Postreplicative Formation of Cohesion Is Required for Repair and Induced by a Single DNA Break

Lena Ström,1 Charlotte Karlsson,1 Hanna Bettis Lindroos,1 Sara Wedahl,1 Yuki Katou,2 Katsuhiro Shirahige,2 Camilla Sjögren*1

Sister-chromatid cohesion, established during replication by the protein complex cohesin, is essential for both chromosome segregation and double-strand break (DSB) repair. Normally, cohesion formation is strictly limited to the S phase of the cell cycle, but DSBs can trigger cohesion also after DNA replication has been completed. The function of this damage-induced cohesion remains unknown. In this investigation, we show that damage-induced cohesion is essential for repair in postreplicative cells in yeast. Furthermore, it is established genome-wide after induction of a single DSB, and it is controlled by the DNA damage response and cohesin-regulating factors. We thus define a cohesion establishment pathway that is independent of DNA duplication and acts together with cohesion formed during replication in sister chromatid–based DSB repair.

The tethering of sister chromatids by the cohesin complex, so-called sister-chromatid cohesion, is essential for chromosome segregation (1). Cohesin consists of Smc1, Smc3, Mcd1 (also called Sccl), and Scc3 and is loaded onto chromosomes before replication by Scc2 and Scc4 (2). The establishment of cohesion requires Ecol1 (also called Ctf7) and occurs in the S phase of the unchallenged cell cycle (3–8). Cohesion then persists until anaphase, when it is resolved by proteolytic cleavage of Mcd1, which is triggered by the degradation of Pds1 (9–11). In addition to its central role in chromosome segregation, replication-established cohesion is needed for double-strand break (DSB) repair in postreplicative cells (12). Cohesin also has to be recruited to the site of damage for efficient repair (13, 14), and de novo cohesion is established in G2/M cells exposed to ionizing irradiation (13). This raises the possibility that chromatin-based DSB repair requires both cohesion formed during replication and postreplicative damage-induced cohesion. It also challenges present concepts that cohesion establishment is tightly connected to chromosome duplication. Therefore, we investigated how damage-induced cohesion is regulated and resolved its function in DSB repair and chromosome segregation.

Central to our investigations are experimental systems in which damage-induced cohesion can be distinguished from cohesion formed during replication (fig. S1, A to C). In one of these systems, smc1-C259 temperature-sensitive cells are first arrested in G2/M at permissive temperature. Thereafter, temperature-resistant, damage-induced cohesion is generated by the expression of wild-type (WT) SMC1 and treatment with γ-irradiation (Fig. 1, A and B, and fig. S1A) (13). We first ascertained that our results were not influenced by the absence of a mitotic spindle in nocodazole-arrested cells (Fig. 1, A and B). Thereafter, we investigated the function of central DNA damage-response proteins in damage-induced cohesion. Mre11 is one of the first proteins that localizes at a DSB (15) and is essential for the recruitment of cohesin to the damage site (16, 17). Accordingly, damage-induced cohesion was compromised in mre11Δ cells (Fig. 1C) (17). Other regulators of the DNA damage response that influence cohesin’s break localization are the Tel1 and Mec1 kinases, which phosphorylate histone 2A (H2A) (in humans, H2AX) (14, 18). Phosphorylated H2A (γ-H2A) marks the DSB and is required for DSB recruitment of cohesin (14). Correspondingly, the formation of damage-induced cohesion was defective in cells lacking Tel1 or Mec1 or in cells expressing nonphosphorylatable H2A (Fig. 1, D to F). However, in the absence of Tel1 or Mec1, substantial amounts of γ-H2A and cohesin still accumulate at a DSB because of the overlapping function of the other kinase (14, 18). This indicates that Tel1 and/or Mec1 could influence cohesion in a way other than through γ-H2A–dependent recruitment of cohesin. The two kinases also activate Rad9, which, in turn, transmits the signal to downstream events in the DNA damage response (19–21). Damage-induced cohesion was, however, unaffected in rad9Δ cells (Fig. 1G), showing that if Mec1 and/or Tel1 affect damage-induced cohesion independently of cohesin recruitment, this pathway does not include the activation of Rad9.

To determine whether replication induced by the repair process influences cohesion, we investigated damage-induced cohesion in rad52Δ cells. Rad52 facilitates the direct interaction between the DNA flanking a DSB and an undamaged homologous template, which is essential for eliciting DNA synthesis at the break (22, 23). Therefore, the unperturbed formation of cohesion in rad52Δ cells (Fig. 1F) shows that, in contrast to the establishment process in unchallenged cells, damage-induced cohesion is independent of ongoing DNA duplication (17).

We observed chromatin separation in a limited region of chromosome V (chr. V) that experiences roughly one DSB per cell at the irradiation dose applied (13). Because cohesin is recruited to only 50 to 100 kb around the DSB (13, 14), the γ-ray–induced cohesion suggests a more general activation of cohesin (Fig. 1, A and B). To investigate whether a single genomic DSB triggers cohesion, we used an unclavable

1Department of Cell and Molecular Biology, Karolinska Institute, 171 77 Stockholm, Sweden. 2Gene Research Centre, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, 226-8501 Yokohama, Japan.
*To whom correspondence should be addressed. E-mail: camilla.sjogren@ki.se

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variant of Mcd1 (Mcd1<sup>UNCL</sup>) that blocks chromatid separation at anaphase when it is present in cohesion-forming complexes (10, 24) (fig. S1C). When Mcd1<sup>UNCL</sup> was expressed alone in G2/M-arrested cells, chr. V separated normally during a release from the arrest. In contrast, chr. V separation did not occur when Mcd1<sup>UNCL</sup> expression was combined with the induction of a homothallic switching (HO)–endonuclease that creates a DSB at the <i>MAT</i> locus on chr. III (Fig. 2, A to C). This inhibition was not due to a cell-cycle delay caused by the break, because Pds1 levels declined concomitantly in both cell populations (Fig. 2B), and HO expression alone left chromatid separation unperturbed (Fig. 2, D and E). Moreover, HO overexpression did not generate DSBs at unspecified sites in the genome, because the chromatids separated normally after the induction of HO and Mcd1<sup>UNCL</sup> in <i>MATα</i> cells (Fig. 2, D and E). As a control of Mcd1<sup>UNCL</sup> function, its chromosomal localization after expression in G2/M was determined and shown to be identical to normally expressed Mcd1 (fig. S2) (8). This finding establishes that a break on chr. III triggers the formation of cohesion on chr. V, demonstrating that a single DSB reactivates cohesion in a genome-wide manner.

We next investigated how genome-wide cohesion is regulated using G2/M-arrested temperature-sensitive <i>smc1-259</i> cells in which WT <i>SMC1</i> and a DSB on chr. III was induced before an upshift in temperature (fig. S1B). This experiment showed that “global” cohesion depends on Mecl and partly on Tel1 and γ-H2A, but not on Rad9 (Fig. 3, A and B, and fig. S3A). In cells lacking γ-H2A, no cohesion is recruited to damaged chromosomes, but cohesion is only partly defective when compared with <i>meclΔ</i> cells (14). This observation indicates that Mecl is required for more than cohesion localization and could be a key factor in the transmission of a signal from damaged to undamaged chromosomes. If so, this transmission is achieved in a Rad9-independent manner. The partial requirement...

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**Fig. 1.** Mre11, Mec1, Tel1, and γH2A, but not Rad9 and Rad52, are required for γ-ray–induced cohesion. (A to H) Chromatid separation at <i>URA3</i> on chr. V in G2/M-arrested cells after γ-irradiation and destruction of S phase–established cohesion, as described in fig. S1A. (A) Chromatid separation in Cdc20-depleted <i>G2/M-arrested smc1-259, GAL:SMC1-13MYC, MET:CDC20</i> cells (CB496). (B) Chromatid separation in nocodazole <i>G2/M-arrested</i> (B) <i>smc1-259, GAL:SMC1-13MYC</i> cells (CB469), combined with (C) <i>mre11Δ</i> (CB478), (D) <i>mec1Δ</i> (CB784), (E) <i>tel1Δ</i> (CB693), (F) <i>hta1-5129stop, hta2-129stop</i> (CB742), (G) <i>rad9Δ</i> (CB696), or (H) <i>rad52Δ</i> (CB571). Gal, galactose addition; IR, irradiation.

**Fig. 2.** A single DSB on chr. III leads to establishment of cohesion on chr. V. <i>MCD1<sup>UNCL</sup></i> was induced in G/M-arrested cells, with or without a concomitant DSB on chr. III. Cells were thereafter released into the next cell cycle under noninducing conditions (fig. S1C). Chromatid separation at <i>URA3</i> on chr. V, the percentage of Pds1-positive cells, and chr. III breakage were determined. (A) Sister-chromatid separation in <i>GAL:MCD1<sup>UNCL</sup>, PDS1-18MYC</i> cells without (CB699) or with <i>GAL:HO</i> (CB507). (B) Pds1-positive cells in (A). (C) Southern blot of chr. III isolated from cells examined in (A). (D) Sister-chromatid separation in <i>GAL:MCD1</i> (CB699), <i>GAL:MCD1 GAL:HO</i> (CB507), <i>GAL:HO</i> (CB524), or <i>MCD1<sup>UNCL</sup> GAL:HO</i> <i>mattΔ</i> (CB586). (E) Analysis of chr. III in cells examined in (D).
**Fig. 3.** Genome-wide cohesion depends both on the DNA damage response and on proteins regulating cohesion function. (A and B) Chromatid separation in G2/M-arrested cells after removal of S phase-established cohesion, in the absence or presence of a DSB on chr. III, as described in fig. S1A. (A) Chromatid separation in smc1-259, GAL1::HO (CB588), and smc1-259 GAL1::SMC1-13MYC GAL1::HO (CB479). (B) Chromatid separation in smc1-259, GAL1::SMC1-13MYC GAL1::HO cells (CB479), combined with tel1Δ (CB815), mec1Δ (CB753), hta1-S129stop, hta2-S129stop (CB740), or rad9Δ (CB813). DSB formation on chr. III is shown in fig. S3B (C to E). Chromatid separation of chr. V in cells containing GAL1::MD1::LUM and GAL1::HO. The experiments were performed as in fig. 2 and fig. S1C, except that the temperature was up-shifted 30 min after the addition of galactose. (C) Wild type (CB507) and scc2-4 (CB573), (D) wild type and smc6-56 (CB537), and (E) wild type and eco1-1 (CB732). (F) Chip-on-in-silico analysis of Scc2 localization on chr. III in the absence (–DSB) or presence (+DSB) of a DSB at the MAT locus. Arrow indicates a DSB. CENIII, chr. III centromere.

**Fig. 4.** Postreplicative function of Eco1 is required for DSB repair but dispensable for chromosome segregation. (A and B) DNA repair and sister-chromatid separation at URA3 in G2/M-arrested WT (CB167) and eco1-1 (CB720) cells. After arrest in G2/M at 21°C, half of the cultures were transferred to 32°C for 30 min, and then all cells were treated with 200 grays of γ-irradiation (IR) (1 gray = 100 rads). At indicated time points and temperatures, samples were withdrawn for analyses of DNA repair by pulsed-field gel electrophoresis (PFGE) and sister-chromatid separation (29). Chromatid separation. (C) Chromosome segregation in the absence (–) and presence (+) of genome-wide damage-induced cohesion. G2/M-arrested WT (CB524) or temperature-sensitive eco1-1 (CB755) cells were treated such that damage-induced cohesion was inhibited in eco1-1 cells (29). A DSB on chr. III was induced in half of the cultures. After release into a G1 arrest, the percentage of cells with a single chr. V, reflecting correct chromosome segregation, was determined.
for Tel1 and γ-H2A in global cohesion could be due to a function in amplifying the signal emanating from the DSB (25). Such a function of γ-H2A is supported by our finding that it covers the entire chr. III after the prolonged break induction that we used in our experiments, but is absent on undamaged chromosomes (fig. S3B).

We also explored the role of cohesion-regulating proteins in genome-wide cohesion by scoring chr. V separation after the induction of Mccl1Δ and a DSB on chr. III in temperature-sensitive scc2-4, smc6-56, or eco1-1 cells. These experiments showed that Scc2, Smc6, and Eco1 are required for global cohesion (Fig. 3, C to E, and fig. S3C).

The prerequisite of Scc2 reveals that genome-wide formation of cohesion requires the loading of cohesion to chromosomes. Loading in G2/M occurs also in the absence of DNA damage, and the cohesins present on chromosomes in this cell-cycle phase are thus a mixture of cohesive and noncohesive complexes (8). Our findings indicate that a DSB triggers an alteration of cohesion or its effectors that activate the cohesive function of the normally unproductive complexes. We investigated whether this is reflected by a change in the localization of Scc2 on undamaged chromosomes, but found that HO expression only induced its accumulation at the DSB (Fig. 3F). This is true also for cohesion (13), showing that genome-wide cohesion is generated without positional changes of cohesin or its loader in undamaged regions of the genome.

The Smc6 protein is part of the cohesin-related Smc5/6 complex, which also is required for sister-chromatid repair, and regulates the localization of cohesin to DNA breaks in human cells (26, 27). In yeast, however, the chromosomal association of Mccl1 was unaltered after the destruction of smc6-56 function in G2/M-arrested cells (fig. S3D). This suggests that the requirement of Smc6 for genome-wide cohesion reflects a more direct influence on cohesin function, which is in accordance with the similar chromosomal localization patterns of cohesin and the Smc5/6 complex (28).

The finding that Eco1 is required for genome-wide cohesion shows that it can act independently of chromosome replication. It also indicates that the damage response removes an inhibitory mechanism and/or reactivates Eco1, thereby allowing cohesion formation in postreplicative cells. Because the eco1-1 mutation leaves the chromosomal association of cohesin unaffected (fig. S4) (4), we examined whether the establishment of damage-induced cohesion and not only chromosomal loading of cohesin is needed for repair (12, 29). The results showed that Eco1, and consequently damage-induced cohesion, is required for postreplicative DSB repair (Fig. 4, A and B). In contrast, the absence of damage-induced cohesion did not interfere with segregation in the presence of functional replication-established cohesion (Fig. 4C). Thus, a possible explanation for genome-wide cohesion is that postreplicative repair requires cohesion at a DSB, and this is achieved by a global activation of the cohesion machinery, leading to de novo cohesion on all chromosomes.

Our investigation characterizes an additional pathway for cohesion establishment that is crucial for DSB repair in postreplicative cells. This pathway generates cohesion on undamaged chromosomes in response to a single DSB, suggesting that the break triggers a diffusible signaling event that activates cohesin and/or Ecol1 via Mccl. Consequently, the establishment of cohesion is not limited to active replication forks and has to occur both before and after DSB formation to repair broken sister chromatids.

References and Notes
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