed mediate the connections between sisters. Nevertheless, further studies will be needed to demonstrate the physiological relevance of cohesin's aggregation activity, especially as it requires a very large excess of cohesin to DNA.

LOADING COHESIN ONTO CHROMOSOMES

Among the most frequent *S. cerevisiae* mutants with cohesion defects are those with mutations in the *SCC2* gene (144, 222). Mutations in its *S. pombe* orthologue *mis4* also cause cohesion defects (52), whereas mutation of the related Nipped B

protein in *Drosophila. melanogaster* causes defects in long-range enhancer promoter interactions (181), and mutation of its orthologue in Ascobolus, called Rad9, causes defects in DNA repair and in meiosis (188). Scc2 is neither stoichiometrically associated with cohesin nor does it appear to associate stably with the same sites on chromatin in vivo (28). In *S. cerevisiae*, Scc2 is stably bound to a 78-kd protein called Scc4, which is also required for sister chromatid cohesion. Inactivation of Scc2 or Scc4 in *S. cerevisiae* (28) or Mis4 in *S. pombe* (52) greatly reduces the amount of cohesin associated with chromosomes, which implies that the Scc2/Scc4 complex is crucial for some as yet ill-defined aspect of cohesin's function. Possibly it catalyzes the formation of complexes between cohesin and chromatin.

Most evidence suggests that cohesin can be loaded onto chromosomes at all stages of the cell cycle apart from mitosis in organisms whose chromosomes are extensively condensed at this stage. Thus, in mammalian cells, cohesin is found stably associated with chromatin throughout interphase; it dissociates from chromosomes during prophase, reassociates during telophase, and remains on chromosomes until cells reenter mitosis (118, 206). In *S. pombe*, where mitotic chromosome condensation is rather modest, Scc1 (Rad21) is associated with chromosomes at all cell cycle stages apart from anaphase (221). In *S. cerevisiae*, where the bulk of cohesin also remains tightly associated with chromosomes until anaphase, cohesin is absent from yeast chromosomes for much of G1 (144). This is due both to a lack of *SCC1* transcription and continued proteolysis by separase during this stage of the cell cycle, and Scc1 readily binds to yeast chromosomes during G1 when ectopically expressed in separase mutants (224). Scc1 can even bind stably to yeast chromosomes when expressed in G2, though it cannot promote sister chromatid cohesion under these circumstances (225). The dependence of cohesin's association with chromosomes on a separate Scc2/Scc4 complex suggests that the structures formed between cohesin and chromatin might have a very special geometry, even when this process occurs outside S phase and does not involve the formation of sister chromatid cohesion. Cohesin is associated with chromosomes in quiescent as well as proliferating mammalian cells, which raises the possibility that it might be a key determinant of chromosome structure during G1 as well as during G2 (206).

ESTABLISHING COHESION DURING DNA REPLICATION

In yeast, whose chromosomes are not visible during mitosis by conventional cytological techniques, sister chromatid cohesion has been measured either using FISH (62) or by visualizing the location of Tet (144) or Lac (201) repressor proteins fused to GFP, which are bound to tandem operator arrays inserted in various locations within the genome. As observed by either method, most if not all sister sequences remain tightly associated, at least at the resolution of light microscopy, from their production during DNA replication until their separation at the onset of anaphase. The only exception to this rule is the precocious separation during

metaphase of sequences within 5 kb of centromeres, which occurs soon after sister kinetochores bi-orient on the mitotic spindle (60, 72, 216). One of the implications of these findings is that sites of sister chromatid cohesion are rather frequent along yeast chromosomes, which fits with the observation that sites associated with cohesin are found every 5 to 10 kb. The situation is more complicated in animal cells where most sequences along chromosome arms can be resolved using FISH in G2 or early M phase cells (189), which suggests that cohesion sites might be much rarer than in yeast. Sister centromere sequences, on the other hand, remain closer to each other, at least until chromosomes align on the spindle during metaphase. There are indications, however, that some sister sequences may be very closely connected even in animal cells, for a brief period after their replication. By measuring whether cells have two or four signals, FISH has been used extensively with a view to determining replication timing (100). The problem with this approach is that "two signals" could arise either because sequences have not yet replicated or because sister sequences are so closely connected that they cannot be distinguished, as is the case in yeast. Indeed, a recent study using BrDU labeling to determine replication timing found that some but not all sequences that were "late" in producing four FISH spots were in fact early replicating (Azuara, Brown & M. Fisher, personal communication). The implication is that some but not all sequences remain closely connected with their sisters for an appreciable period after replication.

Merely looking at the association of sister sequences does not, however, address whether cohesin has established links between them or whether they are connected in a manner capable of resisting the mitotic spindle. It is perfectly conceivable, for example, that sister sequences remain close together soon after their replication due to the intertwining of sister DNA molecules (152) and that cohesin only produces proteinaceous links between chromatids after replication has been completed. An alternative approach has therefore been to address when cohesin is required during the cell cycle. Two studies, one varying the timing of *SCC1* expression during the mitotic cell cycle (225) and a second varying the timing of *REC8* expression during meiosis (239), found that neither gene can fulfil its function when expressed after DNA replication. Cohesion cannot be established between sister chromatids by Scc1 protein produced immediately after replication has been completed even though the protein is fully capable of stably associating with chromosomes under these circumstances. The simplest explanation is that cohesin can only build connections between sisters as they emerge from replication forks. One mechanism by which this could occur is shown in Figure 4*b*. However, these experiments do not exclude the possibility, albeit an improbable one, that cohesin is simply inactive when produced after S phase.

The notion that cohesive structures built by cohesin can only be produced during S phase is supported by the phenotype of *eco1/ctf7* mutants. *ECO1* is an essential gene that is crucial for establishing cohesion between chromatids during S phase (198, 222). The Eco1 protein is neither part of the cohesin complex nor does it stably colocalize with cohesin on chromosomes. *eco1/ctf7* mutants

cannot establish cohesion between chromatids during S phase even though cohesin associates in normal amounts with chromosomes. Furthermore, unlike ts cohesin mutants, shifting ts *eco1* mutants to the restrictive temperature only after they have already undergone DNA replication at the permissive temperature does not destroy sister chromatid cohesion. This implies that Eco1/Ctf 7 may be required to establish cohesion during DNA replication but not to maintain cohesion during G2 or M phases. The partial suppression of *ctf 7* mutants by increased expression of PCNA (198) is also consistent with Eco1 acting during DNA replication. The phenotype of *eco1/ctf 7* mutants implies that the mere presence of cohesin on chromatin while it is being replicated is insufficient to establish cohesion. Though there is as yet no evidence that Eco1/Ctf 7 acts directly on cohesin, Eco1/Ctf 7 presumably facilitates whatever special function cohesin performs soon after the passage of replication forks. Orthologues of Eco1 are found in most if not all eukaryotes, and all contain a C2H2 zinc finger as well as another conserved domain of unknown function. Eco1's homologue in *S. pombe*, called Eso1, is also an essential gene required for sister chromatid cohesion (213). Interestingly, the lethality of *eso1* mutants is suppressed by inactivation of Pds5, which is not an essential protein in *S. pombe* (K. Tanaka, personal communication). This remarkable finding implies that Eco1 cannot be a fundamental component of the cohesion system. It also suggests that Pds5 may have two roles: one that inhibits the establishment of cohesion during DNA replication and another that helps to maintain cohesion during G2. Eso1 is presumably required merely to counteract Pds5's inhibitory function during S phase.

The connection between sister chromatid cohesion and DNA replication has recently been further strengthened by the discoveries that a variant version of replication factor C (RF-C) (65, 128) and a new type of DNA polymerase encoded by the *TRF4* gene (polymerase kappa) (234) have a role in generating cohesion. RF-C is a multisubunit complex essential for loading the ring-shaped DNA polymerase clamp PCNA onto DNA and therefore has a key role in switching DNA polymerases at the replication fork. The genes for all five of its subunits, Rfc1-5, are essential. Yeast contains at least two variants of the RFC complex: one containing the checkpoint protein Rad24 and a second in which Rfc1 is replaced by an Rfc1-related protein called Ctf18 [RF-C(Ctf18)]. The latter contains two further subunits, called Ctf8 and Dcc1, not found in RFC. Deletion of *CTF8*, *CTF18*, or *DCC1* is not lethal but causes high rates of chromosome loss, an accumulation of cells in metaphase due to activation of the mitotic checkpoint, and a partial loss of sister chromatid cohesion. The loss of cohesion in these mutants could be due either to an indirect effect of interfering with DNA replication or to a direct involvement of this alternative RF-C complex in the generation of sister chromatid cohesion. If indeed RF-C(Ctf18) is directly involved, then it is curious that *CTF18* is not an essential gene. One explanation is that cohesion can be generated by two different pathways, one of which does not require RF-C(Ctf18). In this case, it is conceivable that RF-C itself or yet some other variant thereof also performs the same role, albeit inefficiently. If RF-C-like complexes are genuinely involved

in generating sister chromatid cohesion, then a crucial question is whether their cohesion function involves the loading of PCNA and hence polymerase switching or the loading of some other complex such as cohesin.

A role of RF-C(Ctf18) in generating cohesion via some form of polymerase switching raises the issue of whether this switching concerns classes of DNA polymerase already known to be needed for DNA replication in eukaryotes or switching of a new class of polymerase, such as DNA polymerase kappa. Deletion of one (*TRF4*) of two genes encoding this newly discovered polymerase causes high rates of chromosome loss and cohesion defects (234), whereas inactivation of both (*TRF4* and *TRF5*) is lethal and has been reported to prevent DNA replication. However, the lack of budding as well as DNA replication in the double *trf4 trf5* mutant cells raises the possibility that the replication defect might not be genuine but instead be caused by some general cell cycle arrest.

In summary, several independent lines of evidence suggest that cohesin builds special structures during the passage of replication forks that are distinct from those found on unreplicated chromosomes. These structures are fully dependent on Eco1/Ctf 7 and partially dependent on a new form of RF-C. The challenge for the future is to determine the physical form of these structures and how they are built at replication forks. It is increasingly clear that the establishment of cohesion is an integral part of the DNA replication process in eukaryotic cells. If we assume that cohesin is indeed part of the bridge linking sisters, then it will be important in the near future to find direct connections between cohesin and the functions of proteins like Eco1 and RF-C(Ctf18). It will also be important to establish the connection between cohesin and the SWI1 protein from *Arabidopsis*, which is also required for the establishment of cohesion (141).

THE SISTER CHROMATID SEPARATING PROCESS

Sister chromatids are separated from each other in two steps. The first occurs during prophase/prometaphase, when the bulk of sister sequences along chromosome arms are resolved from each other to generate parallel side by side chromatids. The partial immunity to this resolution process of sequences surrounding centromeres is responsible for the central contriction of metaphase chromosomes (207). During undisturbed mitoses, residual connections between chromatid arms hold them together along their entire length and not just at centromeres until the metaphase to anaphase transition. However, the chromatid arm resolution process continues when cells are prevented from embarking on anaphase by surveillance mechanisms that respond to spindle damage. As a result, sister chromatids lose all connection along their arms in cells blocked in metaphase by spindle poisons (155). The classic image of metaphase chromosomes in which chromatids are connected only at a central constriction is therefore largely an artifact of having treated cells with spindle poisons before spreading their chromosomes. It is nevertheless an artifact that emphasizes what are real and important differences

between the processes by which centromeric and arm sequences are separated (177). Those connections between sisters that are resistant to the "prophase" resolution pathway are therefore responsible for holding sisters together while they are aligned on the mitotic spindle during metaphase. The second step in the sister separation process involves the destruction of these residual connections, which only occurs when sister chromatids disjoin at the metaphase to anaphase transition.

To those aware of the difficulties of disentangling ropes, the apparent ease with which eukaryotic cells separate their chromatids during mitosis is nothing short of miraculous. It has long been appreciated that decatenation by Topoisomerase II has an important role both in chromatid resolution during prophase (56) and in sister separation at anaphase (40, 44). To this must now be added the processes by which cohesin dissociates and condensin associates with chromosomes during prophase and cleavage of Scc1 by separase at the onset of anaphase.

SEPARATING SISTERS AT THE METAPHASE TO ANAPHASE TRANSITION

A crucial aspect of the mechanism by which sister chromatids are separated during anaphase was discovered by studying what causes the sudden disappearance of cohesin's Scc1 subunit from yeast chromosomes at the metaphase to anaphase transition. The bulk of Scc1 associated with yeast chromatin during G2 remains tightly associated with chromosomes throughout metaphase but is released at the onset of anaphase due to cleavage at two different sites by a novel cysteine protease called separase (224, 226), which is the product of the *ESP1* gene in *S. cerevisiae* (133) and *CUT1* in *S. pombe* (48). Separase cleavage sites have since been characterized in Scc1's orthologues in *S. pombe* (221) and humans (70), in its meiotic version Rec8 in *S. cerevisiae* (26), and in Slk19 (128), a protein associated with kinetochores and anaphase spindles in *S. cerevisiae* (249). All sites contain arginine at the P1 position, glutamic acid (or more rarely aspartic acid) at the P4 position, and in many cases serines or acidic residues at the P6 position (Table 2). Single mutations that replace the P1 arginine by aspartic or glutamic acid usually abolish cleavage at that site, but replacement of both P1 and P4 amino acids is needed to abolish cleavage at Rec8's second cleavage site in *S. cerevisiae*. In the yeast *S. cerevisiae*, either separase inactivation (using ts *esp1* mutants) or expression of mutant Scc1 proteins that cannot be cleaved at either site (but not mutants lacking only a single site) prevents both Scc1's disappearance from chromosomes and the separation of sister chromatids (224). It is even possible to trigger anaphase in metaphase arrested cells by induction of the foreign TEV protease in cells that express a version of Scc1 in which one of its separase sites has been replaced by that for TEV (226). Cleavage of Scc1 by separase is therefore both necessary and sufficient to trigger anaphase in yeast. Noncleavable versions of Rec8 also prevent chromosome segregation at meiosis I

TABLE 2 A list of known separase cleavage sites

Substrate	Site position	Sequence	Reference
Saccharomyces cerevisiae			
Scc1	268	DNSV**E**QG**R**RLG	224
Scc1	180	DTSL**E**VG**R**RFS	224
Rec8	431	FSSV**E**RG**R**KRA	26
Rec8	453	TRSH**E**YG**R**KSF	26
Slk19	77	DRSI**D**YG**R**SSA	F. Uhlmann, personal communication
Schizosaccharomyces pombe			
Rad21	179	QLSI**E**AG**R**NAQ	221
Rad21	231	QISI**E**VG**R**DAP	221
Rec8	384	TSEV**E**VG**R**DVQ	Y. Watanabe, personal communication
Drosophila melanogaster			
Three rows	865	LQLV**E**PI**R**KQQ	C. Lehner, unpublished
Xenopus laevis			
Xrad21/Scc1	172	MDDR**E**MM**R**EGS	I. Waizenegger, personal communication
Separase	n.d.	DVSI**E**EL**R**GSD	M. Kirschner, personal communication
Separase	n.d.	VTEC**E**VL**R**RDA	M. Kirschner, personal communication
Homo sapiens			
Scc1	172	MDDR**E**IM**R**EGS	I. Waizenegger, personal communication
Scc1	450	PIIE**E**PS**R**LQE	I. Waizenegger, personal communication
Separase	1181	KMSF**E**IL**R**GSD	M. Kirschner, personal communication
Separase	1210	SGEW**E**LL**R**LDS	M. Kirschner, personal communication

(see below). Crucially, this phenotype can be suppressed by the creation of a novel cleavage site elsewhere in the protein (S.B. Buonomo, personal communication), implying that the noncleavable Rec8 is functional in all respects other than its cleavability.

These observations demonstrate what has long been suspected from biophysical studies that microtubules are already straining to pull sister chromatids to opposite poles during metaphase and that they are merely prevented from doing so by cohesion holding sisters together (179). They also suggest that Scc1 is indeed part of the bridge, if not the bridge itself, that holds sisters together and that activation of separase is the long sought after anaphase trigger. The C-terminal fragments of

Scc1 and Rec8 produced by separase in yeast contain either arginine or lysine at their N termini and are rapidly degraded by the N-end rule ubiquitin protein ligase Ubr1 (173). Indeed, their destruction is important for high-fidelity chromosome transmission, possibly because they bind to other cohesin subunits such as Smc1 and form inactive complexes.

Two key questions stem from these studies. First, is cleavage of Scc1 by separase a crucial aspect of anaphase in all eukaryotic organisms? Second, is separase solely responsible for triggering anaphase and if so does it cleave other proteins besides Scc1?

IS CLEAVAGE OF Scc1 UNIVERSAL?

The investigation of Scc1 cleavage by separase in organisms other than yeast has been greatly complicated by the fact that sometimes only a small fraction of the cell's Scc1 protein is cleaved. Furthermore, the rapid degradation of cleavage products (173) means that they can only be readily detected in cultures whose passage through mitosis is highly synchronized. In *S. pombe*, cleavage products of Scc1's orthologue Rad21 are detected at the metaphase to anaphase transition. Furthermore, expression of a Rad21 protein that cannot be cleaved at either site blocks sister chromatid separation (221). The discovery that most cohesin dissociates from chromosomes during prophase in the absence of cleavage in vertebrates raised important doubts whether cohesin could be the glue that holds sisters together during metaphase, let alone be the target for any anaphase trigger (118). However, subsequent studies, both in *D. melanogaster* (235) and in human tissue culture (229) cells, have shown that some cohesin (5% or less of the total pool) remains associated with metaphase chromosomes, in particular in the vicinity of their centromeres, but disappears from chromosomes at the metaphase to anaphase transition (229). Crucially, a similar fraction of Scc1 is cleaved at the metaphase to anaphase transition in Hela cells, which suggests that most if not all the Scc1 that remains associated with chromosomes until metaphase is cleaved at the onset of anaphase (229). Crucially, not only is human Scc1 cleaved by separase in vitro at two sites that resemble those in yeast Scc1 but also expression of mutant Scc1 protein that can be cleaved at neither site interferes with chromatid segregation at anaphase (70).

Though Scc1 cleavage clearly needs to be investigated in a wider variety of organisms, the available data are consistent with the notion that Scc1 cleavage by separase might be a universal aspect of anaphase in eukaryotic cells. If so, then all fully sequenced eukaryotic genomes should encode separase-like proteins containing toward their C termini the amino acid motifs corresponding to the protease's active site. This is indeed the case. They also encode one or more Scc1-like proteins. It is harder in this case to determine merely by sequence analysis whether these proteins contain separase cleavage sites, because amino acids in only three positions are conserved within known sites.

The yeast and human separases are large (160–180 Kd) proteins whose C-terminal domains contain their catalytic residues. These domains are conserved and found at the C termini of all separase orthologues found in GenBank. At the heart of this domain are highly conserved histidine and cysteine residues that are thought to constitute the protease's catalytic dyad. Their mutation abolishes activity (226). The pattern of amino acids immediately surrounding this dyad resembles those of the CD clan of cysteine proteases (27), which includes gingipain, a bacterial protein implicated in tooth decay (46), legumain involved in class II antigen presentation (124), and caspases whose activation triggers programmed cell death in metazoa (45). In the case of gingipain and caspase, whose crystal structures have been determined, the histidine and cysteine residues are held in juxtaposition by a pair of hydrophobic beta sheets, which are predicted to exist in equivalent positions within all separases. Like caspases, acyloxy methyl ketone derivatives of cleavage site hexapeptides act as specific inhibitors of the yeast and human enzymes, at least in vitro (226). The conserved C-terminal domain of all separase orthologues contains extensive amino acid motifs that are unique to separases. Their common ancestry with caspases must therefore predate the common ancestor of eukaryotic cells. Though fungal genomes do not encode caspases, they do all encode a caspase-like protease, called metacaspase, whose function is unknown but is far more similar to caspase than it is to separase (227). It is nevertheless remarkable that the birth and death of eukaryotic cells, two of the most irreversible events in biology, are triggered by related proteases.

DO OTHER PROTEINS NEEDED FOR SISTER SEPARATION FUNCTION VIA SEPARASE?

In the absence of readily fractionable in vitro systems for studying sister chromatid separation, the isolation and characterization of mutants in yeast and flies has been the only avenue by which new players in this process have been identified. Besides separase, genetic studies in *S. cerevisiae*, *S. pombe*, and *D. melanogaster* have identified at least two other types of protein necessary for sister chromatid separation but not for other aspects of cell cycle progression: a protein called Threerows, which has thus far only been found in flies (168, 202), and a class of proteins found in a wide variety of organisms called securins (247). If proteolytic cleavage by separase is indeed the mechanism by which sisters are separated in eukaryotic cells, then Threerows and securins should have some connection with separase. Is this the case?

Securins from *S. cerevisiae* (Pds1) (29), from *S. pombe* (Cut2) (48), from *D. melanogaster* (pimples) (253), and *vertebrates* (PTTG) (253) all bind tightly to separase. Securins are potent inhibitors of separase activity (224) and their proteolysis by the Anaphase-promoting complex shortly before the metaphase to anaphase transition is necessary for sister chromatid separation (see below) (32, 50, 112, 253, 254). They have a key role in ensuring that separase remains

inactive until chromatids are fully aligned on the metaphase spindle. Besides this crucial inhibitory function, securins also have an important role in promoting separase activity. This function is important but not essential in human tissue culture cells (89) and in *S. cerevisiae* (245) but is essential for sister chromatid separation in *S. pombe* (48) and in *D. melanogaster* (202). Whether securins primarily promote separase activity in vivo by targeting the protease to correct cellular locations (29, 90, 109) or more directly by facilitating its adoption of an active conformation (89) is presently unclear. What is clear is that the sister separation defect of cells lacking securins is possibly due to a lack of separase activity.

What about Threerows? Though only found so far in *D. melanogaster*, it too is crucial for sister chromatid separation. Thus, both *Threerows* and *Pimples* mutant embryos accumulate cells in which cell cycle progression has taken place in the absence of sister chromatid separation and which therefore contain four or more (up to 32) chromatids held together at their centromeres (34, 168). Remarkably, Threerows also binds tightly to separase and may be a key regulator of its protease activity in flies (C. F. Lehner, personal communication). The separase protein in flies is about half the size of its orthologues in yeast and vertebrates, and it is possible that the missing N-terminal half of the protein is encoded by a separate polypeptide encoded by Threerows. Though genetic analyses have clearly identified a host of other proteins needed for resolving sisters once anaphase has initiated (for example, Topo II and condensin), separase, securins, and Threerows are the only proteins thus far implicated in initiating the sister separation process. Indeed, the BimB protein, which is required for the completion of mitosis but not for rereplication of DNA in *Aspergillus nidulans*, encodes a separase homologue (127). In conclusion, therefore, several independent genetic investigations of genes required for separating sister chromatids once they have been aligned on the mitotic spindle have all focused on a singe entity: separase.

DOES SEPARASE HAVE OTHER TARGETS BESIDES Scc1?

Though cleavage of Scc1 by the TEV protease is sufficient to trigger the segregation of sisters to opposite spindle poles in yeast, this by no means excludes the possibility that cleavage of other proteins might also facilitate anaphase chromosome movement. Indeed, several lines of evidence suggest that separase also targets proteins concerned with stabilizing anaphase spindles. In both *S. cerevisiae* and *S. pombe*, a sizeable fraction of separase colocalizes with mitotic spindles in a manner that depends on securins and on its own conserved C-terminal protease domain (29, 90, 109). Such an association has not, however, been seen in human cells, where most separase protein appears to be distributed throughout the cytoplasm during mitosis (J. Peters, personal communication). Nevertheless, the association of separase with spindles in yeast might be of functional significance, because the elongated spindles produced by cells induced to undergo anaphase by the TEV protease (cleaving Scc1) are much less stable than those triggered by

overexpression of separase itself (226). This implies that yeast separase has at least two functions during anaphase. By cleaving Scc1, it permits microtubules attached to kinetochores to pull sister chromatids toward opposite spindle poles (known as anaphase A) and at the same time allows the poles themselves to be driven further apart by the elongation, interaction, and sliding apart of microtubules that are not associated with kinetochores (known as anaphase B). Cleavage of some protein other than Scc1 might be required for stabilizing spindle interactions in the midzone where spindles from opposite poles overlap. It might alternatively be required for a stabilization of microtubules necessary for their rapid growth during anaphase B.

Recent work has shown that yeast separase also cleaves Slk19 (204), a protein that both localizes to the spindle midzone during late anaphase and promotes the stability of late anaphase spindles (249). However, Slk19's cleavage by separase is neither essential for stabilizing anaphase spindles in otherwise wild-type cells nor sufficient for stabilizing them in cells triggered to undergo anaphase by the TEV protease. Separase must therefore stabilize anaphase spindles either by cleaving yet another protein or by a separate mechanism that does not involve proteolysis. It is still unclear whether spindle stabilization will prove to be an essential separase function in yeast, because both chromosome segregation and spindle elongation can occur in the absence of separase activity during meiosis I in cells in which crossing over been homologues has been abolished (26). In summary, separase cleaves at least two, if not more, proteins at the metaphase to anaphase transition in yeast.

SEPARASE REGULATION

Mitosis would not function if the destruction of cohesion between sister chromatids preceded their alignment on the mitotic spindle. It is therefore crucial that separase activity be very tightly controlled. As already mentioned, all known separases are bound for much of the cell cycle by a chaperone called securin, whose yeast and human orthologues have been shown to be potent inhibitors of separase's proteolytic activity. In yeast, flies, and vertebrates, securin levels rise during late G1, remain high throughout G2 and early M phase, but drop suddenly shortly before the metaphase to anaphase transition (32, 50, 112, 229, 253). The extent of securin's decline at the onset of anaphase varies amongst cells and organisms. Most if not all is destroyed in budding yeast but at most 50% in fission yeast, and possibly a similar amount in Drosophila. It is therefore conceivable that anaphase onset might only require the destruction of securin that is in the vicinity of chromosomes or mitotic spindles.

The rapid decline of securins at the onset of anaphase is due to proteolysis mediated by a multisubunit ubiquitin protein ligase called the Anaphase-promoting complex (APC) or cyclosome (248). The APC also mediates the ubiquitination and proteolysis of many cell cycle proteins, including A- and B-type cyclins, Polo-like

kinases, and geminin (a regulator of DNA replication). The APC's activity depends on a pair of proteins composed of WD40 repeats called Cdc20 (Fizzy) and Cdh1 (Fizzy related), which are thought to bring substrates to the ligase complex. Cdc20 is only abundant and active during mitosis, whereas Cdh1 is only active during the subsequent G1 period. The proteolysis of securins, cyclins, and geminin shortly before anaphase onset is therefore mediated by APC-Cdc20, whereas their destruction during G1 is mediated by APC-Cdh1. APC-Cdc20 is regulated by the abundance of Cdc20 (which accumulates during G2 and M phase) (192, 241), by phosphorylation of APC subunits (108), by a surveillance mechanism called the spindle checkpoint (6), and by fluctuations in the abundance of an inhibitory protein called Emi1 (175). The spindle checkpoint works by generating an inhibitor of APC-Cdc20 called Mad2 so long as "lagging" chromosomes are present that have not yet properly attached to the spindle. This ensures that securins and cyclins are not degraded and therefore separase not activated precociously. Mutation of the spindle checkpoint is sometimes but not necessarily lethal but invariably causes high rates of chromosome loss, which might contribute to the genesis of tumors in mice (145).

According to this model, the ability to delay Scc1 cleavage (and hence loss of sister chromatid cohesion) in response to spindle damage or lagging chromosomes should be dependent on Mad2, which is needed to inhibit the APC, and on securin, whose persistence is needed to block separase activation. This is indeed the case in budding yeast (5, 87, 245). Whether this will be universally true is less clear because it has been reported that human tissue culture cells whose securin genes have been deleted by homologous recombination can still prevent loss of sister chromatid cohesion when cells are treated with poisons that cause spindle disassembly (89).

Inactivation of the APC prevents the onset of anaphase in cells from budding yeast, fission yeast, worms, flies, and mammalian tissue culture and causes them to arrest in metaphase (248). There can therefore be little question that the APC is essential for initiating anaphase in most if not all eukaryotic cells. Much but not all evidence suggests that its role in this regard is to destroy securin. For example, expression of securins that cannot be recognized and hence destroyed by APC-Cdc20 blocks sister chromatid separation in yeast (32, 50), flies (112), and vertebrate cells (253, 254). This suggests that securin's destruction by the APC is essential for the activation of separase. These findings nevertheless leave unanswered whether physiological levels of securin are sufficient to block anaphase onset and if so, whether securin is the only protein whose destruction by the APC is necessary for anaphase. In flies, a nondegradable securin (pimples) fails to block anaphase when expressed close to physiological levels but does so when expressed at twice this level (112). This could be taken to mean that securin destruction is not in fact required for anaphase in vivo and that the APC's main role is the destruction of some other anaphase inhibitor. It has been suggested, for example, that destruction of cyclin A is necessary (161, 194). An alternative explanation is that a critical concentration of securin is required to prevent separase activation in vivo and that proteolysis by the APC is indeed necessary to reduce securin to

below this level shortly before the onset of anaphase. Budding yeast is the only organism where we have a reasonably definitive answer to this question. The failure of *apc* or *cdc20* mutants to enter anaphase is fully bypassed by deletion of the gene encoding yeast securin (29, 191, 246), which implies that the persistence of securin in *apc* or *cdc20* mutants is entirely responsible for their failure to enter anaphase.

In summary, most evidence is consistent with the notion that proteolysis of securin by the APC is essential for liberating sufficient separase to split sister chromatids. Moreover, inhibition of APC-Cdc20 by Mad2 and the spindle checkpoint prevents securin's destruction in the presence of lagging chromosomes or spindle damage, which in turns delays separase activation.

Ubiquitin-mediated proteolysis of securin is not, however, the sole mechanism that regulates sister separation or the Scc1 cleavage reaction. Somewhat surprisingly, deletion of genes encoding securin is not lethal either in budding yeast or in human tissue culture cells. Yeast or human cells lacking securin are defective in the process of separating sisters, probably due to their lowered separase activity, but neither sister separation nor Scc1 cleavage is precocious (4, 89). Furthermore, the lethality of securin mutants in flies and fission yeast is due to their greater dependence on securin for promoting separase activity and not due to its precocious activation. The implication is that most and possibly all eukaryotic cells possess securin-independent mechanisms that regulate Scc1 cleavage by separase. This is consistent with the finding that there is a 10–20 min delay between securin's decline and the onset of sister chromatid separation in tissue culture cells (J. Pines, personal communication) and with the discovery that disassembly of the actin cytoskeleton in *S. pombe* blocks Scc1/Rad21 cleavage without apparently inhibiting the APC (53).

One such mechanism has recently come to light in yeast, where phosphorylation of Scc1 by the Polo-like kinase Cdc5 greatly facilitates Scc1 cleavage (4). Though not essential for the cleavage of most Scc1 in wild-type cells, Cdc5 is critical for Scc1's cleavage in securin mutants in which separase activity is badly compromised. Phosphorylation by Cdc5 of serine residues six amino acids N-terminal to Scc1's cleavage sites (i.e., in the P6 position) are responsible for part but not all of Cdc5's effect. Serines at the P6 position are conserved in Scc1 orthologues from many but not all eukaryotes. An aspartic acid exists at the equivalent position of the major cleavage site in vertebrate Scc1s (70), which is consistent with the notion that residues at this position must be negatively charged, whether or not due to phosphorylation. Mitosis-specific phosphorylation of Scc1 residues other than those in the P6 position could also regulate the cleavage reaction.

In vertebrate cells, destruction of cyclins as well as securin might be necessary for sister separation. Though previous studies suggested that cyclin degradation might not be required for anaphase in *Xenopus* extracts (79), more recent studies report that expression of nondegradable versions of cyclin A in *Drosophila* (161, 194) or of cyclin B in *Xenopus* extracts (O. Stermmann & M. Kirschner, personal communication) blocks sister separation as well as exit from mitosis. The lack of chromatid disjunction caused by persistent cyclin A in *Drosophila* cannot simply be explained by an interference with APC activity because it does not

affect disappearance either of cyclin B or pimples (securin). In *Xenopus* extracts, the lack of sister chromatid disjunction due to nondegradable cyclin B is largely if not entirely due to the phosphorylation of separase by Cdk1, which inhibits its ability to cleave Scc1 (O. Stermmann & M. Kirschner, personal communication). Thus, nondegradable cyclins no longer block sister separation when *Xenopus* extracts are supplemented with a mutant version of separase that cannot be phosphorylated by Cdk1. These observations suggest that activation of separase in animal cells might require destruction of both securin and cyclins by the APC. Such control of separase by Cdk1 might be responsible for the finding that human cells completely lacking securin (due to deletion of both genes) still block sister separation when the mitotic checkpoint is activated by spindle poisons (89). Direct control of separase by Cdk1 appears to be lacking in yeast because expression even of high levels of nondegradable B-type cyclin does not block anaphase (209) and because deletion of its securin gene *PDS1* permits anaphase to occur in the absence of APC-Cdc20. Inactivation of separase by Cdk1 might therefore not be a universal feature of mitotic control. It may nevertheless help ensure that sister separation never occurs when the APC is inhibited by mitotic surveillance mechanisms.

Yet another potential mechanism for regulating sister separation has been raised by the finding that human separase as well as Scc1 is cleaved around the onset of anaphase (229). The major cleavage site resembles those found in fungal Scc1s and contains a serine at the P6 position (O. Stermmann & M. Kirschner, personal communication; I. Waizenegger & J. M. Peters, personal communication). It is likely that separase cleaves itself upon securin's destruction and possible that this further promotes protease activity, as found for members of the caspase family (45). If so, phosphorylation of cleavage sites within separase itself could also regulate its activity. It is unclear whether such a mechanism also regulates separase in yeast because cleavage of the yeast enzyme has not thus far been detected.

In summary, at least four mechanisms may regulate Scc1's cleavage by separase: ubiquitin-mediated proteolysis of securin, phosphorylation of Scc1 itself, phosphorylation and inhibition of separase by Cdk1, and cleavage of separase. Separase cleavage could either activate the protease or promote its destruction due to the Ubr1 ubiquitin protein ligase (173). Indeed, both mechanisms could cooperate to generate a sudden burst of protease activity soon after securin proteolysis in vertebrate cells. Yet other mechanisms must also exist because something ensures that only Scc1 remaining on metaphase chromosomes is cleaved by separase in vivo. The bulk of Scc1, which dissociates from chromosomes during prophase, is untouched by separase. The multiplicity of mechanisms controlling separase emphasizes the importance of regulating this crucial protease.

RESOLVING SISTERS DURING PROPHASE

By the time chromosomes have aligned on the metaphase plate, the vast majority of chromatin fibers from each sister chromatid arm are packed along two distinct axes, between which there are only tenuous connections (107). This "resolution" of sister sequences coincides with and may indeed be synonymous with "mitotic"

chromosome condensation, which involves an increase in the compaction of chromatin fibers from a single chromatid (76). During prophase, chromosomes emerge from the amorphous mass of chromatin fibers characteristic of interphase cells as undivided "sausages" (207). The continued disentangling of sister DNA sequences from each other during prometaphase subsequently gives rise to the paired sister chromatids that will finally be aligned on the metaphase spindle. A number of different sets of proteins have been implicated in this dramatic chromosome metamorphosis. The first are histones, in particular histone H3, whose phosphorylation is associated with chromosome condensation in some (55, 185) but not all (93) situations. The second is cohesin, which dissociates from chromosome arms during this period (118). The third is condensin, which associates with chromosomes and somehow promotes their compaction during prophase (77). The fourth is Topoisomerase II, which is required to decatenate sister chromatids (44). The fifth are the three mitosis-specific protein kinases, Cdk1, PLK, and Aurora B, which may phosphorylate and thereby regulate the activity of several of the above proteins. However, none of the roles of these different classes of proteins are understood precisely. This list would not, however, be complete without mentioning the key part played by the transcription apparatus. In most if not all cells whose chromosomes undergo a massive increase in their compaction, transcription by all three RNA polymerases is repressed as cells enter mitosis (126). This does not occur in yeast, whose chromosomes do not greatly condense during mitosis. The elimination of transcription is most probably essential for chromosome condensation, but it cannot be the trigger because mitosis-specific condensation clearly takes place in embryos undergoing cleavage divisions during which there is little or no transcription at any stage of the cell cycle.

The degree to which chromatids are resolved during prophase varies tremendously between organisms. The process is undetectable, for example, in yeast, where neither appreciable chromosome compaction nor loss of cohesion between sisters precedes the metaphase to anaphase transition (62). Indeed, it was the absence of the prophase pathway and the persistence of most if not all cohesin on yeast chromosomes during metaphase (144) that made yeast particularly suitable for studying/discovering the separase pathway.

COHESIN DISSOCIATION

Along chromosome arms, mitosis-specific condensation is invariably accompanied by a loss of sister chromatid cohesion. However, these two processes may be uncoupled in the vicinity of centromeres, where chromosomes are compacted without losing sister chromatid cohesion. Given cohesin's persistence at centromeres until metaphase, it is reasonable to suppose that the loss of cohesion along arms is caused by the dissociation during prophase of most cohesin from this region of the chromosome. Cohesin's dissociation takes place in the absence of the APC and thus presumably separase activity (206) and is not accompanied by

Scc1 cleavage (229). The finding that several cohesin subunits are phosphorylated during prophase and prometaphase (83, 120) raises the possibility that the activation of protein kinases such as Cdk1, PLK, and Aurora might trigger dissociation. Cohesin's Scc3 subunit can be phosphorylated by Cdk1 in vitro (120) but there is little or no evidence that Cdk1 is actually required for cohesin's dissociation (206). The Aurora B kinase is also a candidate because of its localization to the interchromatid zone during prometaphase (2). Recent evidence suggests, however, that PLK may be a key player, because its depletion from mitotic *Xenopus* extracts abrogates their ability to remove cohesin from chromosomes without affecting either phosphorylation of histone H3 or association of condensin (I. Sumara & J. M. Peters, personal communication), both of which may depend on Aurora B (55, 85). A role for PLK fits with the finding that this kinase also prepares yeast Scc1 for cleavage by separase. It is therefore conceivable that PLK has at least two crucial roles in chromatid separation, first in dissociating cohesin from chromosome arms during prophase and second in facilitating Scc1cleavage at the metaphase to anaphase transition.

The persistence of cohesin at centromeres during metaphase is most likely crucial for holding sister chromatids together until separase activation triggers the onset of anaphase. This population of cohesin molecules must therefore be refractory to the process that dissociates cohesin from chromosome arms during prophase. The mechanism conferring this protection is not at all understood. Recent observations suggest that the giant filamentous protein Titin, more famous for its role in the sarcomeres of muscle, may have some role in protecting centromeric cohesin from the prophase pathway because sister chromatids separate precociously in *titin* mutants of *Drosophila* (123). In *S. pombe*, methylation of histone H3 and its binding by the HP1-like protein Swi6 is necessary for the recruitment and enrichment of cohesin to outer centromere repeats (R. Aushire, personal communication). HP1 could therefore here have a role in blocking cohesin's dissociation from pericentric heterochromatin during prophase in animal cells.

In summary then, cohesion between chromatids is destroyed in two phases in most but not all eukaryotic cells. It is thought that phosphorylation of cohesin itself or other chromosomal proteins triggers dissociation of cohesin from chromosome arms during prophase, whereas cleavage of Scc1 that persists on chromosomes causes the final loss of cohesion, which triggers chromatid segregation at the onset of anaphase. The loss of cohesion in two steps makes good biological sense. Cohesion between chromatids can only be built once during the cell cycle, presumably following passage of replication forks (225), but it must nevertheless be capable of surviving for long periods of time, as in cells with an extended G2 period. During this phase, cohesion is crucial for double-strand break repair (21, 197) and may also be necessary for maintaining chromosome structure and for regulating gene expression (43).

The amount of cohesin on G2 chromosomes is clearly greater than that required for holding sisters together as they align on the mitotic spindle. Were all of it to remain on chromosomes until metaphase, cells might be unable to disjoin chromatids

as rapidly as they do at the metaphase to anaphase transition. Dissociation of the bulk of cohesin from chromosome arms during prophase enables cells to embark on the difficult task of resolving sister chromatids long before the final connections are severed at the onset of anaphase. It also permits metaphase cells to concentrate their separase on those regions where cohesin persists. By involving proteolysis, the separase pathway is ideally suited for the rapid and irreversible destruction of sister chromatid cohesion, whereas the prophase pathway is designed for the slow but sure disentanglement of the huge network of sister chromatid fibers. Yeast cells may be able to dispense with the prophase pathway because they have a genome that is small enough to be rapidly resolved into two chromatids largely, though not exclusively, by the force of the mitotic spindle alone.

CONDENSIN ASSOCIATION

The impressive acrobatics of chromosome movement mediated by the mitotic spindle tends to eclipse the extraordinary fact that much of the "work" of splitting sister chromatids is actually performed during prophase and pro-metaphase through mechanisms that do not involve microtubules. One of the major tasks facing any cell about to undergo mitosis is that of removing the intertwining of sister DNA molecules created by the conjunction of converging replication forks (208). It has long been recognized that this task is performed by Topoisomerase II (40), but it is still a mystery how this enzyme knows whether to catenate or decatenate. What then provides the directionality of Topo II action? It is clear to anyone who has seen Bayer's film documenting the effortless disengagement of circular chromatids during anaphase (13) that Topoisomerase II has little or no difficulty getting the directionality right when the two DNA strands concerned come under tension due to being pulled in opposite directions. Though never tested, this particular constellation of interlocked DNA strands is presumably the ideal substrate for Topo II. However, the vast majority of intercatenation between sister chromatids is resolved in an equally effortless process during prophase and prometaphase without the help of microtubules (44). One of the many mysteries of mitosis has therefore been the identity of the motor that drives decatenation during this stage.

With ATPases at each of its long coiled coils, condensin is an ideal candidate for this motor. Indeed, the only phenotype of condensin mutants that is consistently found in all organisms is not so much a defect in chromosome condensation but rather a failure to disengage properly sister chromatids during anaphase (19, 110, 200). It is, for example, striking that the first wave of mitotic failures in *D. melanogaster smc4* mutants are not associated with any lengthening of the chromosomal axes, as might be expected if their primary defect were chromosome compaction, but rather the accumulation of anaphase bridges arising from a failure to resolve sister chromatids (200). It is not inconceivable that the primary function of condensin is not condensation per se, which might largely be achieved by the high level coiling of chromatin fibers, but rather chromatid resolution, which clearly

requires a motor to impart directionality to decatenation catalyzed by Topo II. How condensin or any other complex for that matter achieves this goal is still unclear. Such a function is not an obvious consequence of its undoubted ability to introduce positive writhe to DNA in vitro (96) without attributing other important properties to this complex. It is not unreasonable to suppose that condensin somehow achieves this goal by the same route as mitotic spindles, i.e., by bringing interlocked sister DNA molecules under tension. But, how might it perform this?

Chromatid resolution during prophase requires not only tension between sister DNA molecules to drive decatenation but also some mechanism which ensures that only sequences from the same DNA molecule are compacted or condensed together. Chromatid condensation would have little value if it failed to discriminate whether sequences belonged to one or the other chromatid. The process of chromosome condensation is therefore intimately connected with the issue of chromatid identity. What then is the process by which DNA belonging to a single molecule packs along the same axis while that belonging to its sister packs along a parallel but separate one?

There are two fundamentally different ways of thinking about chromatid identity. The first is to propose that chromosomes have an axis or core (often referred to as a scaffold), which exists in some form or another at all stages of the cell cycle, and around which all DNA from a given chromatid is organized. There is considerable evidence that mitotic and meiotic chromosomes do indeed have scaffolds (23, 125, 174), not least of which is the observation that condensin and cohesin, which are known determinants of chromosome structure, are concentrated along axial cores of chromosomes during metaphase (185) and pachytene (104, 165, 171), respectively. Nevertheless, there are potentially four different problems with the notion that a stable scaffold provides chromatid identity. The first is the lack of any serious model for how the immensely long DNA molecules from each chromosome attach to one and only one scaffold. The second is that no single protein has thus far been localized in vivo to a scaffold structure that persists throughout the cell cycle. Cohesin is clearly associated with chromosome axes during meiosis (its role in mitosis in this regard is less clear), whereas condensin is clearly associated with the two chromatid axes of metaphase chromosomes (182). However, neither complex remains associated with chromosomes throughout the cell cycle, condensin being largely absent during interphase and cohesin being largely removed during mitosis (76). Neither complex possesses the continuity required for a stable chromosome core. The third problem concerns how such cores would be replicated and the duplicates cleanly resolved from each other prior to mitosis. The fourth and possibly most serious problem is how recombination between sister DNAs (sister chromatid exchanges) would also recombine axial cores. It is clear, for example, that recombination during G2 gives rise to recombinant chromatids without any trace of discontinuity along the axes of the newly created chromatids. Thus, the axes of mitotic chromatids are determined solely by the chemical continuity of DNA, which can be created anew by recombination, and cannot trace their origin to a pre-existing core.

An alternative way of thinking about chromosome cores is that they emerge de novo from the actions of condensin during mitotic prophase (or cohesin during meiotic prophase). It is clear from cell fusion studies that the cores of mitotic chromosomes can form in the absence of DNA replication (56). Thus, the cytoplasm of mitotic cells induces single chromatids in G1 cells to form chromosomes with a single clearly defined core. The formation of cores does not therefore need some complicated structure established during DNA replication nor does it require a pair of intercatenated chromatids. They arise de novo whenever DNA or rather chromatin comes into contact with active core-forming proteins. According to this view, nucleosomal DNA and not some independent proteinaceous entity is what actually defines a chromatid's axis. The axial core or scaffold is merely a property that emerges from the activities of proteins that package chromatin and help to resolve sister chromatids from each other. If this is correct, eukaryotic chromosomes have two mysterious but interconnected properties: the ability to compact themselves (but not others) around a single axial core and the ability to resolve sister chromatids using a motor other than the mitotic spindle. It is tempting to speculate that condensin might be responsible for both of these. The challenge is to discover its mechanism.

A key question is whether condensin is itself responsible for keeping track of DNA strands (i.e., ensuring that all DNA from a single molecule ends up organized around the same axial core) or whether this crucial property emerges from the activities of other proteins. It is thought, for example, that histones alone can organize DNA into helical 30-nm fibers, in which nucleosomes are wound around a helix, with six nucleosomes per turn (219). These structures alone will tend to segregate self from nonself DNA molecules, as indeed would further coiling to produce yet higher-order fibers. Might then the simple tendency of chromosomes to coil upon themselves be the primary driving force for condensation? Might this process inevitably cause regions where chromatids are intertwined to come under the sort of tension needed to drive efficient decatenation? If so, might condensin, through its known ability to stabilize positive writhe, merely facilitate a reaction that is primarily driven by the properties of nucleosomes? Even if partially correct, at least in outline, this model explains neither why chromatids condense into cyclinders with a defined diameter nor why condensin accumulates along the axial cores of such cylinders. If condensin merely facilitates the coiling of chromatin fibers, then it must do so in a manner that both constrains the degree and form of this coiling. Nevertheless, it is quite possible that condensin has a more active role both in chromosome packaging and chromatid resolution than envisaged by the above model. Without knowing what this particular motor does when presented with a chromatin substrate, one can merely speculate about this role. One possibility is that condensin associates with the bases of small loops or coils of chromatin and enlarges these loops or coils in a processive manner, which ensures that all chromatin within the loop or coil must have been cleanly segregated from all other sequences in the genome. As this process proceeds, neighboring loops or coils would naturally converge, creating an axial core in which the bases of loops or

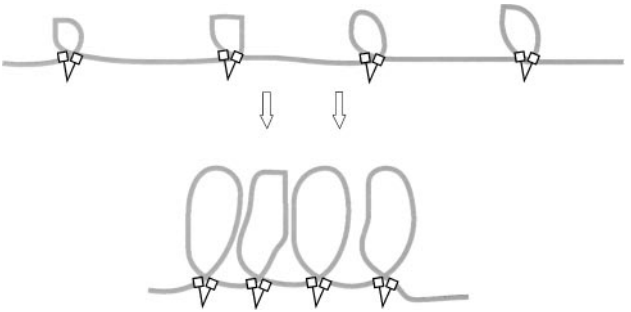


Figure 5 A model for how condensin could form axial cores and thereby help to resolve sister chromatids from each other.

coils containing condensin would alternate with a short linker (Figure 5). I give this example not so much because it is a serious candidate for the function of condensin (or cohesin for that matter) but rather because it illustrates the notion that condensin or molecules like it could have a very active role in folding and resolving chromatids. This model does help to explain many puzzling features of condensin: in particular, its crucial role in chromatid resolution, its accumulation along axial cores, and the curious finding that condensin depletion causes problems with chromatid resolution long before it has any effect on chromatid length. It also neatly explains the origin of the so-called chromosome scaffold with peripheral chromatin loops and how chromatid identity can "emerge" naturally from the actions of molecules that act processively but merely locally on the chromatin fiber. However, it is very difficult to imagine how condensin could actually perform this particular anointed task, especially as its substrate must be chromatin fibers and not naked DNA. It is conceivable that cohesin has a similar function.

In summary, then, the process of sister chromatid separation takes place in two steps in most eukaryotic cells. During the first step, some sort of processive chromatid compaction involving condensin and very probably other regulators of nucleosome packing drives the decatenation of chromatids and packages them around an axial core that contains condensin. The bulk of cohesin dissociates from chromatids as this process proceeds, and little if any remains to connect chromatids along chromosome arms by the time that chromatids are aligned on the metaphase plate. However, cohesin in the vicinity of centromeres, which is largely refractory to the process that removes it from chromatid arms, prevents resolution at centromeres and is capable of providing sufficient cohesion for the alignment of chromatids in a bipolar manner on the mitotic spindle. The second step is triggered by the activation of separase, whose cleavage of cohesin's Scc1 subunit in the vicinity of centromeres permits sisters to be pulled to opposite poles. The force supplied by microtubules now takes over from condensin in driving the decatenation process. Remarkably, the first step of chromatid resolution is almost entirely missing in

yeast, where most if not all cohesin remains on chromosomes until the activation of separase, and chromatids remain tightly connected throughout their length until the metaphase to anaphase transition.

LINKING REREPLICATION WITH CHROMATID SEGREGATION

One of the most characteristic features of the eukaryotic cell cycle is the delay of chromosome reduplication until after chromatids produced by the previous round of DNA replication have been partitioned between daughter cells at mitosis. Now that we understand many of the processes required for sister separation and for DNA replication, we can also begin to understand the broad outlines of the mechanism by which these two crucial events are interlinked. The initiation of DNA replication takes place in two steps (39). The first is the loading at future origins of a hexameric DNA helicase composed of Mcm proteins, which depends on the origin recognition complex (ORC), an Mcm loading factor called Cdc6p, and a cofactor called Cdt1p. The second step is the activation of cyclin-dependent kinases along with the Dbf4-dependent Cdc7 kinase, which together trigger origin unwinding by the Mcm helicase and thereby the loading of DNA polymerase. Because the very same Cdks that trigger origin unwinding also inhibit the loading of Mcm helicase (35, 217), it is not possible for origins to reload Mcm proteins while S phase Cdks remain active, which lasts for most of S phase. Mcm helicases are likewise prevented from loading on origins during G2 and M phase by Cdks containing cyclins A and B (71). In vertebrate cells but possibly not in yeast, an additional mechanism also blocks the formation of prereplication complexes: A protein called geminin (132) accumulates during S or G2, binds to Cdt1, and blocks Mcm loading (243). Thus, preparations for a new round of DNA replication cannot begin until both cyclins and geminin are removed. Because cyclins, geminin, and securin are all destroyed by the Anaphase-promoting complex, preparations for the initiation of DNA replication cannot begin until the process of sister chromatid separation has been initiated.

SEPARATING CHROMATIDS DURING MEIOSIS

During meiotic divisions, two rounds of chromosome segregation following a single round of chromosome duplication give rise to haploid gametes from diploid germ cells. One of the most remarkable aspects of meiotic cells is their ability to undergo two rounds of chromosome segregation using only a single round of DNA replication. To do this, they must undergo the first meiotic division without fully destroying the cohesion established between sister chromatids during premeiotic DNA replication so that the residual cohesion can be utilized at the second meiotic division (149).

The first meiotic division is fundamentally different from the second one and from mitotic divisions (see Figure 2). During mitosis and meiosis II, cells attempt to pull sister kinetochores toward opposite poles of the cell but are prevented from doing so by sister chromatid cohesion until all sister kinetochore pairs have aligned on the spindle, whereupon cleavage of Scc1 or Rec8 by separase destroys this equilibrium and triggers poleward migration. During meiosis I, cells instead attempt to pull toward opposite poles homologous chromosomes, which have been joined together by recombination (158). During this process, sister kinetochores must always attach to microtubules from the same pole (58), known as syntelic or mono-orientation, which is precisely what must be avoided during mitosis. A very similar type of equilibrium is therefore established during the metaphases of meiosis I and mitosis. However, the partners being pulled in opposite directions during meiosis I are homologous chromosomes and not individual chromatids. Meanwhile, chromosome segregation at the onset of anaphase I is triggered by resolution of the chiasmata or crossovers that hold homologues together, which is invariably accompanied by loss of cohesion between sister chromatid arms (122). Another key difference between meiosis I and mitosis is that cohesion between sister chromatids in the vicinity of centromeres is always preserved at anaphase I and persists until finally destroyed at anaphase II (147). This property is not actually necessary for meiosis I but is crucial for meiosis II.

It is clear that chromosome segregation during meiosis largely depends on the same machinery used during mitosis. However, the ability of meiotic cells to reduce chromosome numbers by undergoing two rounds of chromosome segregation after only one round of DNA replication depends on several meiosis-specific innovations. Many of these involve the sister chromatid cohesion apparatus.

DO MEIOTIC AND MITOTIC CELLS USE THE SAME OR A DIFFERENT COHESION MACHINERY?

In budding yeast, all cohesin subunits apart from Scc1 are essential for meiosis I (R. K. Clyne, personal communication; 104). Scc1 declines sharply as cells enter meiosis and is replaced by Rec8, a meiosis-specific variant. Rec8 is normally never expressed in mitotic cells but it is capable of rescuing cells lacking the *SCC1* gene when expressed from the *SCC1* promoter (26, 238). Such cells undergo meiosis with high efficiency and produce largely viable spores, suggesting that Scc1 has little if any role during meiosis (F. Klein, personal communication). Rec8, in contrast, accumulates shortly before premeiotic DNA replication and, along with other cohesin subunits, is essential for maintaining sister chromatid cohesion throughout meiosis (104, 148). A similar though not identical situation prevails in *S. pombe* (238), *C. elegans* (163), mammals (C. Heyting, personal communication), and possibly also in plants (20), in which Scc1s are to a greater or lesser extent replaced by meiosis-specific variants, most of which have been called Rec8. Inactivation of Rec8 in *C. elegans* using RNA interference causes the

appearance of up to 24 chromatids instead of six bivalents prior to the first meiotic division (163). Rec8 is essential for sister chromatid cohesion in the vicinity of centromeres in *S. pombe* (148), but it coexists for much of meiosis I with Scc1 (Rad21), which is relegated to chromosome arms (148). Though the replacement of Scc1s by meiosis-specific variants may be widespread in eukaryotes, it is unclear whether it is a universal phenomenon. Thus far, only a single Scc1-like protein has been detected in the (almost) complete *D. melanogaster* genome (1). Either flies use the same Scc1 subunit for mitosis and meiosis or they possess a second gene, which lurks in their unsequenced heterochromatic pericentric regions.

At least two other cohesin subunits have meiosis-specific variants. The two versions of Scc3 called SA1 and SA2, which are found in most somatic tissues, are replaced, at least in spermatocytes, by a third variant called STAG3 (171). Spermatocytes also express a meiosis-specific version of Smc1, which is called Smc1β and may be the main partner of Smc3, Rec8, and STAG3 during meiosis (C. Heyting, personal communication; 176). The *S. pombe* genome also encodes a meiosis-specific variant of Scc3 called Rec11 (115), which possibly replaces Scc3 along chromosome arms but not at centromeres (Y. Watanabe, personal communication).

Of the other proteins needed for cohesion during mitosis, Pds5's homologue in Sordaria, called Spo76, is essential for maintaining sister chromatid cohesion during diplotene/diakinesis (228), while Scc2 and its homologue in Coprinus, Rad9, are also essential for meiosis (188). The roles of other cohesion proteins such as Eco1/Ctf7and Ctf18 have not yet been investigated.

This list of meiosis-specific cohesin subunit variants (see Table 1) is presumably far from complete. It is, however, already clear that there is considerable variation between organisms in the extent to which mitotic subunits are replaced by meiosis-specific variants, which ranges from the replacement merely of Scc1 by Rec8 in yeast to that of Scc1, Smc1, and Scc3 (SA1 and SA2) by Rec8, Smc1β, and STAG3, respectively, in mammals. These replacements, or in some cases additions, presumably enable cohesin to fulfill many of its functions that are specific to meiotic cells, such as the repair of double-strand breaks using homologous chromatids instead of sisters, the creation of axial cores and synaptonemal complex during pachytene, and the persistence of cohesion at centromeres but not along arms until the second meiotic division.

COHESIN'S ROLE IN RECOMBINATION AND IN BUILDING MEIOTIC AXIAL CORES

It has long been suspected that sister chromatid cohesion has a crucial role in double-strand break repair. G2 cells, for example, are invariably far more resistant to gamma irradiation than are G1 cells (24). Furthermore, in diploid cells, sister chromatids and not homologous ones are clearly the preferred template for repair (91). However, until recently it has not been possible to test whether the greater

radiation resistance of G2 cells is due to the proximity of a sister chromatid with which to repair double-strand breaks or due to other differences between these two cell cycle states, for example in the activity of repair enzymes or checkpoint proteins. The discovery that Rad21 (21), long known to be crucial for double-strand break repair, encoded a cohesin subunit homologous to Scc1 (63, 144) is consistent with the notion that the proximity of sister chromatids does indeed have a key role. Efficient double-strand break repair during G2 or M phase in budding yeast depends not only on cohesin's presence at the time of irradiation but also on its presence during the preceding S phase (197). Thus, the mere presence of cohesin on chromatin, as occurs when Scc1 is synthesized only during G2, is not sufficient for efficient repair. For cohesin to facilitate repair during G2, it must previously have participated in a process that only occurs during S phase, which is presumably the creation of sister chromatid cohesion (197). Cohesion between sisters presumably also prevents double-strand breaks from causing chromosome breakage as well as providing a ready template for repair.

Double-strand break repair has a central role during meiosis, where it is responsible for creating recombinant chromatids and thereby for joining homologues in a manner that permits them rather than sisters to be disjoined at the first meiotic division. During meiosis, the 5′ ends of double-strand breaks created by the Spo11 endonuclease after premeiotic recombination (16, 94) undergo 5′ to 3′ resection to yield 3′-OH single-strand tails, which then invade a homologous chromatid. Repair synthesis and ligation give rise to double Holliday junctions (DHJs) (180, 199). At a later stage, these structures are resolved by cleavage and ligation to yield recombinant molecules with or without exchange of flanking markers (Figure 6). These two outcomes have very different consequences for chromosome segregation. Formation of recombinant chromatids but not gene conversion results in the connection of homologous chromosomes that is so crucial for chromosome segregation at the first meiotic division.

There are several remarkable aspects about this process, which are unique to meiotic cells and are crucial for meiosis. First, the usual preference of mitotic cells to use a sister chromatid for repair is reversed in favor of homologous chromatids (187). Second, double Holliday junctions are far more frequently resolved to form crossovers during meiosis than during mitotic double-strand break repair. Third, the creation of crossovers greatly reduces the probability that neighboring double Holliday junctions will be resolved in a similar manner, a phenomenon that is called crossover interference (102, 151). Fourth, during much of the time that it takes to convert double-strand breaks into crossovers, homologous maternal and paternal chromatids are bound together along their entire lengths (synapsed) to form a structure, unique to meiotic cells, called the synaptonemal complex (SC) (252) (Figure 6).

Electron microscopic analysis of the SC after staining with silver suggests that it is composed of two axial cores, one associated with maternal and the other with paternal sister chromatids, which are connected by a central element composed of a coiled coil protein known as Zip1 in yeast (42) and Scp1 (142, 143) in mammals.

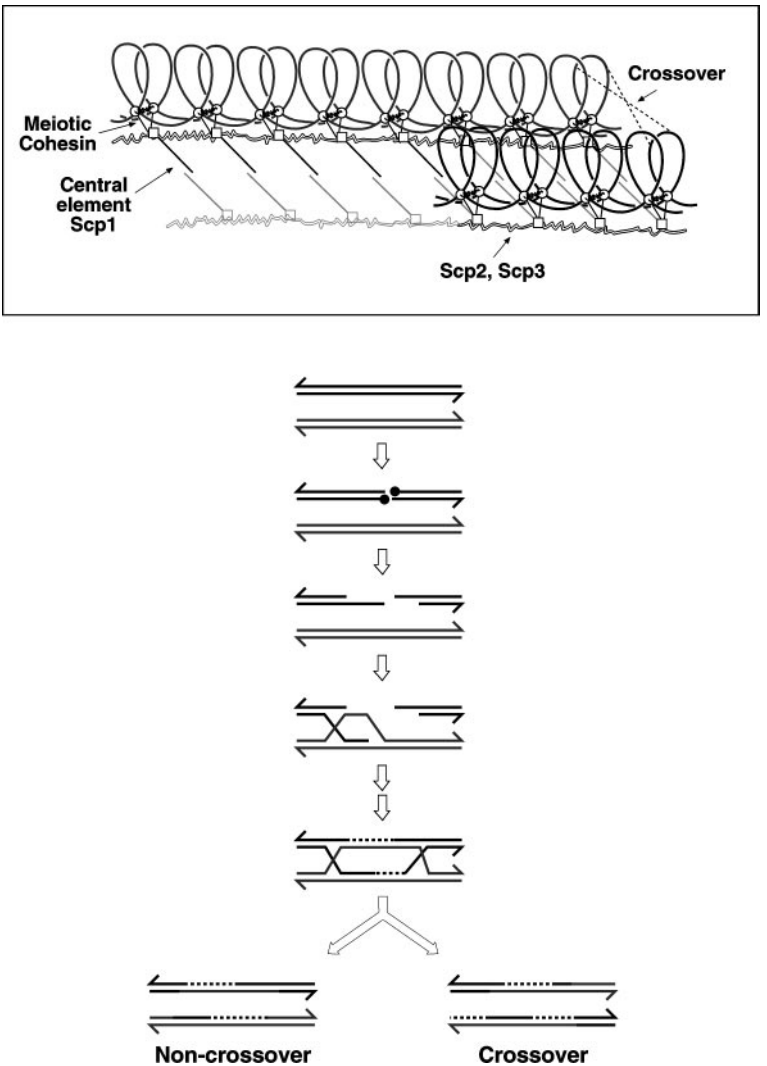


Figure 6 Cohesin and the synaptonemal complex (SC), in which crossing over between sister chromatids take place. Though Scp2 and Scp3 proteins run along the axial cores of the SC, meiotic cohesin composed of Smc1β, Smc3, Scc3-STAG3, and Rec8 lies at the heart of the SC's axial cores. *Below*, formation of double Holliday junctions and their resolution into crossovers, which is initiated by the Spo11 endonuclease.

Dissolution of the synaptonemal complex after the completion of recombination allows maternal and paternal sister chromatid pairs to separate except, of course, in the regions of crossovers, which are easily visible by light microscopy and are called chiasmata (252). The persistence of sister chromatid cohesion at this point ensures that crossovers now connect homologues together (122), which subsequently enables homologue pairs, and not sisters as during mitosis, to be aligned (i.e., pulled in opposite directions) by the meiosis I spindle apparatus (158).

Many if not most of these remarkable aspects of meiotic double strand repair only make sense when one considers that meiosis has two key purposes: to produce recombinant chromatids and to join homologues together via chiasmata so that they and not sisters are disjoined at the first meiotic division, which subsequently permits the formation of haploid progeny when chromatids are disjoined at the second meiotic division.

The formation of recombinant chromatids and the random assortment of centromeres from different chromosomes at the first meiotic division both contribute to the generation of gametes that differ greatly from each other and enable parents to "hedge" their genetic bets. By creating new haplotypes, some of which will lack deleterious mutation combinations, they also enable the cleansing of semideleterious mutations from diploid genomes (105). The purpose of using homologues rather than sisters to repair breaks produced by Spo11 and the resolution of double Holliday junctions as crossovers have obvious roles in halving the number of chromosomes and in producing recombinant chromatids, though the mechanism by which these goals are achieved is far from clear. The purpose of synaptonemal complexes and crossover interference is less obvious. An important clue is that some organisms, such as *S. pombe*, undergo meiosis and reciprocal recombination without forming SCs (11). Because organisms like *S. pombe* lack crossover interference, it is thought that the full synapsis of homologues might be a crucial part of the mechanism by which crossovers interfere with each other. When the number of crossovers per chromosome is low, crossover interference is crucial for ensuring that all chromosomes produce at least one crossover (note that a single crossover is sufficient to join homologues together, as long as there is sufficient cohesion between sister chromatids distal to that crossover). *S. pombe* only possesses three chromosomes along which there are very high rates of recombination, and crossover interference is unnecessary to ensure that each chromosome produces at least one chiasmata. The opposite extreme is found in *C. elegans* where there is rarely if ever more than one crossover per chromosome and crossover interference is therefore extremely high (15).

We have little or no idea how creation of a single crossover manages to inhibit the formation of others along an entire chromosome, as occurs in *C. elegans*. Signals emanating from crossovers, be they mechanical or informational, must be capable of traveling along the entire length of chromosomes and then preventing the resolution of all other double Holliday junctions (DHJs) on the same chromosome as crossovers. For this to occur, meiotic chromosomes must have a defined backbone or axial core along which these interference signals must travel. Furthermore,

crossovers involving only two chromatids must also signal to DHJs elsewhere on the chromosome involving a different pair of chromatids. The synapsis of all four chromatids, as occurs in SC, presumably facilitates this remarkable process.

Because the axial core of meiotic chromosomes may have a central role in providing the correct partner for exchanges (homologues versus sisters) and in mediating crossover interference, characterization of its constituents has been an important goal. Purification of synaptonemal complex from mammals has thus far led to the identification of two meiosis-specific proteins, Scp2 and Scp3, which localize along axial cores (184). Deletion of the gene for Scp3 in mice leads also to the loss of Scp2 from chromosomes and clearly compromises the formation of cores, but it does not eliminate them entirely, suggesting that other proteins lie at the heart of these structures (C. Hoog, personal communication). Indeed, no proteins similar to Scp2 or Scp3 have yet been found in yeast, whose synaptonemal complex also contains two clearly defined axial cores.

There is a growing consensus that meiotic cohesins might be the chief architects and constituents of meiotic axial cores. They both colocalize with cores (C. Heyting, personal communication; C. Hoog, personal communication; 171) and are necessary for the formation of SC (104, 163). Thus, Rec8, Smc1, Smc3, and Scc3 all colocalize with cores during pachytene in yeast (R. K. Clyne, personal communication; 104), whereas Rec8, Smc1β, Smc3, and STAG3 do so in mammals, as does Rec8 in *C. elegans* (163). Whether condensin also participates in formation of the SC's axial cores has not been addressed. Nevertheless, there is a distinct possibility that while the cores of mitotic chromosomes are built by condensin, those of the SC are built largely if not completely by cohesin. If so, this would indicate that cohesin and condensin not only resemble each other structurally but also possess a similar capacity to organize chromatin around axial cores. There is, of course, a crucial difference between the cores of mitotic and meiosis I chromosomes: A single chromatid is organized around the former whereas a pair of sister chromatids is organized around the latter (Figure 6).

Though the cores of meiosis I chromosomes are clearly stabilized by synapsis between homologues (which is mediated by recombination, central element proteins, and by yet other more mysterious pairing mechanisms), rudimentary cores containing cohesin can clearly form in the absence of synapsis (104). In yeast, the ability to produce cores and hence SC depends on the replacement during meiosis of Scc1 by Rec8. Though Scc1 expressed from the *REC8* promoter in cells lacking an intact *REC8* gene can bind to chromatin, produce sister chromatid cohesion, and even support the monopolar attachment of sister kinetochores to meiosis I spindles during meiosis I, it cannot support the formation of SC (223). Whether the replacement of Smc1 by Smc1β and Scc3-SA1/SA2 by STAG3 also contributes to the formation of SC in mammals is not yet known.

Though essential for the formation of axial cores and SC, meiotic cohesin subunits are not, at least in yeast, required for formation of the double-strand breaks that initiate the recombination process (104). In *rec8* or *smc3* mutants, double-strand breaks occur with almost normal kinetics but are poorly repaired

and fail to produce crossovers. The DNA ends produced by Spo11 in these mutants are resected more extensively than in wild-type cells, due presumably to inefficient invasion of homologous chromatids. The damage to DNA caused by this defect is thought to be detected by DNA repair surveillance mechanisms that block the first meiotic division. There is some evidence that double-strand breaks also form in the absence of Rec8 in *C. elegans*. Worms lacking Rec8 due to RNA interference accumulate chromosome fragments and this process is dependent on the Spo11 endonuclease (163). The abnormal repair of double-strand breaks in *rec8* mutants cannot simply be attributed to their defective sister chromatid cohesion because sister chromatids are not usually used to repair breaks during meiosis. Indeed, replacement of Rec8 by Scc1 fails to prevent the block to meiosis due to DNA damage, despite restoring sister chromatid cohesion (223).

In conclusion, the meiosis-specific version of cohesin, containing Rec8 instead of Scc1, is crucial for regulating the repair of double-strand breaks as well as for the formation of SC. Though the topology of meiotic chromosomes is no better understood than their mitotic counterparts, the chromatid fiber must consist of a core from which loops or coils emanate. DNA sequences within loops are presumably far more accessible than those within the core and are therefore most likely to participate in the production of double-strand breaks, though repair of these breaks might be conducted within cores. One of the key functions of cohesin during meiosis may be to organize the chromatid fiber around a cohesin-containing core, to which components like Scp2 and Scp3 might attach and reinforce the axes of meiotic chromosomes during pachytene (Figure 6). It is conceivable that cohesin would also have a similar activity during mitosis were it to remain on chromosomes. Indeed, this may be the explanation for why cohesin appears to have a role in chromosome compaction during mitosis in yeast (63), where the bulk of cohesin remains associated with chromosomes until the onset of anaphase (144).

CHIASMATA

Having regulated the production of crossovers, the SC then dissolves, which causes a dramatic change in the appearance of chromosomes. During pachytene, all four chromatids are held closely together in a single bundle by the SC. Its dissolution severs the connection between homologous chromosomes, except where they are joined by crossovers. Sister chromatids nevertheless remain associated along the entire length of chromosomes, though their proximity can vary considerably between organisms. In yeast, for example, sister chromatids remain very tightly connected (26), whereas in many animals and plants, sister chromatids start to appear as separate entities that have their own axial cores and are only connected with each other at their peripheries (242). In the ovaries of many vertebrates, meiosis is halted for long periods at this stage, which is known as diplotene. High rates of transcription, as witnessed in the famous lampbrush chromosomes of newts

and salamanders, help to produce stockpiles of maternal gene products needed for embryogenesis (54).

In the absence of SC, chiasmata assume responsibility for holding homologous chromosomes together from this stage onwards. Without them, homologous chromosomes would simply drift apart and it would not be possible to align them on opposite poles of the meiosis I spindle, which is precisely what happens when the Spo11 is inactivated in yeast (26, 193) and *C. elegans* (38). In *Drosophila*, there exist alternative mechanisms, which do not use recombination, for the synapsis of homologous chromosomes both during formation of the SC and subsequent to its dissolution (134). Even though it is clearly possible to pair and disjoin homologues without using chiasmata to hold them together, the vast majority of eukaryotic cells choose to use this device, which is presumably more robust than alternative mechanisms. As a consequence, in most eukaryotic organisms recombination is obligatory for chromosome segregation at meiosis I and hence for the formation of viable gametes. This presumably helps to ensure that recombination cannot readily be abandoned when their environment might favor its elimination.

There are two theories for how chiasmata perform their crucial task of holding homologues together (149). According to the first, homologues are bound together in the vicinity of chiasmata by some as yet unidentified substance, known as the chiasma binder. According to the second, homologues are held together by chiasmata due entirely to sister chromatid cohesion that persists distal (with respect to centromeres) to the crossover (122). This second theory is not only more economical than the first but it also explains why sister chromatids remain associated with each other until the onset of anaphase I, whereupon they invariably separate from each other. If sister chromatid cohesion were responsible for the ability of chiasmata to hold homologues together from diplotene until metaphase I, then its destruction would be required to resolve chiasmata at the onset of anaphase I.

The discovery of cohesin has recently permitted this theory to be tested. If it is cohesion between sister chromatids that holds homologues together, then cohesin should be found along the interchromatid region along chromosome arms until the onset on anaphase I, whereupon its removal should trigger loss of sister chromatid cohesion and thereby the resolution of chiasmata. Remarkably, this is precisely what has recently been found. In mammals, Rec8 and STAG3 colocalize to the axis connecting sister chromatids along the entire chromosome (except in the immediate vicinity of chiasmata) during the period from diplotene until metaphase I (C. Heyting, personal communication; 171), as does Rec8 in *C. elegans* (163), and they largely disappear from this location at the first meiotic division (Figure 2). The persistence of "meiotic" cohesin along chromosome arms until the onset of anaphase I clearly contrasts with the behaviour of its mitotic cousin Scc1, which largely if not completely disappears from chromosome arms during prophase and prometaphase of mitosis. The persistence of meiotic cohesin subunits along chromosome arms even during metaphase I is particularly remarkable because meiotic chromosomes shorten and compact considerably during this phase, as they do during the equivalent period of mitosis. Whether the so-called "prophase" pathway

responsible for removing cohesin from chromosome arms during mitosis is completely or only partly inactivated during the period between the onset of diplotene and the onset of anaphase I is unknown. It is not inconceivable that a failure to retain sufficient cohesin along chromatid arms to maintain chiasmata (i.e., to protect it from a process analogous to the mitotic prophase pathway) could contribute to chromosome mis-segregation (and hence to Down's syndrome) during oogenesis in women, whose oocytes enter diplotene around birth and do not enter metaphase I until induced to mature during menstrual cycles (see below).

ALIGNING HOMOLOGUES ON THE MEIOSIS I SPINDLE

One of the central aims of meiosis is to produce gametes with only a single complement of the chromosomes. The formation of haploid cells takes place at the second meiotic division when cells undergo what is in fact a fairly conventional mitotic division. What is unconventional about this division is that it takes place without a preceding round of chromosome duplication. The unique ability of meiotic cells to perform this extraordinary feat is due to peculiarities of their first meiotic division, which differs radically from mitotic divisions in three key aspects: the association through chiasmata of homologous chromosomes and not just sister chromatids, the attachment of sister kinetochores to spindles emanating from the same pole, and the persistence after anaphase I of sister chromatid cohesion in the vicinity of centromeres.

Due to the formation of chiasmata and the persistence of sister chromatid cohesion along chromosome arms, homologous chromosomes and not just sister chromatids are held together when the cell assembles its "meiotic" spindle apparatus. This creates the opportunity for spindles to pull maternal and paternal sister kinetochore pairs in opposite directions and hence to establish an equilibrium during metaphase I in which chiasmata resist traction of homologous chromosomes to opposite poles. One of the great mysteries of meiosis I is how cells avoid attaching sister kinetochores to spindles of opposite polarity, as occurs during mitosis. In the rare cases where this has been studied cytologically, sister kinetochores are seen to be fused into a common structure until their attachment to microtubules but they appear to split into separate structures sometime during metaphase or anaphase I (59). There are suggestions that the unique behavior of meiosis I kinetochores is conferred by the kinetochores themselves and not by the cytoplasm or spindles of these cells. Meiosis I and II cells from grasshoppers can be fused to produce a single cell with two independent spindle apparatuses. When homologous chromosomes attached by chiasmata (that had not yet attached to the spindle) are transferred to a meiosis II spindle, they align normally and disjoin to opposite poles at anaphase at the same time as "native" meiosis II sister chromatid pairs disjoin from each other on the same spindle. These and other similar experiments suggest that sister kinetochores only acquire the ability to attach to spindles with opposing polarity around anaphase I (159). Prior to this point, kinetochores are somehow altered so

that sisters "co-orient" (158) on the spindle. The grasshopper fusion experiments also demonstrate that the signal which triggers the resolution of chiasmata and hence the disjunction of homologues must the same as that which triggers the disjunction of sister chromatids at meiosis II.

In organisms like yeast, it has been possible to address roughly when, during the meiotic process, sister kinetochores become committed to the co-orientation (monopolar or syntelic attachment) characteristic of meiosis I. Cells undergoing meiosis can be returned to media that support vegetative growth. Surprisingly, cells only lose the ability to undergo a mitotic division after they have completed recombination; that is, it is still possible for late pachytene cells that have completed recombination to undergo mitosis instead of meiosis I if transferred to growth media (250). Thus, sister kinetochores become committed to co-orientation sometime between late pachytene and metaphase I.

The search for proteins which ensure that sister kinetochores attach to spindles with the same polarity during meiosis I has largely been undertaken on the premise that such proteins might be specific to meiotic cells. Thus far, three proteins with this property have been identified by genetic studies in yeast: Spo13 and Mam1 from *S. cerevisiae* and Rec8 from *S. pombe*. Inactivation of the *SPO13* gene, which is exclusively expressed in meiotic cells (233), causes about 50% of the chromosomes to undergo an equational rather than a reductional division at the first meiotic division (101, 195). For reasons that are still mysterious, it also prevents cells from undertaking a second meiotic division. The partial separation of sister chromatids to opposite poles at the first meiotic division in *spo13* mutants means that Spo13 is required not only for monopolar attachment but also for preventing the destruction of cohesion in the vicinity of centromeres. This implies either that these two aspects of meiosis I centromeres are intimately connected and conferred by the same set of proteins or that Spo13 has two or more separate functions. The Spo13 protein does not appear to localize exclusively to centromeres/kinetochores (A. Amon & A. Toth, personal communication) and it is therefore likely that Spo13 regulates the overall state of meiotic cells (131) or meiosis I chromosomes rather than any one particular property such as monopolar attachment. Both pairs of sister chromatids from a given homologue tend to behave in the same manner in *spo13* mutants; that is, either both or neither undergoes an equational division (86). This suggests that centromeres acquire a "reductional" state (that will ensure both monopolar attachment and cohesion protection) when all four chromatids are synapsed and can therefore communicate with each other, i.e., some time during pachytene. If so, then Spo13 must be required either to increase the probability that this state will be generated or for maintaining it. Spo13 cannot be an essential part of the monopolar attachment apparatus because this process still occurs in 50% or more of chromosomes in the complete absence of Spo13.

The Mam1 protein has recently been identified in a screen for meiosis-specific genes whose deletion causes chromosome mis-segregation (223). Unlike Spo13, Mam1 localizes to kinetochores from late pachytene until metaphase I. In its

absence, the first meiotic division fails to take place despite the formation of meiotic spindles and the subsequent destruction of securin, which activates separase and removes Rec8 from chromosome arms. The second meiotic division, in contrast, takes place on schedule but does so in the presence of four spindle pole bodies (microtubule organizing centers), which leads to massive chromosome missegregation. Furthermore, the persistence of Rec8 in the vicinity of centromeres until the onset of anaphase I is largely, though possibly not entirely, unaltered in *mam1* mutants, which suggests but does not prove that Mam1 is not essential for protecting cohesion in the vicinity of centromeres. Two pieces of evidence imply that Mam1 is required very specifically to prevent sister kinetochores from attaching to spindles of opposing polarity. First, an appreciable fraction of sister kinetochores separate precociously during its first aborted division, that is, prior to securin's destruction, which is normally closely associated with the onset of anaphase I. This implies that many if not most sister kinetochores come under traction pulling them toward opposite poles during meiosis I. The failure of *mam1* mutants to segregate chromosomes at meiosis I might then be due to their failure to destroy cohesion in the vicinity of centromeres. According to this hypothesis, *mam1* mutants attempt to pull sisters to opposite poles during meiosis I but are merely prevented from doing so by the persistence of cohesion in the vicinity of centromeres. If so, loss of cohesion at centromeres at the same time as it is lost along chromosome arms (see below) should suppress the meiosis I chromosome segregation defect of *mam1* mutants and permit a fully equational division.

The discovery that Scc1 does not persist at centromeres after anaphase I when expressed instead of Rec8 (albeit in a background where recombination has been eliminated by deletion of the *SPO11* gene) and indeed cannot support sister chromatid cohesion past this point suggests that sister chromatid cohesion in the vicinity of centromeres provided by Scc1, unlike that by Rec8, is destroyed along with that along chromosome arms at the onset of anaphase I (223). Remarkably, replacement of Rec8 by Scc1 (in a *spo11* mutant background) suppresses the failure of chromosome segregation during meiosis I in *mam1* mutants and causes all sister chromatid pairs to segregate to opposite spindle poles. These data all point to the Mam1 protein having a highly specific function in regulating the orientation of sister kinetochores during meiosis I. Mam1 is not necessary for the protection of cohesion at centromeres but is essential for monopolar attachment. These two properties of meiosis I centromeres are therefore determined by separate mechanisms, even though there exist mutations, like *spo13*, that affect both. This independence might be widely conserved because inactivation of the MEI-S332 gene in *Drosophila* abolishes retention of centromere cohesion without altering monopolar attachment (117). The Mam1 protein is not well conserved, and it has therefore not yet been possible to identify homologous proteins in other organisms. This will eventually be important for studying the structural basis of monopolar attachment because yeast kinetochores are too small to be observed even at the electron microscopic level.

The last meiosis-specific protein that has been implicated in determining monopolar attachment of sister kinetochores is the Rec8 protein in *S. pombe*. Unlike the situation in *S. cerevisiae*, deletion of *Rec8* in *S. pombe* does not abolish progress through meiosis (148). It is possible that many if not most double-strand breaks can still be repaired in Rec8's absence, due to the persistent expression of Rad21 (the homologue of Scc1), though this has never been investigated. Recombination is indeed reduced but despite this, cells proceed with both meiotic divisions. Another explanation for the very different outcome of deleting Rec8 in *S. pombe* and *S. cerevisiae* is that the former does not make SC and may therefore process its double-strand breaks in a manner that is less dependent on a Rec8-containing form of cohesin. Remarkably, the first meiotic division in *S. pombe rec8* mutants is almost entirely equational, with sister centromeres segregating to opposite poles in 90% or more of cases (238). The implication is that sister kinetochores attach to microtubules of opposing polarity in the absence of Rec8, that Rad21 (Scc1) provides sufficient cohesion between chromatids for their alignment in a bipolar fashion on the meiosis I spindle, and that all cohesion mediated by Rad21 is destroyed by separase at the onset of anaphase I.

At first glance, these data suggest that monopolar attachment in *S. pombe* might be mediated by a very different mechanism from that used by *S. cerevisiae*. Even if Mam1-like proteins exist in *S. pombe*, it is possible to eliminate completely monopolar attachment without directly inactivating such proteins. Is it possible therefore that Rec8 alone is responsible for altering the orientation of sister kinetochores during meiosis in *S. pombe* and that Mam1-like proteins are not required? Furthermore, the monopolar attachment apparatus in *S. pombe* clearly cannot function without Rec8 even when Rad21 is present in the cell, which also differs from *S. cerevisiae* where Scc1 can support monopolar attachment in Rec8's absence (223). It would not be surprising if monopolar attachment of sister kinetochores depended on cohesion between sister chromatids at centromeres, but in *S. cerevisiae* this can equally well be supplied by Scc1 as by Rec8.

Analysis of the distribution of Rad21 (Scc1) and Rec8 on *S. pombe* centromeres has shed some insight into this issue. *S. pombe* centromeres are more complex than those in *S. cerevisiae* and consist of an inner region that is associated with kinetochore proteins like the centromere-specific histone H3 variant Cenp-A and an outer region associated with HP-1-like proteins that regulate chromatin structure (162, 169). Rad21 is found in the outer but not in the inner region during mitosis (221), whereas Rec8 is found in both regions during meiosis I (239). Sister chromatid cohesion along chromosome arms and in the outer centromere regions is presumably sufficient for bipolar attachment of sister kinetochores during mitosis (177) and presumably also during meiosis. The absence of Rad21 from kinetochores themselves during mitosis therefore poses no fundamental problems. Rec8's presence within the kinetochore proper (the inner region) during meiosis I in *S. pombe* is consistent with the notion that monopolar attachment depends on an intimate cohesin-dependent juxtaposition of sister kinetochores. For some reason, Rad21 is incapable of penetrating (or functioning in) this holy sanctuary either

during mitosis or during meiosis and cannot therefore mediate the sister kinetochore cohesion normally performed by Rec8, which would explain why Rec8 is essential for monopolar attachment. Rad21 is nevertheless capable of associating with chromosome arms and provides sufficient cohesion between chromatids for their disjunction to opposite poles in *rec8* mutants at the first meiotic division. It is therefore plausible that, in fact, similar principles govern the monopolar attachment process in both yeasts. The monopolar attachment of sister kinetochores may require not only the activity of Mam1-like proteins, which coordinate sister kinetochores, but also cohesion between sister kinetochores without which Mam1-like proteins cannot even begin to act. Cohesion between sister kinetochores is possibly lacking during mitosis in *S. pombe* but is conferred by Rec8 during meiosis.

It is possible that monopolar attachment during meiosis I also depends on kinetochore proteins that are not specific to meiotic cells. The Bub1 protein kinase, which is associated with kinetochores during mitosis as well as meiosis, also has some role in preventing equational segregation during meiosis I in *S. pombe*. In its absence, chromosomes segregate equationally in about 30% of cells (18). Bub1 is also needed during mitosis (and possibly also during meiosis) for delaying activation of the APC when spindles are damaged or when chromosomes have failed to align on the metaphase plate (the mitotic checkpoint) (84). However, Bub1's role in promoting reductional chromosome segregation at meiosis I has little or nothing to do with its involvement in the mitotic checkpoint because mutation of other crucial components of the mitotic checkpoint such as Mad2 has little or no such effect. Bub1 clearly has multiple functions besides its role in the mitotic checkpoint and is even an essential gene in certain strains of *S. cerevisiae* (K. P. Rabitsch, personal communication). Rec8 is found at centromeres during meiosis I in *S. pombe bub1* mutants but it fails to persist at this location after anaphase I. Bub1 presumably regulates the state of Rec8 at meiosis I centromeres so that it resists destruction at the onset of anaphase I (see below). It is less clear whether this hypothetical alteration of the state of Rec8 in *bub1* mutants might be responsible for reducing, though not eliminating, monopolar attachment. It is possible that Bub1 has multiple functions at the meiosis I centromere and regulates the activity of proteins involved in monopolar attachment independently of its modulation of the state of Rec8. It has also been reported that the Slk19 protein in *S. cerevisiae*, which is associated with centromeres during mitotic metaphase and with the spindle midzone during anaphase, is required to prevent equational chromosome segregation during meiosis I (92). Current evidence does not distinguish whether Slk19 acts primarily to protect cohesion at centromeres, to confer monopolar attachment, or is a more general regulator of the meiotic process.

Proteins like Spo12, which are neither meiosis-specific nor known to be associated with centromeres, are also required to prevent equational chromosome segregation during meiosis I in *S. cerevisiae* (101, 195). Their function in regulating monopolar attachment is still mysterious. Finally, little or nothing is known about the identity of proteins that confer monopolar attachment in animal or plant cells.

RESOLVING CHIASMATA

It has long been recognized that the resolution of chiasmata might be the trigger for the first meiotic division. If sister chromatid cohesion mediated by cohesin's Rec8 subunit is responsible for the ability of chiasmata to hold homologues together, then the first meiotic division could be triggered by the destruction of sister chromatid cohesion, just as occurs in mitosis. This hypothesis is consistent with the loss of sister chromatid cohesion along chromosome arms during anaphase I and with the finding in grasshoppers that homologous chromosomes disjoin at the same time as sister chromatids when transferred to the spindle of a meiosis II cell (159).

The persistence until metaphase I of Rec8 and STAG3 along the axes that lie between sister chromatid arms suggests that the process which removes cohesin from chromosome arms during prophase and prometaphase during mitosis either does not occur or occurs less efficiently during the first meiotic division. A key question is whether cleavage of Rec8 by separase or some other process removes this population of cohesin from chromosome arms at the onset of anaphase I. This issue has thus far only been investigated in *S. cerevisiae*, where several lines of evidence indicate that activation of separase might indeed be the trigger for chiasma resolution and hence anaphase I (26). Separase is required for the first meiotic division and securin is destroyed shortly before the onset of anaphase (183). Furthermore, the bulk of Rec8 is cleaved in a separase-dependent fashion at the onset of anaphase I at two sites, both of which resemble the separase cleavage sites in Scc1. Mutation of both but not just one cleavage site completely blocks meiosis I, even when only one of two copies of *REC8* are mutated. Finally, the block to meiosis I chromosome segregation imposed by separase inactivation or by nondegradable Rec8 is bypassed by deletion of the *SPO11* gene, which indicates that cleavage of Rec8 by separase is only needed for chromosome segregation during meiosis I if crossing over has previously connected homologous chromosomes. In *spo11* mutants homologous chromosomes are segregated by meiosis I spindles to the two poles at random in the complete absence of separase activity (26).

Whether cleavage of Rec8 by separase also triggers anaphase in animal cells is still unclear. On the one hand, there is clear evidence that both the APC and separase are required for meiosis I in *C. elegans*. Mutants with temperature-sensitive mutations in APC subunits arrest in metaphase of meiosis I when shifted to the restrictive temperature (51). Furthermore, inactivation of separase either by mutation or by RNA interference prevents the proper disjunction of homologues at meiosis I (M. Siomos, personal communication). On the other hand, there are indications that meiosis I in *Xenopus* oocytes might not require the APC. Injection of antibodies (166) or antisense RNA (210) directed against the APC activator protein Cdc20 (Fizzy) fails to block meiosis I, despite preventing the proteolysis of cyclin B. Furthermore, neither antibodies against Cdc27, a core APC subunit, nor high levels of the checkpoint protein Mad2, nor a nondegradable form of securin prevented the first meiotic division (166). This raises the possibility that chiasmata in vertebrates might be resolved by a mechanism that requires neither the APC nor separase. A

variation on the pathway that removes cohesin during mitotic prophase is one possibility. However, this process would have to be very differently regulated in that it would have to remain inactive during diplotene, diakinesis, and metaphase I. Given the uncertainties surrounding the use of antibodies and antisense RNA, more rigorous genetic studies will be necessary to resolve whether the APC and separase really are redundant during meiosis I in vertebrates. It would be surprising though not unimaginable if chiasmata were resolved by very different mechanisms in worms and man.

In summary, it is possible though not yet certain that cleavage of Rec8 triggers the first meiotic division just as cleavage of its cousin Scc1 triggers mitotic chromosome segregation. According to this hypothesis, only two key innovations are required to convert a mitotic division into meiosis I during which homologues and not sister chromatids are segregated to opposite poles. These are the formation of chiasmata due to reciprocal recombination and the attachment of sister kinetochores to the same spindle pole. Chromosome alignment comes about because chiasmata resist the attempt of meiosis I spindles to pull homologues to opposite poles. The persistence of meiotic cohesin on chromosome arms until metaphase I, even in organisms that completely remove its mitotic counterpart from chromosome arms during prophase and prometaphase, is responsible for holding homologues together during their alignment on the meiosis I spindle. Control of chromosome arm cohesion must therefore differ between mitosis and meiosis. It is unclear whether this arises from differences between meiotic and mitotic cohesin or from other differences between mitotic and meiotic cells. Little is known about the role of condensin during the process of chiasmata resolution. One suspects that it will be found to bind to meiotic chromosomes during diplotene and diakinesis and to have a key role in resolving sister chromatid arms in preparation for their final separation at the metaphase to anaphase transition.

ACHIASMATE CHROMOSOME SEGREGATION

In many insects, there is little or no recombination during meiosis in the heterogametic sex. For example, meiosis takes place in the complete absence of recombination in *Drosophila* males (232). Despite this, homologues pair and subsequently disjoin at meiosis I. How they do this remains a mystery. Nevertheless, the very fact that they are able to pull off this feat raises questions as to why recombination is an obligate step for meiotic chromosome segregation in most other eukaryotes. There are several possible explanations. Chiasmata might just be a more effective method of holding homologues together. Alternatively, their use for chromosome segregation might provide a mechanism for ensuring that gametes are not produced in the absence of recombination, which has its own independent merits. It is also possible that by linking the production of gametes to the process of recombination, most eukaryotes make it very difficult for themselves to abandon sexual reproduction, which may have short-term advantages but be disastrous in the longer term.

It is clearly important to understand not only how homologues synapse in the absence of recombination but also how they are triggered to disjoin at the onset of anaphase I. The process of homologue pairing may have much in common with the synapsis between homologues that takes place prior to recombination in most if not all eukaryotes. Thus, both homologue pairing and formation of SC take place in mutants defective in the Spo11 endonuclease in *Drosophila* (136) and in *C. elegans* (38). Whether cohesin has a role in this process is unclear. Inactivation of Rec8 in *C. elegans* by RNA interference does not prevent initial alignment of homologues but does prevent SC formation (163).

Whereas homologues fail to remain paired in *spo11* mutants in *C. elegans* after dissolution of the SC, with disastrous consequences for chromosome segregation, they remain associated in *Drosophila* males during their alignment on the meiosis I spindle and then disjoin to opposite poles at anaphase I. Interestingly, the small fourth chromosome in *Drosophila* does not undergo recombination even in females, and its proper segregation presumably depends on the same sort of mechanism that governs segregation during male meiosis. This mechanism, known as distributive pairing, also "kicks in" when autosomes fail to form chiasmata in females. A kinesin-like protein called Nod is crucial for preventing the precocious disjunction of achiasmate homologues in *Drosophila* (3, 251). Nod is neither required during mitosis nor for preventing precocious disjunction of homologues connected by chiasmata (bivalents). Nod and its homologues in *Xenopus*, Xkid, are associated with chromosome arms and are therefore called chromokinesins (9, 49). They are thought to participate in the process by which microtubules unconnected to kinetochores manage to "blow" chromosomes toward the equator of the spindle apparatus. Xkid, for example, is essential for the proper congression of chromosomes to the metaphase plate. It would appear that in Nod's absence, achiasmate connections between homologues are insufficient to resist the tendency of kinetochore-attached microtubules to pull homologues toward opposite poles during metaphase I. Without Nod, chromosome 4 homologues disjoin either prior to or during metaphase I while all other chromosome pairs remain attached by chiasmata and align on the metaphase I spindle (135). Remarkably, Xkid is destroyed at the metaphase to anaphase transition by the APC (49). Furthermore, nondegradable versions block chromosome disjunction in *Xenopus* extracts. If Nod were destroyed likewise, the APC would not only trigger disjunction of chiasmata by activating separase but also the disjunction of achiasmate homologues by triggering destruction of Nod. This could explain how *Drosophila* oocytes manage the remarkable feat of triggering disjunction of chiasmate and achiasmate homologues at around the same time during meiosis I.

RETAINING COHESION AROUND CENTROMERES

Though monopolar attachment of sister kinetochores and the production of chiasmata that are resolved by destruction of arm cohesion can explain the disjunction of homologues and not sister chromatids to opposite poles at meiosis I, they are not

sufficient to explain how meiotic cells then manage to undergo a second round of chromosome segregation without an intervening round of DNA replication. This remarkable feat depends on the retention of cohesion in the vicinity of centromeres at the onset of anaphase I while at the same time destruction of cohesion along chromatid arms triggers resolution of chiasmata. Cohesion retained at centromeres is crucial for aligning sister chromatids on the metaphase II spindle and, in all likelihood, its destruction triggers the disjunction of sister chromatids at the onset of anaphase II (148).

Though necessary for normal chromosome segregation during meiosis II, the retention of cohesion at centromeres is unnecessary for meiosis I. Cohesion between sister centromeres fails to be maintained at the onset of anaphase I in *bub1* mutants in *S. pombe* (18), in *MEI-S322* mutants in *Drosophila* (95), and in strains of *S. cerevisiae* in which Rec8 has been replaced by Scc1 (223), but homologues nevertheless segregate in a (largely) reductional manner at the first meiotic division. This indicates that, once established during metaphase I, the continued attachment of sister kinetochores to spindles from the same pole does not require cohesion to persist after the onset of anaphase I and is sufficient to draw sister centromeres toward the same spindle pole throughout anaphase I. However, loss of cohesion at centromeres does cause sister chromatids to drift apart before they can be aligned on the meiosis II spindle and as a consequence they segregate in random directions to the poles during the second meiotic division.

There are many unanswered questions about the retention of cohesion at centromeres during and after the first meiotic division. How much cohesion must be retained for successful chromatid segregation during meiosis II? By what mechanism is cohesion protected from the process that destroys cohesion along chromatid arms? How is protection propagated from centromeres and what is the signal or seed that initiates the process? How is the propagation of protection blocked by the formation of chiasmata and how far would it propagate away from centromeres in the absence of chiasmata? Finally, how does cohesion at centromeres that had been resistant to dissolution at meiosis I acquire the ability to be dissolved during meiosis II?

A clue as to how much cohesion may be sufficient for meiosis II comes from the study of mitotic cells that have been arrested in a metaphase-like state by treatment with spindle poisons and then triggered to undergo anaphase by their removal. Under these circumstances, cohesion is completely lost from chromosome arms and is only retained within centromeric heterochromatin and yet chromatid segregation takes place with reasonably high fidelity (177). There is no reason to believe that this amount of cohesion would not be equally sufficient for meiosis II.

It has never been clear until recently whether the persistence of cohesion around centromeres is due to this cohesion being of a different or similar nature to that which connects chromatid arms. However, the observation, now in a wide variety of organisms, that Rec8 persists in the vicinity of centromeres until anaphase II while disappearing from chromosome arms at the onset of anaphase I suggests (but does not yet prove) that cohesin mediates cohesion at meiotic centromeres as well as along chromosome arms. For example, Rec8 persists in the vicinity

of centromeres until the onset of anaphase II not only in *S. cerevisiae* (104) and *S. pombe* (238), where this phenomenon was first described, but also in *C. elegans* (163) and in mammals (C. Heyting, personal communication). During meiosis I, centromeric cohesin must resist not only dissociation by processes analogous to the mitotic prophase pathway but also cleavage by separase. Whether the persistence of cohesin at centromeres during meiosis I is due to meiosis-specific differences in its subunit composition or due to changes in its packing (higher-order structure) or modification is not known.

In budding yeast, where it is known that cleavage of Rec8 by separase triggers resolution of chiasmata, there are strong indications that Rec8 in the vicinity of centromeres somehow escapes this fate at meiosis I but nevertheless falls victim at meiosis II. Cleavage of Scc1 during mitosis is known to be both necessary and sufficient to cause its dissociation from chromosomes during anaphase (226). If the same applies to Rec8, then its persistence in the vicinity of centromeres after meiosis I indicates that it must have escaped cleavage by separase. By the same token, Rec8's disappearance from centromeres at the onset of anaphase II indicates that it may be cleaved at this juncture (104). Furthermore, the securin that reaccumulates rapidly after anaphase I is rapidly destroyed just prior to the onset of anaphase II, which implies that separase is activated at the right time to trigger the second as well as the first meiotic division (183). Indeed, given the similarities between mitosis and the second meiotic division, it is hard to believe that chromatid separation is mediated by completely different mechanisms.

It is therefore a reasonable working hypothesis that the retention of centromeric cohesion at anaphase I is due to the resistance of Rec8 to cleavage by separase while similar if not identical molecules on chromosome arms are destroyed at the same time. This resistance must be lost after anaphase I with the result that sister chromatid disjunction can be triggered by a second round of separase activation during meiosis II. To test this model directly, it will clearly be necessary to detect intact Rec8 on meiosis II chromosomes as well as its cleavage at the onset of anaphase II, which has so far not been possible due to the lack of synchrony of meiotic cultures. Whether this model applies to centromeric cohesion in other eukaryotes depends on whether cleavage of Rec8 by separase triggers resolution of their chiasmata. The finding in grasshoppers that meiotic sister chromatids can be disjoined when placed on a meiosis I spindle (159) is certainly consistent with the hypothesis as is the finding that the Rec8 protein retained at *C. elegans* (163) and mammalian (C. Heyting, personal communication) centromeres disappears after anaphase II.

There are several potential mechanisms that could protect Rec8 in the vicinity of centromeres from separase at the first meiotic division. Rec8 might be shielded by factors or modifications that prevent access of the protease, it might be associated with a protein, which, like securin, directly inhibits the protease's activity, or it might fail to be modified in a manner necessary for its cleavage (4). Rec8, like Scc1, might need to be phosphorylated before it can serve as an efficient separase substrate and the kinase responsible for "preparing" Rec8 for cleavage

might be excluded from centromeric chromatin. PLK or other mitotic kinases such as Aurora B or even Cdk1 could play a role in this process. The finding that histone H3 is phosphorylated during maize meiosis only on those chromosomal regions that will imminently lose cohesion suggests that the kinase responsible for this phosphorylation, Aurora B, might be directly involved (93). If phosphorylation of Rec8 by Aurora B were required for its cleavage by separase, then exclusion of Aurora B from centromeres at meiosis I could account for their continued cohesion until anaphase II. It is equally possible that the protection of Rec8 from separase cleavage is mediated by differences in the chromosomal distribution of other types of modification on proteins other than cohesin itself.

The finding that Scc1 can support sister chromatid cohesion and monopolar attachment during meiosis I in *S. cerevisiae* but cannot resist separase in the vicinity of centromeres provides an important clue about Rec8's resistance, at least in yeast (223). Rec8 clearly possesses special properties that are lacking in Scc1 that enable it to be protected from separase in the vicinity of centromeres. It also confirms the notion, first developed from the study of MEI-S332 in *Drosophila*, that the retention of cohesion at centromeres is conferred by a process that is independent of monopolar attachment. Because Rec8 and not Scc1 can be protected, retention of cohesion cannot be due to the general shielding of centromeric chromatin and cohesin complexes associated with it from enzymes like separase.

In the absence of any clear understanding about the biochemical basis for the retention of centromeric cohesion, the identification of proteins with a role in this process by genetics will be invaluable for providing clues as to its mechanism. One might expect that some if not most of the proteins necessary for protecting centromeric cohesion during meiosis would prove to be specific to meiotic cells. Strangely, no such protein has yet been discovered. All three proteins thus far implicated in protecting cohesion at centromeres are also expressed in mitotic cells. The first such protein to be identified was MEI-S332 from *Drosophila*, which associates with the pericentric heterochromatin adjacent to but not coincident with kinetochores from prometaphase I until the onset of anaphase II (117). In its absence, bivalents disjoin normally at anaphase I but sister chromatids soon thereafter separate and mis-segregate at meiosis II. MEI-S332 behaves in similar fashion during mitotic divisions, associating with centromeric chromatin during prometaphase and dissociating at anaphase. It is not, however, required for chromosome segregation during mitosis (150).

MEI-S332's absence from chromosomes until prometaphase (150) suggests that it is not itself part of the sister chromatid cohesion apparatus. Moreover, its presence on chromosomes during metaphase II and during mitotic metaphases implies that it does not directly protect centromeric cohesion from its imminent destruction. Though present on chromosomes during all metaphases, MEI-S332 only protects cohesion at the onset of anaphase I or during a short period thereafter. It is not immediately obvious why a protein with such a role should dissociate from chromosomes when sister chromatids separate during mitosis or meiosis II. MEI-S332's dissociation from chromatids whenever they separate raises the

possibility that it might be caused by the process that triggers sister separation. If so, a conundrum arises: MEI-S332 may both regulate and be regulated by the sister separation process. It is clearly important to establish the mechanism by which MEI-S332 dissociates from chromatids as they separate as well as the mechanism by which it regulates loss of cohesion. Is its dissociation from chromosomes regulated by separase and the APC, by mitotic protein kinases, or by an entirely novel process? Does MEI-S332 regulate loss of cohesion by regulating cleavage of cohesin subunits by separase?

A model that could tie all these phenomena together is that MEI-S332 forms a complex with cohesin on centromeric heterochromatin. Disruption of this complex by cleavage of cohesin's Scc1/Rec8 subunit would explain why MEI-S332 dissociates from chromosomes whenever cohesion is destroyed. During meiosis I but not during mitosis or meiosis II, some as yet mysterious factor or set of conditions alters the MEI-S332/cohesin complex so that it, but not cohesin that has not bound MEI-S332, becomes resistant or inaccessible to separase. Thus MEI-S332 only regulates loss of cohesion during meiosis I but nevertheless dissociates from chromatin at each and every anaphase, except at anaphase I when cohesin is not cleaved in the vicinity of centromeres. Though not essential, MEI-S332 presumably does have some role in mitotic cells, possibly helping to protect cohesion under certain circumstances (150).

The Bub1 protein in *S. pombe* is also required to prevent destruction of cohesion at anaphase I (18). In *S. pombe bub1* mutants, Rec8 fails to persist in the vicinity of centromeres after anaphase I and as a result sister chromatids drift apart before anaphase II. It is unclear whether Bub1 is located at kinetochores (i.e., in the inner centromere region), or in the neighbouring heterochromatin (i.e., in the outer centromere regions). If, as in mammals, Bub1 is located within the kinetochore itself (218), it may be required for sending a signal from the kinetochore to its surrounding heterochromatin—a signal that initiates the formation (and propagation along the chromosome) of cohesion that is resistant to separase. Bub1 is also required for the surveillance mechanism (the mitotic checkpoint) that delays activation of the APC until all chromosomes have correctly aligned on the metaphase spindle. However, Bub1's role in protecting cohesion must be independent of the mitotic checkpoint because *mad2* mutants, which are equally defective in the checkpoint, are not defective in protecting centromeric cohesion. Like MEI-S332, Bub1 is not specific to meiotic cells. Some other meiosis-specific factor is presumably also required for the formation of separase-resistant cohesin at *S. pombe* centromeres during meiosis I.

The last centromeric protein to be implicated in protecting cohesion during meiosis I is Slk19 in *S. cerevisiae*. Slk19 associates with centromeres during mitosis and meiosis but a sizeable fraction of the protein relocates to the midzone of the mitotic spindle during anaphase where it has an important role in stabilizing late anaphase spindles (249). It has been reported that deletion of *SLK19* causes sister chromatids to separate during meiosis I in a high fraction of cells and causes Rec8 to disappear from centromeres precociously (92). If true, Slk19, like Spo13, must be required both for monopolar attachment and for protecting cohesion. Deletion of

SLK19 also prevents a second meiotic division. Because of this and because current studies have not carefully compared the kinetics of meiotic events in wild-type and mutant cells, it is not possible to be sure at this juncture whether the equational division observed in *slk19* mutants is due to precocious sister chromatid separation or due to an aborted reductional division. If the Slk19 protein does indeed have a role in protecting cohesion at centromeres, then a crucial question is whether this function is mechanistically related to its role in stabilizing anaphase spindles or whether these are independent functions of the same protein.

In summary, the ability of centromeric cohesion to resist the process that resolves chiasmata is conferred by a process that is to a considerable extent independent of that which causes the monopolar attachment of sister kinetochores. This is possibly not too surprising when one considers that cohesion is retained within the entire peri-centric heterochromatin whereas monopolar attachment only concerns kinetochores. Thus far, the only meiosis-specific protein to be implicated in retention of cohesion is Rec8, whose replacement by Scc1 in *S. cerevisiae* abolishes the retention of cohesion. It is likely that other meiosis-specific proteins involved in this process await discovery. No meiosis-specific Scc1-like subunit has emerged from sequencing of the *Drosophila* genome. Though it is conceivable that *Drosophila*'s Rec8 gene lurks in hitherto unsequenced heterochromatin regions, it is equally likely that flies use a single Scc1-like protein for mitosis and meiosis. Rec8, for example, is capable of complementing the complete lack of Scc1/Rad21 in both budding and fission yeast, so there is no intrinsic reason why a single gene could not suffice for meiosis and mitosis in flies. If so, then *Drosophila* at least must possess some other meiosis-specific protein that causes the protection of centromeric cohesion during meiosis I.

At least three different types of protein are implicated in protecting centromeric cohesion: (*a*) those residing at kinetochores (like Bub1 in *S. pombe*), which provide a "spatial" signal saying that the adjacent heterochromatin and not that present elsewhere on the chromosome is an appropriate substrate for protection; (*b*) those within centromeric heterochromatin itself (like MEI-S332 in flies), which might facilitate protection from separase; and (*c*) those that are specific to meiotic cells which ensure that this remarkable process only occurs during meiosis I. How the "protected" chromatin propagates along chromosomes and how this is blocked by the formation of chiasmata are genuine mysteries that await further investigation. Though crossovers must be capable of blocking propagation, they do not seem to be necessary to curb its extent to a limited region around the centromere, because the bulk of Rec8 along chromosome arms is still destroyed by separase when recombination has been eliminated by deletion of the *SPO11* gene in *S. cerevisiae* (104).

ANEUPLOIDY IN HUMANS

Mis-segregation of chromosomes during meiosis or mitosis leads to cells with altered numbers of chromosomes, which is known as aneuploidy. Aneuploidy due to mis-segregation during meiosis is usually lethal for mammalian embryos and

is a leading cause of spontaneous miscarriages in humans (61). One third of all spontaneously aborted embryos are trisomic for at least one chromosome. Inheritance of an extra chromosome 21 in humans (trisomy 21) is not lethal but leads to Down's syndrome, the leading single cause of mental retardation. Meanwhile, it has long been recognized that the cells of most malignant solid tumors are highly aneuploid due presumably to frequent chromosome mis-segregation during mitosis (114). Though it has never been established whether chromosome mis-segregation and/or aneuploidy actually promotes the genesis of tumors, it could make a major contribution to the "uncovering" of recessive mutations in tumor suppressor genes. Indeed, mice heterozygous for a *mad2* mutation, which compromises control of the APC by the mitotic checkpoint, are prone to lung tumors (145), though whether this is due to their failure to regulate mitosis as opposed to other steps of the cell cycle controlled by the APC is presently unclear.

Defects in sister chromatid cohesion, resolution, and separation could all contribute to the genesis of aneuploidy. Though essential for mitosis and meiosis, partial, i.e., nonlethal defects, in cohesin or condensin subunits cause very high rates of chromosome loss in yeast (128, 144, 198, 222), and there is no reason to believe that they would not do likewise in humans. Indeed, deletion of the securin gene in a human colon carcinoma cell line with a stable karyotype is sufficient to cause the extreme karyotypic instability characteristic of many malignant colon carcinoma cell lines (89).

Defects in the sister chromatid cohesion apparatus also compromise double-strand break repair both in mitosis and meiosis (21, 104, 197) and could thereby also contribute to the genome instability of somatic tumor cells and to infertility due to defects in gametogenesis. Given that cohesin may have a fundamental role in the organization of interphase chromatin in every cell of our bodies, defects in its activity or regulation could have extremely pleiotropic and damaging consequences.

Because it gives rise to Down's syndrome, there has been extensive investigation of trisomy arising from chromosome mis-segration during meiosis. There are several potential causes: defective recombination leading to a lack of chiasmata (68), instability of chiasmata (i.e., precocious resolution), defects in sister chromatid cohesion that cause chromatids to disjoin before formation of the first meiotic spindle, a failure to retain cohesion between sister centromeres after meiosis I, defects in a back up system that facilitates disjunction of homologues even in the absence of chiasmata (135) or precocious loss of the "reductional" state needed for both monopolar attachment and retention of centromeric cohesion.

Despite extensive study of the etiology of trisomy, no single mechanism has been pinpointed. Several important conclusions have nevertheless been reached (69). First, there is considerable variation in the incidence of trisomy between chromosomes, with that of chromosome 16 by being far the most frequent among spontaneous abortions. Second, the vast majority of trisomies arise due to mis-segregation in oocytes. Third, the frequency of mis-segregation rises steeply with maternal age. Fourth, the majority of segregation errors must have occurred during

the first meiotic division but those occurring at meiosis II are far from negligible especially for particular chromosomes.

Whether or not chromosomes had mis-segregated at the first or second division can to some extent be determined by scoring whether the zygote inherited centromere proximal markers from the same or different grandparents. Maternal sister centromeres normally segregate away from paternal sister centromeres at the first meiotic division. Thus, if one of the two centromeres inherited from a mis-segregating oocyte (there should have been one) has a paternal origin and the other a maternal one, there must have been an error during meiosis I. Possible causes for this "MI" mis-segregation are precocious loss of sister chromatid cohesion prior to alignment on the meiosis I spindle, an equational instead of a reductional division, a lack of chiasmata due to recombination failure, or (precocious) resolution of chiasmata before chromosome alignment on the meiosis I spindle. If, on the other hand, both centromeres derived from the misbehaving oocyte have the same origin, i.e., both are paternal or both maternal, then mis-segregation might have occurred at the second division (MII errors) and could have been caused by a failure to retain cohesion at centromeres after meiosis I or due to misalignment on the meiosis II spindle. However, it is still possible that some so-called "MII" trisomic embryos could have arisen due to abnormalities during meiosis I, if, for example, there had been a precocious loss of sister chromatid cohesion.

The fifth important finding is that many but by no means all trisomies are associated with a lack of recombination. Of embryos with MI errors, 40% of chromosome 21 trisomies were apparently achiasmatic. Furthermore, even when recombination has occurred, it tends to be more telocentric than in controls. It is conceivable that such crossovers are more likely to produce "unstable" bivalents in which sister chromatid cohesion distal to the crossover is insufficient to hold homologues together. However, a failure to recombine seems less likely to contribute to trisomy 16. The finding that achiasmatic trisomies increase in frequency with maternal age is a conundrum because recombination takes place before birth! This raises the possibility that "susceptible" chromosomes arise prenatally and are abnormally processed only much later, possibly when induced to mature. Defects in sister chromatid cohesion could contribute to this phenomenon because cohesion established prenatally during premeiotic recombination could be abnormally processed during subsequent age-dependent oocyte maturation. Furthermore, alterations in the metabolism of meiotic cohesins could contribute to recombination defects as well as sister chromatid cohesion defects.

One of the difficulties in studying human trisomy is that it is impossible to observe either of the meiotic divisions that gives rise to it. One way of overcoming this problem has been to analyze matured oocytes collected from women visiting infertility clinics (8, 244). The premise for this approach has been that some, if not a large fraction, of infertility is caused by meiotic chromosome mis-segregation. Such oocytes, which are arrested in metaphase II prior to fertilization, are far more frequently found to possess extra chromatids than they are to possess an extra pair of sister chromatids. It is unlikely, though still possible, that such chromatids arise

due to a lack of recombination or due to precocious resolution of chiasmata. They are more likely to have arisen either due to an equational division of that chromosome at meiosis I (which would occur if the chromosome's reductional state were lost before meiosis I as occurs in *spo13* mutants in yeast) or to precocious loss of cohesion throughout the chromosome prior to meiosis I or to a failure to retain cohesion between sister centromeres after the first meiotic division. There is some reason to believe that the oocytes that actually give rise to trisomy might derive from this pool of oocytes with extra chromatids because their pattern of aneuploidy resembles that found in aborted fetuses. Future studies will be required to establish whether these findings also apply to oocytes from the female population at large.

SUMMARY

The chromosome movements that constitute mitosis were first properly described in 1880 by Walter Flemming (47). By noting that "the impetus causing chromosomes to split longitudinally acts simultaneously on all of them," Flemming clearly recognized the special nature of the metaphase to anaphase transition. Indeed, the discovery that chromosomes split longitudinally was an important clue that they might carry the hereditary material and that differentiation did not take place through its unequal distribution (129). For over a century, analysis of this process was confined to cytological descriptions, which clearly delineated the various phases of mitotic and meiotic chromosome morphogenesis as well as the crucial role of the spindle apparatus (36, 130, 158, 186). It is remarkable that despite major advances in our understanding of microtubules and their dynamics during the past 25 years (146), we have until very recently remained rather ignorant of the biochemical mechanisms that regulate chromosome morphology. Thus, Miyazaki and Orr-Weaver wrote as recently as 1994 that "It is critical that our understanding of sister chromatid cohesion move to a molecular level. The cell cycle signals that trigger the dissolution of sister chromatid cohesion need to be elucidated and the proteins promoting cohesion isolated" (147). This call for action was a prescient one, as the Anaphase-promoting complex was discovered merely one year later (88, 97, 203).

Though we now have a very clear picture of some of the cell cycle signals that trigger dissolution of sister chromatid cohesion, much remains to be discovered. What, for instance, is the signal that triggers dissociation of cohesin and association of condensin during prophase and by what mechanism does this occur? How is this pathway differently regulated during meiosis, which permits chiasmata to persist until the onset of anaphase I? What protects cohesin in the vicinity of centromeres from the "prophase" pathway during mitosis and from separase at the onset of anaphase I? We still have little or no idea how securin regulates separase activity, how unoccupied kinetochores inhibit the APC, or even what determines APC activation in the absence of such surveillance mechanisms. We also have

little idea about the mechanism that to some limited extent confines anaphase triggers, such as active APC or separase, to a single mitotic spindle (14, 178, 231). Though many of the proteins that mediate sister chromatid cohesion (cohesin) and resolution (condensin) have been identified, we have a poor grasp, if any, as to the mechanism by which they function, without which it will not be possible to understand how cleavage of Scc1 and Rec8 triggers the destruction of cohesion and hence the onset of anaphase. The complete determination of genome sequences has tended to lull us into a false sense that we understand chromosomes. They are one of the cell's most complex organelles and one about which we remain hugely ignorant.

Even when we have answers to these questions, the mystery of how the mitotic and meiotic process evolved will remain. There is a strong tendency in evolutionary biology to view current cellular mechanisms as the result of past accidents. Though many if not most of the details might be attributable to contingency, there is an underlying logic to the mitotic and meiotic process that clearly "awaited" discovery once the first genomes had arisen (153). There is only a single solution to the blind men's riddle and nature sooner rather than later came up with it. Its evolution was therefore no accident. Far more mysterious is the pathway that facilitated its selection. The conundrum is a familiar one to evolutionary biology. Mitosis requires two fundamental processes, neither of which is much use without the other. The spindle apparatus presumably evolved first for transporting molecules around the cell and for controlling cell morphology, whereas the sister chromatid cohesion apparatus presumably evolved for double-strand break repair. Only when both of these key innovations were in place could they together be used for segregating chromosomes.

Meiosis is in a way less mysterious because the only fundamental extra innovation required was a method for systematizing crossing over between homologous chromosomes, which enables sister chromatid cohesion to hold homologous chromosomes together on the first meiotic spindle. The suggestion that sisters only separate at the second division in order to avoid "sister killers" (64) does not fully take into account the actual mechanisms by which chromatids are segregated during meiosis and how this might have evolved from mitosis. It could merely be a curious accident, from which most eukaryotic organisms profit, that sister killers are indeed minimized by the meiotic process that was most readily derived from what may have been pre-existing mitotic processes. Whether we will ever be able to answer these "why" questions will depend on the survival of missing links that confirm, for instance, that mitosis did indeed precede meiosis. Sadly, the superiority of sexual reproduction may have ensured their demise. No existing eukaryotic phylum lacks meiosis. It is, however, equally possible that the simpler process (mitosis) in fact evolved from the more complicated one (meiosis). Though this may appear counterintuitive, it is actually easier to imagine rudimentary cohesion/spindle apparatuses being used initially on a sporadic basis for sexual purposes before they were sufficiently refined for disseminating genomes in an efficient manner during cell proliferation. In the absence of missing links,

we will have to remain content with addressing "how" questions, of which plenty remain.

ACKNOWLEDGMENTS

I would like to thank Hannes Tkadletz for help with drawing the figures, Alex Schleiffer for compiling the table of separase cleavage sites, Jan-Michael Peters for comments on the manuscript, Diane Turner for help preparing this manuscript, Jean-Louis Sikorav for telling me about the riddle of the blind men and their socks, and all those who kindly communicated information prior to publication.

LITERATURE CITED

1. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–95
2. Adams RR, Carmena M, Earnshaw WC. 2001. Chromosomal passengers and the (aurora) ABCs of mitosis. *Trends Cell Biol.* 11:49–54
3. Afshar K, Barton NR, Hawley RS, Goldstein LS. 1995. DNA binding and meiotic chromosomal localization of the Drosophila nod kinesin-like protein. *Cell* 81:129–38
4. Alexandru G, Uhlmann F, Mechtler K, Poupart MA, Nasmyth K. 2001. Phosphorylation of the cohesin subunit Scc1 by polo/cdc5 kinase regulates sister chromatid separation in yeast. *Cell* 105:459–72
5. Alexandru G, Zachariae W, Schleiffer A, Nasmyth K. 1999. Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage. *EMBO J.* 18:2707–21
6. Amon A. 1999. The spindle checkpoint. *Curr. Opin. Genet. Dev.* 9:69–75
7. Deleted in proof
8. Angell R. 1997. First meiotic-division nondisjunction in human oocytes. *Am. J. Hum. Genet.* 61:23–32
9. Antonio C, Ferby I, Wilhelm H, Jones M, Karsenti E, et al. 2000. Xkid, a chromokinesin required for chromosome alignment on the metaphase plate. *Cell* 102: 425–35
10. Deleted in proof
11. Bahler J, Wyler T, Loidl J, Kohli J. 1993. Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. *J. Cell Biol.* 121:241–56
12. Bai X, Peirson BN, Dong F, Xue C, Makaroff CA. 1999. Isolation and characterization of SYN1, A RAD21–like gene essential for meiosis in Arabidopsis. *Plant Cell* 11:417–30
13. Bajer A. 1958. Cine-micrographic studies on mitosis in endosperm IV. *Exp. Cell Res.* 14:245–56
14. Bajer AS, Mole-Bajer J. 1972. Spindle dynamics and chromosome movements. *Int. Rev. Cytol. Suppl.* 3:1–271
15. Barnes TM, Kohara Y, Coulson A, Hekimi S. 1995. Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans*. *Genetics* 141: 159–79
16. Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P. 1997. An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* 386:414–17

17. Bernander R. 2000. Chromosome replication, nucleoid segregation and cell division in archea. *Trends Microbiol.* 8:278–83
18. Bernard P, Maure JF, Javerzat JP. 2001. Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation. *Nat. Cell Biol.* 3:522–26
19. Bhat MA, Philp AV, Glover DM, Bellen HJ. 1996. Chromatid segregation at anaphase requires the barren product, a novel chromosome-associated protein that interacts with Topoisomerase II. *Cell* 87: 1103–14
20. Bhatt AM, Lister C, Page T, Fransz P, Findlay K, et al. 1999. The DIF1 gene of Arabidopsis is required for meiotic chromosome segregation and belongs to the REC8/RAD21 cohesin gene family. *Plant J.* 19:463–72
21. Birkenbihl RP, Subramani S. 1992. Cloning and characterization of rad21 an essential gene of Schizosaccharomyces pombe involved in double strand break repair. *Nucleic Acids Res.* 20:6605–11
22. Blat Y, Kleckner N. 1999. Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell* 98:249–59
23. Boy de la Tour E, Laemli UK. 1988. The metaphase scaffold is helically folded: sister chromatids have predominantly opposite helical handedness. *Cell* 55:937–44
24. Brunborg G, Williamson DH. 1978. The relevance of the nuclear division cycle to radiosensitivity in yeast. *Mol. Gen. Genet.* 162:277–86
25. Deleted in proof
26. Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K. 2000. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. *Cell* 103:387–98
27. Chen J-M, Rawlings ND, Stevens RAE, Barrett AJ. 1998. Identification of the active site of legumain links it to caspases, clostripain and gingipains in a new clan of cysteine endopeptidases. *FEBS Lett.* 441:361–65
28. Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, et al. 2000. Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. *Mol. Cell* 5:243–54
29. Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K. 1998. An Esp1/Pds1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell* 93:1067–76
30. Deleted in proof
31. Cohen-Fix O, Koshland D. 1997. The anaphase inhibitor of *Saccharomyces cerevisiae* Pds1p is a target of the DNA damage checkpoint pathway. *Proc. Natl. Acad. Sci. USA* 94:14361–66
32. Cohen-Fix O, Peters J-M, Kirschner MW, Koshland D. 1996. Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev.* 10:3081–93
33. Comings DE. 1966. Centromere: absence of DNA replication during chromatid separation in human fibroblasts. *Science* 154:1463–64
34. D'Andrea RJ, Stratman R, Lehner CF, John UP, Saint R. 1993. The three rows gene of *Drosophila melanogaster* encodes a novel protein that is required for chromosomes disjunction during mitosis. *Mol. Cell Biol.* 4:1161–74
35. Dahmann C, Diffley JF, Nasmyth KA. 1995. S-phase-promoting cyclin-dependent kinases prevent re-replication by inhibiting the transition of replication origins to a pre-replicative state. *Curr. Biol.* 5:1257–69
36. Darlington CD. 1939. *The Evolution of Genetic Systems*. New York: Basic Books
37. Dej KJ, Orr-Weaver TL. 2000. Separation anxiety at the centromere. *Cell Biol.* 10:392–99
38. Dernburg AF, McDonald K, Moulder G,

Barstead R, Dresser M, Villeneuve AM. 1998. Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* 94:387–98
39. Diffley JF. 2001. DNA replication: building the perfect switch. *Curr. Biol.* 11: R367–70
40. Dinardo S, Voelkel KA, Sternglanz RL. 1984. DNA topoisomerase mutant of *S. cerevisiae*: topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. *Proc. Natl. Acad. Sci. USA* 81:2616–20
41. Donachie WD. 1993. The cell cycle of *Escherichia coli*. *Annu. Rev. Microbiol.* 47:199–230
42. Dong H, Roeder GS. 2000. Organization of the yeast Zip1 protein within the central region of the synaptonemal complex. *J. Cell Biol.* 148:417–26
43. Donze D, Adams CR, Rine J, Kamakaka RT. 1999. The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. *Genes Dev.* 13:698–708
44. Downes CS, Mullinger AM, Johnson RT. 1991. Inhibitors of DNA topoisomerase II prevent chromatid separation in mammalian cells but do not prevent exit from mitosis. *Proc. Natl. Acad. Sci. USA* 88: 8895–99
45. Earnshaw WC, Martins LM, Kaufmann SH. 1999. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem.* 68:383–424
46. Eichinger A, Beisel HG, Jacob U, Huber R, Medrano FJ, et al. 1999. Crystal structure of gingipain R: an Arg-specific bacterial cysteine proteinase with a caspase-like fold. *EMBO J.* 18:5453–62
47. Flemming W. 1879. Beiträge zur Kenntnisse der Zelle und ihrer Lebenserscheinungen. *Arch. Mikrosk. Anat.* 18:151–259
48. Funabiki H, Kumada K, Yanagida M. 1996. Fission yeast Cut1 and Cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes. *EMBO J.* 15:6617–28
49. Funabiki H, Murray AW. 2000. The Xenopus chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell* 102:411–24
50. Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yanagida M. 1996. Cut2 proteolysis required for sister-chromatid seperation in fission yeast. *Nature* 381: 438–41
51. Furuta T, Tuck S, Kirchner J, Koch B, Auty R, et al. 2000. EMB-30: an APC4 homologue required for metaphase-to-anaphase transitions during meiosis and mitosis in *Caenorhabditis elegans*. *Mol. Biol. Cell* 11:1401–19
52. Furuya K, Takahashi K, Yanagida M. 1998. Faithful anaphase is ensured by Mis4, a sister chromatid cohesin molecule required in S phase and not destroyed in G1 phase. *Genes Dev.* 12:3408–18
53. Gachet Y, Tournier S, Millar JBA, Hyams JS. 2001. A MAP kinase-dependent actin checkpoint ensures proper spindle orientation in fission yeast. *Nature* 412: 352–55
54. Gall JG, Murphy C, Callan HG, Wu ZA. 1991. Lampbrush chromosomes. *Methods Cell Biol.* 36:149–66
55. Giet R, Glover DM. 2001. Drosophila aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J. Cell Biol.* 152:669–82
56. Gimenez-Abian JF, Clarke DJ, Devlin J, Gimenez-Abian MI, De la Torre C, et al. 2000. Premitotic chromosome individualization in mammalian cells depends on topoisomerase II activity. *Chromosoma* 109:235–44
57. Glaser P, Sharpe ME, Raether B, Perego M, Ohlsen K, Errington J. 1997. Dynamic, mitotic-like behavior of a bacterial protein required for accurate chromosome partitioning. *Genes Dev.* 11:1160–68

58. Goldstein LSB. 1980. Mechanisms of chromosome orientation revealed by two meiotic mutants in *Drosophila melanogaster*. *Chromosoma* 78:79–111
59. Goldstein LSB. 1981. Kinetochore structure and its role in chromosome orientation during the first meiotic division in male *D. melanogaster*. *Cell* 25:591–602
60. Goshima G, Yanagida M. 2000. Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. *Cell* 100:619–33
61. Griffin DK. 1996. The incidence, origin, and etiology of aneuploidy. *Int. Rev. Cytol.* 167:263–96
62. Guacci V, Hogan E, Koshland D. 1994. Chromosome condensation and sister chromatid pairing in budding yeast. *J. Cell Biol.* 125:517–30
63. Guacci V, Koshland D, Strunnikov A. 1997. A direct link between sister chromatid cohesion and chromosome condensation revealed through analysis of MCD1 in *S. cerevisiae*. *Cell* 91:47–57
64. Haig D, Grafen A. 1991. Genetic scrambling as a defence against meiotic drive. *J. Theor. Biol.* 153:531–58
65. Hanna JS, Kroll ES, Lundblad V, Spencer FA. 2001. *Sacharomyces cerevisiae* CTF18 and CTF4 are required for sister chromatid cohesion. *Mol. Cell Biol.* 21: 3144–58
66. Hartman T, Stead K, Koshland D, Guacci V. 2000. Pds5p is an essential chromosomal protein required for both sister chromatid cohesion and condensation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 151:613–26
67. Hartwell LH, Weinert TA. 1989. Checkpoints: controls that ensure the order of cell cycle events. *Science* 246:629–34
68. Hassold T, Arbruzzo M, Adkins K, Griffin D, Merrill M, et al. 1996. Human aneuploidy: incidence, origin, and etiology. *Environ. Mol. Mutagen.* 28:167–75
69. Hassold T, Hunt P. 2001. To err (meiotically) is human: the genesis of human aneuploidy. *Nat. Rev. Genet.* 2:280–91
70. Hauf S, Waizenegger I, Peters JM. 2001. Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. *Science.* In press
71. Hayles J, Fisher D, Woollard A, Nurse P. 1994. Temporal order of S phase and mitosis in fission yeast is determined by the state of the p34cdc2-mitotic B cyclin complex. *Cell* 78:813–22
72. He X, Asthana S, Sorger PK. 2000. Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. *Cell* 101:763–75
73. Deleted in proof
74. Hirano T. 1998. SMC protein complexes and higher-order chromosome dynamics. *Curr. Opin. Cell Biol.* 10:317–22
75. Hirano T. 1999. SMC-mediated chromosome mechanics: a conserved scheme from bacteria to vertebrates? *Genes Dev.* 13:11–19
76. Hirano T. 2000. Chromosome cohesion, condensation, and separation. *Annu. Rev. Biochem.* 69:115–44
77. Hirano T, Kobayashi R, Hirano M. 1997. Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E, and a Xenopus homolog of the Drosophila Barren protein. *Cell* 89:511–21
78. Hirano T, Mitchison TJ. 1994. A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. *Cell* 79:449–58
79. Holloway SL, Glotzer M, King RW, Murray AW. 1993. Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* 73:1393–402
80. Holmes VF, Cozzarelli NR. 2000. Closing the ring: links between SMC proteins and chromosome partitioning, condensation, and supercoiling. *Proc. Natl. Acad. Sci. USA* 97:1322–24
81. Deleted in proof

82. Hopfer KP, Karcher A, Shin DS, Craig L, Arthur LM, et al. 2000. Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double strand break repair and the ABC-ATPase superfamily. *Cell* 101:789–800
83. Hoque MT, Ishikawa F. 2001. Human chromatid cohesin component hRad21 is phosphorylated in M phase and associated with metaphase centromeres. *J. Biol. Chem.* 276:5059–67
84. Hoyt MA, Trotis L, Roberts BT. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 66:507–17
85. Hsu J-Y, Sun Z-W, Li X, Reuben M, Tatchell K, et al. 2000. Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* 102:279–91
86. Hugerat Y, Simchen G. 1993. Mixed segregation and recombination of chromosomes and YACs during single-division meiosis in spo13 strains of *Saccharomyces cerevisiae*. *Genetics* 135:297–308
87. Hwang LH, Lau LF, Smith DL, Mistrot CA, Hardwick KG, et al. 1998. Budding yeast Cdc20: a target of the spindle checkpoint. *Science* 279:1041–44
88. Irniger S, Piatti S, Michaelis C, Nasmyth K. 1995. Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* 81:269–78
89. Jallepalli PV, Waizenegger I, Bunz F, Langer S, Speicher MR, et al. 2001. Securin is required for chromosomal stability in human cells. *Cell* 105:445–57
90. Jensen S, Segal M, Clarke DJ, Reed SI. 2001. A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1. *J. Cell Biol.* 152:27–40
91. Kadyk LC, Hartwell LH. 1992. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. *Genetics* 132: 387–402
92. Kamieniecki RJ, Shanks RMQ, Dawson DS. 2000. Slk19p is necessary to prevent separation of sister chromatids in meiosis I. *Curr. Biol.* 10:1182–90
93. Kaszás É, Cande WZ. 2000. Phosphorylation of histone H3 in correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin. *J. Cell Sci.* 113:3217–26
94. Keeney S, Giroux CN, Kleckner N. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* 88:375–84
95. Kerrebrock AW, Miyazaki WY, Birnby D, Orr-Weaver TL. 1992. The Drosophila mei-S332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. *Genetics* 130:827–41
96. Kimura K, Rybenkov VV, Crisona NJ, Hirano T, Cozzarelli NR. 1999. 13S condensin actively reconfigures DNA by introducing global positive Writhe: implications for chromosome condensation. *Cell* 98:239–48
97. King RW, Peters J, Tugendreich S, Rolfe M, Hieter P, Kirschner MW. 1995. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 81:279–88
98. Deleted in proof
99. Deleted in proof
100. Kitsberg D, Selig S, Brandeis M, Simon I, Keshet I, et al. 1993. Allele-specific replication timing of imprinted gene regions. *Nature* 364:459–63
101. Klapholz S, Esposito RE. 1980. Recombination and chromosome segregation during the single division meiosis in SPO12–1 and SPO13–1 diploids. *Genetics* 96:589–611
102. Kleckner N. 1996. Meiosis: How could

it work? *Proc. Natl. Acad. Sci. USA* 93: 8167–74
103. Deleted in proof
104. Klein F, Mahr P, Galova M, Buonomo SBC, Michaelis C, et al. 1999. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell* 98:91–103
105. Kondrashov AS. 1994. The asexual ploidy cycle and the origin of sex. *Nature* 370: 213–16
106. Koshland D, Hartwell L. 1987. The structure of sister minichromosome DNA before anaphase in *Saccharomyces cerevisiae*. *Science* 238:1713–16
107. Koshland D, Strunnikov A. 1996. Mitotic chromosome condensation. *Annu. Rev. Cell Dev. Biol.* 12:305–33
108. Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters J-M. 2000. Mitotic regulation of the APC activator proteins Cdc20 and Cdch1. *Mol. Biol. Cell* 11:1555–69
109. Kumada K, Nakamura T, Nagao K, Funabiki H, Nakagawa T, Yanagida M. 1998. Cut1 is loaded onto the spindle by binding to Cut2 and promotes anaphase spindle movement upon Cut2 proteolysis. *Curr. Biol.* 8:633–41
110. Lavoie BD, Tuffo KM, Oh S, Koshland D, Holm C. 2000. Mitotic chromosome condensation requires Brn1p, the yeast homologue of Barren. *Mol. Biol. Cell* 11: 1293–304
111. Deleted in proof
112. Leismann O, Herzig A, Heidmann S, Lehner CF. 2000. Degradation of Drosophila PIM regulates sister chromatid separation during mitosis. *Genes Dev.* 14: 2192–205
113. Lemon KP, Grossman AD. 2000. Movement of replicating DNA through a stationary replisome. *Mol. Cell* 6:1321–30
114. Lengauer C, Kinzler KW, Vogelstein B. 1997. Genetic instability in colorectal cancers. *Nature* 386:623–27
115. Li YF, Numata M, Wahls WP, Smith GR. 1997. Region-specific meiotic recombination in *Schizosaccharomyces pombe*: the rec11 gene. *Mol. Microbiol.* 23:869–78
116. Lin DC, Grossman AD. 1998. Identification and characterization of a bacterial chromosome partitioning site. *Cell* 92:675–85
117. Lopez JM, Karpen GH, Orr-Weaver TL. 2000. Sister-chromatid cohesion via MEI-S332 and kinetochore assembly are separable functions of the Drosophila centromere. *Curr. Biol.* 10:997–1000
118. Losada A, Hirano M, Hirano T. 1998. Identification of Xenopus SMC protein complexes required for sister chromatid cohesion. *Genes Dev.* 12:1986–97
119. Losada A, Hirano T. 2001. Intermolecular DNA interactions stimulated by the cohesin complex in vitro. Implications for sister chromatid cohesion. *Curr. Biol.* 11:268–72
120. Losada A, Yokochi T, Kobayashi R, Hirano T. 2000. Identification and characterization of SA/Scc3p subunits in the Xenopus and human cohesin complexes. *J. Cell Biol.* 150:405–16
121. Lowe J, Cordell SC, van den Ent F. 2001. Crystal structure of the SMC head domain: an ABC ATPase with 900 residues antiparallel coiled-coil inserted. *J. Mol. Biol.* 306:25–35
122. MacGuire MP. 1990. Sister chromatid cohesiveness: vital function, obscure mechanism. *Biochem. Cell Biol.* 68:1231–42
123. Machado C, Andrew DJ. 2000. D-Titin: a giant protein with dual roles in chromosomes and muscles. *J. Cell Biol.* 151:639–52
124. Manoury B, Hewitt EW, Morrice N, Dando PM, Barrett AJ, Watts C. 1998. An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. *Nature* 396:695–99
125. Marsden MP, Laemmli UK. 1979. Metaphase chromosome structure: evidence for a radial loop model. *Cell* 17:849–58
126. Martinez-Balbas MA, Dey A, Rabindran

SK, Ozato K, Wu C. 1995. Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* 83:29–38

127. May GS, McGoldrick CA, Holt CL, Denison SH. 1992. The bimB3 mutation of *Aspergillus nidulans* uncouples DNA replication from the completion of mitosis. *J. Biol. Chem.* 267:15737–43
128. Mayer ML, Gygi SP, Aebersold R, Hieter P. 2001. Identification of RFC (Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in *S. cerevisiae*. *Mol. Cell* 7:959–70
129. Mayr E. 1982. *The Growth of Biological Thought*, p. 677. Cambridge: Harvard Univ. Press
130. Mazia D. 1961. *Mitosis and the Physiology of Cell Division*, pp. 77–412. New York: Academic
131. McCarroll RM, Esposito RE. 1994. SPO13 negatively regulates the progression of mitotic and meiotic nuclear division in *Saccharomyces cerevisiae*. *Genetics* 138:47–60
132. McGarry TJ, Kirschner MW. 1998. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* 93:1043–53
133. McGrew JT, Goetsch L, Byers B, Baum P. 1992. Requirement for ESP1 in the nuclear division of *S. cerevisiae*. *Mol. Biol. Cell* 3:1443–54
134. McKim KS, Green-Marroquin BL, Sekelsky JJ, Chin G, Steinberg C, et al. 1998. Meiotic synapsis in the absence of recombination. *Science* 279:876–78
135. McKim KS, Hawley RS. 1995. Chromosomal control of meiotic cell division. *Science* 270:1595–601
136. McKim KS, Hayashi-Hagihara A. 1998. mei-W68 in *Drosophila melanogaster* encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes Dev.* 12:2932–42
137. Deleted in proof
138. Megee PC, Mistrot C, Guacci V, Koshland D. 1999. The centromeric sister chromatid site directs Mcd1p binding to adjacent sequences. *Mol. Cell* 4:445–50
139. Melander Y. 1950. Studies on the chromosomes of Ulophysema Öresundense. *Heriditas* 36:233–55
140. Melby TE, Ciampaglio CN, Briscoe G, Erickson HP. 1998. The symmetrical structure of structural maintainance of chromosomes (SMC) and MukB proteins: Long, antiparallel coiled coils, folded at a flexible hinge. *J. Cell Biol.* 142:1595–604
141. Mercier R, Vezon D, Bullier E, Motomayor JC, Sellier A, et al. 2001. SWITCH1 (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. *Genes Dev.* 15:1859–71
142. Meuwissen RL, Meerts I, Hoovers JM, Leschot NJ, Heyting C. 1997. Human synaptonemal complex protein 1 (SCP1): isolation and characterization of the cDNA and chromosomal localization of the gene. *Genomics* 39:377–84
143. Meuwissen RL, Offenberg HH, Dietrich AJ, Riesewijk A, van Iersel M, Heyting C. 1992. A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J.* 11: 5091–100
144. Michaelis C, Ciosk R, Nasmyth K. 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* 91:35–45
145. Michel LS, Liberal V, Chatterjee A, Kirchwegger R, Pasche B, et al. 2001. MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* 409:355–59
146. Mitchison TJ, Salmon ED. 2001. Mitosis: a history of division. *Nat. Cell Biol.* 3:E17–21
147. Miyazaki WY, Orr-Weaver TL. 1994. Sister-chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet.* 28:167–87
148. Molnar M, Bahler J, Sipiczki M, Kohli J. 1995. The rec8 gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and

sister-chromatid cohesion during meiosis. *Genetics* 141:61–73
149. Moore DP, Orr-Weaver TL. 1998. Chromosome segregation during meiosis: building an unambivalent bivalent. *Curr. Top. Dev. Biol.* 37:263–99
150. Moore DP, Page AW, Tang TT, Kerrebrock AW, Orr-Weaver TL. 1998. The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. *J. Cell Biol.* 140:1003–12
151. Muller HJ. 1916. The mechanism of crossing-over. *Am. Nat.* 50:193–221
152. Murray AW, Szostak JW. 1985. Chromosome segregation in mitosis and meiosis. *Annu. Rev. Cell Biol.* 1:289–315
153. Nasmyth K. 1995. Evolution of the cell cycle. *Philos. Trans. R. Soc. London Ser. B* 349:271–81
154. Nasmyth K. 1999. Separating sister chromatids. *Trends Biochem. Sci.* 24:98–104
155. Nasmyth K, Peters JM, Uhlmann F. 2000. Splitting the chromosome: cutting the ties that bind sister chromatids. *Science* 288:1379–85
156. Neuwald AF, Hirano T. 2000. HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. *Genome Res.* 10:1445–52
157. Nicklas RB, Ward SC. 1994. Elements of error correction in mitosis: microtubule capture, release, and tension. *J. Cell Biol.* 126:1241–53
158. Östergren G. 1951. The mechanism of coorientation in bivalents and multivalents. *Hereditas* 37:85–156
159. Paliulis LV, Nicklas RB. 2000. The reduction of chromosome number in meiosis is determined by properties built into the chromosomes. *J. Cell Biol.* 150:1223–31
160. Panizza S, Tanaka T, Hohchwagen A, Eisenhaber F, Nasmyth K. 2000. Pds5 cooperates with cohesin in maintaining sister chromatid cohesion. *Curr. Biol.* 10:1557–64
161. Parry DH, O'Farrell PH. 2001. The schedule of destruction of three mitotic cyclins can dictate the timing of events during exit from mitosis. *Curr. Biol.* 11:671–83
162. Partridge JF, Borgstrom B, Allshire RC. 2000. Distinct protein interaction domains and protein spreading in a complex centromere. *Genes Dev.* 14:783–91
163. Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J. 2001. A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. *Genes Dev.* 15:1349–60
164. Pearson CG, Maddox PS, Salmon ED, Bloom K. 2001. Budding yeast chromosome structure and dynamics during mitosis. *J. Cell Biol.* 152:1255–66
165. Pelttari J, Hoja M, Yuan L, Liu JG, Brundell E, et al. 2001. A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. *Mol. Cell Biol.* 21:5667–77
166. Peter M, Castro A, Lorca T, Le Peuch C, Magnaghi-Jaulin L, et al. 2001. The APC is dispensable for first meiotic anaphase in Xenopus oocytes. *Nat. Cell Biol.* 3:83–87
167. Deleted in proof
168. Philp AV, Axton JM, Saunders RD, Glover DM. 1993. Mutations in the *Drosophila melanogaster* gene three rows permit aspects of mitosis to continue in the absence of chromatid segregation. *J. Cell Sci.* 106:87–98
169. Pidoux AL, Allshire RC. 2000. Centromeres: getting a grip of chromosomes. *Curr. Opin. Cell Biol.* 12:308–19
170. Deleted in proof
171. Prieto I, Suja JA, Pezzi N, Kremer L, Martinez C, et al. 2001. Mammalian STAG3 is a cohesin specific to sister chromatid arms during meiosis I. *Nat. Cell Biol.* In press
172. Deleted in proof
173. Rao H, Uhlmann F, Nasmyth K, Varshavsky A. 2001. Degradation of a cohesin

subunit by the N-end pathway is essential for chromosome stability. *Nature* 410: 955–59
174. Rattner JB, Lin CC. 1985. Radial loops and helical coils coexist in metaphase chromosomes. *Cell* 42:291–96
175. Reimann JD, Freed E, Hsu JY, Kramer ER, Peters JM, Jackson PK. 2001. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the Anaphase promoting complex. *Cell* 105:645–55
176. Revenkova E, Eijpe M, Heyting C, Gross B, Jessberger R. 2001. A novel meiosis-specific isoform of mammalian SMC1. *Mol. Cell Biol.* In press
177. Rieder CL, Cole R. 1999. Chromatid cohesion during mitosis: lessons from meiosis. *J. Cell Sci.* 112:2607–13
178. Rieder CL, Khodjakov A, Paliulis LV, Fortier TM, Cole RW, Sluder G. 1997. Mitosis in vertebrate somatic cells with two spindles: implications for the metaphase/anaphase transition checkpoint and cleavage. *Proc. Natl. Acad. Sci. USA* 94: 5107–12
179. Rieder CL, Salmon ED. 1998. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8:310–17
180. Roeder GS. 1997. Meiotic chromosomes: it takes two to tango. *Genes Dev.* 11:2600–21
181. Rollins RA, Morcillo P, Dorsett D. 1999. Nipped-B, a Drosophila homologue of chromosomal adherins, participates in activation by remote enhancers in the cut and Ultrabithorax genes. *Genetics* 152:577–93
182. Saitoh N, Goldberg IG, Wood ER, Earnshaw WC. 1994. ScII: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. *J. Cell Biol.* 127:303–18
183. Salah SM, Nasmyth K. 2000. Destruction of the securin Pds1p occurs at the onset of anaphase during both meiotic divisions in yeast. *Chromosoma* 109:27–34
184. Schalk JA, Dietrich AJ, Vink AC, Offenberg HH, van Aalderen M, Heyting C. 1998. Localization of SCP2 and SCP3 protein molecules within synaptonemal complexes of the rat. *Chromosoma* 107: 540–48
185. Schmiesing JA, Gregson HC, Zhou S, Yokomori K. 2000. A human condensin complex containing hCAP-C-hCAP-E and CNAP1, a homolog of Xenopus XCAP-D2, colocalizes with phosphorylated histone H3 during the early stage of mitotic chromosome condensation. *Mol. Cell Biol.* 20:6996–7006
186. Schrader F. 1944. *Mitosis*. New York: Columbia Univ. Press. 110 pp.
187. Schwacha A, Kleckner N. 1997. Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. *Cell* 90:1123–35
188. Seitz LC, Tang K, Cummings WJ, Zolan ME. 1996. The *rad9* gene of *Coprinus cinereus* encodes a proline-rich protein required for meiotic chromosome condensation and synapsis. *Genetics* 142:1105–17
189. Selig S, Okumura K, Ward DC, Cedar H. 1992. Delineation of DNA replication time zones by fluorescence in situ hybridization. *EMBO J.* 11:1217–25
190. Shelby RD, Hahn KM, Sullivan KF. 1996. Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells. *J. Cell Biol.* 135: 545–57
191. Shirayama M, Toth A, Galova M, Nasmyth K. 1999. APC(Cdc20) promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. *Nature* 402:203–7
192. Shirayama M, Zachariae W, Ciosk R, Nasmyth K. 1998. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/Fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J.* 17:1336–49
193. Shonn MA, McCarroll R, Murray AW.

2000. Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. *Science* 289:300–3
194. Sigrist S, Jacobs H, Stratmann R, Lehner CF. 1995. Exit from mitosis is regulated by Drosophila fizzy and the sequential destruction of cyclins A, B and B3. *EMBO J.* 14:4827–38
195. Simchen G, Hugerat Y. 1993. What determines whether chromosomes segregate reductionally or equationally in meiosis? *BioEssays* 15:1–8
196. Deleted in proof
197. Sjoegren C, Nasmyth K. 2001. Sister chromatid cohesion is required for postreplicative double strand break repair in *Saccharomyces cerevisiae*. *Curr. Biol* 11: 991–95
198. Skibbens RV, Corson LB, Koshland D, Hieter P. 1999. Ctf 7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes Dev.* 13:307–19
199. Smith KN, Nicolas A. 1998. Recombination at work for meiosis. *Curr. Opin. Genet. Dev.* 8:200–11
200. Steffensen S, Coelho PA, Cobbe N, Vass S, Costa M, et al. 2001. A role for Drosophila SMC4 in the resolution of sister chromatids in mitosis. *Curr. Biol.* 11:295–307
201. Straight AF, Belmont AS, Robinett CC, Murray AW. 1996. GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr. Biol.* 1599–608
202. Stratmann R, Lehner CF. 1996. Separation of sister chromatids in mitosis requires the Drosophila pimples product, a protein degraded after the metaphase/anaphase transition. *Cell* 84:25–35
203. Sudakin V, Ganoth D, Dahan A, Heller H, Hershko J, et al. 1995. The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell* 6:185–98
204. Sullivan S, Lehane C, Uhlmann F. 2001. Slk19 cleavage. *Nat. Cell Biol.* In press
205. Deleted in proof
206. Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters J-M. 2000. Characterization of vertebrate cohesin complexes and their regulation in prophase. *J. Cell Biol.* 151:749–62
207. Sumner AT. 1991. Scanning electron microscopy of mammalian chromosomes from prophase to telophase. *Chromosoma* 100:410–18
208. Sundin O, Varshavsky A. 1981. Arrest of segregation leads to accumulation of highly intertwined catenated dimers: dissection of the final stages of SV40 DNA replication. *Cell* 25:659–69
209. Surana U, Amon A, Dowzer C, McGrew J, Byers B, Nasmyth K. 1993. Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J.* 12:1969–78
210. Taieb FE, Gross SD, Lewellyn AL, Maller JL. 2001. Activation of the anaphase-promoting complex and degradation of cyclin B is not required for progression from meiosis I to II in Xenopus oocytes. *Curr. Biol.* 11:508–13
211. Takizawa CG, Morgan DO. 2000. Control of mitosis by changes in the subcelluluar location of cyclin-B1-Cdk1 and Cdc25C. *Curr. Opin. Cell Biol.* 12:658–65
212. Deleted in proof
213. Tanaka K, Yonekawa T, Kawasaki Y, Kai M, Furuya K, et al. 2000. Fission yeast Eso1p is required for establishing sister chromatid cohesion during S phase. *Mol. Cell. Biol.* 20:3459–69
214. Deleted in proof
215. Tanaka T, Cosma MP, Wirth K, Nasmyth K. 1999. Identification of cohesin association sites at centromeres and along chromosome arms. *Cell* 98:847–58
216. Tanaka T, Fuchs J, Loidl J, Nasmyth K. 2000. Cohesin ensures bipolar attachment

of microtubules to sister centromeres and resists their precocious separation. *Nat. Cell Biol.* 2:492–99
217. Tanaka T, Knapp D, Nasmyth K. 1997. Loading of an MCM protein onto DNA replication origins is regulated by Cdc6p and CDKs. *Cell* 90:649–60
218. Taylor SS, McKeon F. 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* 89:727–35
219. Thomas JO. 1984. The higher order structure of chromatin and histone H1. *J. Cell Sci. Suppl.* 1:1–20
220. Tippet DH, Pickett-Heaps JD, Leslie RL. 1980. Cell division in two large pennate diatoms Hantzchia and Nitzchia III. A new proposal for kinetochore function during prometaphase. *J. Cell Biol.* 86:402–16
221. Tomonaga T, Nagao K, Kawasaki Y, Furuya K, Murakami A, et al. 2000. Characterization of fission yeast cohesin: essential anaphase proteolysis of Rad21 phosphorylated in the S phase. *Genes Dev.* 14:2757–70
222. Toth A, Ciosk R, Uhlmann F, Galova M, Schleifer A, Nasmyth K. 1999. Yeast cohesin complex requires a conserved protein, Eco1p (Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes Dev.* 13:320–33
223. Toth A, Rabitsch KP, Galova M, Schleiffer A, Buonomo SB, Nasmyth K. 2000. Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I. *Cell* 103:1155–68
224. Uhlmann F, Lottspeich F, Nasmyth K. 1999. Sister chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1p. *Nature* 400: 37–42
225. Uhlmann F, Nasmyth K. 1998. Cohesion between sister chromatids must be established during DNA replication. *Curr. Biol.* 8:1095–101
226. Uhlmann F, Wernic D, Poupart MA, Koonin E, Nasmyth K. 2000. Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell* 103:375–86
227. Uren GA, O'Rourke K, Aravind L, Pisabarro TM, Seshagiri S, et al. 2000. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of whic plays a key role in MALT lymphoma. *Mol. Cell* 6:961–67
228. van Heemst D, James H, Pöggeler S, Berteaux-Lecellier V, Zickler D. 1999. Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programms. *Cell* 98:261–71
229. Waizenegger I, Hauf S, Meinke A, Peters JM. 2000. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell* 103:399–410
230. Deleted in proof
231. Wakefield JG, Huang JY, Raff JW. 2000. Centrosomes have a role in regulating the destruction of cyclin B in early Drosophila embryos. *Curr. Biol.* 10:1367–70
232. Walker MY, Hawley RS. 2000. Hanging on to your homolog: the roles of pairing, synapsis and recombination in the maintenance of homolog adhesion. *Chromosoma* 109:3–9
233. Wang HT, Frackman S, Kowalisyn J, Esposito RE, Elder R. 1987. Developmental regulation of SPO13, a gene required for separation of homologous chromosomes at meiosis I. *Mol. Cell Biol.* 7:1425–35
234. Wang Z, Castano IB, Fitzhugh DJ, De Las Penas A, Adams C, Christman MF. 2000. Pol Kappa: a DNA polymerase required for sister chromatid cohesion. *Science* 289:774–79
235. Warren WD, Steffensen S, Lin E, Coelho P, Loupart M, et al. 2000. The Drosophila RAD21 cohesin persists at the centromere region in mitosis. *Curr. Biol.* 10:1463–66
236. Deleted in proof
237. Deleted in proof
238. Watanabe Y, Nurse P. 1999. Cohesin Rec8

is required for reductional chromosome segregation at meiosis. *Nature* 400:461–64

239. Watanabe Y, Yokobayashi S, Yamamoto M, Nurse PN. 2001. Pre-meiotic S phase is linked to reductional chromosome segregation and recombination. *Nature* 409:359–63
240. Webb CD, Teleman A, Gordon S, Straight A, Belmont A, et al. 1997. Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. *Cell* 88:667–74
241. Weinstein J. 1997. Cell cycle-regulated expression, phosphorylation, and degradation of p55Cdc. A mammalian homolog of CDC20/Fizzy/slp1. *J. Biol. Chem.* 272: 28501–11
242. Whitehouse HLK. 1973. *Towards An Understanding of the Mechanism of Heredity*. London: Arnold
243. Wohlschlegel JA, Dwyer BT, Dhar SK, Cvetic C, Walter JC, Dutta A. 2000. Inhibition of eukaryotic DNA replication by geminin binding to Cdt1. *Science* 290:2309–12
244. Wolstenholme J, Angell RA. 2000. Maternal age and trisomy—a unifying mechanism of formation. *Chromosoma* 109: 435–38
245. Yamamoto A, Guacci V, Koshland D. 1996. Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* 133:85–97
246. Yamamoto A, Guacci V, Koshland D. 1996. Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathways. *J. Cell Biol.* 133:99–110
247. Yanagida M. 2000. Cell cycle mechanisms of sister chormatid separation; roles of Cut1/separin and Cut2/securin. *Genes Cells* 5:1–8
248. Zachariae W, Nasmyth K. 1999. Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Dev.* 13:2039–58
249. Zeng X, Kahana JA, Silver PA, Morphew MK, McIntosh JR, et al. 1999. Slk19p is a centromere protein that functions to stabilize mitotic spindles. *J. Cell Biol.* 146:415–25
250. Zenvirth D, Loidl J, Klein S, Arbel A, Shemesh R, Simchen G. 1997. Switching yeast from meiosis to mitosis: double-strand break repair, recombination and synaptonemal complex. *Genes Cells* 2:487–98
251. Zhang P, Hawley RS. 1990. The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the nod locus. *Genetics* 125:115–27
252. Zickler D, Kleckner N. 1999. Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* 33:603–754
253. Zou H, McGarry TJ, Bernal T, Kirschner MW. 1999. Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. *Science* 285:418–22
254. Zur A, Brandeis M. 2001. Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. *EMBO J.* 20:792–801

Annual Review of Genetics
Volume 35, 2001

CONTENTS

HYPOVIRUSES AND CHESTNUT BLIGHT: EXPLOITING VIRUSES TO UNDERSTAND AND MODULATE FUNGAL PATHOGENESIS, *Angus L. Dawe and Donald L. Nuss* 1

GENETICS AND THE FITNESS OF HYBRIDS, *John M. Burke and Michael L. Arnold* 31

RECOMBINATIONAL DNA REPAIR OF DAMAGED REPLICATION FORKS IN *ESCHERICHIA COLI*: QUESTIONS, *Michael M. Cox* 53

SIR FRANCIS GALTON AND THE BIRTH OF EUGENICS, *Nicholas W. Gillham* 83

BUILDING A MULTICELLULAR ORGANISM, *Dale Kaiser* 103

THE INHERITANCE OF GENES IN MITOCHONDRIA AND CHLOROPLASTS: LAWS, MECHANISMS, AND MODELS, *C. William Birky, Jr.* 125

THE ACTION OF MOLECULAR CHAPERONES IN THE EARLY SECRETORY PATHWAY, *Sheara W. Fewell, Kevin J. Travers, Jonathan S. Weissman, and Jeffrey L. Brodsky* 149

CHROMATIN INSULATORS AND BOUNDARIES: EFFECTS ON TRANSCRIPTION AND NUCLEAR ORGANIZATION, *Tatiana I. Gerasimova and Victor G. Corces* 193

ANIMAL MODELS OF TUMOR-SUPPRESSOR GENES, *Razqallah Hakem and Tak W. Mak* 209

HOMOLOGOUS RECOMBINATION NEAR AND FAR FROM DNA BREAKS: ALTERNATIVE ROLES AND CONTRASTING VIEWS, *Gerald R. Smith* 243

MECHANISMS OF RETROVIRAL RECOMBINATION, *Matteo Negroni and Henri Buc* 275

THE GENETIC ARCHITECTURE OF QUANTITATIVE TRAITS, *Trudy F. C. Mackay* 303

NEW PERSPECTIVES ON NUCLEAR TRANSPORT, *Arash Komeili and Erin K. O'Shea* 341

TRANSLATIONAL REGULATION AND RNA LOCALIZATION IN *DROSOPHILA* OOCYTES AND EMBRYOS, *Oona Johnstone and Paul Lasko* 365

CONSERVATION AND DIVERGENCE IN MOLECULAR MECHANISMS OF AXIS FORMATION, *Sabbi Lall and Nipam H. Patel* 407

REGULATION OF GENE EXPRESSION BY CELL-TO-CELL COMMUNICATION: ACYL-HOMOSERINE LACTONE QUORUM SENSING, *Clay Fuqua, Matthew R. Parsek, and E. Peter Greenberg* 439

MODELS AND DATA ON PLANT-ENEMY COEVOLUTION, *Joy Bergelson, Greg Dwyer, and J. J. Emerson* 469

BIOLOGY OF MAMMALIAN L1 RETROTRANSPOSONS, *Eric M. Ostertag and Haig H. Kazazian, Jr.* 501

DOES NONNEUTRAL EVOLUTION SHAPE OBSERVED PATTERNS OF DNA VARIATION IN ANIMAL MITOCHONDRIAL GENOMES?, *Anne S. Gerber, Ronald Loggins, Sudhir Kumar, and Thomas E. Dowling* 539

IDENTIFICATION OF EPILEPSY GENES IN HUMAN AND MOUSE, *Miriam H. Meisler, Jennifer Kearney, Ruth Ottman, and Andrew Escayg* 567

MOLECULAR GENETICS OF HEARING LOSS, *Christine Petit, Jacqueline Levilliers, and Jean-Pierre Hardelin* 589

GENETIC ANALYSIS OF CALMODULIN AND ITS TARGETS IN *SACCHAROMYCES CEREVISIAE*, *Martha S. Cyert* 647

DISSEMINATING THE GENOME: JOINING, RESOLVING, AND SEPARATING SISTER CHROMATIDS DURING MITOSIS AND MEIOSIS, *Kim Nasmyth* 673

EPITHELIAL CELL POLARITY AND CELL JUNCTIONS IN *DROSOPHILA*, *Ulrich Tepass, Guy Tanentzapf, Robert Ward, and Richard Fehon* 747

INFORMED CONSENT AND OTHER ETHICAL ISSUES IN HUMAN POPULATION GENETICS, *Henry T. Greely* 785

G. LEDYARD STEBBINS AND THE EVOLUTIONARY SYNTHESIS, *Vassiliki Betty Smocovitis* 803

INDEXES

Subject Index 815

Cumulative Index of Contributing Authors, Volumes 31–35 851

Cumulative Index of Chapter Titles, Volumes 31–35 854

ERRATA

An online log of corrections to *Annual Review of Genetics* chapters may be found at http://genet.AnnualReviews.org/errata.shtml