reefs it is no longer dominant (10). It is the largest coral species in this region, has very slow recruitment (18), and is also highly susceptible to disease that can kill 500-year-old colonies within months, with recovery unlikely for decades.

In the eastern tropical Pacific, a high proportion of corals have been affected by warming events. However, subsequent monitoring has shown reefs are recovering in most areas across the region (19). Indian Ocean corals were the most affected by the 1998 warming event with two subsequent bleaching events in some places. Many of the shallow reefs have lost their three-dimensional rugosity, with cascading trophic and ecological effects including subsequent loss of fish populations (20). Other reefs are recovering their structure, but the time to complete recovery may range to decades and will be highly dependent on future climatic and local disturbance regimes.

The epicenter of marine biodiversity in the Indo-Malay-Philippine archipelago, the Coral Triangle (11, 21), has the highest proportion of Vulnerable and Near Threatened coral species (Fig. 2, C and D). The chronic nature of anthropogenic disturbance in many parts of this region is compounded by the effects of climate change.

Corals in oceanic islands of the Pacific generally have the lowest proportion of threatened species (Fig. 2), and Hawaiian reefs have been spared extensive coral loss from bleaching or disease (22–25). However, Hawaii is an isolated archipelago with high levels of endemism (23), and several rare endemic species may prove especially vulnerable to future threats.

Our analysis indicates that the extinction risk for many corals is now much greater than it was before recent massive bleaching events. Whether corals actually go extinct this century (12) will depend on the continued severity of climate change, the extent of other environmental disturbances, and the ability of corals to adapt. If bleaching events become very frequent, many species may be unable to reestablish breeding populations before subsequent bleaching causes potentially irreversible declines, perhaps mimicking conditions that led to previous coral extinctions (13). If corals cannot adapt, the cascading effects of the functional loss of reef ecosystems will threaten the geologic structure of reefs and their coastal protection function and have huge economic effects on food security for hundreds of millions of people dependent on reef fish. Our consensus view is that the loss of reef ecosystems would lead to large-scale loss of global biodiversity.

References and Notes
10. Methods are available as supporting online material on Science Online.

Eco1-Dependent Cohesin Acetylation During Establishment of Sister Chromatid Cohesion
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Replicated chromosomes are held together by the chromosomal cohesin complex from the time of their synthesis in S phase onward. This requires the replication fork–associated acetyl transferase Eco1, but Eco1’s mechanism of action is not known. We identified spontaneous suppressors of the thermosensitive eco1−I allele in budding yeast. An acetylation-mimicking mutation of a conserved lysine in cohesin’s Smc3 subunit makes Eco1 dispensable for cell growth, and we show that Smc3 is acetylated in an Eco1-dependent manner during DNA replication to promote sister chromatid cohesion. A second set of eco1−I suppressors inactivate the budding yeast ortholog of the cohesin destabilizer Wap1. Our results indicate that Eco1 modifies cohesin to stabilize sister chromatid cohesion in parallel with a cohesin establishment reaction that is in principle Eco1-independent.

The cohesin complex provides sister chromatid cohesion from the time of DNA replication onward until mitosis (M) (1, 2). A number of cohesin establishment factors that do not themselves form part of the cohesive structure that linking sister chromatids (3–9) ensure that cohesin engages in productive linkages between sister chromatids during the synthesis phase (S phase), the period of DNA replication in the cell cycle. Of these factors, Eco1/Ctfl7 is the only known essential protein. In its absence, cohesin associates with chromosomes before, during, and after S phase apparently normally (3, 10), yet cohesin between sister chromatids is not established. Eco1 is a replication fork–associated acetyl transferase (10–12), suggesting a mechanistic link between replication-fork progression and cohesin establishment. How Eco1 promotes sister chromatid cohesion, and the role of its acetyl transferase activity in this process, have remained unclear.

When streaking eco1−I thermosensitive budding yeast cells (3) at their restrictive temperature, we noticed among the dying cells the outgrowth of colonies that had gained resistance to Eco1 inactivation (Fig. 1A) (13). Backcrossing of 20 such colonies revealed that spontaneous mutations in three complementation groups, a to c, outside the ECO1 locus, conferred thermoresistant growth. We


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tested whether the suppressor mutations restored sister chromatid cohesion in eco1-1 cells by analyzing the green fluorescent protein (GFP)-marked URA3 locus in metaphase-arrested cells (Fig. 1B). Mutations in all three complementation groups markedly reduced the cohesion defect in the eco1-1 background, suggesting that they compensate for loss of Eco1 function in sister chromatid cohesion.

We identified the eco1-1 suppressor mutations from the hybridization pattern of genomic DNA on oligonucleotide tiling arrays by means of the SNPsscanner algorithm (14). Three members of the largest complementation group, b1 to b3, showed hybridization differences, as compared with the eco1-1 parental strain, within the RAD61 gene (Fig. 2A). Two of these strains contained nonsense mutations at the predicted positions, leading to premature termination of the open reading frame. Cells lacking Rad61 are radiation sensitive and show a weak defect in sister chromatid cohesion (15, 16). Rad61 is a homolog of human and fission yeast Wapl, a protein that destabilizes cohesin on interphase chromosomes (17–19). Similar to its human counterpart, Rad61 physically associates with cohesin. It colocalizes with cohesin along chromosomes, and its absence leads to increased cohesin association with chromosomes (fig. S1). This suggests that Rad61 is the budding yeast ortholog of Wapl, and we propose that RAD61 may also be called WPL1.

WPL1 is a nonessential gene, and the early stop codon in suppressor b1 suggested that loss of Wp1 function may rescue eco1-1 cells. Consistently, deletion of WPL1 noticeably reduced the cohesion defect in eco1-1 cells (Fig. 2B), in agreement with observations in human cells (18). We next asked whether WPL1 deletion allowed survival of cells entirely lacking Eco1. We analyzed haploid progeny after sporulation of a heterozygous diploid eco1Δ/eco1-1 wpl1Δ/Δ strain. As expected, eco1A spores were inviable, but eco1A wpl1Δ double-mutant spores were frequently recovered (Fig. 2C). Thus, Eco1’s essential function for cell survival becomes dispensable in the absence of Wpl1. Sister chromatid cohesion in eco1Δ wpl1Δ double-mutant cells was compromised when compared with wild-type or wpl1Δ single mutants, but improved compared with eco1-1 cells at the restrictive temperature (Fig. 2B). This demonstrates that cohesion between sister chromatids is established without Eco1 when Wpl1 is absent, although less efficiently than in Eco1’s presence.

Eco1 not only promotes cohesion establishment during S phase, but also during repair of DNA double-strand breaks during G2 (20, 21). These findings open the possibility that Eco1 fulfills its truly essential role in response to DNA damage. We therefore tested the sensitivity to radiation-induced DNA breaks of eco1-1, wpl1Δ, and eco1-1 wpl1Δ cells. In response to 150 grays of γ-radiation, growth of wpl1Δ cells was only marginally affected, consistent with its sensitivity only to high doses of radiation (Fig. 2D) (15). eco1-1 cells showed marked sensitivity to this level of exposure, which was almost entirely rescued by deletion of WPL1. Assuming that the radiation sensitivity of eco1-1 cells stems at least in part from Eco1’s postreplicative role in double-strand break repair, this result suggests that Eco1 becomes dispensable for DNA repair in the absence of Wpl1.
The single mutation obtained in eco1-1 suppressor complementation group a mapped to the gene encoding the cohesin subunit Smc3 (Fig. 3A). Sequencing revealed a single nucleotide change, resulting in a lysine-to-asparagine alteration at amino acid 113 (K113N). When reintroduced into the genome, like Wpl1 deletion, the SMC3K113N mutation allowed cell growth in the absence of Eco1 (Fig. 3B), indicating an important role of lysine K113 in Eco1-dependent cohesion establishment. Smc3K113 is conserved in species from yeast to human; the residue likely emerges from a surface loop on the Smc3 adenosine triphosphatase (ATPase) head domain (fig. S2) (22).

The biophysical properties of an asparagine side chain resemble those of acetylated lysine, suggesting that K113 may be an acetylation target. To test this, we immunopurified Smc3 from budding yeast and subjected it to mass spectrometric analysis. The fragmentation spectrum of a peptide containing K113 showed that this residue is acetylated, as is the neighboring conserved lysine K112 (Fig. 4A). To analyze whether the cell cycle acetylation occurs, we immunopurified Smc3 from a synchronously cell culture and analyzed its acetylation status by Western blotting with an α-acetyl lysine antibody (Fig. 4B). Smc3 acetylation was hardly detectable in the prereplicative phase (G1), but increased at the time of S phase. Acetylation remained strong throughout the period between S phase and M (G2), and diminished again as cells entered anaphase. The signal was reduced when K113 was replaced by arginine and abolished when both K112 and K113 were mutated (fig. S3). This suggests that acetylation at these two residues is recognized by the α-acetyl lysine antibody and that they are the main acetylation sites within Smc3.

To analyze whether Smc3 acetylation during S phase depends on Eco1, we compared wpl1Δ and eco1Δ wpl1Δ cells in the above time-course experiment (Fig. 4B). The timing of Smc3 acetylation and deacetylation during the cell cycle was indistinguishable between wild-type and wpl1Δ cells. In contrast, no Smc3 acetylation was detect-
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 counteracted 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 counteracts 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 Smc3K113R 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 cohesion 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 acetyl transferase during the establishment of sister chromatid cohesion. The ring-shaped cohesion 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 cohesion's ring-opening reaction during loading onto DNA (25, 26). Acetylation of these lysines could modulate cohesion's interaction with Wpl1, to prevent Wpl1 from destabilizing the cohesion ring. Alternatively, acetylation could reinforce interactions within cohesion 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 cohesion ring—may provide the underlying basis for sister chromatid cohesion. In wild-type cells, Eco1 modifies cohesion 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.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/321/5888/563/DC1
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 Smc3p subunit of cohesin is acetylated by the Eco1p acetyltransferase at two evolutionarily conserved residues, promoting the chromatin-bound cohesin to tether sister chromatids. Smc3p acetylation is induced in S phase after the chromatin loading of cohesin and is suppressed in G1 and G2/M. Smc3 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) (13–17). 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 DSBRs in G2/M phase (7–10).