

Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1

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Cohesion between sister chromatids is established during DNA replication and depends on a multiprotein complex called cohesin. Attachment of sister kinetochores to the mitotic spindle during mitosis generates forces that would immediately split sister chromatids were it not opposed by cohesion. Cohesion is essential for the alignment of chromosomes in metaphase but must be abolished for sister separation to start during anaphase. In the budding yeast *Saccharomyces cerevisiae*, loss of sister-chromatid cohesion depends on a separating protein (separin) called Esp1 and is accompanied by dissociation from the chromosomes of the cohesin subunit Scc1. Here we show that Esp1 causes the dissociation of Scc1 from chromosomes by stimulating its cleavage by proteolysis. A mutant Scc1 is described that is resistant to Esp1-dependent cleavage and which blocks both sister-chromatid separation and the dissociation of Scc1 from chromosomes. The evolutionary conservation of separins indicates that the proteolytic cleavage of cohesin proteins might be a general mechanism for triggering anaphase.

The separation of sister chromatids at the metaphase-to-anaphase transition is one of the most dramatic events of the eukaryotic cell cycle. As cells enter mitosis, chromosome condensation during prometaphase resolves the bulk of each chromatid's chromatin from that of its sister^{1,2}. Chromatids nevertheless remain paired along their entire length during the attachment of chromosomes to the mitotic spindle. Cohesion between sisters resists the pulling forces exerted by microtubules attached to sister kinetochores³ and thereby ensures that sister chromatids attach to microtubules emanating from opposite spindle poles^{4,5}. It has long been suspected that destruction of sister-chromatid cohesion, rather than a major change in traction exerted by the spindle, is responsible for the sudden separation of sister chromatids at the metaphase-to-anaphase transition^{3,4}. It is not known what triggers this event in the eukaryotic cell cycle.

There are important clues as to the molecular nature of the cohesive structures that holds sisters together and the mechanism by which it is suddenly broken at the onset of anaphase⁶. In *S. cerevisiae*, cohesion between sister chromatids depends on a multisubunit complex, called cohesin, which contains at least four subunits: Scc1, Scc3, Smc1 and Smc3 (refs 7–9). Cohesion is established during DNA replication with the help of Scc2 and Eco1 (refs 9–11). A similar cohesin complex has been implicated in sister-chromatid cohesion in *Xenopus* extracts².

In yeast, there is a sudden change in the state of cohesin at the metaphase-to-anaphase transition: two cohesin subunits, Scc1 and Scc3, suddenly disappear from chromosomes at the point when sister chromatids separate^{7,9}. The dissociation of Scc1 from chromosomes and the separation of sister chromatids both depend on a 'separin' protein called Esp1 (ref. 12). The existence of Esp1 homologues in many eukaryotes, including humans, suggests that separins have a fundamental and conserved role in chromosome segregation^{13–15}.

For much of the cell cycle, Esp1 is tightly bound by the anaphase inhibitor Pds1 (ref. 12), whose destruction at the metaphase-to-anaphase transition is triggered by ubiquitination due to the anaphase-promoting complex (APC)¹⁶. The APC requires an

activator protein, Cdc20, to mediate Pds1 destruction¹⁷. In *S. cerevisiae*, the only role of the APC in promoting sister separation is to destroy Pds1 (refs 12, 18). We now investigate the mechanism by which Esp1 dissolves sister-chromatid cohesion once it has been liberated from Pds1.

Esp1 controls Scc1 chromosome association

The displacement of Scc1 from chromosomes at the metaphase-to-anaphase transition might be a direct effect of Esp1 activity. Alternatively, it might just be a consequence of sister-chromatid separation initiated by Esp1. We therefore examined the mechanism that prevents the association of Scc1 with chromosomes during early G1 phase. Scc1 is destroyed during anaphase and is normally not resynthesized until late G1 in the next cell cycle⁷. However, even when Scc1 is synthesized in early-G1 cells from the galactose-inducible *GALI-10* promoter, it fails to bind stably to chromosomes¹⁰. We repeated this experiment using cells arrested in a G1-like state with the mating pheromone α -factor (Fig. 1a). Scc1, induced during pheromone arrest, accumulated in the nuclei of most cells, but bound to chromosomes only weakly, if at all. Furthermore, the protein rapidly disappeared from cells after expression was shut off by addition of glucose (Fig. 1b). When the experiment was repeated with *esp1-1* mutant cells, Scc1 bound to chromosomes with high efficiency and remained associated even after synthesis was terminated (Fig. 1b). This result indicates that Esp1 prevents the stable association of Scc1 with chromosomes during G1 phase, in addition to causing dissociation of Scc1 from chromosomes when sister chromatids separate. Esp1 may therefore have a direct role in removing Scc1 from chromosomes.

An *in vitro* Scc1-dissociation assay

To investigate the mechanism by which Esp1 causes Scc1 to dissociate from chromosomes, we assayed this process *in vitro* (Fig. 2). A crude preparation of yeast chromatin¹⁹, isolated from cells arrested in a metaphase-like state by nocodazole, was incubated with soluble extracts from *esp1-1* mutant cells that either had or had not been induced to overexpress wild-type Esp1 from the *GALI-10*

promoter. After incubation with both types of extract, the chromatin fraction was again separated from the supernatant by centrifugation and the levels of haemagglutinin(HA)-epitope-tagged Scc1 in chromatin and supernatant fractions were analysed by SDS-PAGE and subsequent immunoblotting. About 70% of the total Scc1 in nocodazole-blocked cells is tightly associated with chromatin⁹ and is therefore present in the starting chromatin fraction, which served as substrate. Most Scc1 remained in the chromatin fraction following incubation with extract prepared from *esp1-1* mutant cells, but almost all disappeared from the chromatin fraction after incubation with extract containing overexpressed Esp1 (Fig. 2). Surprisingly, Scc1 induced to dissociate from chromatin by Esp1 appeared in the supernatant fraction as a cleaved product (Fig. 2). Both dissociation of Scc1 from chromatin and its cleavage were inhibited by Pds1 translated in reticulocyte lysate, but not by a control lysate (Fig. 2). We also detected a small amount of Scc1-cleavage activity in extracts prepared from cells not overexpressing Esp1 if they were arrested in G1, and in extracts from cycling cells lacking Pds1 (data not shown). Esp1 also caused ~50% of the Scc3 cohesin subunit⁹ to dissociate from chromatin without cleavage. The association of Smc proteins and histone H2B1 with chromatin was unaffected by Esp1-containing extracts (data not shown).

We next investigated the requirements for Scc1 dissociation *in vitro*. It has been suggested that a transient calcium wave in mitotic cells might trigger sister-chromatid separation²⁰, but Scc1 cleavage was unaltered by addition of the calcium chelator EGTA or by an excess of free calcium. The reaction was also not inhibited by inhibitors of kinases or phosphatases (data not shown). This suggests that the dissociation of Scc1 from metaphase chromatin *in vitro* is neither induced by calcium nor by *de novo* phosphorylation/ dephosphorylation. ATP depletion of extracts or addition of the proteasome inhibitor LLNL did not prevent Scc1 cleavage (data not shown), indicating that Scc1 cleavage is probably not due to APC-mediated ubiquitination. We attempted to characterize the proteolytic activity by using protease inhibitors from the four known classes, and found that Scc1 cleavage was only inhibited by high concentrations (10 mM) of *N*-ethylmaleimide.

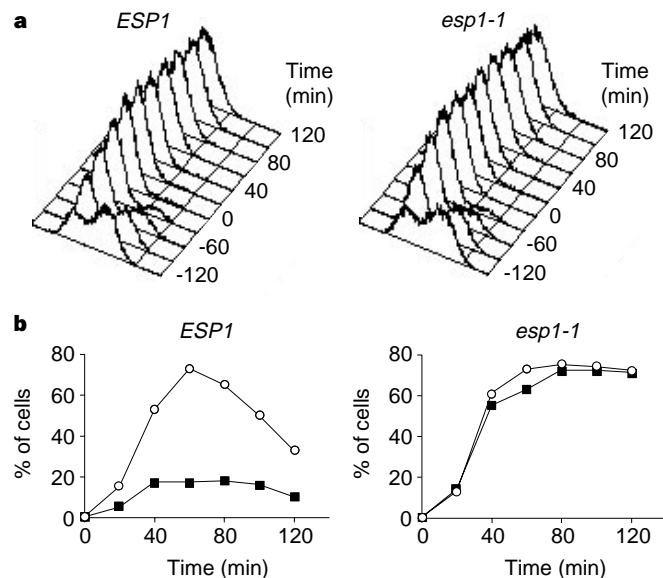


Figure 1 Esp1-dependent chromosome association of Scc1 in G1. **a**, Strains K7466 (*MAT α ESP1 SCC1 GAL-SCC1myc18*) and K7468 (*MAT α esp1-1 SCC1 GAL-SCC1myc18*) were arrested in G1 with α -factor for 120 min (time point zero). FACSscan analysis showed that cells stayed arrested during the experiment. **b**, Scc1-Myc18 was induced for 60 min, then cells were transferred to medium containing glucose to repress Scc1-Myc18. Expression of Scc1-Myc18 was seen by whole-cell *in situ* staining (circles), and chromosome binding of Scc1-Myc18 was observed by using chromosome spreads (squares).

Scc1 cleaved at anaphase onset *in vivo*

To test whether Esp1-induced cleavage of Scc1 also occurs *in vivo* at the onset of anaphase, we used a yeast strain in which expression of the APC-activator Cdc20 is under the control of the galactose-inducible *GAL1-10* promoter²¹. Cells from this strain were arrested in metaphase by incubation in galactose-free medium and then induced to undergo synchronous anaphase by addition of galactose. Sister-chromatid separation, visualized by the binding to *tet* operators close to CenV of *tet* repressor tagged with green fluorescent protein (GFP) (ref. 7), occurred in most cells within 15 min of Cdc20 induction, and Scc1 dissociated from chromosomes at a similar rate (Fig. 3a, b). We detected a small amount of Scc1 cleavage product of the same size as that seen *in vitro* in cycling cells, but not in cells arrested in metaphase. The cleavage product appeared 15 min after release into anaphase, simultaneously with sister-chromatid separation and the dissociation of Scc1 from chromosomes (Fig. 3c). Full-length Scc1 remaining in cells at this time might originate from the soluble pool of Scc1, whose cleavage is unnecessary for sister separation. Soluble Scc1 in the supernatant fraction of chromatin preparations makes a poor substrate in our *in vitro* cleavage assay (data not shown).

To establish whether cleavage of Scc1 during anaphase depends on Esp1, we compared wild-type and *esp1-1* mutant cells after their release from *GAL-CDC20* arrest at 35°C. The extent of sister-chromatid separation, Scc1 dissociation from chromosomes (data not shown), and Scc1 cleavage (Fig. 3d) was greatly reduced in the *esp1-1* mutant. We conclude that Esp1 promotes cleavage of Scc1 and its dissociation from chromosomes both *in vivo* and *in vitro*.

A cleavage-resistant Scc1

To determine whether Esp1-mediated cleavage of Scc1 is a cause or a consequence of its dissociation from chromosomes, we first identified the Scc1-cleavage site, with a view to producing a cleavage-resistant mutant. The C-terminal Scc1-cleavage product in anaphase cells (Fig. 3) was immunoprecipitated from cell extracts. Amino-terminal sequence analysis of the fragment showed that cleavage had occurred between a pair of arginine

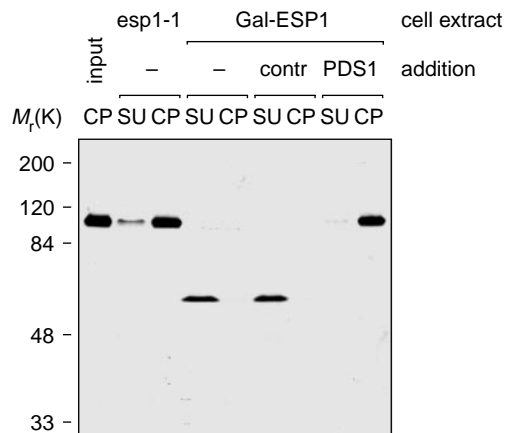


Figure 2 *In vitro* assay for Scc1 dissociation from chromatin. Chromatin was prepared as described¹⁹ from strain K7563 (*MAT α SCC1-HA6*) arrested in metaphase by nocodazole treatment. Proteins in the chromatin preparation were resolved by SDS-PAGE; Scc1-HA6 was detected by western blotting (input). This chromatin preparation was resuspended in the indicated extracts, with or without addition of 50% (v/v) *in vitro* translation reactions, as indicated. After incubation, aliquots of the supernatant fraction (SU) and the chromatin fraction (CP) of each reaction were analysed.

residues at positions 268 and 269. The first of these arginine residues was then mutated to aspartic acid (R268D), tagged at the C terminus with HA epitopes, and expressed from the *GAL1-10* promoter: expression of the mutant protein had little effect on cell proliferation. To test whether the R268D mutation had abolished cleavage, we used chromatin from cells expressing it as a substrate in the Esp1 assay. We found that there was no cleavage at site 268, but that the mutant protein was still cleaved in an Esp1-dependent manner (Fig. 4a). The C-terminal cleavage product was now about 10K larger. To identify the second cleavage site, we looked for sequences in Scc1 that are similar to those around the C-terminal cleavage site and found a 5-out-of-7 amino-acid match at position 180 (Fig. 4b). We mutated the arginine before this putative cleavage site to aspartate (R180D).

We next compared the effect of expressing wild-type Scc1, R180D and R268D single-mutant proteins, and the R180D/R268D double-mutant protein from the *GAL1-10* promoter. Neither the single-mutant proteins nor the wild-type protein greatly affected cell proliferation, but expression of the double-mutant protein was lethal (data not shown). We used chromatin from cells transiently expressing the double-mutant protein in our Esp1 assay and found that the R180D/R268D double-mutant protein (Scc1RR-DD) was no longer cleaved. Furthermore, it failed to dissociate from chromosomes (Fig. 4a). The small amount of 'leakage' of Scc1 from chromatin into the supernatant was Esp1-independent (data not shown).

To obtain the N- and C-terminal cleavage products simultaneously, we used as a substrate chromatin from a strain expressing Scc1 tagged N-terminally with a Myc epitope and C-terminally with HA epitope (Fig. 4b). Esp1-mediated cleavage produced a single HA-tagged cleavage fragment but two Myc-tagged fragments, the smaller of which was more abundant (Fig. 4c). These results indicate that all molecules were cleaved at the C-terminal site but not all of them were cleaved at the N-terminal site. A similar pattern of N-terminal cleavage fragments was obtained during anaphase *in vivo* (data not shown).

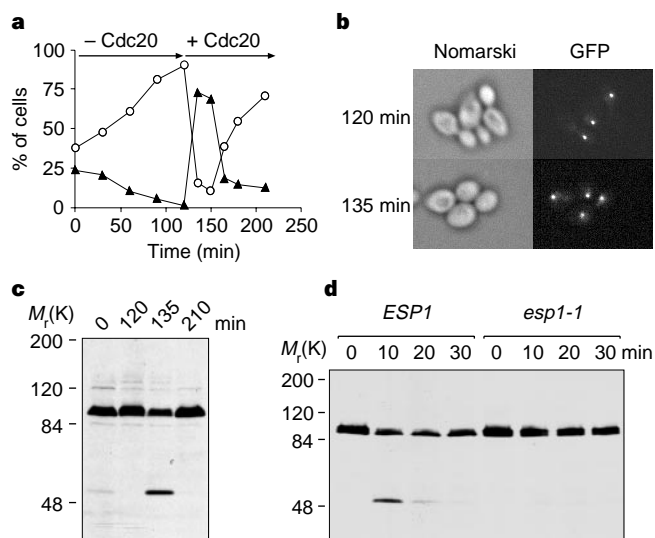


Figure 3 Scc1p cleavage at anaphase onset *in vivo*. **a**, Metaphase arrest and release using Cdc20 depletion of strain K7677 (*MAT α cdc20 Δ GAL-CDC20 Scc1-HA3 TetR-GFP TetOs*). Scc1-HA3 bound to chromosomes (circles), and the fraction of cells containing two separated GFP dots (triangles). **b**, Examples of cells in the arrest at 120 min, and 15 min after release. **c**, Western blot analysis of Scc1-HA3 in whole-cell extracts prepared from cells at the indicated time points. **d**, As **c**, except that strain K7677 and K8054 (*MAT α esp1-1 cdc20 Δ GAL-CDC20 Scc1-HA3 TetR-GFP TetOs*) were arrested and released at 35°C.

Non-cleavable Scc1 and sister separation

To investigate why cells expressing the non-cleavable Scc1RR-DD double mutant cannot proliferate, we used centrifugal elutriation to isolate G1 cells from a culture growing without expression of Scc1RR-DD, which were then incubated in the presence and absence of galactose to induce Scc1RR-DD from the *GAL1-10* promoter (Fig. 5). To minimize the duration of mutant protein expression, cells grown in the presence of galactose were transferred to glucose-containing medium after 135 min, when most cells had replicated their DNA (Fig. 5a). In the absence of galactose, sister separation and the dissociation from chromosomes of endogenous Myc-tagged Scc1 occurred simultaneously about 60 min after DNA replication (Fig. 5b-d). Transient expression of Scc1RR-DD, tagged with HA epitope, almost completely prevented sister-chromatid separation (Fig. 5b) but did not affect binding and dissociation of endogenous wild-type Scc1 (Fig. 5c). The mutant protein remained tightly associated with chromosomes long after the endogenous wild-type protein had disappeared (Fig. 5c, d). Scc1RR-DD bound to chromosomes immediately following induction in G1, when wild-type Scc1 is prevented from binding by Esp1 (compare to Fig. 1 and

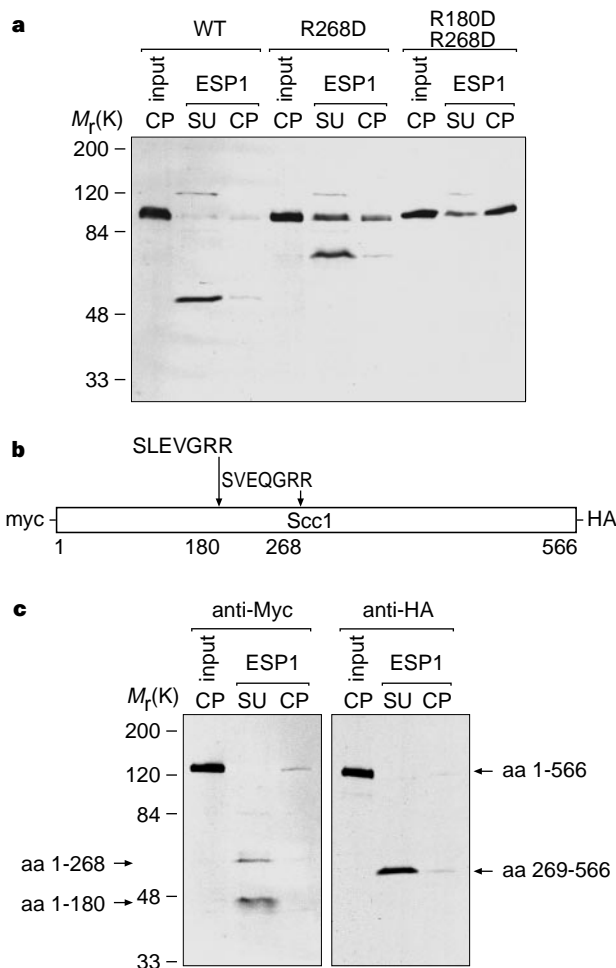


Figure 4 Characterization of the Scc1 cleavage sites. **a**, Strains K8097 (*MAT α Scc1-myc18 GAL-Scc1-HA3 TetR-GFP TetOs*), K8099 (*MAT α Scc1-myc18 GAL-Scc1(R268D)-HA3 TetR-GFP TetOs*) and K8101 (*MAT α Scc1-myc18 GAL-Scc1(R180D, R268D)-HA3 TetR-GFP TetOs*) were grown in medium containing 2% raffinose. Expression of the respective Scc1 variant was induced for 4 h, and cells were arrested by nocodazole treatment. Chromatin was prepared and used in the Esp1 assay. The 120K bands are HA-cross-reacting proteins. WT, wild type. **b**, The cleavage sites in Scc1. **c**, Chromatin was prepared from strain K7768 (*MAT α myc9-Scc1-HA6*). Scc1 (ref. 8) and the derived cleavage fragments (aa, amino-acid residues) migrate abnormally slowly during SDS-PAGE, as shown by western blotting with the indicated antibodies.

ref. 10). The failure of Scc1RR-DD to dissociate from chromosomes was not an artefact due to transient overexpression of the protein from the *GAL1-10* promoter, because wild-type Scc1 expressed similarly dissociates from chromosomes with normal kinetics¹⁰.

Expression of the Scc1RR-DD mutant protein caused a transient delay of cytokinesis for 20 min (Fig. 5b), after which cells divided

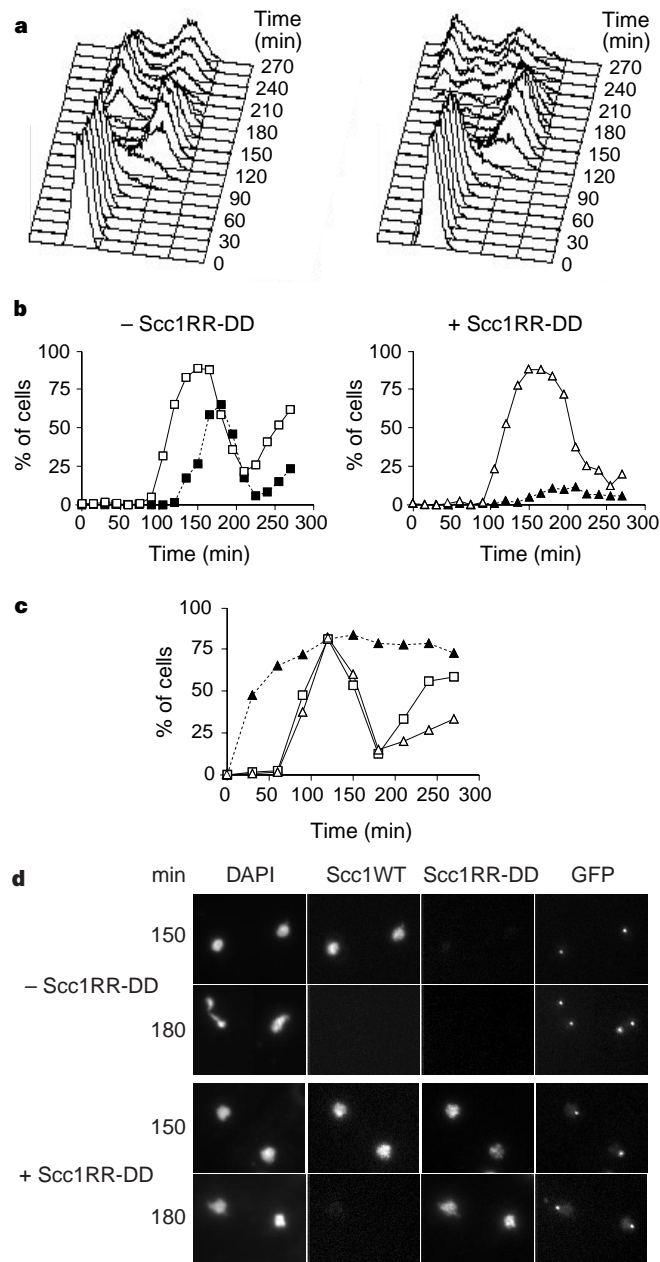


Figure 5 Expression of non-cleavable Scc1 prevents sister chromatid separation. **a**, DNA content as unbudded G1 cells of strain K8101 were released into the cell cycle with or without induction of the Scc1RR-DD mutant. **b**, Budding index (without Scc1RR-DD, open squares; with Scc1RR-DD, open triangles) and percentage of cells with separated sister chromatids (filled symbols). **c**, Scc1 chromosome association. Endogenous wild-type Scc1-Myc18 in cells without (squares) and with Scc1RR-DD expression (open triangles). Scc1RR-DD was tagged with HA epitopes (filled triangles). **d**, Examples of chromosome spreads at 150 min in metaphase and at 180 min when most cells of the control culture had undergone anaphase. DNA was stained with DAPI, Scc1-Myc18 was detected with rabbit anti-Myc antiserum and Cy5-conjugated secondary antibody, Scc1RR-DD-HA3 was detected with mouse monoclonal antibody 16B12 and Cy3-conjugated secondary antibody. Sister chromatids of chromosome V were visible by the GFP dots.

without having separated sister chromatids. Progeny with abnormal DNA contents were produced (Fig. 5a), resembling *esp1-1* mutant cells incubated at the restrictive temperature²². The dissociation from chromosomes of wild-type protein on cue shows that Esp1 activity was not impaired in these cells. The failure of sister chromatids to separate even when Esp1 was active also prevented elongation of the mitotic spindle (data not shown), consistent with the idea that loss of cohesion triggers anaphase.

To determine the phenotype of single- and double-mutant Scc1 proteins when expressed from the natural *SCC1* promoter, we transferred the mutations to *SCC1* carried on a centromeric vector. Plasmids carrying the wild-type or either of the single-mutant genes transformed wild-type and *scc1-73* strains and complemented the temperature-sensitive phenotype of the *scc1-73* mutation. No transformants expressing the R180D/R268D double mutant were obtained (data not shown). Together, our results indicate that cleavage of Scc1 at one of two sites is necessary both for sister-chromatid separation and for the dissociation of Scc1 from chromosomes.

Non-cleavable Scc1RR-DD is functional

To test whether Scc1RR-DD is fully functional apart from its non-cleavability, we first checked whether the double-mutant protein could establish cohesion by itself between sister chromatids in the absence of endogenous Scc1 function, and then whether cohesion established by Scc1RR-DD was dependent on other cohesin subunits. We used centrifugal elutriation to isolate G1 cells of *scc1-73* and *smc3-42* mutant strains⁷ that could express Scc1RR-DD from the *GAL1-10* promoter, then incubated them in the presence or absence of Scc1RR-DD at 35 °C, a restrictive temperature for both mutations. In the absence of galactose, sister chromatids separated prematurely in both mutants and failed to segregate to opposite poles of the cell⁷ (Fig. 6). Expression of Scc1RR-DD suppressed premature sister-chromatid separation in *scc1-73* mutant cells but not in *smc3-42* cells (Fig. 6). Thus, Scc1RR-DD alone fulfils the cohesion function of Scc1. The cohesion due to Scc1RR-DD depends on Smc3, as does that produced by wild-type Scc1,

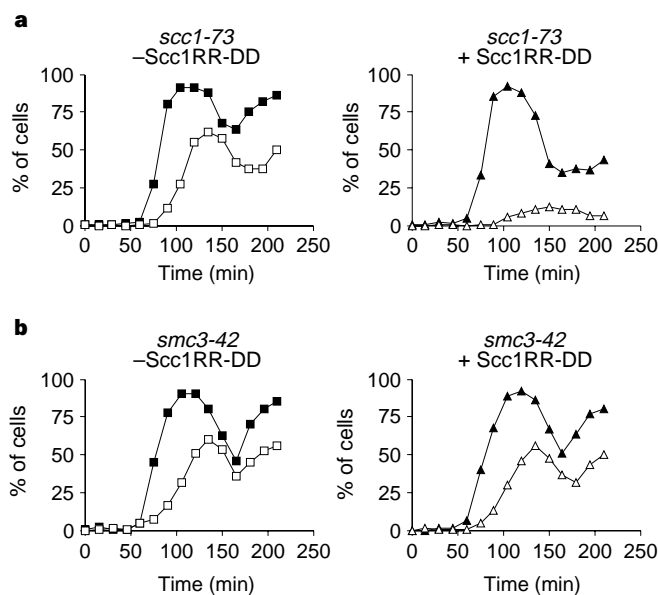


Figure 6 Scc1RR-DD is a functional Scc1 variant. **a**, G1 cells of strain K8103 (*MATa scc1-73 GAL-SCC1(R180D,R268D)-HA3 TetR-GFP TetOs*) were released at 35 °C into medium containing or lacking galactose. The budding index (filled symbols) and the percentage of cells containing separated sister chromatids (open symbols) are shown. **b**, As **a**, except that strain K8149 (*MATa smc3-42 SCC1-myc18 GAL-SCC1(R180D,R268D)-HA3 TetR-GFP TetOs*) was used.

suggesting that Scc1RR-DD differs from wild-type only in its susceptibility to cleavage by Esp1.

Cohesin cleavage triggers anaphase

Our results indicate how cohesion between sister chromatids might be destroyed at the onset of anaphase in yeast (Fig. 7a). As Scc1 is required to maintain cohesion until the metaphase-to-anaphase transition⁷⁻⁹, its disappearance from chromosomes should destroy cohesion. We have shown that cleavage of Scc1 mediated by the separin Esp1 at the exact point when sister chromatids separate is necessary for sister-chromatid separation, for dissociation of Scc1 from chromosomes, and for the movement of spindle poles to opposite ends of the cell. We propose that sister separation in yeast is triggered by the sudden cleavage of Scc1, although what brings this about is not fully understood. Proteolysis of Pds1 shortly before the metaphase-to-anaphase transition is important: Pds1 inhibits Esp1-dependent cleavage *in vitro* and is destroyed suddenly by APC^{Cdc20} *in vivo* so that sister chromatids can separate^{16,17}. The inhibition of APC^{Cdc20} by Mad2 that occurs when chromosomes fail to attach correctly to the mitotic spindle^{18,23,24} prevents the destruction of Pds1 and so blocks the onset of anaphase (hence the dubbing of Pds1 and homologues like Cut2 (ref. 25) as securins). Although destruction of Pds1 is necessary for sister separation, it is not sufficient. The disappearance of Scc1 from chromosomes is tightly regulated in yeast cells lacking Pds1 (ref. 26), so a second pathway must exist that regulates either Esp1 activity or the susceptibility of Scc1 to Esp1-dependent cleavage.

Given that Smc1 and Smc3 may form an antiparallel heterodimer²⁷ and that the link between sisters is a symmetrical, we suggest that Scc1 and Scc3 hold the two Smc1/3 heterodimers together, with each being bound to sister DNA molecules²⁸ (Fig. 7a); separin could then cleave sister chromatids apart. Cleavage also destabilizes

the association of Scc1 with the rest of the cohesin complex. Our results do not indicate whether it is cleavage of Scc1, its subsequent dissociation from the cohesin complex, or both combined that triggers sister-chromatid separation.

Is cohesin cleavage general?

To investigate whether Scc1 was separin's only target, we searched for yeast proteins containing the Scc1 cleavage-site consensus sequences SxExGRR. Only one protein gave a convincing match: we call this protein Rec8 (ref. 29) on the basis of its homology to the *rec8* gene product of fission yeast (Fig. 7b). Rec8 is a member of the Scc1 family and contains two SxExGRK motifs; it is only expressed in meiotic cells and, unlike Scc1, is essential for sister-chromatid cohesion during meiosis I and II (ref. 30; and F. Klein, S. Buonomo and K.N., unpublished results). Rec8 seems to replace Scc1 during meiosis, and cleavage of Rec8 might be crucial for separating sister chromatids during meiosis.

We have not been able to detect Scc1 cleavage motifs in homologous proteins from animals, but the equivalent protein in fission yeast, Rad21 (ref. 31), does contain two near matches in a similar region of the protein to those from Scc1 (Fig. 7b). Most cohesin in animal cells dissociates from chromosomes during prometaphase². A key question for the future is therefore whether the target for animal cell separins is a small fraction of the cohesin pool that persists on metaphase chromosomes or some other cohesion protein.

Is the Esp1 separin a protease?

Although we have not directly determined the identity of the protease that cleaves Scc1, our finding that the cleavage activity in extracts is roughly proportional to their Esp1 concentration (data not shown) raises the possibility that Esp1 is itself the protease. Esp1 and its homologues in other organisms are all large proteins of

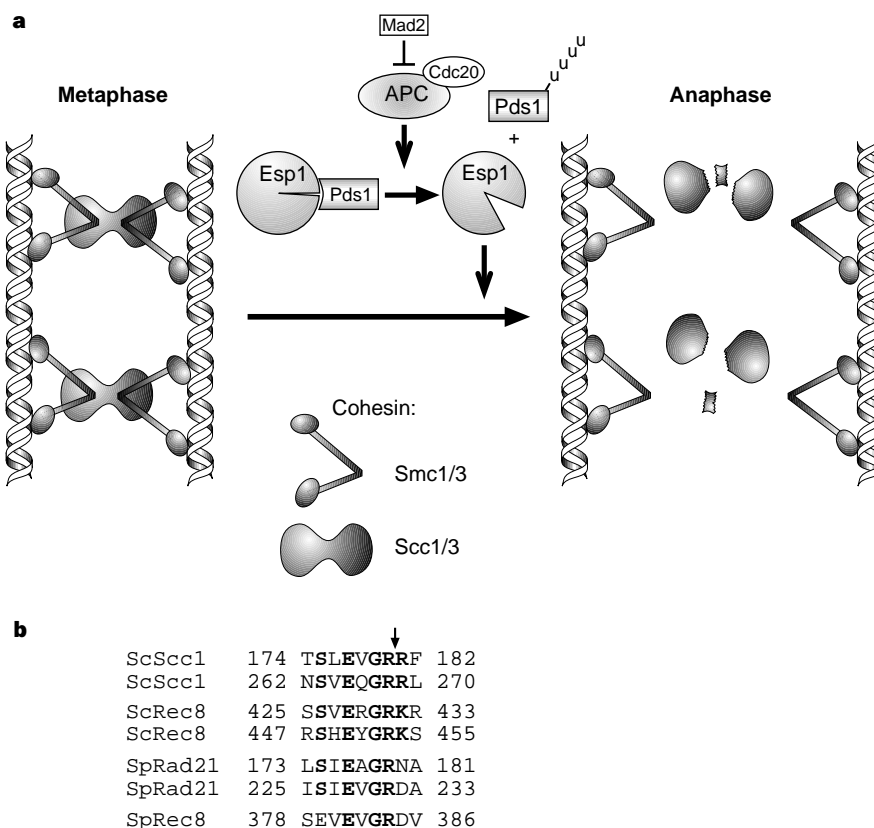


Figure 7 Model for separin action on cohesin, and conservation of potential cohesin cleavage sites. **a**, Proteolytic cleavage of one of cohesin's subunits is necessary for sister separation, suggesting that cohesin complexes link sister chromatids. Mad2 is included in the scheme as a known inhibitor of APC^{Cdc20}

(ref. 24). **b**, Alignment of known and putative cohesin cleavage sites. Sequence motifs similar to the consensus between the two Scc1 cleavage sites were searched for using the HMMER algorithm³⁶.

relative molecular mass ~200K (refs 13, 15, 22, 32). Most of their sequences are not highly conserved, but they all contain a conserved C-terminal domain, which might be responsible for a proteolytic activity. This 'separin domain' does not resemble any known protease. However, the insensitivity of the cleavage reaction to many known protease inhibitors suggests that the protease may have a novel mechanism of action. If Esp1 is not itself the protease, then it might instead be an allosteric effector of a protease, which either resides on chromatin or is present in the soluble fraction. Indeed, Esp1 might have functions in addition to Scc1 cleavage³⁷. It will be necessary to purify Esp1 and provide it with a more clearly defined substrate to answer these questions. □

Methods

Plasmids and strains. The Scc1 coding sequence was cloned under control of the *GAL1-10* promoter into a YIplac128 derived vector³³. A DNA fragment encoding 3 tandem HA epitopes was inserted into a *NotI* restriction site introduced by PCR at the C terminus of *SCC1*. Site-directed mutagenesis was performed by exchanging restriction fragments from Scc1 with PCR fragments obtained using primers containing the desired nucleotide changes.

All strains used were derivatives of W303. Epitope tags at the C terminus of the endogenous Scc1p were generated by a PCR one-step tagging method (W. Zachariae and K.N., unpublished results). The Myc-epitope tag at the N terminus of endogenous Scc1 was obtained by integration of a N-terminally tagged portion of Scc1 at the *SCC1* locus. Strains expressing Scc1-Myc18, Esp1, and Cdc20 under control of the *GAL1-10* promoter have been described^{7,12,21}.

Cell-growth and cell-cycle experiments. Cells were grown in complete medium³⁴ at 25 °C unless otherwise stated. Strains expressing Cdc20, Esp1 or Scc1 from the *GAL1-10* promoter were grown in complete medium containing 2% raffinose as carbon source. The *GAL1-10* promoter was induced by adding 2% galactose. A G1-like arrest was achieved by adding 1 µg ml⁻¹ of the pheromone α-factor to the medium. For metaphase arrest, 15 µg ml⁻¹ nocodazole was added with 1% DMSO. Metaphase arrest due to Cdc20 depletion was obtained in cells with Cdc20 under control of the *GAL1-10* promoter by shifting to medium containing raffinose only. For release from the arrest, 2% galactose was added back to the culture.

In vitro assay for Esp1 activity. A crude Triton X-100-insoluble chromatin preparation was obtained from yeast cells as described¹⁹. The pelleted chromatin was resuspended in yeast cell extracts that had been prepared similarly to the supernatant fraction of the chromatin preparation. Routinely, one tenth volume of an ATP regenerating system (50 mM HEPES/KOH, pH 7.5, 100 mM KCl, 10 mM MgCl₂, 10 mM ATP, 600 mM creatine phosphate, 1.5 mg ml⁻¹ phosphocreatine kinase, 1 mM DTT, 10% glycerol) was added. (This addition was later found not to be essential.) Reactions were incubated for 10 min at 25 °C with gentle shaking, and stopped on ice. The chromatin fraction was separated again from the supernatant by centrifugation, and resuspended in buffer EBX¹⁹. Equivalent aliquots of supernatant and chromatin pellet were analysed by SDS-PAGE and western blotting. HA-tagged Scc1 was detected using monoclonal antibody 16B12, Myc-tagged Scc1 with monoclonal antibody 9E10. As overexpression of Esp1 from the *GAL1-10* promoter is toxic to cells, extracts with overproduced Esp1 were prepared 2 h after induction with galactose of a culture pregrown in medium containing raffinose only.

Protein sequencing of the Scc1 cleavage site. The C-terminal Scc1 cleavage fragment was isolated from strain K7756 (*MATa cdc20Δ GAL-CDC20 SCC1-myc18*). Cells synchronized in anaphase were obtained as described for Fig. 3. Protein extract of 5 × 10⁹ cells was prepared by breakage with glass beads in breakage buffer (50 mM HEPES/KOH, pH 7.5, 100 mM KCl, 2.5 mM MgCl₂, 1 mM DTT, 0.25% Triton X-100, 0.1% SDS, plus protease inhibitors). Myc-epitope-tagged protein was immunoprecipitated with 20 µg anti-Myc 9E11 monoclonal antibody, resolved on SDS-PAGE and transferred to a PVDF membrane³⁵. N-terminal sequencing of the band corresponding to the Scc1 cleavage fragment was performed using an Applied Biosystems 492A sequencer. The amino-acid sequence was RLGESIM, corresponding to the Scc1 amino-acid residues from position 269.

Chromosome spreading. Analysis of DNA content and chromosome spreading have been described⁷, but spheroplastation to prepare cells for spreading was at 37 °C for only 5 min.

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