REVIEW

Segregating Sister Genomes: The Molecular Biology of Chromosome Separation

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During cell division, each daughter cell inherits one copy of every chromosome. Accurate transmission of chromosomes requires that the sister DNA molecules created during DNA replication are disentangled and then pulled to opposite poles of the cell before division. Defects in chromosome segregation produce cells that are aneuploid (containing an abnormal number of chromosomes)—a situation that can have dire consequences. Aneuploidy is a leading cause of spontaneous miscarriages in humans and is also a hallmark of many human cancer cells. Recent work with yeast, *Xenopus*, and other model systems has provided new information about the proteins that control chromosome segregation during cell division and how the activities of these proteins are coordinated with the cell cycle.

The inheritance by daughter cells of complete copies of their genome is central to the process of cell proliferation. To perform this remarkable feat, cells must first disentangle sister DNA molecules created during DNA replication and then ensure that they are segregated to opposite poles of the cell before division (Fig. 1). When repeated each time cells divide, this process ensures that most if not all cells in our bodies contain both copies of the genomes inherited from our parents. One of the consequences is that differential gene expression rather than selective gene transmission is responsible for cell differentiation.

How sister genomes are segregated with high fidelity to opposite poles of the cell is also a matter of great biomedical interest. Defects in genome segregation in somatic cells in all likelihood contribute to oncogenesis, whereas defects during meiosis generate trisomies, the most prevalent of which, Down syndrome, is caused by an extra copy of chromosome 21. Recent progress in characterizing proteins such as cohesin, condensin, separase, and the aurora B kinase, which control chromosome behavior during mitosis, is paving the way to a molecular understanding of genome transmission.

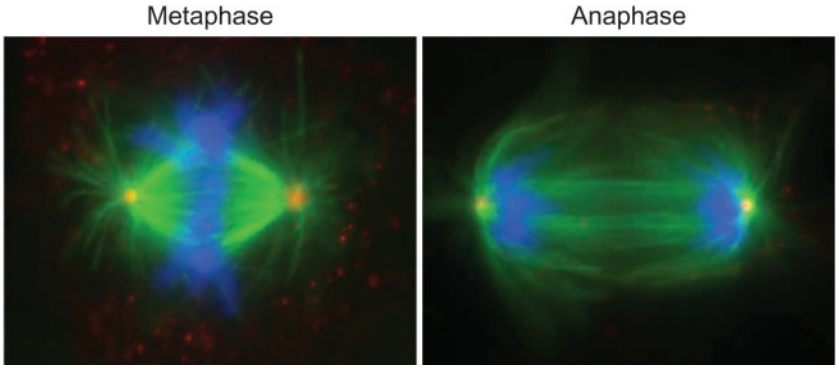

Fig. 1. Metaphase-to-anaphase transition in rat kangaroo PtK2 cells. α-Tubulin, green; centrosomes, red; DNA stained with 4′,6-diamidino-2-phenylindole, blue.

Sister Chromatid Cohesion Is a Crucial Aspect of Mitosis

In bacteria, where chromosomes are replicated from a single origin of DNA replication, movement of chromatids to the poles coincides with ongoing chromosome duplication. There is some debate as to whether nascent sister DNA sequences remain associated for any appreciable length of time, except possibly at the unique termini of DNA replication (*1, 2*). Nascent DNAs emerge from opposite faces of a stationary replisome and are organized into compact nucleoids with the help of DNA gyrase, which produces negative supercoils, and SMC (structural maintenance of chromosomes) proteins, which are thought to organize chromosomal DNA into large coils (*3*). Bacterial SMC proteins are composed of long (50 nm) antiparallel coiled coils with an ABC-like adenosine triphosphatase (ATPase) at one end and a half-hinge or junction domain at the other (*4*). Homotypic interactions between junctions (*5*) produce V-shaped dimers, each of whose two heads contains an ABC-like ATPase domain (Fig. 2A). Non-SMC proteins interacting with SMC heads are also crucial for SMC function (*6*). Work on Rad50-like proteins, which are related to SMCs, suggests that binding of adenosine triphosphate (ATP) promotes association of the SMC dimer's two head domains (and an alteration in their conformation) and that this is a precondition for ATP hydrolysis (*7*) (Fig. 2A). A similar situation prevails for bacterial SMC proteins, each of whose two heads can independently bind ATP but not hydrolyze it efficiently without interacting (*8*).

In eukaryotes, cohesion between sister chromatids, which is generated during the replication process, holds sisters together long after replication is complete and makes possible a totally new principle for chromosome segregation. This process, known as mitosis, involves the attachment of sister chromatids to microtubules of opposite polarity (amphitelic attachment) and their traction to opposite poles of the cell, known as bi-orientation (Figs. 1 and 3). Microtubules usually attach to chromosomes at unique loci called centromeres (Fig. 3). By providing a force that counteracts that exerted by microtubules, sister chromatid cohesion is an essential aspect of the bi-orientation process (see below) (*9, 10*). The eventual destruction of cohesion when all sister chromatid pairs have attached in an amphitelic manner triggers the segregation of sisters to opposite poles of the cell at the metaphase-to-anaphase transition (*11*). Because of sister chromatid cohesion, segregation of chromosomes can take place long after their duplication is complete.

Cohesin and Condensin

Cohesion in eukaryotic cells is mediated by a multisubunit complex called cohesin, which binds to chromosomes from telophase until the onset of anaphase in the next cell cycle (*12, 13*). Cohesin contains an SMC heterodimer formed by heterotypic interactions between the hinge domains of two different SMC proteins, called Smc1 and Smc3 (*5*). These are bound by a third protein, Scc1 (also called Mcd1 and Rad21), whose cleavage by a cysteine protease called separase triggers poleward movement of sisters at the metaphase-to-anaphase transition. Scc1 in turn binds a fourth cohesin subunit, Scc3, which has two orthologs in mammals called SA1 and SA2 (Fig. 2).

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Cohesin has been postulated to connect sister DNA molecules through the binding of its two heads to each sister DNA molecule (*14, 15*). However, the recent finding that the NH_2- and COOH-terminal domains of Scc1 bind, respectively, to the Smc3 and Smc1 heads of the Smc1/3 heterodimer (*5*) suggests that cohesin forms a large proteinaceous ring within which DNA strands could be trapped. In electron micrographs of soluble cohesin, non-SMC subunits (presumably Scc1 and/or Scc3) are found associated with the SMC heterodimer's heads and appear to promote their association (*15*). These findings raise the possibility that cohesin holds sisters chromatids together by embracing both within a single ring. If so, then the connection between sisters may be a topological rather than a chemical one. This notion explains why cohesin does not bind avidly to DNA on its own (*16*) but is nevertheless so tightly associated with chromosomes that it cannot be eluted by 1.5 M KCl (*17, 18*). It also neatly explains how cohesin is so readily released from chromosomes by cleavage of its Scc1 subunit (*19*) (Fig. 2B). If this topological model proves correct, then future investigations must focus on how DNA enters cohesin's ring in the first place. The ring must have a gate, whose opening and shutting might depend on the ATPase activity of Smc head domains.

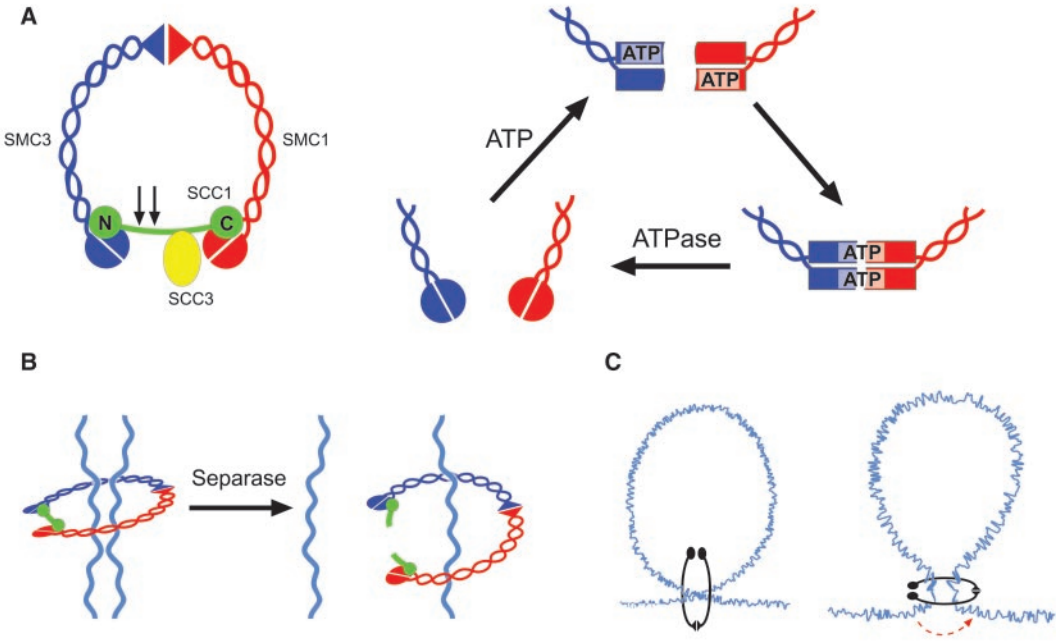

Fig. 2. Structure of cohesin and a possible mechanism by which it might hold sister chromatids together. (**A**) Smc1 (red) and Smc3 (blue) form intramolecular antiparallel coiled coils, which are organized by hinge or junction domains (triangles). Smc1/3 heterodimers are formed through heterotypic interactions between the Smc1 and Smc3 junction domains. The COOH terminus of Scc1 (green) binds to Smc1's ABC-like ATPase head, whereas its NH_2 terminus binds to Smc3's head, creating a closed ring. Scc3 (yellow) binds to Scc1's COOH-terminal half and does not make any direct stable contact with the Smc1/3 heterodimer. Scc1's separase cleavage sites are marked by arrows. Cleavage at either site is sufficient to destroy cohesion. By analogy with bacterial SMC proteins, it is expected that ATP binds both the Smc1 and Smc3 heads, alters their conformation, and possibly brings them into close proximity. By altering Scc1's association with Smc heads, ATP binding and/or hydrolysis could have a role in opening and/or closing cohesin's ring. (**B**) Cohesin could hold sister DNA molecules together by trapping them both within the same ring. Cleavage of Scc1 by separase would open the ring, destroy coentrapment of sister DNAs, and cause dissociation of cohesin from chromatin. (**C**) Smc-containing complexes other than cohesin could also function via chromatid entrapment. Condensin, for example (black), could organize mitotic chromosomes by trapping supercoils. It and/or other related complexes could hold distant loci together (arrow) and thereby facilitate the function of long-range enhancers and silencers of transcription.

In eukaryotic cells, the bulk of cohesin dissociates from chromosomes during the early stages of mitosis—between prophase, when chromosomes start to condense, and prometaphase when they bi-orient on the mitotic spindle. This dissociation is thought to be independent of separase and is accompanied by the splitting of chromosomes into two morphologically defined chromatids (chromatid individualization), which takes place along chromosome arms but not in the neighborhood of centromeres (*18, 20*) (Fig. 4).

At about the same time, a related complex, called condensin (*21*), binds to the axes of both chromatids and organizes chromosomal DNA in a manner that is essential for sister chromatid disentanglement (*22–26*). Cells lacking condensin fail to separate sisters properly during anaphase after removal of cohesin (*27*). Like cohesin, condensin is composed of a pair of SMC proteins, Smc2 and Smc4, which form a heterodimer whose heads bind three non-SMC proteins (*15, 21*). It has recently been suggested that condensin might help to create and/or maintain the coiling of chromosomal DNA. If so, it might also form rings through which strands from the same chromosome fiber pass as they cross over at the base of a coil (*13*) (Fig 2C). Its ability to promote positive writhe (*28*) could come about by "trapping" supercoils with a defined chirality.

Condensin also contributes to mitosis-specific chromosome compaction. Incubation of unreplicated sperm chromatin in mitotic extracts from *Xenopus* induces formation of fibrous-like chromatin threads, and this process clearly depends on condensin (*21*). Strangely, inactivation of condensin in flies and worms appears to have little or no effect on the axial length of their chromosomes during metaphase (*25, 26*). If the persistence of condensation in these mutants is not simply due to residual condensin activity (which remains a distinct possibility), then the observation raises the possibility that mitosis-specific chromatin compaction might be driven by local changes in nucleosome packing in addition to chromatin's reorganization by condensin. Condensin's main role may be to organize the coiling topology of individual chromatids. The transition from a situation in which many higher order connections between chromatin fibers are mediated by cohesin to one in which these are replaced by condensin-mediated connections between neighboring coils on the same chromatid is possibly an essential aspect of sister chromatid disentanglement (Fig. 4).

The fraction of cohesin that persists on chromosomes until metaphase—mainly at centromeres (*29, 30*) but possibly also all along the interface between sisters (*31*)—is responsible for holding sisters together while they bi-orient during prometaphase. The eventual cleavage of this fraction by separase is thought to trigger anaphase (*11, 32*). Separase is tightly regulated. For most of the cell cycle, it is bound by an inhibitory chaperone called securin (*33, 34*), whose destruction by a ubiquitin protein ligase called the anaphase-promoting complex or cyclosome (APC/C)

takes place only after all chromatid pairs have aligned correctly on the mitotic spindle (*35, 36*) (Fig. 3).

Viewed in this light, the process by which sister chromatids disengage from each other occurs in two distinct phases in eukaryotic cells. The first phase, which involves cohesin's dissociation from and condensin's association with chromosomes, occurs in the complete absence of microtubules and yet is capable of separating sister sequences by up to 0.5 μm. This phase may not be unlike the segregation of bacterial nucleoids and may involve similar processes. The second phase involves the traction of sister molecules to opposite poles of the cell by microtubules in a process that appears to be unique to eukaryotic cells and requires sister chromatid cohesion.

What Drives Chromatid Individualization During Prophase?

In recent years, the chromosomal acrobatics mediated by microtubules during the second phase of mitosis have tended to eclipse the equally impressive first phase. That has not always been the case. The discovery that chromatid individualization heralded the forthcoming division of the cell laid the foundations for the hypothesis that heredity is mediated by chromosomes. At the time of its discovery, this finding was considered so important that mitosis was initially divided into two phases: one before (prophase) and another after (metaphase) this transition (*37*). Despite recent progress in understanding how the second phase of chromatid segregation is triggered by the APC/C and separase at the metaphase-to-anaphase transition, we have remained largely ignorant about what triggers chromatid individualization during prophase. The finding that cohesin is phosphorylated as cells enter mitosis (*38–40*) raises the possibility that its dissociation from chromosomes might be triggered by one of several mitosis-specific kinases—for example, Cdk1, Aurora A, Aurora B, or Polo-like kinases (PLK). If so, which kinase is responsible and is the same kinase also responsible for triggering chromosome condensation? A major impediment in the search for the critical mitotic kinase is their multifunctionality. Both PLK and Cdk1 are essential for transit of the cell from G_2 to the mitotic state, which confounds the study of their role during mitosis itself.

To circumvent this problem, Sumara *et al.* (*41*) studied *Xenopus* extracts, which are capable of cycling between cell cycle states. The advantage of in vitro systems is that proteins can be depleted not only from interphase extracts but also from those that have already entered mitosis, which is difficult if not impossible to achieve with intact cells (unless one has highly specific chemical inhibitors). As might be expected, depletion of mitotic kinases had no effect on the loading of cohesin onto chromosomes during interphase. The bulk of this cohesin dissociated from chromatin when it was placed in mitotic extracts, but it did not dissociate from those depleted of PLK, even though these extracts clearly remained in a mitotic state with high Aurora B and Cdk1 activities. The PLK antibodies had not caused removal of any crucial factor besides PLK itself because readdition of active but not inactive PLK fully restored the ability of depleted extracts to promote cohesin's dissociation. In contrast, depletion of Aurora A or Aurora B had no effect. PLK was also found to be necessary for the dissociation of cohesin from chromatin in extracts forced into a mitotic state by okadeic acid (a phosphatase inhibitor), which occurs in the complete absence of mitotic cyclins. Therefore, Cdk1 is neither sufficient nor necessary for promoting cohesin's dissociation.

The case for PLK's intimate involvement in this process was strengthened by the findings that addition of constitutively active PLK to interphase extracts triggered dissociation, that pure PLK was capable of phosphorylating several cohesin subunits in vitro, and that phosphorylation of cohesin by PLK in vitro severely reduced its ability to bind to chromatin when added to interphase extracts. To determine whether cohesin alone or some other protein is PLK's target during this process requires identifying PLK's phosphorylation sites on cohesin and demonstrating that nonphosphorylatable mutant cohesin complexes no longer dissociate from chromosomes during prophase.

Although depletion of PLK blocked cohesin's dissociation, it had little or no effect on the association of condensin or on the phosphorylation of histone H3 by Aurora B, two other chromosomal events that occur during prophase. As a result, chromosomes accumulated with high levels of both cohesin and condensin, a situation that normally does not arise during undisturbed mitoses. Interestingly, this was accompanied by a failure of chromosomes to individualize from the amorphous mass of interphase chromatin. Although loading of condensin and dissociation of cohesin normally coincide during prophase, these two processes are clearly regulated differently. Whereas the latter depends on PLK, the former might be regulated by Cdk1, which is known to increase condensin's ability to impart writhe to circular DNAs in vitro (*42*).

One of the remaining mysteries about cohesin's dissociation from chromosomes during prophase is what prevents this process from proceeding to completion, which would cause precocious sister separation and thereby nondisjunction during anaphase. Small amounts of cohesin remain associated with interchromatid axes (*31*) right up until metaphase. Even larger amounts remain within centromeric heterochromatin, which does not individualize into two chromatids until separase is

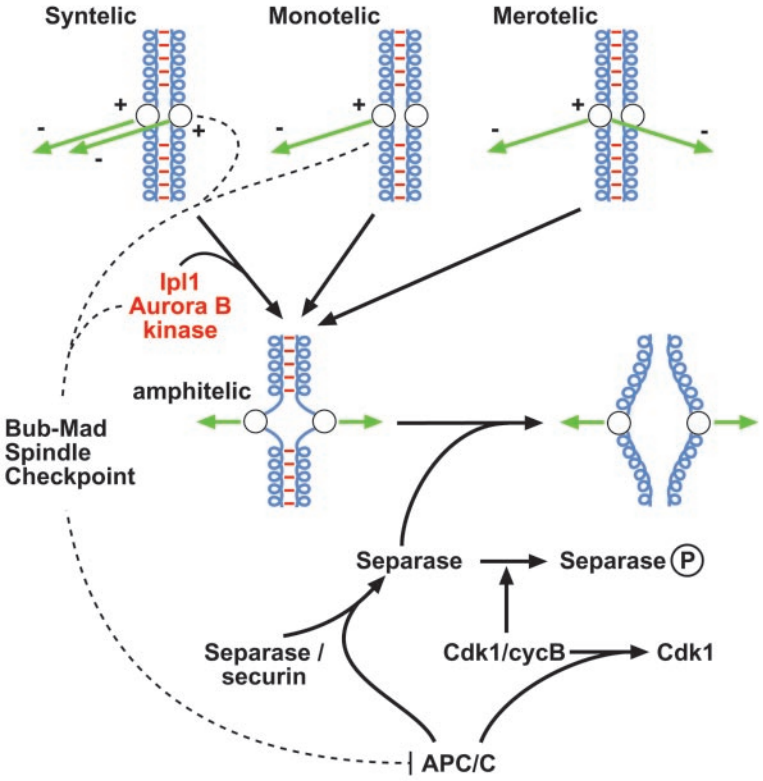

Fig. 3. The anaphase-promoting complex induces amphitelically attached chromatids to segregate to opposite poles by destroying both cyclin B and securin. The Ipl1/Aurora B kinase both eliminates syntelically attached chromatid pairs and promotes inhibition of APC/C when centromeres fail to come under tension. Chromatin, blue; microtubules, green; centromeres, open circles; cohesin, red.

activated. A key question is whether cohesin's persistence at centromeres is due to its failure to be phosphorylated by PLK or to other factors specific to centromeric heterochromatin that render cohesin's dissociation refractory to phosphorylation by PLK. The latter is more likely, because efficient cleavage of Scc1 at the onset of anaphase may also depend on its phosphorylation by PLK, as found in yeast (*40*). Finding the centromeric heterochromatin proteins that prevent cohesin's dissociation from this region of the chromosome is clearly a priority.

What Is Special About Centromeric Cohesion?

A clue to the identity of such proteins has recently come from studies of the fission yeast *Schizosaccharomyces pombe*, whose centromeres encompass 30 to 100 kilobases of DNA (*43*). At the heart of this region lies a central domain associated with kinetochore proteins such as the centromere-specific histone H3 variant CenpA. It is presumed that this is the region that attaches to microtubules. This domain is surrounded by repetitive sequences called the outer repeats, which contain few genes and are transcriptionally silenced by a process that involves binding of the heterochromatin protein HP1 (Swi6 in *S. pombe*) to nucleosomes whose histone H3 has been methylated on lysine 9 by the Suvar3-9 methyltransferase (*44*) (Clr4 in *S. pombe*). Swi6 and Clr4 are required not only for silencing transcription at centromeres but also for efficient chromosome segregation. Sister chromatids disjoin synchronously and move rapidly to opposite poles soon after activation of separase in wild-type cells, but individual chromatids occasionally "lag" in the middle of the cell in *swi6* or *clr4* mutants (*45*). This is thought to be caused either by unstable connections between spindles and kinetochores or by the attachment of individual kinetochores to spindles from both poles (merotelic attachment; Fig. 3).

Several pieces of evidence suggest that chromosome lagging in *swi6* mutants might be due to defective cohesin function (*46*). First, a search for mutants that were synthetic lethal with deletion of *swi6* largely yielded mutants with defective sister chromatid cohesion (*47*). Thus, the normally temperature-sensitive allele of Scc1 (*rad21-K1*) is inviable at all temperatures when combined with a *swi6* deletion. Second, chromosomes that lag in a manner similar if not identical to that of *swi6* mutants occur frequently in cohesin mutants growing at the permissive temperature. Finally, although present throughout *S. pombe* chromosomes, cohesin is clearly enriched in outer repeats of their centromeres—that is, in regions bound by Swi6p.

Remarkably, recruitment of cohesin to the centromeric outer repeats largely if not completely depends on Swi6 and Clr4 (*46, 48*). To address whether the lack of cohesin causes loss of centromeric cohesion, Bernard *et al*. (*46*) arrested *S. pombe* cells in metaphase by inactivating the APC/C. Because this manipulation prevents Cdk1 inactivation and securin destruction, cells arrest in a state in which microtubules strain to split sisters but are prevented from doing so by cohesion, which has remained intact due to the lack of separase activation. Deletion of *swi6* in such cells caused the parting of sister chromatids in the vicinity of centromeres but not along chromosome arms. The implication is that, by recruiting cohesin to centromeres, Swi6 strengthens cohesion in this crucial region of the chromosome. Recruitment could be mediated by a direct interaction between Swi6 and cohesin. In support of this notion, cohesin's Scc3 subunit (known as Psc3) has been found to bind a GST-Swi6 fusion protein added to *S. pombe* extracts and Swi6 and Psc3 interact in a two-hybrid assay (*48*). Cohesin is also recruited by Swi6 to other chromosomal loci to which Swi6 binds, such as telomeres and mating-type loci.

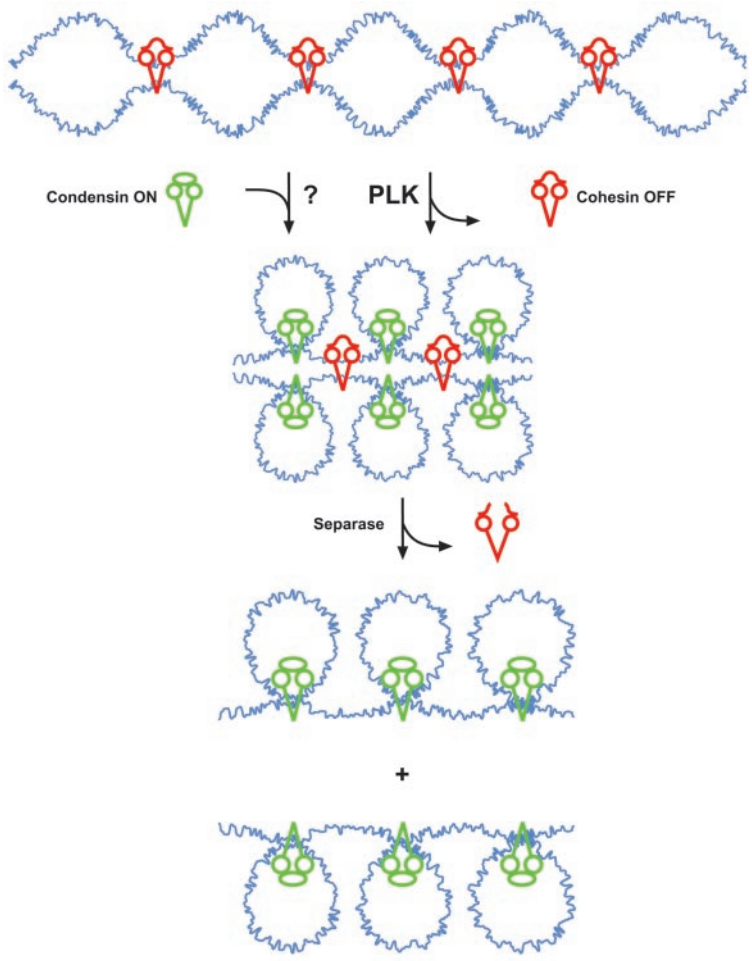

Fig. 4. Chromatid individualization as cells enter mitosis involves dissociation of most cohesin (red symbols) from chromosomes, which is regulated by PLK. This process coincides with condensin's (green symbols) association with chromosomes and their compaction. Cohesin remaining on chromosomes, largely at centromeres, is then cleaved by separase at the metaphase-to-anaphase transition.

An unaddressed question is whether the lagging chromatids in *swi6* and *rad21* mutants arise because of a lack of sister chromatid cohesion or because cohesin might have an additional function at centromeres. Bernard *et al*. suggest that an especially robust form of cohesion may force sister kinetochores to face in opposite directions, which would improve their chances of being captured by microtubules of opposing polarity. However, it is equally plausible that cohesin and/or cohesion has a direct role in the signaling mechanism that abolishes kinetochore-microtubule connections that do not generate tension (see below). Whether HP-1 promotes cohesin's persistence at centromeres until metaphase in animal cells remains to be investigated.

Orienting Sisters on the Mitotic Spindle (Bi-orientation)

One of the great mysteries about the mitotic process is how cells ensure that sister kinetochores attach to microtubules with opposing polarities (Fig. 3). Although all microtubules attach to kinetochores via their plus ends, those attached to sister kinetochores must extend toward opposite poles of the cell. When this bi-orientation is successfully achieved, sister chromatids are pulled in opposite directions but fail to come apart because of the action of cohesin. The result is that chromatin in the

vicinity of centromeres comes under tension (*9, 49, 50*), which is thought to have some role in shutting off a Mad2- and Aurora B (Ipl1)–dependent checkpoint (Fig. 3) that prevents activation of separase until all chromatid pairs have aligned on the metaphase plate (*51, 52*).

Generation of tension within centromeric chromatin might also play a key role in the process by which cells prevent sister kinetochores from attaching to microtubules extending to the same pole (syntelic attachment). Recent evidence suggests that the Aurora B–like protein kinase Ipl1 (*53*) has a crucial role in promoting bi-orientation in yeast (*54, 55*). In its absence, sister kinetochores frequently attach to the same pole; as a consequence, sister chromatids are segregated to the same daughter cell, with disastrous consequences.

One of the peculiarities of the yeast mitotic apparatus is that microtubules connect kinetochores to spindle poles throughout the yeast cell cycle. Spindle pole bodies (SPBs) duplicate conservatively to produce old and new SPBs. If chromosome duplication is prevented, then the kinetochore-SPB connections with which cells start off the cell cycle are disrupted. In the process of their reattachment, unreplicated kinetochores end up attached to old and new SPBs with equal probabilities. Remarkably, inactivation of the Ipl1/Aurora B kinase prevents the detachment of unreplicated kinetochores from old SPBs, and all 16 chromatids segregate with the old SPB into buds (*55*). The implication is that Ipl1 may be an integral part of a correction mechanism that eliminates syntelically attached chromosomes (Fig. 3). If so, then understanding the mechanism by which amphitelic attachment (and the resulting tension) either shuts off the Ipl1 kinase or renders microtubule-kinetochore attachments refractory to its action becomes of paramount importance. It is crucial to establish whether the homologous protein kinase in animal cells also promotes bi-orientation by eliminating syntelic attachment and to investigate how the rules of the game are altered during meiosis I, when homologous chromosomes and not sister chromatids must bi-orient.

Regulating Sister Chromatid Separation

It has long been recognized that the movement of sister chromatids to opposite poles during anaphase is a fairly synchronous process (*56*). This is because, once activated, separase appears to act globally on all chromatid pairs. Thus, any chromatid pair that has not yet bi-oriented on the mitotic spindle is unable to do so once separase activation destroys any residual sister chromatid cohesion. Therefore, all eukaryotic cells possess elaborate mechanisms that regulate the timing of separase activation and prevent it from occurring in the presence of chromatid pairs that have not yet bi-oriented. One of these is a surveillance mechanism known as the spindle checkpoint (Fig. 3), which detects unattached chromosomes and syntelically attached chromosomes (and possibly also chromosomes that have attached to only one pole, known as monotelic attachment) and blocks destruction of both cyclin B and securin by the APC/C (*57*). The persistence of cyclin B keeps Cdk1 active, which maintains cells in a mitotic state in which chromosomes remain condensed and the nuclear membrane remains absent, whereas the persistence of securin, which binds to and inhibits separase, prevents cleavage of Scc1. Unattached or misattached chromosomes trigger the production of a complex containing the Mad1, Mad2, Mad3, and Bub3 proteins, which binds to the APC/C's activator protein Cdc20 (*58*) and thereby blocks ubiquitination of both securin and cyclin B, but, strangely, not ubiquitination of cyclin A. Exactly how production of the Mad/Bub Cdc20 inhibitor is stimulated by unattached kinetochores remains a mystery. According to one model, unattached kinetochores provide sites for production of the inhibitor, from which it then dissociates and inhibits APC/C-Cdc20 function throughout the cell.

In yeast, the inhibition of securin destruction and not that of cyclins is responsible for blocking separase activation. Thus, mutant cells that lack securin still block cyclin B destruction in the presence of spindle poisons but fail to block cleavage of Scc1 by separase, which results in loss of sister chromatid cohesion (*59, 60*). Scc1 cleavage continues to be cell cycle regulated in yeast securin mutants (growing in the absence of spindle poisons), at least partly due to its dependence on Scc1's phosphorylation by PLK (*40*). The securin gene can also be deleted in human tissue culture cells (*61*) and even in mouse embryos without causing lethality (*62, 63*). Surprisingly, mammalian cells that lack securin are still capable of blocking the loss of sister chromatid cohesion when treated with spindle poisons. These cells must either block separase activation by a securin-independent mechanism or protect centromeric Scc1 from cleavage—for example, by preventing its phosphorylation by PLK.

Recent work, again with *Xenopus* extracts, implicates the former mechanism (although it does not exclude the latter) (*64*). Stemmann *et al*. started with the observation that the addition of nondegradable cyclin B blocks sister chromatid separation in *Xenopus* extracts. Previous work had suggested otherwise (*65*), but it turns out that the result depends crucially on the amount of cyclin B added to the extracts. Small amounts of cyclin B (40 to 80 nM) prevent disassembly of the mitotic spindle, chromosome decondensation, and reformation of nuclei but do not prevent sister chromatid separation. When raised to 120 nM or higher, nondegradable cyclin B also blocks sister separation. This is not due to a lack of APC/C activity because both securin and an NH_2-terminal fragment of cyclin B, which is an efficient APC/C substrate, are degraded with equal kinetics in extracts with both low and high cyclin concentrations.

To test whether the lack of chromatid disjunction might be due to inactivity of separase, the authors transferred separase-securin complexes attached to Sepharose beads into these extracts. Although the securin moiety was degraded in both low and high cyclin concentrations, separase left on the beads was active only when retrieved from the extract with the low concentration of cyclin. The implication is that high levels of cyclin B/Cdk1 activity prevent separase activation despite securin destruction. Separase isolated from mitotic cells was found to be phosphorylated at eight different serine/threonine residues but only one of these, Ser^{1126}, proved to be of functional significance. When this residue was mutated to alanine, separase failed to be inactivated by high levels of Cdk1 activity but could nevertheless be inhibited by securin. Furthermore, unlike the wild-type protein, the Ser^{1126} to Ala mutant was able to trigger separation of human sister chromatids when it was added to mitotic *Xenopus* extracts containing high levels of nondegradable cyclin B. Ser^{1126} is quantitatively phosphorylated during metaphase and becomes at least partly dephosphorylated upon anaphase onset. Future work should nevertheless address whether the level of cyclinB/Cdk1 activity needed to inhibit separase is reached under physiological circumstances.

The conclusion from these experiments is that at least two mechanisms prevent separase activation while chromosomes are in the process of bi-orienting. During this period, the mitotic checkpoint, and possibly other mechanisms, prevents the APC/C from destroying both securin and cyclin B. The persistence of either protein keeps separase inhibited, one by causing its phosphorylation at Ser^{1126} and the other by binding to and inhibiting the protease domain (see below). The discovery of this mechanism clearly raises the question of why the cell uses two apparently redundant mechanisms to control separase. Is control by both mechanisms simply more robust or might there exist circumstances when one but not the other mechanism is called into play? Whatever the answer, the eukaryotic cell clearly places a high premium in controlling this crucial protease.

Structure, Activation, and Evolution of Separase

Most separases are large (180 to 250 kD) proteins with a highly conserved COOH-terminus. This region is predicted to contain a catalytic domain common to caspases and hemoglobinases (and hence called the CH fold), which is composed of four parallel β sheets linked by α helices. At the ends of two of the β sheets lie conserved histidine and cysteine residues that constitute the pro-

tease's catalytic dyad. A clue about the evolutionary origin of separases has recently come from the discovery that the highly conserved region immediately COOH-terminal to the CH fold of separases is also found in a class of putative bacterial proteases called the Het F family but is lacking in caspases and hemoglobinases (*66*). The absence of certain key residues within the CH folds of separases suggests that they are more highly derived than their Het F cousins, which implies that they evolved from a member of this family and not the other way around. Therefore, it has been proposed that separases may be descended from a prokaryotic enzyme that entered the eukaryotic genome by lateral transfer, possibly as a consequence of the symbiosis with the α-proteobacterium that gave rise to mitochondria. If correct, this proposal implies that mitosis may have evolved only after the symbiosis that gave rise to mitochondria.

The mechanism by which removal of securin activates separase remains unclear. Like caspases, separases in vertebrate cells undergo autocleavage upon their activation by the APC/C. However, this cleavage does not occur in yeast separase, either in vitro or in vivo (*67*). Moreover, mutations that reduce the extent of vertebrate separase cleavage have little or no effect on its activation by the APC/C (*68*). In flies, where separase appears to have split into two proteins—SSE containing the protease domain and Three rows—the latter also undergoes cleavage upon activation of the protease. Cleavage of Three rows does not appear to be necessary for separase activity but instead may contribute to its inactivation during telophase (*69*). Autocleavage of separase in vertebrate cells may have a similar role. In yeast, the entire NH_2-terminal domain of separase is essential for the activity of its COOH-terminal catalytic domain. Securin binds to both NH_2- and COOH-terminal domains and hinders access of substrates to the catalytic cleft. Its removal appears to permit an interaction between NH_2-terminal and COOH-terminal domains, which is necessary for the binding of substrate (*70*).

Splitting Sisters During Meiosis

During meiosis I, cohesion between sister chromatid arms is essential for holding homologs together after their recombination to produce chiasmata. In yeast, this cohesion is destroyed by separase-mediated cleavage of a meiosis-specific variant of Scc1 called Rec8 (*71*). Cleavage of Rec8 along chromosome arms occurs at the onset of anaphase I, but Rec8 in the vicinity of centromeres is spared this fate until the onset of anaphase II. The persistence of centromeric cohesion during meiosis I is essential for chromatid segregation during meiosis II. The mechanism underlying this differential treatment of cohesion along chromosome arms and at centromeres remains obscure. However, in the nematode *Caenorhabditis elegans*, where separase is also required for meiosis I (*72*), it has been suggested that the differential phosphorylation of Rec8 by the aurora B protein kinase Air2 might ensure that only Rec8 distal to chiasmata is cleaved at the first division (*73, 74*). Whether Rec8 cleavage is used to resolve chiasmata in vertebrates has recently been called into question, because the APC/C is thought to be dispensable for meiosis I in *Xenopus* oocytes (*75, 76*). Further experiments are required to settle this important issue. As long ago as 1909, Janssens pointed out that the first meiotic division was fundamentally similar to mitosis because it also involved the equational division of chromosomes (*77*). It would be strange if fundamentally different mechanisms were involved.

Conclusions

With the discovery of cohesin and condensin, the chromosomal mechanisms behind genome transmission have recently become amenable to molecular/mechanistic analysis. A pair of SMC proteins lie at the core of both of these complexes. Such proteins also play key roles in the segregation of bacterial chromosomes and presumably function through a mode of action that evolved long before histones. Although the fundamental geometry of these extraordinary proteins has finally been settled, the mechanisms by which they hold sisters together and help to disentangle them remains obscure. It has recently been suggested that they act by creating topologically enclosed domains that trap DNA strands, a novel proposal that remains to be tested. How cohesin is removed in two steps during mitosis and is gradually replaced by condensin is currently under scrutiny. The second step, cleavage of cohesin's Scc1 subunit by a thiol protease called separase, is subject to several control mechanisms that prevent the dissolution of cohesion before all chromatid pairs have bi-oriented on the mitotic spindle. PLK appear to have a crucial role in regulating the removal of cohesin during prophase and in stimulating cleavage of its Scc1 subunit by separase at the metaphase-to-anaphase transition Understanding how PLK and Cdk1 together orchestrate the transformation of interphase chromatin into compacted and individualized sister chromatids ready to be parted by separase remains a huge challenge for future studies.

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REVIEW

Connecting Chromosomes, Crisis, and Cancer

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Cancer is a disease of impaired genome stability. The molecular forces that maintain genome integrity and sense altered chromosome structure are invariably subverted in cancer cells. Here, we explore the contrasting contributions of telomeres in the initiation and suppression of cancer and review the evidence supporting a role for telomere dysfunction as a mechanism driving the radical chromosomal aberrations that typify cancer genomes. Recent work suggests that passage of cells through crisis in the setting of deactivated DNA damage checkpoints provides a mutational mechanism that can generate the diverse genetic alterations required for cancer initiation. A greater understanding of telomere-induced crisis and the cell's crisis management mechanisms should guide the rational development of new therapeutics for cancer and other disorders.

The genetic paradigm that now forms the foundation of our view of cancer pathogenesis has its deepest roots in the early cytogenetic analyses of cancer cells [reviewed in (*1*)]. Aberrant mitoses first noted by von Hansemann in 1890 (*2*) inspired Boveri's seminal concept of cancer as a genetic disease of somatic cells driven by chromosomal imbalances (*3*). This genetic hypothesis received experimental support from Muller's discovery that ionizing radiation, an agent already recognized as a potent carcinogen, also had mutagenic activity (*4*). Subsequently, Muller and McClintock began to explore the special role of chromosomal termini in the maintenance of chromosome structure (*5, 6*)—efforts that, years later, led to an integrated view of telomere dynamics in chromosomal stability and cancer [reviewed in (*7*)].

That genetic instability helps drive the development of cancer has emerged as a core concept in modern biology—continually reinforced by the increased incidence of neoplasia observed in human genetic disorders (and their animal models) of compromised genome stability [reviewed in (*8*, *9*)]. In such disorders, genetic instability endows incipient cancer cells with the molecular alterations that deactivate growth arrest and apoptotic checkpoints and permits the engagement of pathways essential for immortal growth. Indeed, the identification of the molecular mechanisms governing genome integrity has been a central focus in the field of cancer. Disruption of these mechanisms in cancer cells is manifested as defects in mitotic checkpoints, impaired nonhomologous end-joining, imprecise DNA replication, and so forth (*8*, *10*). The relative contribution of each of these mechanisms to the genome instability encountered in the majority of human cancers, particularly epithelial cancers, is not well understood. Here, we review the mounting experimental evidence that telomere dysfunction figures prominently in the evolution of cancer, providing a potential mechanism that enables cells to reach a critical threshold of cancer-promoting genetic changes during the formative stages of neoplastic transformation.

Telomeres, the structure at the ends of linear chromosomes, have long been recognized as critical for the maintenance of chromosomal integrity (*5*, *6*). The replication of linear chromosomes presents a special challenge that stems from the inability of conventional DNA polymerases to complete synthesis of chromosomal ends (*11*, *12*). Thus, as cells divide, this "end replication problem" results in the eventual reduction of telomeres to a short critical length that elicits the activation of cellular checkpoints not unlike those provoked by DNA damage [reviewed in (*13–15*)]. In human cell cultures, short telomeres result in activation of the Hayflick limit (Mortality Stage 1 or senescence), and the cells stop dividing [reviewed in (*16*)]. However, the Hayflick limit can be readily breached by inactivation of the p53 and Rb growth inhibitory pathways. Continued proliferation of cells beyond the Hayflick limit and further telomere erosion exacerbate telomere dysfunction and associated genomic instability, culminating in a period of massive cell death aptly termed "cellular crisis" (or Mortality Stage 2) (*16*).

The Hayflick limit presents a block to normal cell growth in culture, but because cancer cells invariably acquire Rb and p53 pathway defects, it has been difficult to document a direct role for shortened telomere–induced senescence in tumor suppression in vivo [reviewed in (*17*)]. We favor the hypothesis that crisis plays a more prominent role than senescence in tumorigenesis. Although crisis is a potent barrier to immortal growth in culture, the massive genetic instability associated with this state may well be the mechanism by which the rare cells surviving crisis acquire the constellation of genetic alterations needed for malignant transformation (*18–22*). These rare cells emerge from crisis by activating telomere maintenance mechanisms—most commonly by expression of the specialized ribonucleoprotein complex, telomerase (*18*). Telomerase consists of a catalytic telomerase reverse transcriptase (TERT) that synthesizes a sequence (TTAGGG in humans and mice) at the ends of chromosomes by using an RNA template encoded by the *telomerase RNA component* (*TERC*) gene (*23*). In human cells and tissues, the presence of telomerase activity correlates well with the level of *TERT* gene transcription, although additional levels of regulation such as RNA processing and posttranslation modification may also be important (*23*). In humans, *TERT* gene expression is limited

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