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GENE EXPRESSION

Expression homeostasis during DNA replication

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Genome replication introduces a stepwise increase in the DNA template available for transcription. Genes replicated early in S phase experience this increase before late-replicating genes, raising the question of how expression levels are affected by DNA replication. We show that in budding yeast, messenger RNA (mRNA) synthesis rate is buffered against changes in gene dosage during S phase. This expression homeostasis depends on acetylation of H3 on its internal K56 site by Rtt109/Asf1. Deleting these factors, mutating H3K56 or up-regulating its deacetylation, increases gene expression in S phase in proportion to gene replication timing. Therefore, H3K56 acetylation on newly deposited histones reduces transcription efficiency from replicated DNA, complementing its role in guarding genome stability. Our study provides molecular insight into the mechanism maintaining expression homeostasis during DNA replication.

The synthesis of mRNA depends on protein factors binding to the DNA template. During the cell cycle, DNA dosage increases at discrete times in S phase, whereas cell volume increases continuously, introducing considerable temporal variations in DNA concentration. How these variations in DNA level affect mRNA synthesis was examined in classical studies (*1*). In bacteria, mRNA production follows gene dosage, so that the expression of each gene increases rapidly after its replication (*2–4*). By contrast, experiments in eukaryotic cells, ranging from yeast to mammals (*5–7*), indicate a limited dependency of gene expression on DNA dosage, prompting the hypothesis that transcription of newly replicated DNA is transiently repressed (*8*).

We extended previous studies, which measured total mRNA synthesis (*9, 10*), or focused on individual genes (*11, 12*) by directly comparing the expression of early- versus late-replicating genes during S phase. If replicated loci produce more mRNA than unreplicated ones, then expression of genes that replicate early should increase relative to the expression of late-replicating genes during S phase (fig. S1A). In contrast, we find that the relative expression of early- versus late-replicating genes remained relatively constant in budding yeast, progressing synchronously through S phase after release from α-factor or hydroxyurea (HU) arrest and did not correlate

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with DNA replication timing (Fig. 1, A and B, and figs. S1 and S2). Further, the synthesis rates of early-replicating genes increased by only ~20% relative to late-replicating genes, significantly less than the ~70% increase in DNA content (Fig. 1A). We also examined cells arrested in the beginning of S phase after 3-hour treatment with HU. Despite the stable increase in DNA content of early-replicated genes, their expression increased by a mere 5% relative to that of late nonreplicated genes, suggesting that buffering under this S phase–arrested condition is even stronger than in cycling cells (Fig. 1C and fig. S8D). Taken together, our results are consistent with previous studies showing that during S phase, DNA dosage has a limited influence on mRNA synthesis rates.

In contrast to mRNA levels, the binding of RNA polymerase II to DNA did correlate with DNA content in HU-arrested cells and after release into S phase. Still, the increase in PolII binding to replicated genes (30%) was lower than expected by the increase in DNA content (Fig. 2A and fig. S3). In HU-arrested cells, early-replicated genes were depleted of elongating PolII (Fig. 2B). However, this difference was specific to HU arrest and disappeared upon release, before the completion of replication. Therefore, reduced PolII binding to replicated DNA may partially account for the buffering of gene expression, with additional differences in elongation capacity that increases buffering in HU-arrested cells.

We hypothesized that chromatin regulators may suppress transcription from replicated DNA. To identify such factors, we examined a published data set describing how individual deletions of 165 chromatin-associated factors affect the genome-wide expression profile (*13*). Deleting a factor that limits transcription from replicated DNA will increase gene expression in proportion to the time at which the gene is replicated in S phase, so that early-replicated genes will increase in expression more than genes replicated late. We therefore searched for mutants in which gene expression levels were (on average) negatively correlated with gene replication timing (Fig. 3A). Of the three mutants showing the strongest correlation between gene expression and replication timing, two were involved in H3 acetylation: the acetyltransferase Rtt109 and its histone chaperone cofactor Asf1 (*14–16*). A similar effect was detected in expression data from fission yeast deleted of the Asf1 paralogue (fig. S4A) (*17, 18*). The third candidate, Tos4, is a less-characterized putative transcription factor (*19*). All three genes increase in expression during G1, just before DNA replication (*20*).

The correlation between gene expression and replication timing were highly significant in all three mutants, but the difference in expression between early- and late-replicated genes was small. This is expected, as measurements were taken in asynchronous cultures in which only a minority of cells are in S phase. To amplify the difference in mRNA levels between early- and late-replicated genes, we profiled gene expression in all three mutants synchronized by HU (Fig. 3B). In both Δrtt109 and Δasf1 cells (but not Δtos4), expression levels correlated with DNA content, with genes that were already replicated showing 24 to 28% increase in expression relative to the nonreplicated genes. We next measured the mRNA levels and synthesis rates in Δrtt109 cells progressing synchronously through S phase. Indeed, expression and synthesis rates of early-replicated genes increased transiently during mid–S phase relative to late-replicating genes (Fig. 3C and fig. S5). Therefore, Rtt109 is required for buffering mRNA synthesis during DNA replication (Fig. 3C, red line).

A similar loss of buffering was observed for Δasf1 (Fig. 3D) and also for Δtos4 cells (fig. S7). The latter is particularly notable because Δtos4 did not abrogate buffering in HU-arrested conditions, consistent with an additional buffering mechanism acting upon HU arrest. To examine whether the three candidates act through the same pathway, we measured gene expression in the double deletions Δrtt109 Δasf1 and Δrtt109Δtos4. The increased expression of early-replicated genes was similar to the single deletions for the two pairs, suggesting that the three genes function
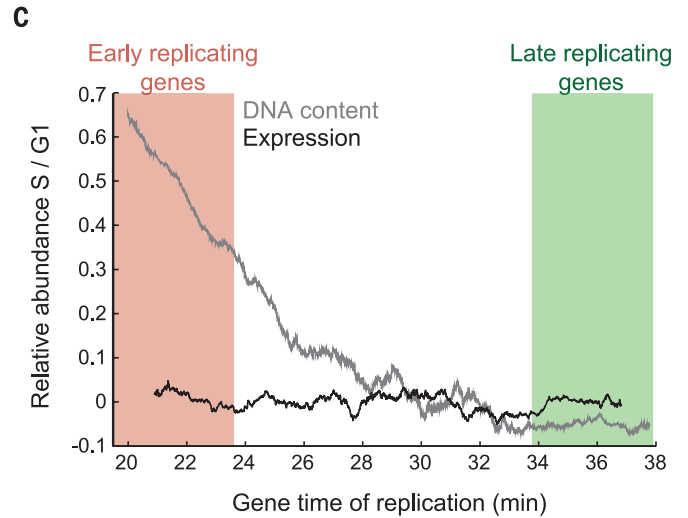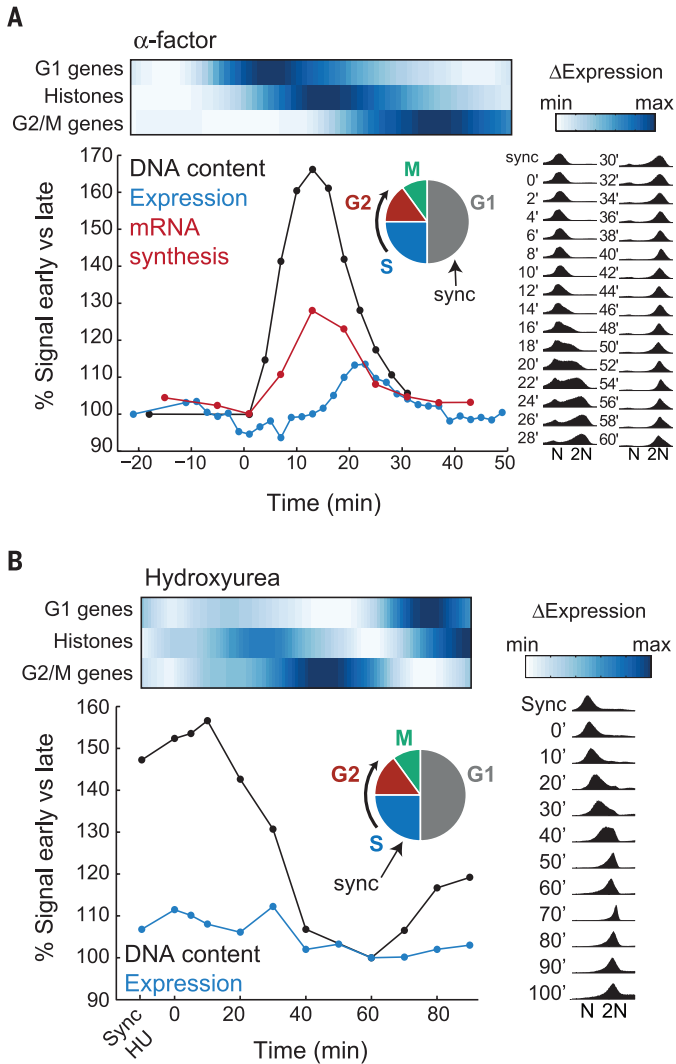
Fig. 1. mRNA synthesis rate is buffered against changes in DNA content during S phase. (A and B) Expression during S phase. Average increase in DNA content (black), expression levels (blue), and mRNA synthesis rate (red) of early-replicating genes relative to late-replicating ones, for cells progressing synchronously through S phase, after release from α-factor (A) or HU (B) arrest. Synchronized progression along the cell cycle was verified by total DNA content profile using fluorescence-activated cell sorting (right panel) and average expression (relative to G1) of cell-cycle genes (top panel) (see also table S4). In (A), G1/S transition is defined as time-point 0. (C) Expression in HU-arrested cells. Genes were ordered by their time of replication, taken from Yabuki et al. (29). Shown is the log2 relative DNA content (black) and gene expression (blue) normalized by G1 (α-factor arrest), averaged over a 500-gene sliding window.

Fig. 2. RNA polymerase binding increases on replicated genes. (A) PolII binding during S phase. The ratio between PolII binding of early- versus late-replicating genes (purple) as measured by chromatin immunoprecipitation sequencing (ChIP-Seq). A line is presented to guide the eye, omitting the 10-min time point. DNA content (black) and gene expression (blue) were sampled in the same experiment and shown also in Fig. 1B. See fig. S3A for other antibodies. (B) PolII phosphorylation on Ser2 and Ser5. The log2 ratio of Ser2P (initiating PolII) versus Ser5P (elongating PolII) abundance on early- (red) and late- (green) replicated genes in HU-arrested cells and after their release into S phase as measured by ChIP-Seq. Error bars represent standard error.
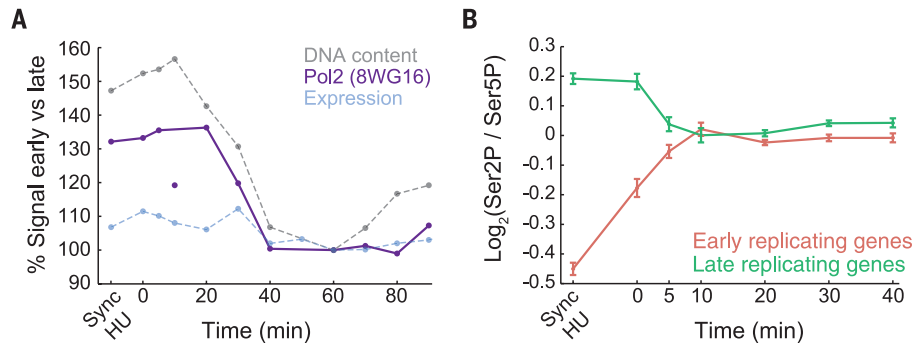
through the same pathway (Fig. 3, D and E, and figs. S6A and S7).

Rtt109 acetylates histone H3 on two residues, K56 and K9, and Asf1 is required for both functions (21). To differentiate which of these residues is responsible for the reduced transcription efficiency of replicated DNA, we considered mutants in which K56 or K9 were replaced by residues that mimic constant nonacetylation (lysine

to alanine, K→A) or constant acetylation (lysine to glutamine, K→Q) (22). Mutating K9 did not affect buffering of early-replicated gene expression in HU-arrested cells (Fig. 4A). In contrast, the relative expression of early-replicating genes was significantly higher in cells mutated for K56 (average 34%, compared with a 5% increase in wild-type cells). As expected, both modifications (K→A and K→Q) eliminated the asymmetry be-

tween late- and early-replicated genes. Consistently, buffering was also lost upon overproducing the H3K56ac-specific histone deacetylases, Hst3 and Hst4 (23) (Fig. 4B and fig. S8). Therefore, H3K56 acetylation is required for the reduced gene expression from newly replicated DNA.

In summary, we find that Rtt109/Asf1-dependent H3K56ac suppresses transcription from newly replicated DNA during S phase, thereby maintaining
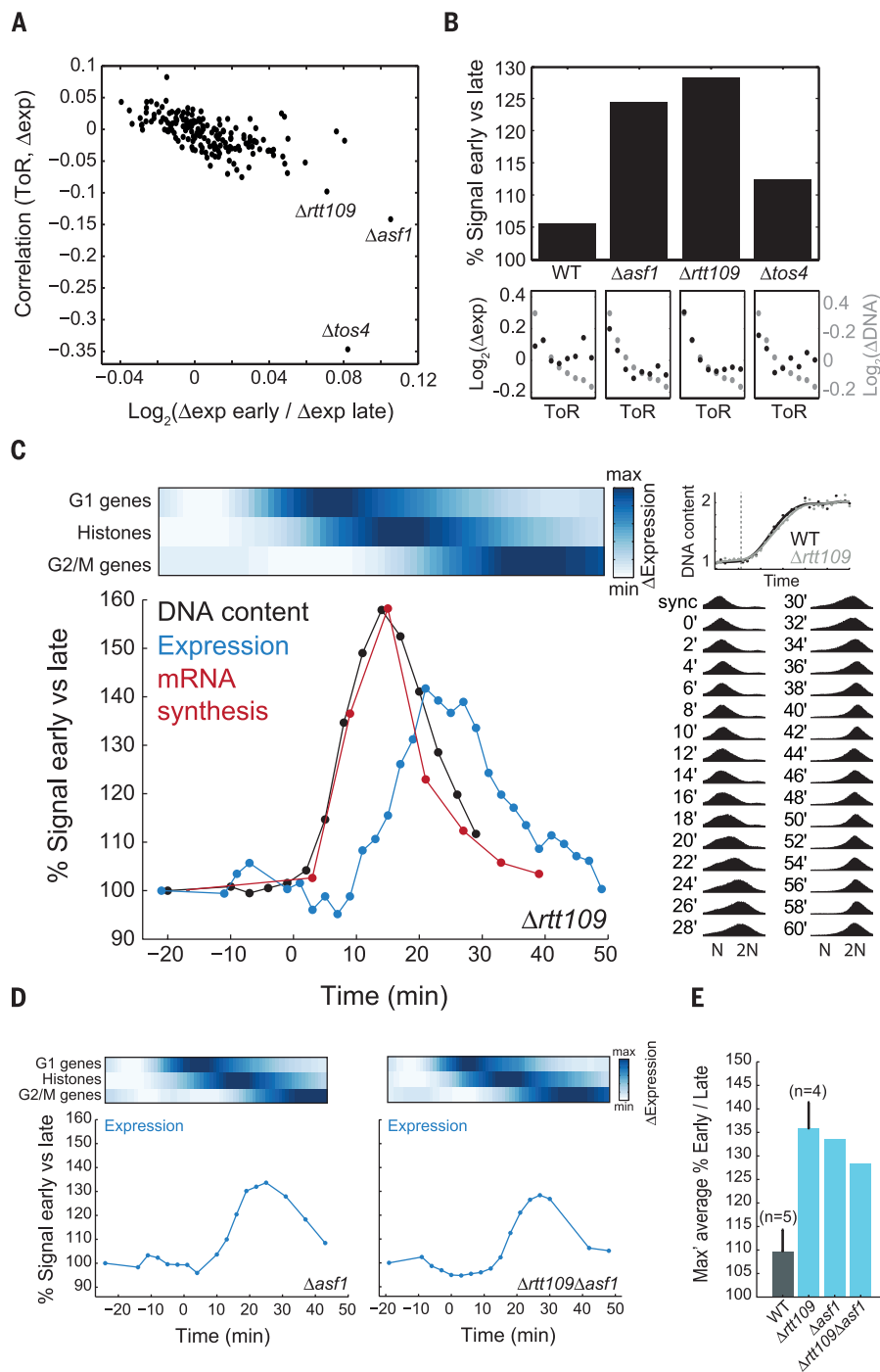
Fig. 3. Rtt109 and Asf1 are required for expression homeostasis during S phase. (**A**) Expression in asynchronous cultures. Each dot represents a single mutant. Correlation between replication timing and change in gene expression is shown on the *y* axis, and the difference in the expression of early-and late-replicating genes is shown on the *x* axis. The two measures are expected to be correlated. Expression data from a data set by Lenstra *et al.* (*13*). (**B**) Expression in HU-synchronized cells. Increase in the expression of replicated genes (early) relative to nonreplicated (late) ones for the indicated strains (top), in cells arrested with HU for 3 hours. Correlations of gene expression with replication timing is also shown (bottom). (**C**) Expression during S phase in Δ*rtt109*. Same as in Fig. 1A for Δ*rtt109* after α-factor synchronization. Average DNA content from Δ*rtt109* and wild-type (WT) are plotted along the time course (top right) (DNA content from WT same as in Fig. 1A). (**D** and **E**) Expression during S phase in mutants. The average increase in expression levels (blue) of early-replicating genes relative to late-replicating ones is depicted for the indicated mutants progressing through S phase after release from α-factor arrest. The maximal increase in the expression of early- versus late-replicated genes is summarized in (E) over 5 and 4 repeats for WT and Δ*rtt109*, respectively (see also figs. S7B and S8D).
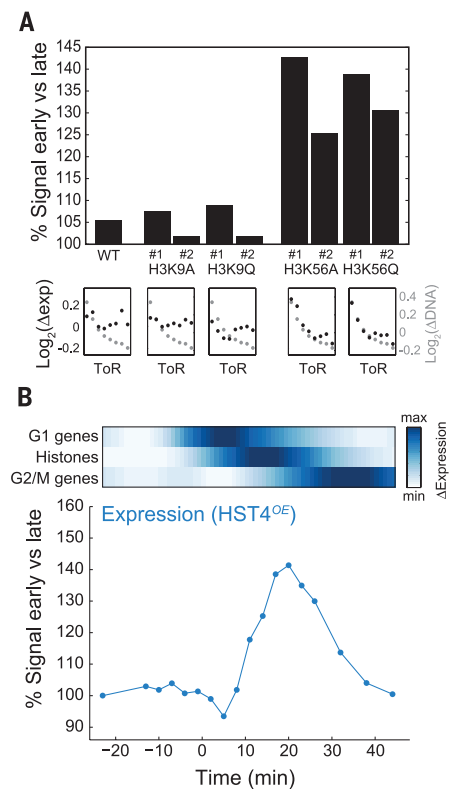

Fig. 4. H3K56 acetylation is required for expression homeostasis during S phase. (**A**) Expression in HU-synchronized cells of H3K56 or H3K9 point mutations. The indicated strains were HU-arrested for 3 hours. Shown is the increase in expression of replicated genes (early) relative to nonreplicated (late) ones for the indicated strains (top). Correlations of gene expression with the replication timing are also shown (bottom). (**B**) Expression during S phase in a strain overexpressing Hst4: the average increase in gene expression levels of early-replicating genes relative to late-replicating ones for cells overexpressing Hst4, progressing synchronously through S phase after release from α-factor arrest (see also table S4). A milder overexpression of Hst3 led to an intermediate effect (fig. S8C).

expression homeostasis during this time when the DNA dosage of different genes transiently differs. H3K56 is an internal site that is acetylated on newly synthesized histones before incorporation onto DNA (*15, 24*) (fig. S8E). Previous studies associated this modification with active transcription of specific genes (*25*), showing that it promotes nucleosome disassembly (*26*). H3K56ac, however, is primarily a marker of replicated DNA during S phase (*27*) (fig. S11), when it promotes nucleosome assembly and guards genome stability (*14, 16, 28*). Our study ascribes a complementary role to H3K56ac in maintaining expression homeostasis during S phase.

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TRANSCRIPTION

Multiplexed protein-DNA cross-linking: Scrunching in transcription start site selection

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In bacterial transcription initiation, RNA polymerase (RNAP) selects a transcription start site (TSS) at variable distances downstream of core promoter elements. Using next-generation sequencing and unnatural amino acid–mediated protein-DNA cross-linking, we have determined, for a library of 4^{10} promoter sequences, the TSS, the RNAP leading-edge position, and the RNAP trailing-edge position. We find that a promoter element upstream of the TSS, the "discriminator," participates in TSS selection, and that, as the TSS changes, the RNAP leading-edge position changes, but the RNAP trailing-edge position does not change. Changes in the RNAP leading-edge position, but not the RNAP trailing-edge position, are a defining hallmark of the "DNA scrunching" that occurs concurrent with RNA synthesis in initial transcription. We propose that TSS selection involves DNA scrunching prior to RNA synthesis.

D uring bacterial transcription initiation, RNA polymerase (RNAP) holoenzyme binds to promoter DNA through sequence-specific interactions with core promoter elements, unwinds a turn of promoter DNA to form an RNAP-promoter open complex (RPo) with an unwound "transcription bubble," selects a transcription start site (TSS), and aligns the TSS template-strand nucleotide with the RNAP active center (1). There is variability in the position of the TSS relative to core promoter elements (2–6). The mechanistic basis for this variability has remained unclear. In addition, although DNA-sequence determinants for TSS selection within the TSS region have been defined (2), it has remained unclear whether there also are DNA-sequence determinants for TSS selection outside the TSS region.

To investigate whether there are sequence determinants for TSS selection outside the TSS region, we applied a next-generation-sequencing approach that enables comprehensive analysis of sequence determinants during transcription: "massively systematic transcript end readout" (MASTER) (2). MASTER entails generating transcripts from a library of bar-coded randomized sequences and sequencing transcript ends (fig. S1) (2). In previous work, we defined TSS-region sequence de-terminants for TSS selection, using a library containing all 4^7 (~16,000) sequences at positions 4 to 10 base pairs (bp) downstream of the –10 element of a consensus bacterial promoter (MASTER-N7; Fig. 1A and fig. S1) (2). Here, to define effects on TSS selection of sequences outside the TSS region, we analyzed a template library containing all 4^{10} (~1,000,000) sequences at positions 1 to 10 bp downstream of the –10 element, extending the randomized sequence to include the "discriminator" (7–10), located between the TSS region and the –10 element (MASTER-N10; Fig. 1A and fig. S1). Results of MASTER-N10 analysis reveal that the discriminator affects TSS selection (Fig. 1, B to D; figs. S2 to S4; and table S1). Changes in the discriminator change TSS selection by up to ~3 bp (Fig. 1D and fig. S4) and change the mean TSS, averaged over the ~16,000 templates analyzed for each of the 64 discriminator sequences, by ~1 bp (Fig. 1B). Discriminators having a purine at each position (RRR), particularly GGG, favor TSS selection at upstream-shifted positions, whereas discriminators having a pyrimidine at each position (YYY), particularly CCT, favor TSS selection at downstream-shifted positions (modal TSS for RRR, 7 bp downstream of –10 element; modal TSS for YYY, 8 bp downstream of –10 element; Fig. 1, B and C, and fig. S2B). Results from MASTER-N10, where the discriminator is GTG, match results from MASTER-N7, where the discriminator is GTG, demonstrating the reproducibility of the approach (Fig. 1, C and D, and fig. S2B). We conclude that the discriminator is a determinant of TSS selection.

A conserved region of transcription initiation factor σ, "σ region 1.2" ($\sigma_{1.2}$), makes sequence-specific protein-DNA interactions with the nontemplate strand of the discriminator in the transcription bubble in RP$_O$ (7, 8). These interactions confer specificity for GGG (7–9). To determine whether sequence-specific $\sigma_{1.2}$-discriminator interactions affect TSS selection, we used MASTER-N10 to compare wild-type σ to a σ derivative having alanine substitutions that disrupt sequence-specific discriminator-$\sigma_{1.2}$ interactions: $\sigma_{1.2}$-mut (7, 11). The results show that disrupting $\sigma_{1.2}$-discriminator interactions markedly alters TSS selection for templates containing a GGG discriminator, resulting

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Editor's Summary

Doubling DNA but not expression

As the genome replicates, and before the cell divides, the copy number of the replicated portions of the genome doubles. In bacteria and archaea, gene expression tracks with gene dosage, both of which increase after DNA replication. Voichek *et al.*, however, show that an increase in DNA dosage after replication does not increase gene expression in budding yeast. This expression buffering is mediated by the acetylation of newly synthesized histone H3 deposited on the replicated DNA. This acetylation helps suppress transcription from the excess DNA.

Science, this issue p. 1087

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