A Molecular Determinant for the Establishment of Sister Chromatid Cohesion
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able in the eco1Δ wpl1Δ strain. The Eco1 dependence is consistent with Eco1 directly acetylating Smc3. Furthermore, Wpl1 does not appear to counteract Eco1-dependent Smc3 acetylation. Rather, a destabilizing effect of Wpl1 on sister chromatid cohesion might be countered by Smc3 acetylation. Smc3 acetylation at the time of S phase was substantially reduced, but not abolished, when DNA replication was prevented by depletion of the replication initiation factor Cdc6 (fig. S4). This suggests that Smc3 acetylation is facilitated when Eco1 moves along chromosomes as part of the replisome (10, 12) but that additional cell-cycle regulation of Eco1, or of a deacetylase that counters Eco1, contributes to S-phase specificity of Smc3 acetylation.

If Eco1 promotes establishment of sister chromatid cohesion by acetylating Smc3, then preventing acetylation by a lysine-to-arginine (K113R) substitution should interfere with cohesion establishment. Cells containing the smc3K113R mutation were viable but displayed pronounced defects in sister chromatid cohesion (Fig. 4C). Cohesion was also compromised in smc3K113N cells, albeit to a lesser extent, which suggests that although asparagine 113 compensates for the requirement of Eco1, it does not support sister chromatid cohesion to the same degree as acetylated lysine. Eco1 is essential, and if its sole function in S phase is Smc3 acetylation, we would expect nonacetylatable Smc3 to cause lethality. Consistent with this expectation, cells harboring smc3K112,113R, with both acetylated lysines replaced by arginine, were no longer viable (fig. S5). Viability was restored by deletion of WPL1, indicating that Smc3K112,113R is in principle proficient in sister chromatid cohesion.

In an accompanying study, Ünal et al. similarly describe Eco1-dependent Smc3 acetylation during S phase (23). These authors confirm that Smc3K112,113R supports cohesion association with budding yeast chromosomes in a manner apparently indistinguishable from wild-type Smc3, yet fails to promote sister chromatid cohesion. In contrast with our results, a single Smc3K113R mutation interfered with DNA binding and did not support cell viability. The reason for this difference is not known but could be due to different Wpl1 levels in the two strain backgrounds used for our studies. Together, our results using the Smc3K112,113R mutants suggest that Smc3 acetylation is not required for DNA binding but that Eco1-dependent acetylation of at least one of the two neighboring lysines K112 and K113 is essential to stabilize chromosome-bound cohesin at the time of cohesion establishment. Acetylation of both lysines might act in part redundantly, because an acetylation mimicking K112N mutation, like K113N, allowed growth of eco1Δ cells at restrictive temperature, albeit not of eco1Δ cells (fig. S6). These results are consistent with the idea that Eco1 acts as an acetyltransferase during the establishment of sister chromatid cohesion.

The ring-shaped cohesin complex is thought to bind DNA by topological embrace (1, 24). K112 and K113 emerge from the Smc3 ATPase head, where ATP hydrolysis is instrumental for cohesin’s ring-opening reaction during loading onto DNA (25, 26). Acetylation of these lysines could modulate cohesin’s interaction with Wpl1, to prevent Wpl1 from destabilizing the cohesin ring. Alternatively, acetylation could reinforce interactions within cohesin to render it Wpl1-resistant. That the latter may be the case is suggested by our observation that Eco1 strengthens sister chromatid cohesion even in the absence of Wpl1. The position of K112 and K113 could also allow regulation of an interaction with the Smc hinge, on the opposite side of the ring, that has been implicated in ring opening (27, 28). In the absence of both Eco1 and Wpl1, the fundamental mechanism for pairing sister chromatids during DNA replication remains intact. Reactions that are innate to the DNA replication process—for example, passage of the replication fork through the cohesin ring—may provide the underlying basis for sister chromatid cohesion. In wild-type cells, Eco1 modifies cohesin during DNA replication, a prerequisite for stable sister chromatid cohesion, but this can be uncoupled from S phase by WPL1 deletion or the smc3K113N mutation. We cannot exclude that the primary benefit of Wpl1-dependent cohesion regulation pertains to cohesion function outside of sister chromatid cohesion (29).

References and Notes
13. Materials and methods are available as supporting material on Science Online.
30. We thank L. Kruglyak for providing the SNPscanner software; M. Ocampo-Hafalla for the cohesin chromatin immunoprecipitation data in fig. S1; Ünal, D. Koshland, K. Shihahige, and M. Singleton for discussions and sharing unpublished results; and members of our laboratory for comments on the manuscript. This work was supported by a European Molecular Biology Organization long-term fellowship to S.H. and by the Human Frontier Science Program. The microarray data reported in this manuscript can be obtained from Gene Expression Omnibus with accession number GSE11527.

Supporting Online Material
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Materials and Methods
Figs. S1 to S6
References
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A Molecular Determinant for the Establishment of Sister Chromatid Cohesion
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Chromosome segregation, transcriptional regulation, and repair of DNA double-strand breaks require the cohesin protein complex. Cohesin holds the replicated chromosomes (sister chromatids) together to mediate sister chromatid cohesion. The mechanism of how cohesion is established is unknown. We found that in budding yeast, the head domain of the Snc3p subunit of cohesin is acetylated by the Eco1p acetyltransferase at two evolutionarily conserved residues, promoting the chromatin-bound cohesin to tether sister chromatids. Snc3p acetylation is induced in S phase after the chromatin loading of cohesin and is suppressed in G2 and G2/M. Snc3 head acetylation and its cell cycle regulation provide important insights into the biology and mechanism of cohesion establishment.

Sister chromatid cohesion is required for faithful chromosome segregation and for efficient DNA double-strand break (DSB) repair and is mediated by the cohesin protein complex (Fig. 1A) (1–4). Chromatin loading per se is not sufficient for cohesion to tether sister chromatids (3, 5, 6). Eco1p (also known as Ctf7p) must act on the chromatin-bound cohesin to promote the establishment of sister chromatid cohesion both during S phase and in response to DSBs in G2/M phase (7–10).
Eco1p possesses acetyltransferase activity (II–13). A mutant form of Eco1p, eco1 (R222G, K223G) (Fig. S1), purified from bacteria has almost no detectable catalytic activity in vitro (I/). In yeast, eco1 (R222G, K223G) cells are defective only in DSB-induced cohesion in G2/M (9, 14). Thus, Eco1p acetylation of cohesin seemed required for DNA damage–induced cohesion but not S-phase cohesion (9). However, the S-phase conclusion was challenged by two observations. First, we found that eco1p (R222G, K223G) purified from yeast extracts has auto-acetyltransferase activity in vitro (Fig. S2A). Second, eco1 mutants lacking the acetyltransferase domain are inviable, a phenotype of cells defective in S-phase cohesion (Fig. S2B). Together, these results suggest that the Eco1p acetyltransferase activity is required for establishing sister chromatid cohesion during S phase, and that the eco1 (R222G, K223G) protein must have sufficient acetyltransferase in vivo to carry out this function. The eco1p (R222G, K223G) protein may be unable to promote DSB-induced cohesion in G2/M for several reasons. For instance, its acetyltransferase activity might be reduced such that it is unable to overcome an antagonizing activity (like the activity of a deacetylase), which accrues after exit from S phase. Alternatively, DSB-induced cohesion may require acetylation of DNA damage–specific targets in G2/M that are recognized poorly by the mutant protein.

Potential acetyltransferase targets include the four cohesin subunits Smc1p, Smc3p, Mcd1p (also known as Scc1p or Rad21p), and Scc3p (Fig. 1A), as well as the cohesin-associated factor Pds5p (15, 17–19). Mcd1p, Pds5p, and Scc3p are acetylated by Eco1p in vitro (II). We immunoprecipitated cohesin from extracts of asynchronous wild-type and eco1 (R222G, K223G) cells and observed a single acetylated band of ~150 kD, which we demonstrated to be Smc3p (Fig. 1, B and C, and Fig. S2C). In eco1-203 cells at the nonpermissive temperature, acetylated Smc3p was barely detectable (Fig. 1D and fig. S3) but was restored to wild-type levels in the presence of a plasmid-borne ECO1 (Fig. 1D). In addition, Smc3p immunoprecipitated from yeast was acetylated in vitro by a recombinant Eco1p (Fig. 1E). Therefore, Smc3p is a bona fide substrate of the Eco1p acetyltransferase.

Liquid chromatography–mass spectrometry (LC-MS) revealed eight acetylated lysine residues in Smc3p (Fig. 1F and fig. S4). We initially focused on the conserved lysine (K) residues and mutated them either as a pair (K112, K113) or individually.

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**Fig. 1.** Eco1p acetylates the Smc3p subunit of the cohesin complex. See (27) for experimental details and strains used for each experiment. (A) Cohesin architecture. (B) An acetylated protein immunoprecipitates with Mcd1p. WB, Western blot; IP, immunoprecipitation; HA, hemagglutinin. (C) Identification of the acetylated protein as Smc3p. α-AcK, antibody to acetylated lysine. (D) Smc3p acetylation in eco1-203. Ac-Smc3, acetylated Smc3p; CEN, centromeric plasmid. (E) Recombinant Eco1p acetylates Smc3p in vitro. Ac-CoA, acetyl-coenzyme A. (F) Alignment of the Smc3 orthologs using ClustalW. The amino acid numbers above the alignment correspond to the budding yeast Smc3p; # indicates the acetylated lysine residues. Abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

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**Fig. 2.** Smc3p acetylation is necessary for the establishment of sister chromatid cohesion. (A) Spot test for growth. Triangles indicate decreasing concentration of cells in spots. 5-FOA, 5-fluoroorotic acid. (B) smc3p (K112R, K113R) binding to chromosomes. Each inset is a magnified view. Asterisk indicates the magnified field. DAPI, 4´,6´-diamidino-2-phenylindole. (C) smc3p (K112R, K113R) binding to cohesin-associated regions. (D) Cohesion establishment defect in smc3 (K112R, K113R). Violet area corresponds to S phase. (E) Suppression of the cohesion establishment defect in eco1-203. Error bars indicate SD; n = 3.
(K931) to arginine (R), a structurally similar amino acid that cannot undergo acetylation. The K112R, K113R mutation, but not the K931R mutation, failed to complement smc3 and support growth (Fig. 2A), which suggests that sister chromatid cohesion requires K112, K113 acetylation. Moreover, in the smc3 (K112R, K113R) mutant, as in the eco1 mutant, cohesion associated with chromatin (Fig. 2, B and C) but failed to establish cohesion (Fig. 2D) (7, 8). This phenotypic similarity between smc3 (K112R, K113R) and eco1 strongly suggests that K112, K113 acetylation by Eco1p promotes chromatin-bound cohesin to become cohesive [also reported in (22)]. Because both the Eco1p acetyltransferase and its functionally relevant acetylation sites to assess their contribution to cohesion establishment. Only the K113R mutation failed to support growth and establish cohesion (Fig. 2E and fig. S5A). Surprisingly, unlike cohesion in the eco1 or smc3 (K112R, K113R) mutants, cohesion in the smc3 (K113R) mutant failed to associate stably with chromosomes (fig. S6, B and C). One explanation for this instability is that K113R continues to be acetylated at K112. Indeed, the monoacetylated K112 was recovered from wild-type cells (Fig. S4). This monoacetylated form may normally be generated from the diacetylated K112, K113 as part of the Wapl-dependent mechanism for dissociating cohesin from chromatin in interphase and prophase (24–26). By mutating K113R, the monoacetylated K112 would be generated inappropriately, causing precocious cohesion removal.

If acetylation of K112 and K113 are the only functionally important targets of Eco1p in S phase, then changing them to an acetyl-mimic glutamine (Q) (23) should allow cells to establish cohesion without Eco1. The presence of an ectopic copy of smc3 (K112Q, K113Q) restored sister chromatid cohesion in the eco1 mutant (Fig. 2E). K113 seems to be the more critical target of the Eco1p acetyltransferase, because smc3 (K113Q) suppresses the cohesion defect of eco1 to the same extent as smc3 (K112Q, K113Q). However, in both smc3 (K113Q) and smc3 (K112Q, K113Q), cohesion and growth were only partially rescued (Fig. 2E and fig. S5A).

This partial suppression might reflect the presence of additional targets or the incomplete mimic of the acetylated state by glutamine substitution. Alternatively, forcing Smc3p to be acetylated constitutively could compromise sister chromatid cohesion because cohesion becomes active at the wrong time or in the wrong context.

Individual substitution mutants were generated for K112, K113, and the remainder of the Smc3 acetylation sites to assess their contribution to cohesion establishment. Only the K113R mutation failed to support growth and establish cohesion (fig. S5, B and C). Acetylated K112 is undetectable in G1, where cohesin accumulates during S phase, is relatively constant until G2/M, and then diminishes when Mec1p is degraded at the onset of anaphase to dissolve cohesion. Analysis of its spatial regulation shows that K112 acetylation also requires the Scc2p/Scc4p (30)–dependent loading of cohesin on chromatin (Fig. 3C). This requirement for loading might have explained the low K112 acetylation in G1, because cohesion normally does not load at this time in budding yeast. However, even when we induced the premature loading of cohesin onto chromosomes in G1 (Fig. 3D), K112 acetylation still remained greatly reduced relative to S-phase cells (Fig. 3E). Thus, Eco1p-dependent acetylation of Smc3p, minimally at K112 and likely at K113, occurs only upon entry into S phase and after chromatin loading of cohesin.

This regulation of Smc3 acetylation may serve several biological functions. It may ensure robust cohesion by ensuring that cohesin becomes cohesive only after binding chromatin and only in the presence of an emerging sister chromatid. In addition, it may allow cells to mark a subset of cohesins to generate two functional cohesin pools. Indeed, evidence for two pools of cohesin with different chromatin-binding properties has been found in mammalian cells (31). We suggest that cohesins with acetylated Smc3p may be stably bound to chromosomes and locked in their cohesive state, thereby maintaining cohesion for chromosome segregation. Cohesins lacking Smc3p acetylation may be an uncommitted reservoir that can be targeted to de novo regions of the genome to respond to dynamic processes like transcription or DNA repair.

Fig. 3. Smc3p acetylation is cell cycle–regulated. (A and B) K112 acetylation during and after G2 (A) or S phase (B). (C) K112 acetylation in scc2; asyn, asynchronous. (D) Induction of cohesin loading during G2. (E) K112 acetylation status in G1–loaded cohesin.

References and Notes
ERdj5 Is Required as a Disulfide Reductase for Degradation of Misfolded Proteins in the ER

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Membrane and secretory proteins cotranslationally enter and are folded in the endoplasmic reticulum (ER). Misfolded or unassambled proteins are discarded by a process known as ER-associated degradation (ERAD), which involves their retrotranslocation into the cytosol. ERAD substrates frequently contain disulfide bonds that must be cleaved before their retrotranslocation. Here, we found that an ER-resident protein ERdj5 had a reductase activity, cleaved the disulfide bonds of misfolded proteins, and accelerated ERAD through its physical and functional associations with EDEM (ER degradation–enhancing α-mannosidase–like protein) and an ER-resident chaperone BiP. Thus, ERdj5 is a member of a supramolecular ERAD complex that recognizes and unfolds misfolded proteins for their efficient retrotranslocation.

In eukaryotic cells, membrane and secretory proteins are cotranslationally translocated into the endoplasmic reticulum (ER), acquire N-glycans and disulfide bonds, and become folded with the help of ER-resident molecular chaperones. Correctly folded proteins exit the ER and traffic through the Golgi to their final destinations. However, if these proteins fail to acquire their correct conformation, they are recognized by ER “quality-control” mechanisms (1). Terminally misfolded proteins are retrotranslocated from the ER into the cytosol for degradation via the ubiquitin-proteasome system, a process known as ER-associated degradation (ERAD) (1). The enzymatic modification of the N-linked Glc3Man9GlcNAc2–oligosaccharide of glycoproteins (i.e., glucose, mannose, and N-acetylglucosamine) is recognized not only by the calnexin-calreticulin cycle for productive folding (1), but also by ER degradation–enhancing α-mannosidase–like protein EDEM (Hml1/Mnlp1 in yeast), or Yos9 (yeast ositoscora9) for ERAD (2). EDEM enhances the degradation of misfolded proteins in a mannosine-trimming–dependent manner (3). EDEM accepts Man3GlcNAc2 substrates from calnexin (4, 5) and also associates with the transmembrane proteins Derlin-2 and 3 (6), which turn in association with the cytosolic p97 complex. However, EDEM itself does not bind properly folded proteins, nor does it affect their secretion or degradation (7).

Disulfide bonds not only stabilize protein tertiary structure, but in the ER, they also create large oligomers of misfolded proteins (8) that may not be accommodated by the retrotranslocation channel. Thus, the reduction of such disulfide bonds is required for the unfolding and retrotranslocation of misfolded proteins. ERAD is accelerated by treatment with redutants, such as dithiothreitol (DTT), and is inhibited by oxidants (7, 9). No protein having reductase activity in the ER has been reported to be involved in ERAD. To elucidate the precise role of EDEM in ERAD, we screened for EDEM-binding ER proteins by an ER-membrane yeast two-hybrid system (ER-MYTHS) (10, 11). In this method, the ER luminal portion of the yeast Ire1p was replaced by rat EDEM as bait and tested against a library of ER proteins as prey. Association of prey and bait causes oligomerization of Ire1p and activates its cystolic ribonuclease activity, which leads to splicing of the mRNA of the transcriptional activator HAC1 and, in turn, results in induction of the reporter gene (ADE2 in this study) under control of unfolded protein–responsive elements (UPRE).

In a screen of a focused library of ER-resident proteins, we identified ERdj5 (JPDI) (12, 13) as an EDEM-binding protein (fig. S1A). The ERdj5 family is comprised of five ER proteins, each containing a DnaJ domain (14, and ERdj5 is the only member that has thio/oxidoreductase-like domains with CXXC motifs (active cysteines with various amino acid residues between them) (Fig. 1A) (15). The specificity of the binding of ERdj5 to EDEM was confirmed with ER-MYTHs by using ERdj5 as bait (fig. S1B), and EDEM did not bind to any other ERdj proteins, including ERdj3 and ERdj4 (fig. S1C).

We purified recombinant mouse ERdj5 from Escherichia coli (E. coli) (Fig. 1B) and determined its reductase activity using oxidized insulin as a substrate. ERdj5 catalyzed the reduction of the insulin disulfide bonds in a dose-dependent manner in the presence of reduced glutathione (GSH), although the specific activity of ERdj5 was about one-third that of recombinant human protein disulfide reductase (PDH) (Fig. 1C). This reductase activity was not displayed by ERdj5/SS mutant, in which all cysteines of the four CXXC motifs of ERdj5 are replaced by serines (Fig. 1C). In addition, ERdj5 had neither oxidase nor isomerase activity for ribonuclease A (RNase A) and lysozyme (fig. S2).

Recombinant ERdj5 was incubated with different ratios of GSH and oxidized glutathione (GSSG) in order to determine its redox potential. The redox equilibrium constant K of ERdj5 was determined from the alkylation of free cysteines with a fluorescent maleimide (Fig. 1D). The apparent equilibrium constant Kapp of ERdj5 (190 mM) was ~100 times that of the redox state of the ER lumen (0.5 to 2.3 mM) (Fig. 1E), consistent with ERdj5’s having only reductase activity. Because the redox state of the ERdj5/SS mutant did not change within the measured redox ranges (Fig. 1E), we could attribute the observed Kapp values of ERdj5 to its CXXC motifs. The Kapp of ERdj5 is...