Sister chromatid cohesion and genome stability in vertebrate cells

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Abstract
For successful eukaryotic mitosis, sister chromatid pairs remain linked after replication until their kinetochores have been attached to opposite spindle poles by microtubules. This linkage is broken at the metaphase–anaphase transition and the sisters separate. In budding yeast, this sister chromatid cohesion requires a multi-protein complex called cohesin. A key component of cohesin is Scc1/Mcd1 (Rad21 in fission yeast). Disruption of the chicken orthologue of Scc1 by gene targeting in DT40 cells causes premature sister chromatid separation. Cohesion between sister chromatids is likely to provide a substrate for post-replicative DNA repair by homologous recombination. In keeping with this role of cohesion, Scc1 mutants also show defects in the repair of spontaneous and induced DNA damage. Scc1-deficient cells frequently fail to complete metaphase chromosome alignment and show chromosome segregation defects, suggesting aberrant kinetochore function. Consistent with this, the chromosomal passenger protein, INCENP (inner centromere protein) fails to localize to centromeres. Survivin, another passenger protein and one which interacts with INCENP, also fails to localize to centromeres in Scc1-deficient cells. These results show that cohesion maintains genomic stability by ensuring appropriate DNA repair and equal chromosome segregation at mitosis.

Sister chromatid cohesion
To ensure the equal transmission of genetic material to both daughter cells following mitotic division, eukaryotic anaphase does not begin until paired sister chromatids are attached to opposite spindle poles by their kinetochores. For this to occur in a co-ordinated manner, sister chromatids must remain linked after replication, so that the sisters cannot both move to a single pole [1–4]. In Saccharomyces cerevisiae, this linkage, termed sister chromatid cohesion, requires a multi-protein complex called cohesin. Cohesin contains at least four subunits: Scc1p/Mcd1p (Rad21 in fission yeast), Scc3p, Smc1p, and Smc3p [5–8]. In budding yeast, Scc1 is cleaved during the metaphase–anaphase transition, in a reaction that requires a cysteine protease called ‘separase’ (also known as Esp1p; Cut1p in fission yeast). It then dissociates from chromosomes in anaphase, leading to sister chromatid separation [5,9–11]. In contrast, in metazoans, a cleavage-independent pathway removes cohesin from chromosome arms during prophase, whereas a separase-dependent pathway cleaves centromeric cohesin at the metaphase–anaphase transition [12–15]. The latter event appears to be responsible for sister chromatid separation in anaphase, as has been demonstrated in fission yeast [16]. Current models for anaphase suggest that Scc1 cleavage destroys the connection between sister chromatids thus allowing microtubules to pull the chromatids to opposite poles [4].

Chromosomal passengers
Chromosomal passengers are a class of proteins that show a complex and dynamic localization during mitosis. They associate along the chromosome axis during prophase, concentrate at the centromere at metaphase and move from the centromere to the central region of the mitotic spindle at anaphase [17]. One of these is Aurora B, one of a number of essential protein kinases that regulate progression through mitosis [18]. Aurora B is required for chromosome alignment at the metaphase plate and for completion of cytokinesis in metazoan cells ([19–23]; reviewed in [24]). It is also involved somehow in mitotic chromosome condensation, but the extent of this involvement is unclear [14,20,22,25]. Other chromosomal passengers include the inner centromere protein (INCENP) and survivin, both of which physically interact with Aurora B. INCENP or survivin deficiency causes Aurora B mislocalization and cellular defects indistinguishable from those occasioned by Aurora B deficiency, while the loss of Aurora B prevents both INCENP and survivin from behaving normally [19,21,22,26–29].

Conditional inactivation of SCC1 in chicken DT40 cells
Gene targeting in the hyper-recombinogenic chicken DT40 B-lymphoid cell line was used to disrupt the SCC1 locus,
with cell viability being sustained by the expression of a repressible transgene [30]. Upon repression of the Scc1 transgene, a significantly increased distance between sisters is seen in interphase and mitosis, confirming that Scc1 is necessary for sister chromatids to remain closely connected in vertebrate cells. Neither DNA replication nor chromosome condensation appears to be dependent on sister chromatid cohesion.

Scc1−/− cells stop proliferating shortly after SCC1 repression and cells begin to accumulate in G2/M with extensive cell death occurring slightly later, suggesting that Scc1 is essential for the timely completion of mitosis. The bulk of the mitotically arrested cells are blocked in prometaphase, with most of the chromosomes aligned on a metaphase plate, but with a significant minority scattered throughout the spindle. While small numbers of Scc1−/− cells do manage to exit mitosis, these cells consistently show high levels of chromosome missegregation [30], suggestive of defects in either kinetochore attachment or function. Kinetochore assembly in the absence of Scc1 appears to be normal, as judged by the binding of the constitutive kinetochore proteins CENP-H and CENP-C and the signalling component Mad2 [30]; however, the distribution of the chromosomal passenger protein INCENP is abnormal—it binds to chromosomes, but fails to target normally to the inner centromere [30]. Its interactor, survivin, also mislocalizes in the absence of Scc1, showing that the chromosomal passenger complex fails to behave normally in the absence of cohesin (C. Morrison, P. Vagnarelli and W.C. Earnshaw, unpublished work). These observations are consistent with findings in Rad21/Scc1-depleted Schizosaccharomyces pombe, which exhibit defective targeting to mitotic chromosomes of the chromosomal passengers, Bir1/Cut17 (a putative survivin homologue) and the Aurora kinase [31].

Spindle–kinetochore attachment and mitotic checkpoint

The spindle checkpoint, which monitors either tension between sister centromeres or their occupancy by spindles [32,33], is activated by any sister kinetochores that have not established bipolar attachments to the spindle apparatus. Since the activation of separase is dependent on the activity of the anaphase-promoting complex/cyclosome [9–11,34–36], the control of the anaphase-promoting complex/cyclosome by the spindle checkpoint integrates metaphase chromosome alignment with the onset of anaphase. The cohesin enriched at centromeres or the centromere-proximal region [37–39], appears to have an essential role in generating a dynamic tension between microtubules and sister chromatids in yeast [40–42]. In animal cells, this tension is required for the establishment of stable kinetochore–microtubule attachments [32,43–45] and appears to be involved in down-regulating the spindle checkpoint. It is noteworthy that the centromeres of the unaligned chromosomes in Scc1-deficient prometaphase cells are positive for Mad2 and BubR1 proteins ([30]; P. Vagnarelli, C. Morrison, H. Dodson, E. Sonoda, S. Takeda and W.C. Earnshaw, unpublished work), which is indicative of spindle-checkpoint signalling, thereby explaining the mitotic arrest seen in the absence of cohesin.

Cohesion in interphase nuclei facilitates homologous recombinational DNA repair

Rad21 from fission yeast was originally identified from a radiation-sensitive mutant [46] and genetic studies showed that Scc1–Rad21 is involved in DNA double-strand break (DSB) repair [47,48]. Previous work in the DT40 system showed that homologous recombination plays an important role in repairing DSBs arising during DNA replication and those induced by irradiation in the late S–G2 phases [49]. The level of spontaneously occurring chromosomal breaks was only slightly increased in Scc1−/− cells relative to Scc1+ cells, which appears to be consistent with there being no detectable problems in S phase [30]. Both the level of ionizing radiation-induced chromosomal aberrations and the sister chromatid exchange frequency, which we have previously shown to be mediated by homologous recombination, were significantly increased in Scc1−/− cells when compared with Scc1+ cells. Thus, the loss of cohesion between sisters undermines the efficiency of DSB repair in interphase nuclei. The simplest model for this is that the increased distance between sister chromatids in the absence of cohesion reduces the efficiency of the search for appropriate sequences for repair by homologous recombination. Similarly, Scc1–Rad21 is involved in DSB repair in yeast [48,50], suggesting that this is an evolutionarily conserved function of sister chromatid cohesion.

Complex role for cohesin in controlling chromosome bi-orientation

The question of how cohesin is required for chromosomes to make a proper bipolar attachment to the mitotic spindle remains open. Significantly, budding-yeast cohesin also facilitates the bipolar attachment of centromeres to the spindle microtubules [42]. Classic micromanipulation experiments from Nicklas and Staehly [51], which revealed that tension is an important factor in bi-orientation, provide one explanation. Our results, that Scc1 is required for the proper targeting of the chromosome passenger complex to the inner centromere, may provide an additional element to explain the misalignment of metaphase chromosomes in Scc1-deficient DT40 cells. Previous studies of INCENP depletion by RNA interference (RNAi) in Drosophila cells found that the protein is absolutely required for chromosomes to achieve a stable bipolar attachment to the spindle (i.e. metaphase alignment) [19]. The role of INCENP in promoting bipolar attachment remains unknown, but may involve the action of Aurora B kinase, a binding partner of INCENP [29], which we have shown to be also required for INCENP targeting to centromeres in Drosophila cells [19]. The present results thus raise the very intriguing possibility that the chromosomal passengers function downstream of Scc1 to regulate kinetochore activity, thereby suggesting that cohesins may function not only as structural links between sister
chromatids, generating tension, but also have other roles in mitotic regulation.

Interestingly, even in the absence of functional Scc1, sister chromatids do not separate completely in most cells. The mechanism(s) underlying this sister chromatid association remains unknown. This could be due to an alternative system for linking of sister chromatids, or to the persistence of low levels of residual Scc1 that are not detected by the methods used. A possible alternative system is that provided by the DNA intercatenations that are generated during replication. This notion is supported by recent evidence showing topoisomerase II activity to be a substrate for Aurora B kinase in vitro [52], along with results showing that topoisomerase II activity is implicated in ensuring that the principal tension-stabilizing DNA intercatenations that are generated during replication.

Conclusion
The interactions described recently between the cohesin complex and the chromosomal passenger complex in yeast and in vertebrate cells are providing a new level of complexity in the control of mitosis. In addition to ensuring the proximity of a template for repair of DNA damage by homologous recombination during interphase, the cohesin complex is also required for the accurate alignment of mitotic chromosomes on the metaphase plate. This implicates cohesin in genome stability at both the DNA level and at the chromosome level.

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