Together until separin do us part

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Loss of sister-chromatid cohesion triggers chromosome segregation. Several recent reports show that the protease Esp1 cleaves the cohesin subunit Scc1/Mcd1 to induce sister-chromatid segregation in yeast and vertebrates. This finding indicates that cohesin cleavage may control sister-chromatid separation in all eukaryotes.

To generate two daughter cells with exactly the same complement of chromosomes it is critical that the duplicated genetic material (the sister chromatids) remains tightly associated before chromosome segregation and promptly dissociates as chromosome division commences during anaphase (reviewed in refs 1, 2). The first breakthrough in understanding how sister-chromatid separation is controlled came from the identification of cohesin, a protein complex that is required to hold sister chromatids together until the onset of chromosome segregation[1,2]. The finding that a component of cohesin, Scc1/Mcd1, is proteolytically cleaved at the onset of anaphase and that this cleavage is required for sister-chromatid separation to occur marked the second breakthrough in understanding chromosome segregation[3].

Uhlmann *et al.*[4] now report that cleavage of Scc1/Mcd1 is sufficient to induce sister-chromatid separation, and have identified Esp1 (also known as separin or separase) as the protease that carries out this cleavage. Whether proteolytic cleavage of Scc1/Mcd1 homologues is responsible for initiating sister-chromatid separation in other eukaryotes and in specialized cell cycles such as meiosis was, until recently, unclear. However, Waizenegger *et al.*[5] have now shown that proteolytic cleavage of Scc1/Mcd1 also occurs in vertebrate cells and depends on Esp1. In addition, Tomonaga *et al.*[6] have demonstrated that in fission yeast, cleavage of Rad21 (a homologue of Scc1/Mcd1) is essential for chromosome segregation. Finally, Buonomo *et al.*[7] report that cleavage of Rec8, the meiotic brother of Scc1/Mcd1, is required for chromosome segregation during meiosis in budding yeast. Together, these findings point towards a universal mechanism for controlling chromosome division in eukaryotes.

A 14S protein complex known as cohesin assembles onto DNA during S phase and holds the duplicated DNA together until the onset of chromosome segregation[1,2]. The subunit composition of cohesin complexes varies between cell types[1,2,8–11] (Table 1). In budding yeast, cohesin consists of Smc1, Smc3, Scc1/Mcd1 and Scc3 during mitosis, but Rec8 replaces Scc1/Mcd1 in cells undergoing meiosis. In meiosis in fission yeast, Rec8 replaces Rad21 around centromeres. Vertebrate cohesin contains Smc1, Smc3, Scc1 and either SA1 or SA2 (homologues of Scc3). Both types of cohesin co-exist in all cell types that have been analysed so far, but SA2-containing cohesin predominates in somatic tissues, whereas in *Xenopus* embryos SA1-containing complexes are the most abundant. A meiosis-specific Scc3 homologue, SA3/STAG3, has also been identified. Whether the various cohesin subtypes have different functions or differ in their regulation is, at present, unclear. However, it is tempting to speculate that, at least, meiosis requires different types of cohesin, to accommodate exchange of genetic material between homologous chromosomes and retention of sister-chromatid cohesion at centromeres beyond the first meiotic division (see below, reviewed in ref. 12). The finding that overexpression of *rad21*$^+$ cannot compensate for the loss of its meiotic brother *rec8*$^+$ is consistent with this idea[13].

In 1999, Uhlmann *et al.*[3] showed that cleavage of the cohesin subunit Scc1/Mcd1 is required for chromosome segregation and is dependent on Esp1. However, it was unclear whether this cleavage is indeed the trigger for sister-chromatid separation and whether Esp1 is the protease that is responsible for cleavage. In their recent paper, Uhlmann and colleagues have now answered these questions. They replaced one of the two cleavage sites in Scc1/Mcd1 with the cleavage site of the Tobacco-etch virus (TEV) protease and expressed the TEV protease in cells containing this modified Scc1/Mcd1 as the sole source of Scc1/Mcd1. Under conditions in which Esp1 was inactive because of high levels of its inhibitor Pds1, expression of the TEV protease was sufficient to induce sister-chromatid separation, demonstrating that Scc1/Mcd1 cleavage can initiate chromosome segregation. Further evidence that Esp1 cleaves Scc1/Mcd1 came from the finding that its carboxy terminus shares sequence similarity with CD clan cysteine proteases. With this lead in hand, Uhlmann and colleagues then showed that purified Esp1 is capable of cleaving recombinant Scc1/Mcd1 *in vitro*, whereas Esp1 containing a mutation at the predicted active site was not. Finally, peptide inhibitors designed to covalently bind to the active site of Esp1 also prevented Scc1/Mcd1 cleavage. Thus, in budding yeast, the mechanism for triggering sister-chromatid separation and subsequent anaphase is now clear. A ubiquitination machinery called the anaphase-promoting complex/cyclosome (APC/C), in association with Cdc20, targets the Esp1 inhibitor Pds1 (Cut2 in fission yeast, securin in mouse) for degradation (reviewed in ref. 14). This allows Esp1 to cleave Scc1/Mcd1, and chromosome segregation ensues (Fig 1a).

Table 1 Cohesin subunits

Saccharomyces cerevisiae	*Schizosaccharomyces pombe*	*Drosophila melanogaster*	*Xenopus laevis*	*Homo sapiens*
Scc1/Mcd1 or **Rec8**	Rad21 or **Rec8**	Rad21	XRAD21	Scc1
Scc3	Psc3	SA	SA1 or SA2	SA1 or SA2 or **SA3 (STAG3)**
Smc1	Psm1	?	XSMC1	SMC1
Smc3	Psm3	?	XSMC3	SMC3

Meiosis-specific cohesin subunits are in bold

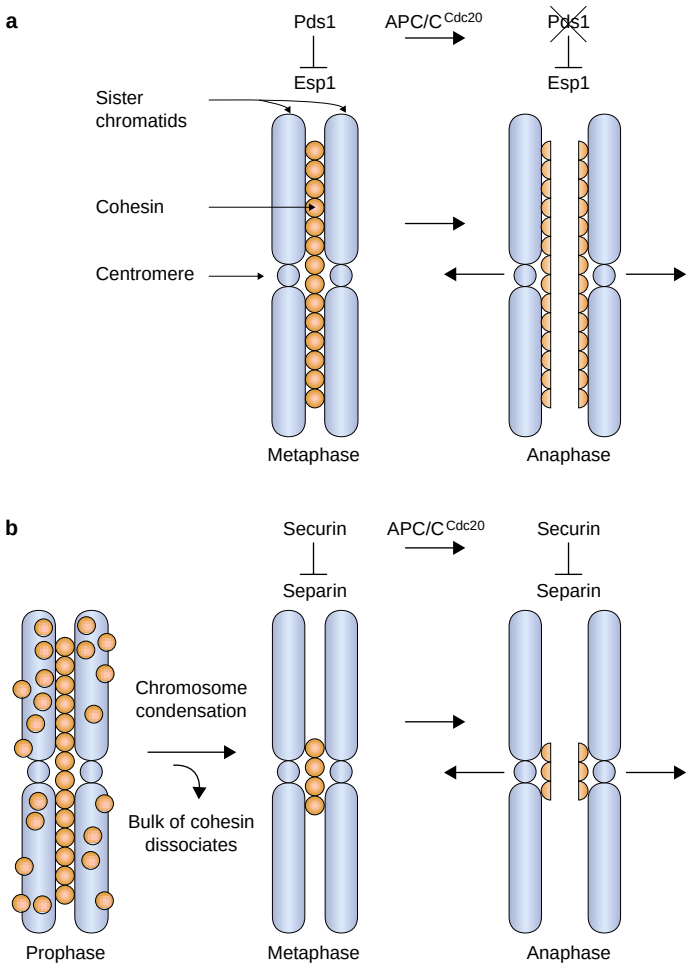

Figure 1 **Control of sister-chromatid cohesion in yeast and vertebrates.** a, **In budding yeast, sister chromatids (blue) are held together by cohesin complexes (orange) at metaphase. Degradation of Pds1 by APC/C^{Cdc20}-dependent proteolysis releases Esp1, allowing it to cleave Scc1/Mcd1 and leading to the initiation of anaphase.** b, **In vertebrates, the bulk of cohesin dissociates from chromosomes during prophase, perhaps as a result of chromosome condensation[5,8]. A small amount of cohesin remains on chromosomes, predominantly around centromeres. This pool of cohesin is cleaved by separin at the metaphase–anaphase transition. Activation of separin at this cell-cycle transition is brought about by destruction of securin by APC/C^{Cdc20}.**

Several reports now show that the mechanism that controls sister-chromatid separation, as discovered in budding yeast, is conserved among eukaryotes. Tomonaka *et al.*[6] report that fission-yeast Rad21 is cleaved at the metaphase–anaphase transition, and that Rad21 containing mutations at its cleavage sites prevents the onset of anaphase, at least when overexpressed. In vertebrates, the bulk of cohesin dissociates from chromosomes during prophase and not at the onset of anaphase[8–10,15]. However, Losada *et al.*[8] and Waizenegger *et al.*[5], have now shown that a small pool of cohesin remains associated with chromosomes, predominantly around centromeric regions until metaphase, but dissociates from chromosomes during anaphase (Fig. 1b). Having established that a small pool of cohesin remains associated with chromosomes until the onset of anaphase, Waizenegger and colleagues investigated whether vertebrate Scc1 is cleaved. Scc1 fragments were present during, but not before, the onset of anaphase and, importantly, cleavage occurred only after activation of APC/C^{Cdc20} and degradation of securin. Furthermore, purified separin that was liberated from its inhibitor securin — this was accomplished by incubating separin in mitotic extracts so that securin was degraded by APC/C^{Cdc20} — cleaved Scc1 *in vitro*. Together, these results indicate that separin also cleaves Scc1 in vertebrates. However, the ultimate proof will be to determine whether human Scc1, rendered resistant to cleavage by separin (for example, by mutating the cleavage site(s) within Scc1), prevents sister-chromatid separation. Why the bulk of cohesin dissociates from chromatin during prophase and how a small pool of cohesin is kept on chromosomes, particularly around centromeres, until the onset of anaphase are also critical questions that remain to be addressed. *Drosophila* Mei-S332 is an excellent candidate for preventing the dissociation of cohesin from centromeric regions. This protein localizes to regions around the centromeres of mitotic and meiotic chromosomes until the onset of anaphase and anaphase II, respectively. Furthermore, loss of Mei-S332 function causes premature loss of sister-chromatid cohesion during meiosis[16,17].

Cohesin not only controls chromosome segregation during the mitotic cell cycle, but also during the specialized meiotic cell cycle. The meiotic cycle consists of one phase of DNA replication followed by two nuclear divisions. Recombination (the exchange of genetic material) and segregation of homologous chromosomes occur during the first nuclear division (meiosis I); sister chromatids are then segregated during the second nuclear division (meiosis II). Buonomo *et al.*[7] have now shown that in budding yeast, Esp1-dependent cleavage of Rec8 is also required for chromosome segregation during both meiotic divisions, indicating that, at least in this organism, the mechanism that controls sister-chromatid separation is conserved between mitosis and meiosis. Having shown that Rec8 is like Scc1 and is cleaved in an Esp1-dependent manner, Buonomo and colleagues were in a position to test an old hypothesis. In contrast to mitosis, sister-chromatid cohesion is lost in a stepwise manner during meiosis — chromosome-arm cohesion is lost during meiosis I and centromeric cohesion during meiosis II (reviewed in ref. 12). It had been hypothesized that loss of chromosome-arm cohesion is required for crossover recombination events to be resolved and for homologous chromosomes to segregate (reviewed in ref. 18). Expression of a Rec8 mutant that was resistant to Esp1 cleavage led to arrest in metaphase I, demonstrating that loss of arm cohesion is indeed required for segregation of homologous chromosomes. Loss of arm cohesion during meiosis I might, however, be regulated differently in vertebrates. On page 83 of this issue Peter *et al.*[19] report that in *Xenopus* oocytes APC/C^{Cdc20}, which is critical for separin activation, is not required for chromosome segregation during meiosis I. As the bulk of cohesin in vertebrates dissociates from chromosomes during prophase and only persists on chromosomes around centromeres[5,8], cohesin cleavage may not be necessary for dissolving arm cohesion in

vertebrates.

Despite the fact that the mechanisms that trigger sister-chromatid separation seem to be conserved among eukaryotes, *Xenopus* separin does not seem to cleave human Scc1 (ref. 5). Although this initial observation needs to be extended and trivial explanations excluded, this finding raises exciting possibilities for the development of anti-fungal and anti-helminthic drugs. If it is indeed the case that separins from yeast and *Plasmodium* (the genus that causes malaria) only cleave yeast Scc1/Mcd1 and *Plasmodium* Scc1 respectively, this would faciliate the development of species-specific inhibitors for various separins.

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