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Damage-induced reactivation of cohesin in postreplicative DNA repair

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Summary

Cohesin establishes sister-chromatid cohesion during S phase to ensure proper chromosome segregation in mitosis. It also facilitates postreplicative homologous recombination repair of DNA double-strand breaks by promoting local pairing of damaged and intact sister chromatids. In G₂ phase, cohesin that is not bound to chromatin is inactivated, but its reactivation can occur in response to DNA damage. Recent papers by Koshland's and Sjögren's groups describe the critical role of the known cohesin cofactor Eco1 (Ctf7) and ATR checkpoint kinase in damage-induced reactivation of cohesin, revealing an intricate mechanism that regulates sister-chromatid pairing to maintain genome integrity.^(1,2)

Introduction

The cellular response to, and subsequent repair of, DNA damage is a critical cellular process that maintains genome integrity. Disruption of DNA double-strand break (DSB) repair frequently results in genomic instability (i.e. chromosomal translocations or aneuploidy). One way that the cell responds to DSBs is through the homologous recombination (HR) repair pathway. HR repair occurs in S/G₂ phase following DNA replication since an intact sister chromatid acts as a template for the repair.⁽³⁾ Studies by the Koshland and Sjögren groups offer new insight into the pivotal role of the cohesin complex and its associated factors in this process.^(1,2)

Essential function of cohesin in genome-wide sister-chromatid cohesion

Cohesin is a conserved essential multiprotein complex required for sister chromatid cohesion.^(4–6) Sister-chromatid cohesion, the pairing of two sister chromatids following DNA replication, is prerequisite for the proper alignment and segregation of chromosomes during cell division. Cohesin consists of the structural maintenance of chromosomes (SMC) proteins SMC1 and SMC3 as a heterodimer with the two non-SMC components Rad21 (also called Mcd1 or Scc1) and Scc3 (SA).⁽⁷⁾ SMC proteins contain ATPase motifs within their conserved head and tail globular domains. ATP binding and hydrolysis by SMC proteins were shown to be essential for complex formation and chromatin association of cohesin, respectively.^(8,9)

In *S. cerevisiae*, cohesin is loaded onto chromosomes during G₁ phase, which requires the heterodimeric cohesin loading factor Scc2–Scc4.⁽¹⁰⁾ Chromatin immunoprecipitation (ChIP) coupled with genomic microarray (ChIP on chip) analyses revealed that cohesin accumulates at the centromere regions and binds to chromosome arms at intervals of 9–13 kb.^(11–15) Cohesin-binding sites exhibit no obvious sequence specificity and were found to cluster in regions of transcriptional convergence.^(14,15) Scc2-binding sites are often different from cohesion-binding sites suggesting that cohesin may slide from its initial Scc2–Scc4-mediated loading location. In metazoans, genome-wide cohesin loading occurs at the end of mitosis during telophase, which also requires their Scc2 and Scc4 homologs.^(16–18) In addition, pre-replication factors, including ORC, Cdc6, Cdt1 and MCM2-7, were shown to be required for loading of Scc2–Scc4 and subsequent cohesin binding to chromatin in *Xenopus*.^(16,17)

The establishment of sister-chromatid cohesion is distinct from the initial loading of cohesin and takes place during S phase concomitant with DNA replication. Studies in *S. cerevisiae* provided much insight into the factors involved in this process. Eco1 (also called Ctf7) was identified to be essential for the establishment of sister chromatid cohesion during S phase.^(19,20) Eco1 (Ctf7) is an acetyltransferase, and was shown to acetylate the non-SMC subunits of cohesin in vitro.⁽²¹⁾ However, the acetyltransferase activity appears to be dispensable for the establishment of genome-wide sister chromatid cohesion during S phase.⁽²²⁾ Thus, it is unclear how Eco1 (Ctf7) participates in this process. Eco1 (Ctf7) and its human homolog ESCO2 were shown to interact with PCNA in yeast and in human cells, suggesting a connection to the DNA replication machinery.⁽²³⁾ In fact, several DNA replication-related factors were genetically identified to be involved in sister chromatid cohesion in yeast. These include the Ctf18–RFC complex, the DNA polymerase α -associating Ctf4, Trf4 (DNA polymerase κ), and PCNA, further suggesting the coupling of DNA replication and cohesion.^(23–27) However, the precise molecular mechanism of the cohesion process is not understood. Importantly, cohesin newly expressed after the completion of DNA replication fails to establish sister chromatid cohesion despite its loading onto chromatin.^(25,28) Consistent with the apparent coupling of DNA replication and establishment of sister chromatid cohesion, the results indicate that, once DNA replication is finished, it is too late to establish sister chromatid cohesion. Intriguingly, it was found that DNA damage induction can alleviate this restriction (see below).

Cohesin's role in postreplicative repair

Studies in *S. cerevisiae* and chicken cells indicated the role of cohesin in postreplicative DNA repair.^(29,30) Immunostaining analysis of laser-induced damage in human cells and ChIP analysis of the HO-endonuclease-induced DSB site in yeast revealed that cohesin specifically accumulates at the damage sites, suggesting a specific function of cohesin in DNA repair.^(28,31,32)

Cohesin accumulation at the damage site requires Scc2–Scc4, indicating that the loading mechanism is similar to that for the genome-wide cohesin binding following mitosis (Fig. 1a).^(28,32) In addition, in both human cells and yeast, cohesin recruitment to the damage site is dependent on the Mre11–Rad50 complex, one of the first factors to recognize and

accumulate at broken DNA ends.^(28,31,32) Cohesin physically interacts with the Mre11 complex in an interphase-specific manner in human cells, which may be important for the initial recruitment of cohesin to the DSB sites.⁽³¹⁾ Cohesin clustering in the area surrounding the damage site coincides with and requires H2A phosphorylation (γ H2A) in yeast, suggesting that checkpoint signaling also plays an important role.⁽³²⁾ Whether cohesin directly or indirectly binds to phosphorylated H2A remains to be investigated.

Although genome-wide cohesin loading onto chromatin occurs in telophase, cohesin recruitment to the damage site is S/G₂-specific in human cells.⁽³¹⁾ Because cohesin loading at the damage sites appears to correlate with the presence of sister chromatids, it was speculated that cohesin establishes local sister chromatid cohesion at the damage sites to facilitate HR repair by promoting homologous pairing of damaged and intact sister chromatids.⁽³¹⁾ By inactivating the temperature-sensitive (ts) mutant cohesin involved in genome-wide sister chromatid cohesion and complementing it with inducible temperature-resistant cohesin to spotlight the behavior of the newly recruited cohesin in G₂ phase, it was elegantly demonstrated in *S. cerevisiae* that cohesin at the damage site in G₂ phase is indeed capable of promoting sister chromatid cohesion.⁽²⁸⁾ Studies in yeast using HO endonuclease and in human cells using the I-SceI endonuclease to induce DSBs demonstrated that cohesin is specifically involved in HR between the two sister chromatids, but not in intra-chromosomal gene conversion.^(32,33)

Genome-wide reactivation of cohesin in response to DNA damage

In the absence of any damage, newly expressed cohesin in G₂ phase goes to its normal binding sites but is unable to establish sister chromatid cohesion in yeast.^(25,28) In contrast, cohesin at damage sites can establish sister chromatid cohesion, suggesting that cohesin function is somehow reactivated in response to DNA damage.⁽²⁸⁾ How this occurs was unknown. The new papers by Koshland's and Sjögren's laboratories provide exciting answers to this question.^(1,2) One of the key findings detailed in both studies is that reactivation of cohesin is not limited to the damage site, but occurs genome-wide if there is newly synthesized and unincorporated cohesin available. Both groups demonstrated that a limited number of DSBs on one chromosome is sufficient to induce de novo cohesion on other chromosomes, suggesting that a transacting signal spreads from the initial DSB to the entire nucleus.

Factors required for reactivation of cohesin in response to DSBs

By looking at the establishment of sister chromatid cohesion using the ts mutant complementation system described above⁽²⁸⁾ on a chromosome different from the one that contained the HO endonuclease-induced DSB(s), both groups were able to define the factors required for cohesin reactivation. Separation or cohesion of sister chromatids was visualized by the artificial tethering of GFP to specific chromosome subregions using either the Tet- or Lac-repressor/operator system or fluorescent in situ hybridization.^(1,2) Specifically, they found that damage-induced genome-wide reactivation of cohesin requires Eco1 (Ctf7), the Smc5–Smc6 complex, Mre11 and ATR (Mec1 in *S. cerevisiae*) (Fig. 1b), and, to a lesser extent, ATM (Tel1 in *S. cerevisiae*) and γ H2A.^(1,2)

Eco1 (Ctf7)

Eco1 (Ctf7) is the factor required for generation of genome-wide sister chromatid cohesion during S phase (see above). Although Eco1 (Ctf7) was shown to accumulate at the DSB site,⁽³⁴⁾ it is not required for cohesin recruitment to the damage site.^(1,34) Therefore, similar to the genome-wide cohesin loading and establishment of sister chromatid cohesion, initial cohesin binding at the DNA damage site and subsequent establishment of sister chromatid cohesion may be two separate processes. Interestingly, although the acetyltransferase activity of Eco1 (Ctf7) is not necessary for the sister chromatid cohesion established in S phase, damage-induced postreplicative cohesion of sister chromatids requires this activity.⁽²⁾ Currently, the reason for this differential requirement for the catalytic activity of Eco1 (Ctf7) in S and G₂ phases is unknown. Importantly, overexpression of the catalytically active Eco1 (Ctf7) in G₂ phase was sufficient to reactivate cohesin even in the absence of DNA damage, indicating that Eco1 (Ctf7) is the limiting factor for reactivation of cohesin in G₂ phase.⁽²⁾ How the sister chromatid cohesion activity of cohesin is normally suppressed in G₂ phase, and how cohesin can be reactivated by Eco1 (Ctf7) in response to DNA damage, will require additional investigation.

The Smc5–Smc6 complex

The Smc5–Smc6 complex is another SMC-containing complex that plays an important role in HR repair.^(33,35,36) Recently, this complex was shown to be required for cohesin accumulation at I-SceI endonuclease-induced DSB sites (detected by ChIP) in human cells.⁽³³⁾ However, a Chip-on-chip analysis found no apparent effect of a Smc6 mutation on Mcd1 (Scc1/Rad21) localization to damage sites in *S. cerevisiae*.⁽¹⁾ Currently, the reason for these differences is unknown. Interestingly, MMS21 (also called Nse2), a non-SMC subunit of the Smc5–Smc6 complex, functions as an E3 SUMO ligase, and this activity is required for efficient DNA repair.^(37–39) Its substrates include the NHEJ factor Ku70 as well as Rad21 and SA2, two non-SMC subunits of cohesin in human cells.^(33,39) It remains to be tested whether SUMOylation of cohesin subunits by MMS21 (Nse2) is necessary for cohesin targeting to the damage site and/or establishment of sister chromatid cohesion.

Mre11 and ATR (Mec1)

Mre11 is also required for cohesin recruitment to the damage site, and it is currently unclear how it is involved in reactivation of cohesin. It may be important for the initial damage recognition and subsequent signaling to ATR. ATR, one of the PI3 kinase-related protein kinases, plays a critical role in DNA damage checkpoint responses.⁽⁴⁰⁾ ATM and ATR are known to share several downstream target proteins, albeit with some distinct preferences. ATM is the primary kinase activated by DSBs, and ATR was shown to be downstream of ATM in DSB-induced checkpoint signaling in S/G₂ phases.⁽⁴¹⁾ It was also shown that SMC1 is an important target of ATM for the DSB-induced S phase checkpoint.⁽⁴²⁾ Thus, it is curious that DSB damage-induced sister chromatid cohesion requires ATR more than ATM. It is unclear at this point whether Eco1 (Ctf7) is a direct target of ATR and whether the Smc5–Smc6 complex is involved in this process. Further studies should provide interesting insight into ATR's specific role in genome-wide DSB-responsive sister chromatid cohesion.

Conclusion and future perspectives

Recent studies revealed the significant roles of specific histone modifications and chromatin remodeling complexes in DSB response and repair, indicating the importance of chromatin structural organization at the damage site.⁽⁴³⁾ The work described here reveals a new regulatory mechanism involving the cohesion cofactor Eco1 (Ctf7) and the chromosome organization complex cohesin as a guardian of genome integrity distinct from its classical function in chromosome segregation.

Interestingly, ESCO2, a human homolog of Eco1 (Ctf7), was found to be the gene responsible for the developmental disorder Roberts syndrome (RBS).⁽⁴⁴⁾ RBS chromosomes exhibit premature centromere separation and heterochromatin puffing, indicative of a sister chromatid cohesion and chromosome organization defect. While there is only one Eco1 (Ctf7) in yeast, there are two other homologs in human cells in addition to ESCO2, called ESCO1 and SAN. All three homologs were shown to function in sister chromatid cohesion.^(45,46) Whether one or more of them has a role in damage-induced sister chromatid cohesion remains to be determined. Furthermore, unlike in yeast, the majority of cohesin in higher eukaryotes dissociates from chromosomes by the action of Aurora B and Polo-like kinases at the G₂–M transition.^(47,48) It will be interesting to see whether reactivation of cohesin in G₂ phase in response to damage may be important for preventing premature dissociation of cohesin as part of the G₂–M checkpoint mechanism.

The studies by Koshland's and Sjögren's groups contribute significantly towards understanding the mechanism and regulation of cohesin in DNA repair. Comparative analyses of replication-coupled and damage-induced sister chromatid cohesion processes should provide further insight into the basic molecular mechanism of sister chromatid cohesion and its regulation in the maintenance of genome integrity.

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Abbreviations

DSB	DNA double-strand break
HR	homologous recombination
SMC	structural maintenance of chromosomes
ChIP	chromatin immunoprecipitation
ChIP on chip	ChIP coupled with genomic microarray
γH2A	phosphorylated H2A

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Figure 1. Schematic models for the assembly of cohesin and associated factors at double-strand breaks (DSBs), and ATR- and Eco1 (Ctf7)-dependent reactivation of cohesin in G₂ phase. **a:** In response to DNA DSB damage, cohesin is recruited to the damage site in a S/G₂ phase-specific manner. This is dependent on the Mre11 complex (Mre11–Rad50–Nbs1), the cohesion-loading factor (Scc2–Scc4), and γ H2A. In the case of human cells, the Smc5–Smc6 complex is also required for cohesin recruitment to DSBs. For establishment of sister chromatid cohesion, cohesin requires additional factors, including Eco1 (Ctf7), the Smc5–Smc6 complex, and ATR (see **b**). **b:** In G₂ phase, DSBs induce ATR activation, which leads to reactivation of cohesin by Eco1 (Ctf7). Mre11 and the Smc5–Smc6 complex may be involved in the same pathway. This process requires the acetyltransferase activity of Eco1 (Ctf7). The targets of ATR-mediated phosphorylation and Eco1 (Ctf7)-mediated acetylation are unknown.