reflect multiple, partially overlapping mechanisms for rapid promoter turnover (Fig. 4, B to D). Some enrichments suggest clear hypotheses about the mechanism for rapid turnover (e.g., rapid histone replacement at Swi/snf-regulated promoters may well be a consequence of Swi/snf action), whereas other enrichments are less illuminating (e.g., what causes rapid replacement at nuclear pores?).

Many features of hot nucleosomes (including Htz1, tRNA genes, nuclear pore association, and Rap1 and Reb1 sites) are associated with boundaries that block heterochromatin spreading in yeast (*21*–*24*). How do boundaries block lateral spreading (*25*) of chromatin states? Suggested mechanisms include long gaps between nucleosomes, or recruited acetylases that compete with spreading deacetylation (*26*, *27*). The rapid H3 replacement at boundary-associated regions suggests an alternative hypothesis: that constant replacement of nucleosomes serves to erase a laterally spreading chromatin domain before it spreads any further (fig. S12). To investigate the role of Htz1 (whose role in boundary function is poorly understood) in histone replacement, we measured Flag-H3 incorporation in *htz1*Δ mutants, finding globally slowed H3 incorporation but few locus-specific effects (*14*). Further experiments will be required to untangle this relationship and to evaluate the role of rapid turnover at chromatin boundaries.

We have measured H3 replacement rates throughout the yeast genome, finding that nucleosomes over coding regions are replaced at high transcription rates, although most turnover occurs over promoters and small RNA genes. What function is served by histone replacement at promoters? Rapid turnover could transiently expose occluded transcription factor binding sites or it could ensure, by erasure of promoter chromatin marks, that transcriptional reinitiation occurs only in the continuing presence of an activating stimulus. Whatever the function, one important implication is that steady-state localization studies of histone marks could be confounded by dilution with histones carrying the average modification levels of the free histone pool, making dynamic or genetic studies key to deciphering any instructive roles of histone marks in transcriptional control. Finally, rapid turnover occurs at chromatin boundaries [see also (*28*)]. We propose that erasure of histone marks (or associated proteins) by rapid turnover delimits the spread of chromatin states. We further speculate that the widespread histone turnover at promoters throughout the compact yeast genome could serve, in a sense, to "expand" the genome by preventing chromatin states of adjacent genes from affecting each other.

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Histone Replacement Marks the Boundaries of cis-Regulatory Domains

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Cellular memory is maintained at homeotic genes by cis-regulatory elements whose mechanism of action is unknown. We have examined chromatin at *Drosophila* homeotic gene clusters by measuring, at high resolution, levels of histone replacement and nucleosome occupancy. Homeotic gene clusters display conspicuous peaks of histone replacement at boundaries of cis-regulatory domains superimposed over broad regions of low replacement. Peaks of histone replacement closely correspond to nuclease-hypersensitive sites, binding sites for Polycomb and trithorax group proteins, and sites of nucleosome depletion. Our results suggest the existence of a continuous process that disrupts nucleosomes and maintains accessibility of cis-regulatory elements.

Chromatin can be differentiated by the replication-independent replacement of one histone variant with another (*1*). For example, histone H3.3 is deposited throughout the cell cycle, replacing H3 that is deposited during replication (*2*–*4*). Unlike replication-coupled assembly of H3, which occurs in gaps between old nucleosomes on daughter helices, the insertion of H3.3 is preceded by disruption of preexisting histones during transcription and other active processes (*3*, *5*). We have previously shown that H3.3 replacement profiles resemble those for RNA polymerase II (*2*), which suggests that gradual replacement of H3.3 occurs in the wake of transiting polymerase to repair disrupted chromatin (*1*). Here, we ask whether histone replacement and nucleosome occupancy are also distinctive at cis-regulatory elements.

Log-phase *Drosophila melanogaster* S2 cells were induced to produce biotin-tagged H3.3 for two or three cell cycles (*2*). DNA was extracted from streptavidin pull-down assay and input material, labeled with Cy3 and Cy5 dyes, and cohybridized to microarrays. To provide a standard, we profiled biotin-tagged H3-containing chromatin in parallel. Analysis of H3.3/H3 levels over the entire *3*R chromosome arm revealed that the ~350-kb bithorax complex (BX-C) region displays the lowest H3.3/H3 ratio of any region of comparable size on *3*R, and the Antennapedia

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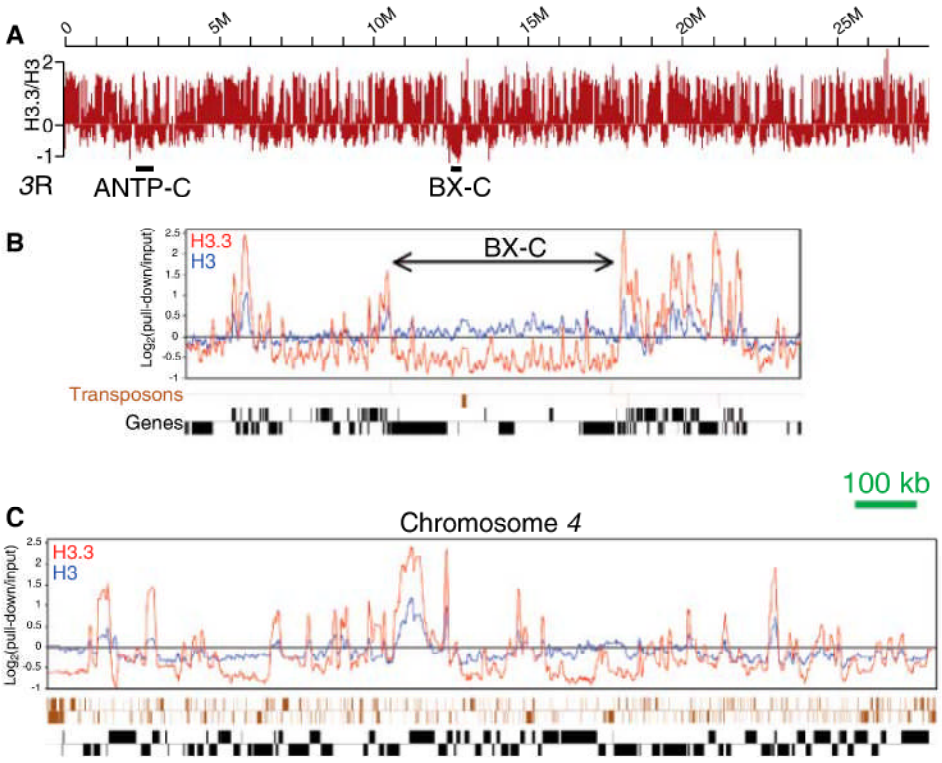


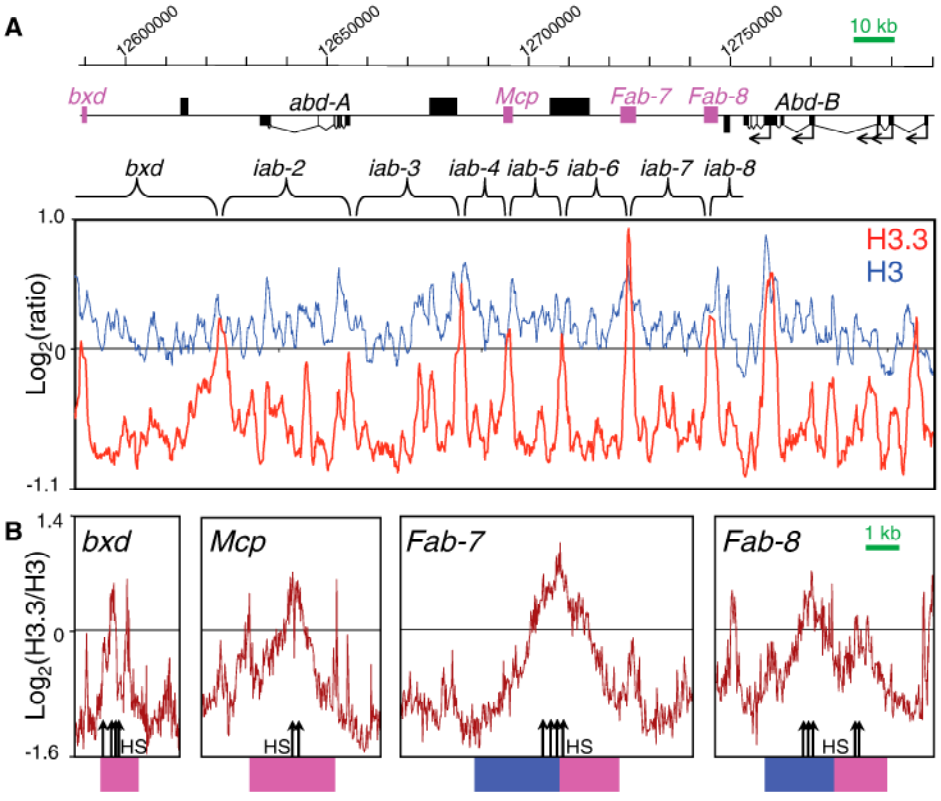
Fig. 1. The BX-C is extensively depleted in H3.3. (**A**) H3.3/H3 log-ratio profile of chromosome arm *3*R. Orientation is proximal (0 on base-pair scale) to distal (28 Mb). The location of the two homeotic gene clusters are indicated with horizontal bars. (**B**) H3.3 (red) and H3 (blue) profiles for the BX-C and flanking regions. (**C**) Same as (B) for chromosome *4*. The locations of annotated transposons (brown boxes) and genes (black boxes) are shown beneath each panel, oriented 5′-to-3′ above the line and 3′-to-5′ below. Profiles are displayed as moving averages, with a 1-kb (A) or a 2.5-kb (B and C) window, in 100-bp intervals.

homeotic gene complex (ANTP-C) also displays an unusually low H3.3/H3 ratio (Fig. 1A). Low H3.3/H3 ratios at the homeotic gene clusters are attributable to infrequent histone replacement, and not to low nucleosome occupancy, because H3.3 levels at the BX-C are far below the median ($\log_2 = 0$) for all of *3*R, whereas H3 levels are slightly above the median overall (Fig. 1B). Even the heterochromatic chromosome *4* (*6*) includes only shorter (~100-kb) stretches that are as depleted in H3.3 as the BX-C (Fig. 1C).

A close-up view of the BX-C *iab* region reveals the presence of several prominent H3.3 peaks (Fig. 2A). Notably, the seven highest peaks correspond to the functional boundaries of the seven proximal-to-distal cis-regulatory domains that regulate the *abd-A* (*iab2* to *iab4*) and *Abd-B* (*iab5* to *iab8*) homeotic genes successively from anterior to posterior in the abdomen (*7*). Conspicuous peaks of H3.3 also correspond to the *bxd* Polycomb response element (PRE) and to promoters within the *Abd-B* gene, which is known to be active in S2 cells (*8*, *9*). Therefore, each of the most prominent H3.3 peaks in the region corresponds to a previously defined cis-regulatory element. Our findings are likely to be general, because in budding yeast, promoters and boundaries are also sites of intense histone replacement (*10*).

A characteristic feature of both boundaries and PREs in the BX-C is that they span deoxyribonuclease I (DNaseI)–hypersensitive sites in a variety of cell types, including S2 cells (*11*). To better delineate histone replacement patterns in the vicinity of hypersensitive sites, we tiled the entire BX-C at 20-bp resolution (fig. S1).

Fig. 2. Conspicuous histone replacement at cis-regulatory domain boundaries and DNaseI-hypersensitive sites within the BX-C. (**A**) Map and histone profiles for the abdominal and flanking regions of the BX-C, including four well-mapped PRE-boundaries [magenta boxes (*11*, *13*, *25*)]. The scale at top indicates genomic location on *3*R, with genes and PRE-boundaries indicated on the line below. The five *Abd-B* promoters are marked with horizonal arrows. H3.3 (red) and H3 (blue) $\log_2$-ratio profiles are displayed as 1-kb moving averages. Boundaries between adjacent cis-regulatory domains are indicated with brackets above the log-ratio plots. (**B**) H3.3/H3 log ratios (brown) are shown for the PREs (magenta) and boundaries (blue) for which DNaseI-hypersensitive sites have been mapped (indicated with vertical arrows).



The *bxd*, *Mcp*, *Fab-7*, and *Fab-8* PRE-boundaries each encompass conspicuous peaks of H3.3 abundance (Fig. 2B) that closely correspond to all the known nuclease-hypersensitive sites within the region (*11*–*13*). Nuclease hypersensitivity identifies sites of relatively accessible DNA, so that their correspondences to peaks of histone replacement suggest that continuous disruption of nucleosomes exposes cis-regulatory DNA relative to surrounding regions.

PRE-boundary elements in the BX-C and other regions are binding sites for multiple Polycomb group (PcG) proteins, which have been mapped in an S2 cell line at high resolution (*8*). If the process that disrupts nucleosomes also facilitates PcG binding, then we would expect a correspondence between peaks of PcG binding and peaks of H3.3. Indeed, when we compared H3.3 profiles with those for Enhancer-of-zeste (EZ) and Posterior-sex-combs (PSC) PcG proteins, we found that all 10 peaks of PcG binding in the abdominal region are also local peaks of H3.3 (Fig. 3A and table S1). Likewise at the ANTP-C, all 13 peaks of PcG binding in the *Scr-Antp* region correspond to high levels of H3.3 (Fig. 3B and table S1). H3.3 enrichment at PcG-binding sites is not attributable to higher nucleosome occupancy, because essentially identical results were obtained for H3.3/H3 profiles (figs. S2 and S3).

Not all PREs in the BX-C are found to be sites of PcG binding; for example, neither *Fab-7* nor *Fab-8* is detectably bound by EZ or PSC (*8*). The fact that all PcG sites are peaks of histone replacement, but not vice versa, suggests that histone replacement at PREs and boundaries is constitutive and independent of the expression of the homeotic genes that they regulate. For example, *Abd-B* is expressed at high levels in S2 cells and displays the typical H3.3 5′ peak for an active gene (Fig. 2A), whereas *Ubx* and *abd-A* are nearly inactive (*8*, *9*), yet the PREs and boundaries regulating all three genes are sites of conspicuous histone replacement over a low background.

We also examined histone replacement averaged over the 175 genomewide EZ+PSC peaks outside of the BX-C and ANTP-C (table S1) and observed an H3.3 peak centered over the PcG maximum (Fig. 3C and fig. S4). Therefore, the strong association between PcG protein binding and histone replacement is not limited to homeotic gene clusters. The genomewide H3.3 peak is higher than that for the BX-C and ANTP-C, presumably because other PcG-binding sites are not superimposed over such deep H3.3 valleys (fig. S5).

The colocalization of PcG-binding sites and local peaks of H3.3 suggests that the process that disrupts nucleosomes locally maintains the accessibility of cis-regulatory DNA to PcG proteins. If so, then there should be a lower average occupancy of nucleosomes over sites of PcG protein binding than over their surrounding regions (*8*, *14*). To test this possibility, we hybridized nucleosomal DNA and fragmented genomic DNA on the same microarrays and measured nucleosomal/genomic DNA log ratios. Around peak regions of EZ+PSC binding, nucleosomal DNA was clearly depleted on average (Fig. 3D and table S1), similar to the depletion seen for active gene promoters (*2*) (fig. S1), and essentially the same results were obtained with different methods for genomic DNA fragmentation (fig. S6). We conclude that the correspondence between histone replacement and nucleosome depletion is a genomewide feature of PcG-binding sites.

In *Drosophila*, many cis-regulatory elements, including PREs and boundaries, are bound by the trxG proteins, Zeste and GAGA factor (GAF) (*15*). To test the possibility that histone replacement is enhanced and nucleosome occupancy is reduced where Zeste protein preferentially binds, we aligned 390 Zeste-binding sites identified by high-resolution chromatin immunoprecipitation (ChIP) combined with tiling microarrays (ChIP-chip profiling) (*16*) and averaged log ratios of H3.3/H3 and nucleosome occu-

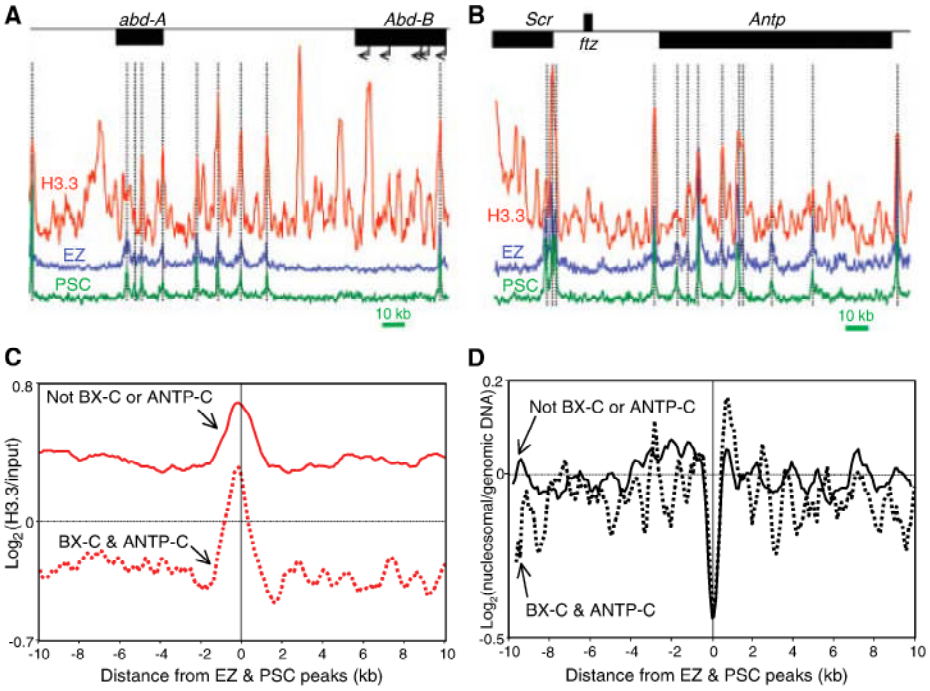
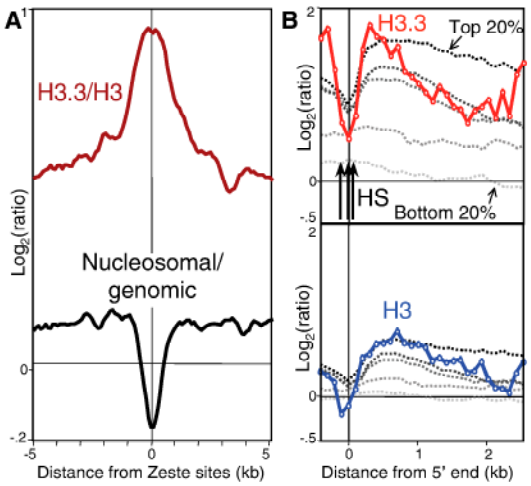


Fig. 3. Sites of PcG binding correspond to local peaks of histone replacement and reduced nucleosome occupancy. Comparison of the H3.3 log-ratio profile to EZ and PSC profiles (*8*) at (**A**) the BX-C (from Fig. 2A) and (**B**) the ANTP-C. Locations of prominent EZ and PSC peaks are marked with vertical dotted lines. Arbitrary scaling was used to facilitate visual comparison between H3.3/H3 log ratios and linear EZ and PSC profiles. Actual scales are shown in fig. S5. (**C**) H3.3 log-ratio profiles aligned around EZ+PSC peaks for the BX-C and ANTP-C regions (dotted line) and for the remainder of the genome (solid line), showing moving averages using a 500-bp window. (**D**) Same as (C), except showing the moving averages for nucleosomal/genomic DNA log-ratio profiles.

Fig. 4. Binding sites for trxG proteins and poised promoters are associated with conspicuous histone replacement and reduced nucleosome occupancy. (**A**) Average H3.3/H3 and nucleosome occupancy log-ratio profiles aligned at 390 Zeste-binding sites. (**B**) Average H3.3 (red) and H3 (blue) log ratios at uninduced heat shock genes. Dotted gray lines are histone profiles for all annotated genes on *3R*, shown in decreasing intensity from the most active (top 20%) to the least active (bottom 20%) gene sets. Very similar H3.3 profiles were obtained in three different experiments (fig. S10).



pancy. We observed a prominent maximum of histone replacement and a sharp minimum of nucleosome occupancy centered over the point of alignment (Fig. 4A and table S2). Similar results were obtained for predicted GAF sites (figs. S7 and S8), which suggests that nucleosome disruption is a general feature of trxG protein DNA-binding sites. H3.3 enrichment at PcG- and trxG protein–binding sites results from a replication-independent replacement process, because essentially identical profiles were obtained for $H3.3^{core}$, which lacks the N-terminal tail and does not assemble during replication (fig. S9).

Like *Fab-7* and *Fab-8*, heat shock gene promoters are prominent sites of GAF binding, nuclease hypersensitivity, and reduced nucleosome occupancy (*17*). Heat shock protein Hsp70 genes are constitutively "poised" for rapid induction, but do not produce detectable mRNAs in the uninduced state. We aligned Hsp70 genes at their 5′ ends and averaged H3.3 and H3 profiles. For comparison, we averaged similarly aligned H3.3 and H3 profiles for all 2165 genes on *3*R with known 5′ and 3′ ends, divided into quintiles based on expression levels. H3.3 patterns were similar to those of highly active genes (Fig. 4B and fig. S10), with histone replacement levels peaking on either side of heat shock promoters. As do transcriptionally active gene promoters (*2*), heat shock genes display prominent H3.3 and H3 dips in abundance that are attributable to partial nucleosome depletion (*17*). Constitutive histone replacement also appears to be a feature of poised promoters in vertebrates, because H3.3 is strongly enriched in the upstream region of the chicken folate receptor gene, regardless of whether the gene is active or inactive (*18*).

What process maintains the chromatin of cis-regulatory elements in a state of flux? Many DNA-binding and chromatin-binding proteins involved in gene regulation display short residence times on DNA (*19*), and some mouse transcription factors show dynamic behavior at their functional binding sites (*20*, *21*). A model for this process has been proposed, involving alternating cycles of nucleosome disruption by a Brahma-related SWI/SNF chromatin-remodeler and transcription factor binding (*21*). The binding of PcG and trxG proteins is also dynamic (*22*, *23*), and we propose that a similar cycle of nucleosome disruption and factor binding takes place at boundaries and PREs. Nucleosome disruption by SWI/SNF remodeling complexes would occasionally evict nucleosomes (*24*) and transiently expose DNA, which would become available to other diffusible factors, including PcG proteins. The continued local presence of nucleosome remodelers would result in another cycle of remodeling, nucleosome depletion, nuclease hypersensitivity, and histone replacement at the site. This model could account for the diversity of trxG proteins (*15*), which include DNA-binding proteins (Zeste and GAF), nucleosome remodelers (Brahma and Kismet), and histone methyltransferases (Trithorax and Ash1) that are specific for H3K4, a modification that is highly enriched on H3.3. The resulting dynamic process would allow for proteins that promote opposite epigenetic outcomes to act at common cis-regulatory sites.

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Supporting Online Material

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Anaphase Onset Before Complete DNA Replication with Intact Checkpoint Responses

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Cellular checkpoints prevent mitosis in the presence of stalled replication forks. Whether checkpoints also ensure the completion of DNA replication before mitosis is unknown. Here, we show that in yeast *smc5-smc6* mutants, which are related to cohesin and condensin, replication is delayed, most significantly at natural replication-impeding loci like the ribosomal DNA gene cluster. In the absence of Smc5-Smc6, chromosome nondisjunction occurs as a consequence of mitotic entry with unfinished replication despite intact checkpoint responses. Eliminating processes that obstruct replication fork progression restores the temporal uncoupling between replication and segregation in *smc5-smc6* mutants. We propose that the completion of replication is not under the surveillance of known checkpoints.

Eukaryotes have acquired cellular mechanisms that prevent or delay progression through the cell cycle when DNA is damaged (*1*). These mechanisms are referred to as checkpoints. Completion of DNA replication before mitotic entry is thought to be subjected to regulation by a checkpoint (*1*), because premature entry into mitosis would be detrimental to the integrity of the genome. Such a replication-completion checkpoint should prevent mitosis by sensing the persistence of unreplicated DNA or ongoing fork progression in an otherwise normal S phase.

The arguments supporting the existence of a replication-completion checkpoint derive from observations demonstrating that budding yeast cells activate a reversible checkpoint when cells are treated with the drug hydroxyurea (HU) (*2*). However, the checkpoint response to HU is caused by the accumulation of single-stranded DNA on replication forks rather than unreplicated DNA (*3*–*5*). Indeed, indirect evidence from several studies suggests that yeast cells might lack a replication-completion checkpoint (*6*–*8*).

The Smc5-Smc6 complex is related to cohesin and condensin and functions in DNA repair (*9*). Cells expressing the *smc5-smc6* mutant alleles showed S phase– and anaphase-entry times similar to those of wild-type (WT) cells (Fig. 1A and fig. S1A), and the central checkpoint kinase Rad53 was activated only after the first mitosis under nonpermissive conditions (*10*). The ribosomal DNA (rDNA) array in the