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Relicensing of Transcriptionally Inactivated Replication Origins in Budding Yeast*[S]

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DNA replication origins are licensed in early G_1 phase of the cell cycle where the origin recognition complex (ORC) recruits the minichromosome maintenance (MCM) helicase to origins. These pre-replicative complexes (pre-RCs) remain inactive until replication is initiated in the S phase. However, transcriptional activity in the regions of origins can eliminate their functionality by displacing the components of pre-RC from DNA. We analyzed genome-wide data of mRNA and cryptic unstable transcripts in the context of locations of replication origins in yeast genome and found that at least one-third of the origins are transcribed and therefore might be inactivated by transcription. When investigating the fate of transcriptionally inactivated origins, we found that replication origins were repetitively licensed in G_1 to reestablish their functionality after transcription. We propose that reloading of pre-RC components in G_1 might be utilized for the maintenance of sufficient number of competent origins for efficient initiation of DNA replication in S phase.

Recent studies indicate that active transcription at the loci of replication origins disrupts their function, primarily affecting the assembly of the pre-replicative complexes (pre-RCs)[2] at origins. For example, experiments using a yeast meiosis-specific gene *MSH4*, containing an active replication origin ARS605, revealed that transcription of *MSH4* inhibited the activity of ARS605 by removing ORC from the origin (1). In addition, it has been shown that replication efficiency of autonomously replicating sequences (ARSs) is in negative correlation with transcriptional activity at these loci (2–4). In the genome of budding yeast, the majority of replication origins are located in intergenic regions that are not directly involved in transcription of protein-coding genes, suggesting that transcription is generally detrimental to origin function (5). However, recent studies have revealed that bidirectional initiation of transcription from many promoters is common in eukaryotes, leading to the transcription of intergenic regions and production of cryptic unstable transcripts (CUTs) (6–11). The widespread transcription of noncoding DNA and the compact nature of yeast genome raise the possibility that a large fraction of replication origins might be transcribed on regular basis, which could lead to their inactivation before cells enter the S phase.

In this study we present data indicating that about one-third of replication origins in budding yeast genome are transcribed, and we also confirm that transcription disrupts replication origin function by inhibiting pre-RC formation. In addition, we show that transcriptionally inactivated origins can be relicensed in G_1 after repression of their transcription and that these origins are used for initiation of replication in the following S phase. We propose that replication origins suffer from transcriptional stress genome-wide. However, continuous relicensing of origins in G_1 can keep them functional for initiation of replication and might ensure the presence of sufficient amount of competent origins in the S phase.

EXPERIMENTAL PROCEDURES

Computational Analysis of Replication Origin Transcription—Coordinates for 336 confirmed yeast replication origins were downloaded from OriDB (12). The data of CUTs from SAGE analysis were retrieved from the study by Neil *et al.* (7). Tiling array measurements of transcriptional activity were also retrieved from the latter analysis. Yeast open reading frame coordinates (SGD version 1.01) were downloaded from the Ensembl database (version 57) via the BioMart interface (13). CUTs of the Δ*rrp6* strain were included in this analysis. For every replication origin, CUTs of both strands were counted that fully or partially overlapped with the coordinates of the origin. Replication origins were classified into three groups: untranscribed (0 overlapping CUTs), moderately transcribed (1–10 overlapping CUTs), and extensively transcribed (11–43 overlapping CUTs). Tiling array data of the Δ*rrp6* strain were analyzed as follows. First, the baseline transcription rate of noncoding regions was determined using the signal intensity of probe sets located in intergenic regions, *i.e.* outside ORFs. To detect replication origins with significantly higher transcription rate, one-sided *t* tests were used; *t* tests quantified the difference of means of baseline transcription rate and intensity of probe sets within the coordinates of a given replication origin. Replication origins with insufficient tiling array signal (<10 probe sets) were excluded from the analysis. *p* values for the remaining 326 origins were corrected for multiple testing (False Discovery Rate, $p = 0.05$). Based on the above, replication origins were classified into three groups: untranscribed ($p > 0.05$), moderately transcribed ($p < 0.05$),

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Table 1.

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[2] The abbreviations used are: pre-RC, pre-replicative complex; ARS, autonomously replicating sequence; CUT, cryptic unstable transcript.

TABLE 1
Names and genotypes of yeast strains used in the study

Strain	Genotype
AKY421	W303 MATa *GAL-VPS13-3kb-ARS605::TRP1 ORC2-3×E4tag::spHIS5*
AKY431	W303 MATa *GAL-VPS13-3kb-ARS607::TRP1 ORC2-3×E4tag::spHIS5*
AKY441	W303 MATa *GAL-VPS13-3kb-ARS609::TRP1 ORC2-3×E4tag::spHIS5*
AKY461	W303 MATa *GAL-VPS13-3kb-ARS409::TRP1 ORC2-3×E4tag::spHIS5*
AKY445	W303 MATa *GAL-VPS13-3kb-ARS609::TRP1 MCM4-3×E4tag::spHIS5*
AKY465	W303 MATa *GAL-VPS13-3kb-ARS409::TRP1 MCM4-3×E4tag::spHIS5*
AKY542	W303 MATa *GAL-VPS13-3kb-ARS609::TRP1 CDC45-3×E4tag::spHIS5 bar1Δ::hphMX6*
AKY551	W303 MATa *GAL-VPS13-3kb-ARS605::TRP1 MCM4-3×E4tag::spHIS5 bar1Δ::hphMX6*
AKY552	W303 MATa *GAL-VPS13-3kb-ARS607::TRP1 MCM4-3×E4tag::spHIS5 bar1Δ::hphMX6*
AKY553	W303 MATa *GAL-VPS13-3kb-ARS609::TRP1 MCM4-3×E4tag::spHIS5 bar1Δ::hphMX6*
AKY555	W303 MATa *GAL-VPS13-3kb-ARS409::TRP1 MCM4-3×E4tag::spHIS5 bar1Δ::hphMX6*
AKY653	W303 MATa *GAL-VPS13::TRP1 ORC2-3×E4tag::spHIS5 bar1Δ::hphMX6*

and extensively transcribed ($p < 10^{-5}$). Normalized genome-wide *in vivo* nucleosome occupancy data for optimal growth conditions (Yeast Peptone Dextrose) were retrieved from Ref. 14. Nucleosome depletion of replication origins was quantified using one-sided *t* tests by comparing average genome-wide nucleosome density with origin-specific densities. Resulting *p* values were corrected for multiple testing (False Discovery Rate, $p = 0.05$) and classified into nucleosome-depleted origins ($p <= 0.05$) and origins with genome-wide average nucleosome density ($p > 0.05$).

Yeast Strains—All *Saccharomyces cerevisiae* strains were congenic with W303. To create *GAL-VPS13-ARS* loci, the ARSs were inserted into the coding region of the *GAL-VPS13* (15) 3 kb downstream from the beginning of *VPS13* coding sequence. The inserted chromosomal positions of amplified ARSs were as follows: ARS605 (Chr6:135943–136180); ARS607 (Chr6:199329–199515); ARS609 (Chr6:256257–256446); ARS409 (Chr4:212368–212673) (*Saccharomyces Genome Database*). For detection of replication proteins, a C-terminal triple E4 tag was fused to *ORC2*, *MCM4*, or *CDC45* loci in *GAL-VPS13-ARS* strains. For efficient α-factor arrest, the *BAR1* gene was also deleted in some of those strains. For reference, E4-tagged *ORC2* strain with no ARS insertion in *GAL-VPS13* gene was also made. Genotypes of the strains used in this study are summarized in Table 1.

Chromatin Immunoprecipitation (ChIP) Assay—Cells were grown in yeast extract-peptone (YP) medium containing 2% glucose, galactose, or raffinose as a carbon source for designated periods of time before fixation for the ChIP assay. For cell cycle arrest experiments, α-factor mating pheromone (Zymo Research) was used with a final concentration of 5 μM, and cell cycle status during the experiment was confirmed by flow cytometry analysis. ChIP assays were performed as described previously (16). Whole cell extract from 10^7 cells was used for ChIP assays with antibodies directed against RNAPII (4H8; Upstate Biotechnology) or anti-E4 tag (1E2; Icosagen). Co-precipitated DNA was analyzed by quantitative PCR using an ABI Prism 7900HT real-time PCR system under standard conditions (40 cycles; 95 °C for 15 s and 60 °C for 1 min). 5× Hot FIREPol EvaGreen qPCR mix (Solis BioDyne) was used. PCRs were done with primer pairs covering coding region of *VPS13* gene (3 kb), ARS605, ARS607, ARS609, ARS409, and the chromosome IX telomere region for normalization. RNAPII was normalized to the amount of RNAPII on the constitutively expressed *FBA1* gene. The sequences of primers are available on request.

Flow Cytometry—For flow cytometry analysis, 0.5 ml of yeast culture was fixed in 10 ml of 70% ethanol overnight at 4 °C and stained with propidium iodide (5 μg/ml) in the presence of RNase H (10 μg/ml) for 30 min at room temperature in the dark and washed afterward in PBS. Cells were subsequently analyzed using a FACS Calibur flow cytometer.

Analysis of Replication Intermediates by Two-dimensional Gel Electrophoresis—Preparation of chromosomal DNA and neutral two-dimensional agarose gel electrophoresis were carried out as described previously (17). 5 μg of nuclear DNA was cleaved with 40 units of NheI (Fermentas) for 4 h at 37 °C. Restriction fragments were separated on the first dimension in 0.4% Tris-Borate-EDTA agarose gels at 0.9 V/cm and 22 °C for 18 h, and second dimension gels were run in 1% TBE-agarose with 300 ng/ml ethidium bromide at 3 V/cm and 4 °C for 14 h. Gels were blotted to nylon membranes by alkaline transfer and hybridized by incubation for 10 h at 65 °C in 7% SDS, 250 mM $NaHPO_4$ (pH 7.2), 1 mM EDTA, and 0.1% BSA followed by three washes of 15 min at 65 °C with 5% SDS, 40 mM $NaHPO_4$ (pH 7.2), 1 mM EDTA. Hybridization probe was prepared by PCR and covers nucleotides 2573–3259 of *VPS13* ORF. Blots were exposed to Storage Phosphor Screens for 3 days, and signals were detected with a Typhoon Trio PhosphorImager (GE Healthcare).

RESULTS

Transcription of Replication Origins in Budding Yeast Genome—To find out whether replication origins are transcribed in yeast, we analyzed the expression data of CUTs and regular transcripts from Neil *et al.* (7) in the context of replication origin positioning defined as "confirmed ARS" dataset in *S. cerevisiae* OriDB (12). Altogether, 336 replication origins were used. First, we analyzed the tiling array data of total transcriptome and compared the transcription of replication origins to the average level of noncoding DNA transcription genome-wide. This analysis revealed that transcription of more than 10% of replication origins was statistically significant (Fig. 1*A*). However, this approach probably underestimated the fraction of transcribed replication origins because widespread transcription of noncoding DNA in yeast cells can significantly increase the average level of background signal that was calculated for this analysis. To find out more pre-

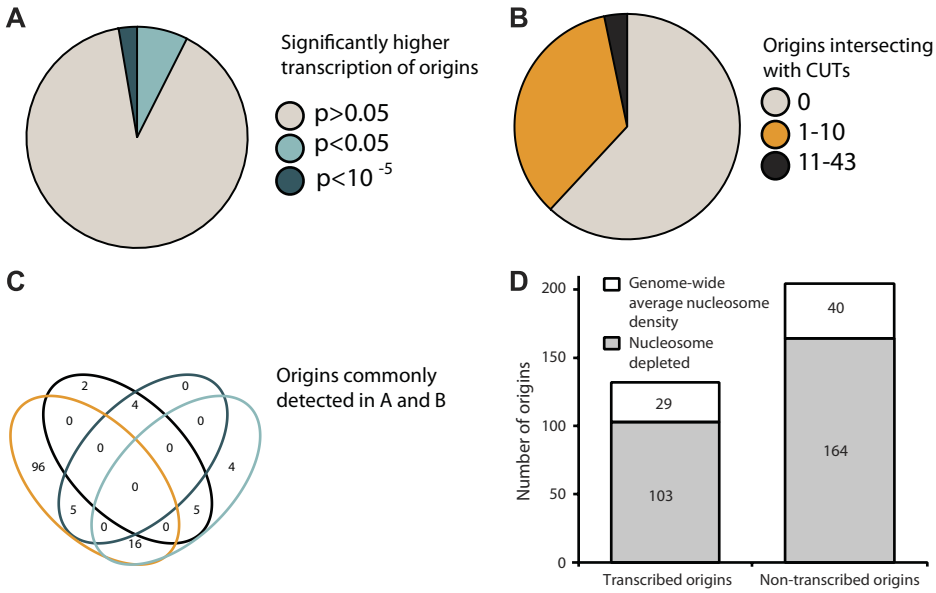

FIGURE 1. **Transcription of replication origins.** Data from Ref. 7 were analyzed in the context of replication origin positioning. *A*, tiling array data analysis of total transcriptome overlapping replication origins. Replication origin transcription higher than average transcription of noncoding regions is expressed. Replication origins were classified into three groups: untranscribed ($p > 0.05$), moderately transcribed ($p < 0.05$), and extensively transcribed ($p < 10^{-5}$). *B*, CUT-enriched SAGE tags overlapping replication origins. Replication origins are classified into three groups: untranscribed (0 overlapping CUTs), moderately transcribed (1–10 overlapping CUTs), and extensively transcribed (11–43 overlapping CUTs). *C*, representation of *A* and B on Venn diagram. *D*, nucleosome occupancy at replication origins quantified using one-sided *t* tests by comparing average genome-wide nucleosome density with origin-specific nucleosome densities. Resulting *p* values were classified into nucleosome-depleted origins ($p <= 0.05$) and origins with genome-wide average nucleosome density ($p > 0.05$).

cisely whether noncoding transcription covers the loci of replication origins, we also analyzed the genome-wide locations of CUTs, defined by the SAGE analysis (7). In this case, more than one-third of origins showed at least one CUT-enriched SAGE tag in the dataset, indicating that a significant number of replication origins were transcribed as CUTs (Fig. 1*B*). Importantly, this analysis revealed 98 additional transcribed origins that were not identified by the tiling array analysis of total transcriptome (Fig. 1*C*). We also analyzed the nucleosome occupancy data from (14) to explore whether transcriptional activity in ARS loci could be related to nucleosome density in these regions. Regardless of their transcriptional activity, ~80% of replication origins were located in regions where the occupancy of nucleosomes was lower than in the genome in average (Fig. 1*D*). This finding was in accordance with recent genome-wide study of nucleosome occupancy at loci of replication origins showing that ARS consensus sites contain nucleosome-depleted regions that are flanked by positioned nucleosomes (18).

Altogether, these results indicate that transcription of replication origins is common in yeast genome and the origins are transcribed predominantly as CUTs. Although most of the origins in the yeast genome are located in noncoding regions with no or low detectable transcriptional activity, the loci of several efficient and active replication origins, like ARS214, ARS305, ARS432.5, and ARS519, are highly transcribed as CUTs (supplemental Table 1). Because active transcription is antagonistic to replication origin functioning (1–3, 5), a mechanism to rescue transcriptionally inactivated origins must exist, particularly because some active origins are highly transcribed.

Formation of Pre-RC in a Transcriptionally Active Locus—To study the influence of transcription on replication complex formation, we inserted different ARS elements (ARS605, ARS607, ARS609, and ARS409) into a galactose-inducible *GAL-VPS13* gene 3 kb downstream from the transcription start site (Fig. 2*A*) and used ChIP to monitor the binding of pre-RC proteins to *GAL-VPS13-ARS* loci. As insertion of ARS sequences might directly or indirectly terminate the RNAPII-dependent transcription, we first verified that RNAPII can transcribe through the ARS sequences inserted into the *GAL-VPS13* locus. We induced *GAL-VPS13-ARS* transcription by galactose and determined the distribution of RNAPII in the loci. RNAPII was able to transcribe through the ARS sequences as it was detected in the locus both before (at 2.6 kb) and after (at 3.5 kb) the ARS insertion point (Fig. 2*B*). Although we did observe ~30% weaker signal of RNAPII at 3.5 kb compared with the 2.6-kb probe, the level of the signal reduction was identical in *GAL-VPS13* and *GAL-VPS13-ARS* strains (Fig. 2*B*), reflecting the spontaneous abortive transcription in the locus (15).

To monitor pre-RC formation in *GAL-VPS13-ARS* and natural ARS loci, the binding of Orc2 and Mcm4 proteins to the origin regions was determined. We observed ~6–12-fold enrichment of Orc2 binding to different *GAL-VPS13-ARS* loci when transcription was repressed, whereas no Orc2 signal was detected in *GAL-VPS13* locus without ARS insertion (Fig. 2*C*). In accordance with previous findings (1), we also found that in conditions of active transcription, the recruitment of ORC complexes to *GAL-VPS13-ARS* loci was significantly reduced compared with nontranscribed conditions (Fig. 2*C*). For reference, the binding of Orc2 protein was also deter-

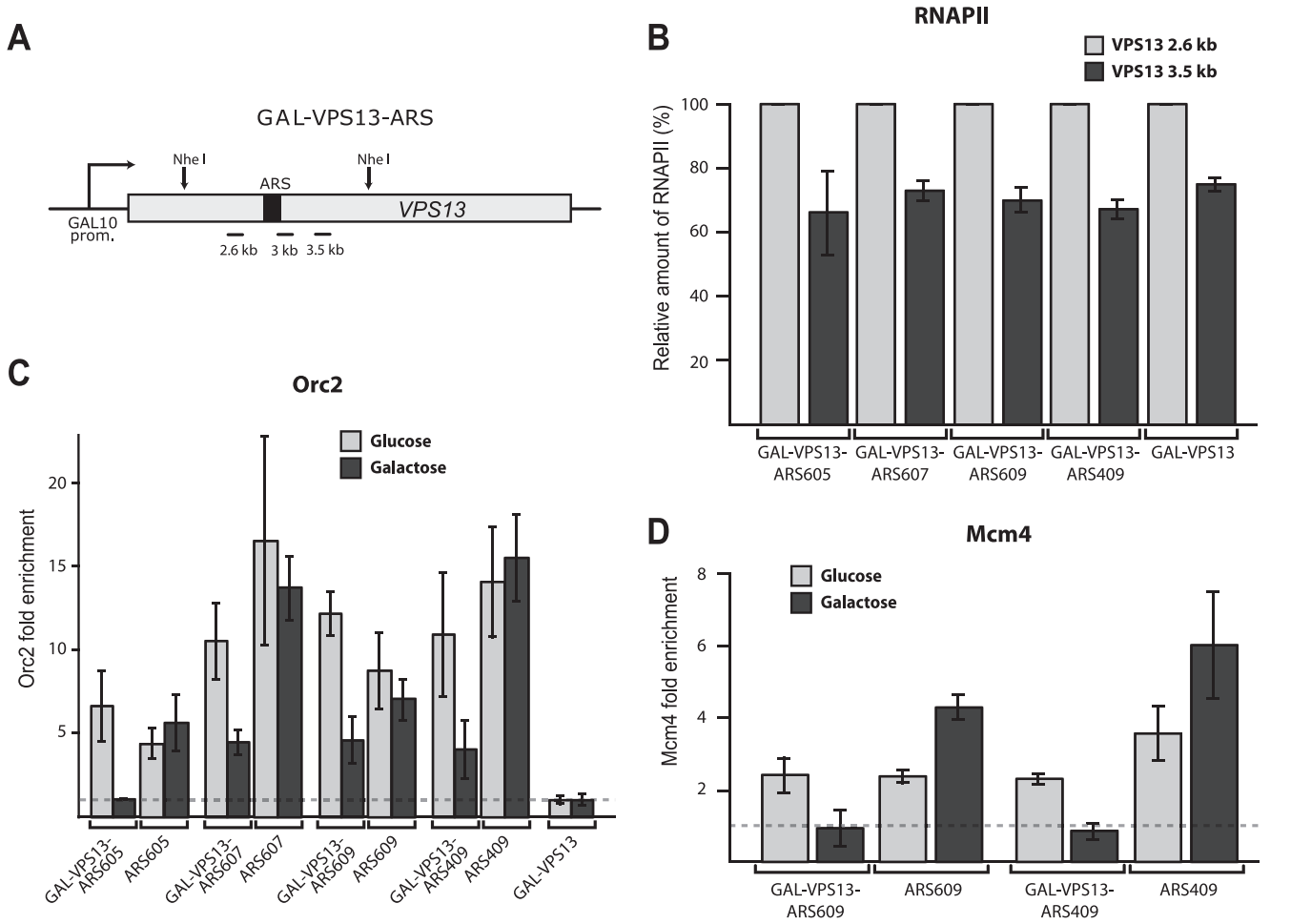

FIGURE 2. **Influence of transcription on pre-RC formation.** *A*, schematic representation of the *GAL-VPS13* locus with ARS sequences inserted at 3 kb from the *VPS13* promoter. *Horizontal lines* beneath the gene region indicate the positions (2.6, 3.0, and 3.5 kb) of PCR probes used in ChIP assays. Locations of NheI restriction sites, used for two-dimensional gel analysis of replication intermediates, are indicated. *B*, presence of RNAPII in *GAL-VPS13-ARS* loci upstream (2.6 kb) and downstream (3.5 kb) of the ARS insertion point. For reference, the *GAL-VPS13* strain without ARS insertion was used. *C*, Orc2 binding to 3 kb of *GAL-VPS13-ARS* and to genuine ARS605, ARS607, ARS609, ARS409 loci. Orc2 binding in cells grown overnight in YP-glucose (*GAL-VPS13-ARS* transcription OFF) was compared with Orc2 binding in cells grown overnight in YP-galactose (*GAL-VPS13-ARS* transcription ON). For reference, the *GAL-VPS13* strain without ARS insertion was used. *D*, Mcm4 binding (in YP-glucose and YP-galactose) to 3 kb of *GAL-VPS13-ARS609* and *GAL-VPS13-ARS409* strains. Mcm4 binding to genuine ARS609 and ARS409 loci was also determined for reference in both media.

mined in corresponding ARS sequences in their natural chromosomal positions. The overall levels of Orc2 signals in natural ARS sites were very similar to those found in transcriptionally repressed *GAL-VPS-ARS* loci. However, no significant changes of Orc2 binding were monitored in different growth media in natural ARS loci, indicating that the removal of the ORC from *GAL-VPS13-ARS* was a result of transcriptional activity in the locus (Fig. 2*C*).

Next we performed analogous experiments with MCM helicase component Mcm4. Similarly to Orc2, Mcm4 also bound *GAL-VPS13-ARS* loci when transcription was repressed, and it was removed upon transcriptional induction of the locus in two selected strains (Fig. 2*D*), indicating that the origins were efficiently licensed in transcriptionally inactive *GAL-VPS13-ARS* loci. Corresponding binding of Mcm4 on natural chromosomal positions was not lost when transcription in *GAL-VPS13-ARS* was activated (Fig. 2*D*).

To understand further the temporal dynamics of transcription-related removal of ORC and MCM, the *GAL-VPS13-ARS607* locus was induced with galactose for 120 min and then repressed with glucose for 30 min. Almost all of the ORC and MCM complexes were removed from *GAL-VPS13-ARS607* within 120 min of transcription, and surprisingly, both complexes were quickly reloaded to the locus after repression of the gene (Fig. 3, *A* and *B*). Almost half of the pre-repressed amounts of ORC and MCM were achieved within the first 10 min, and 75% of both complexes were rebound after 30 min (Fig. 3, *A* and *B*). Reference signals were also obtained from the natural chromosomal locus of ARS607, and no significant changes in different media were monitored during the time course of the experiment (Fig. 3, *A* and *B*). As expected, the amount of the RNAPII in *GAL-VPS13-ARS607* locus was in reverse correlation with the amounts of pre-RC components (Fig. 3*C*).

Relicensing of a Transcriptionally Inactivated Origin—The striking speed of foremost MCM reloading after transcriptional repression led us to question whether the MCM complex can be reloaded instantly after transcription stops, or is a transition through the S phase necessary for MCM reloading and relicensing of origins in *GAL-VPS13-ARS* loci. To clarify

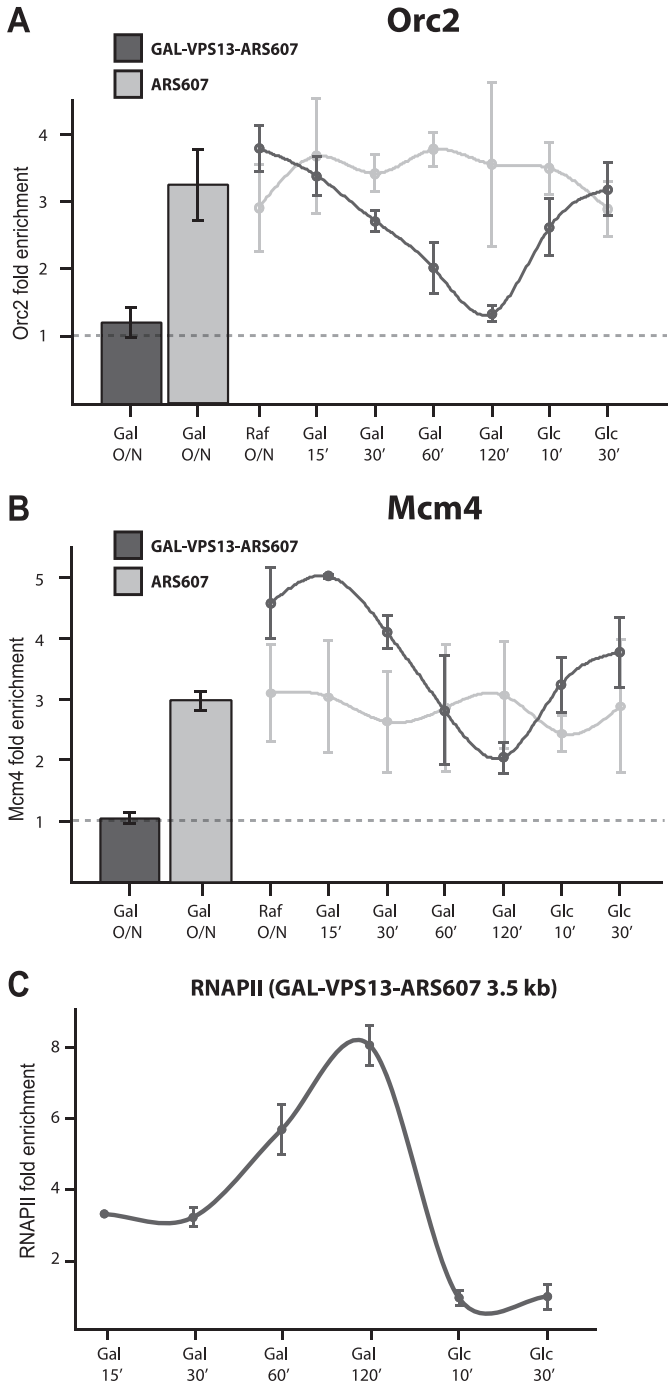

FIGURE 3. **Dynamics of transcriptional inactivation of replication origins.** Cells grown in YP-raffinose overnight were shifted to YP-galactose and afterward to YP-glucose. Samples were taken after growth for 15, 30, 60, and 120 min in YP-galactose and after 10 and 30 min in YP-glucose. *A*, ChIP-determined Orc2 binding on 3 kb of *GAL-VPS13-ARS607* and on genuine ARS607 in YP-raffinose, YP-galactose, or YP-glucose at the indicated times. *B*, Mcm4 binding on 3 kb of *GAL-VPS13-ARS607* and on genuine ARS607 in YP-raffinose, YP-galactose, or YP-glucose at the indicated times. *C*, RNAPII binding on 3.5 kb of the *GAL-VPS13-ARS607* strain in YP-galactose and YP-glucose at the indicated times.

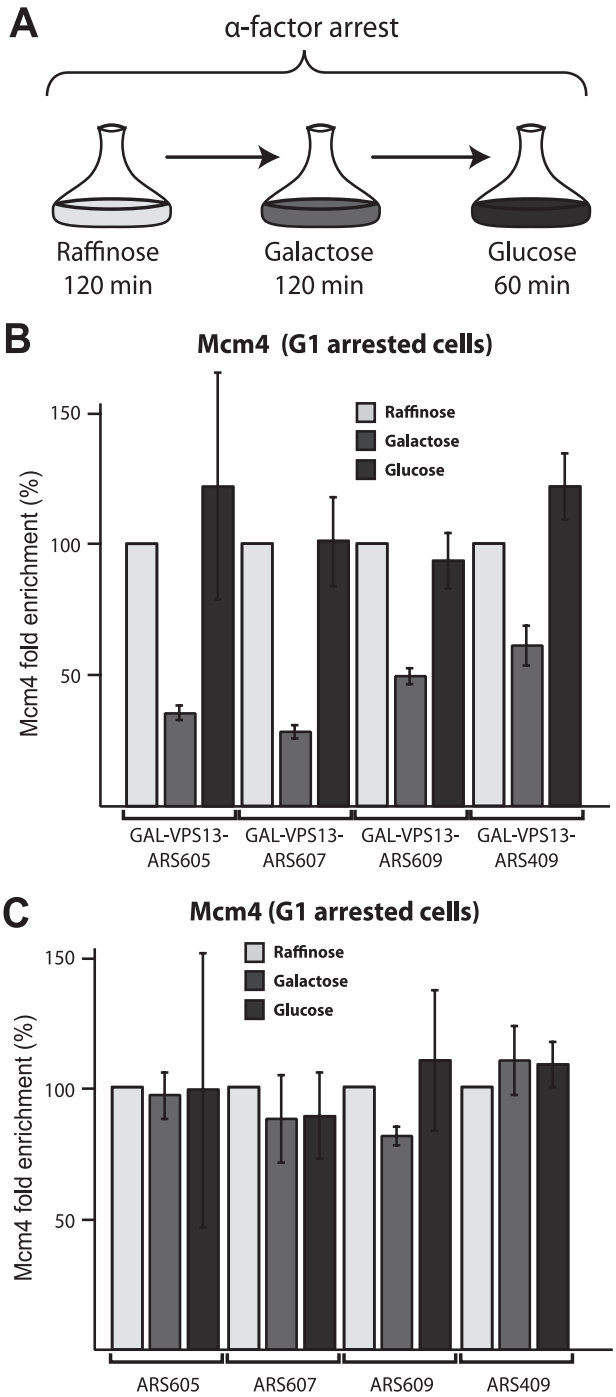

FIGURE 4. **Relicensing of transcriptionally inactivated replication origins.** *A*, schematic representation of the experiment. Cells were arrested with α-factor in YP-raffinose for 2 h, then shifted to α-factor containing YP-galactose for 2 h, and after that shifted to α-factor containing YP-glucose for 1 h. Samples were collected after 2 h in YP-raffinose, 2 h in YP-galactose, and 1 h in YP-glucose. *B*, binding of Mcm4 on 3 kb of *GAL-VPS13-ARS* strains in α-factor-arrested cells. *C*, binding of Mcm4 to genuine ARS605, ARS607, ARS609, and ARS409 loci in α-factor-arrested cells.

this issue we arrested cells with α-factor and monitored the removal and reloading of Mcm4 in conditions where cells were continuously arrested in G_1 (Fig. 4*A*). In all strains used, MCM was removed from *GAL-VPS13-ARS* loci in response to transcription induction (Fig. 4*B*). When transcription was subsequently repressed, but cells were restrained in G_1 arrest, MCM was reloaded to *GAL-VPS13-ARS* loci (Fig. 4*B*). After 60 min of repression, the level of MCM in *GAL-VPS13-ARS* loci was fully recovered in G_1-arrested cells, indicating that transition through S phase is unnecessary for MCM reloading. For reference, the binding of MCM on natural chromo-

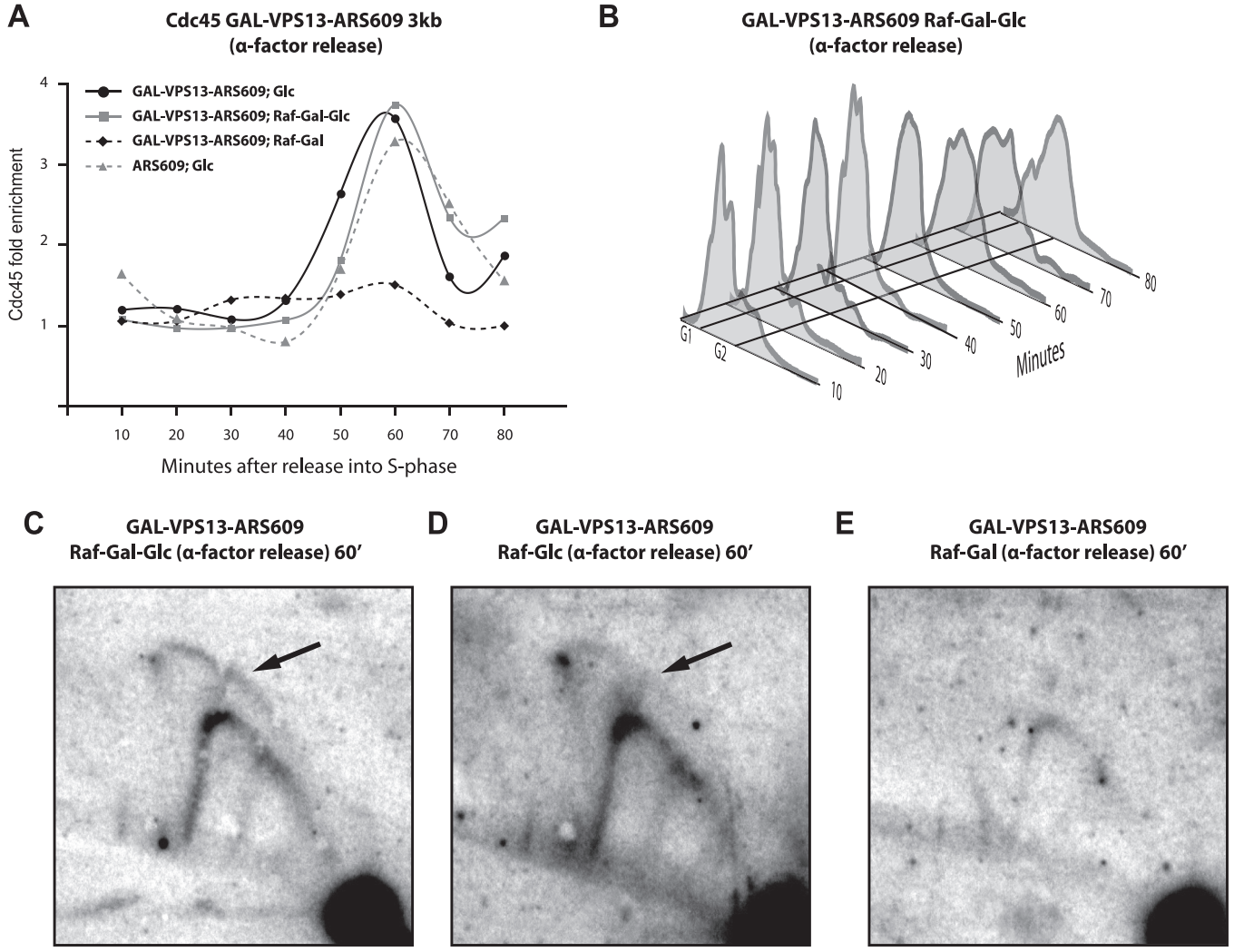

FIGURE 5. **Replication firing from a relicensed origin.** *A*, Cdc45 binding on 3 kb of *GAL-VPS13-ARS609* and genuine *ARS609* loci after release into S phase. Cells were released into S phase after arrest in transcriptionally repressing conditions (*Glc*), after transcription of the *GAL-VPS13-ARS609* locus in G_1 arrest (*Raf-Gal*), or after relicensing of the origin in G_1-arrested cells (2 h of transcription followed by 1 h of repression, *Raf-Gal-Glc*). For reference, Cdc45 binding to native ARS609 locus was determined in glucose-grown cells (*ARS609; Glc*). *B*, cell cycle analysis of *GAL-VPS13-ARS609* Raf-Gal-Glc sample shown on *A*. *C–E*, two-dimensional gel electrophoresis analysis of DNA replication intermediates at *GAL-VPS13-ARS609* locus. *C–E*, cells were released into S phase after relicensing of the origin in G_1-arrested cells (*C*), after G_1 arrest in transcriptionally repressing conditions (*D*), or after continuous transcription of *GAL-VPS13-ARS609* locus in G_1-arrested cells (*E*). *Arrows* mark bubble arcs indicative of origin firing in the *GAL-VPS13-ARS609* locus.

somal positions was also obtained and no MCM removal or reloading was detected during the course of the experiment (Fig. 4*C*).

Having established that transcriptionally removed MCM complexes can be reloaded in G_1, we wanted to find out whether relicensed origins were functional and able to initiate replication in the following S phase. To monitor origin firing in the S phase, we detected the recruitment of the Cdc45 protein to the origin. Cdc45 has been shown to associate with origins at the time of replication initiation, and its binding to the origins has been utilized as a reliable marker of origin firing (19–23). We arrested cells with α-factor and removed pre-RC proteins by transcription. Then we allowed relicensing of origins in repressing medium for 60 min and after that released cells to S phase. Cdc45 binds to *GAL-VPS13-ARS609* locus ~60 min after release from G_1 arrest (Fig. 5*A*; sample *Raf-Gal-Glc*), indicating that replication was initiated from relicensed origin in S phase of the same cell cycle (Fig. 5*B*).

The timing and efficiency of Cdc45 recruitment to relicensed origin were similar to the *GAL-VPS13-ARS609* locus that has been continuously kept in transcriptionally repressing conditions (Fig. 5*A*; sample *Glc*). However, when cells were released from the G_1 block without prior repression of *GAL-VPS13-ARS609* transcription, no recruitment of Cdc45 to the locus was observed (Fig. 5*A*; sample *Raf-Gal*). For reference, Cdc45 recruitment to the natural chromosomal locus of ARS609 was also determined. Cdc45 associates with native ARS609 locus also ~60 min after release into the S phase (Fig. 5*A*; sample *ARS609; Glc*), indicating similar firing time of native and *GAL-VPS13-ARS609* loci.

To confirm further that Cdc45 recruitment to relicensed origin reflects its firing, we also analyzed the patterns of DNA replication intermediates in *GAL-VPS13-ARS609* loci that have grown in transcriptionally active or repressed conditions or have been relicensed in G_1. The presence of a bubble arc in two-dimensional DNA electrophoresis analysis confirmed

firing of relicensed origin in *GAL-VPS13-ARS609* locus (Fig. 5*C*). As expected, origin firing was also detected in cells that were grown continuously in transcriptionally repressing conditions (Fig. 5*D*), but not in cells where transcription of *GAL-VPS13-ARS609* was active before and during release form G_1 arrest (Fig. 5*E*).

DISCUSSION

Our results reveal that at least one-third of replication origins in yeast genome are transcriptionally active, despite their intergenic location. We also confirm that RNAPII-dependent transcription inhibits pre-RC formation and thus abolishes replication origin firing. Nevertheless, our results show that transcription-coupled inactivation of replication origins is reversible in G_1, and pre-RC components can be quickly reloaded to origins when transcription stops. Relicensing of transcriptionally inactivated origins fully restores their functionality and leads to their firing in S phase.

Several recent studies suggest that active transcription abolishes replication origin firing by inhibiting pre-RC formation (1, 4). Although the majority of replication origins in budding yeast are located in intergenic regions of the genome, recent data show that transcription of noncoding regions is widespread in yeast (6–9). To estimate the fraction of annotated replication origins that are regularly transcribed, we reanalyzed the genome-wide data of yeast transcripts (7) in the loci of replication origins (12). Our analysis shows that at least one-third of the replication origin sequences are transcribed on regular basis, mostly as CUTs (Fig. 1). In addition, several replication origins are located in otherwise transcriptionally active regions that have significant transcriptional overlap due to normal gene expression (Fig. 1). However, the replication origin placement in yeast genome seems to favor transcriptionally more silent regions, as the number of heavily transcribed origins is significantly smaller than the ones transcribed occasionally (Fig. 1). Although direct transcription through the origins disrupts their function, the replication machinery might benefit from transcription-coupled remodeling and modifications of the chromatin that might help pre-RC components to access DNA and facilitate the initiation of replication. The fact that many replication origins are transcribed raises question about the fate of transcribed origins. As there is an excess of potential origins on chromosomes, cells can afford the inactivation of some of them under normal circumstances. Indeed, not all origins fire in a single S phase, and even the most efficient origins do not fire in every S phase (24). However, the activity of those dormant origins becomes critical in stress conditions, when the movement of replication forks initiated from major origins stalls or slows down (25). An efficient alternative to the transcription-coupled loss of functional origins would be reestablishing of pre-RCs on origins after transcription. To investigate this possibility, we inserted different replication origins into the coding region of an inducible gene and determined the dynamics of pre-RC components in this locus. Our results show that both ORC and MCM complexes were quickly reloaded to origins as soon as transcription was shut down (Figs. 3 and 4), indicating the possible rescue of replication origins by this mechanism. Importantly, in G_1-arrested cells pre-RCs can reform on origins without transition through S phase. After release from G_1 arrest, DNA replication initiation protein Cdc45 was recruited to relicensed origin (Fig. 5*A*), and DNA replication was initiated in S phase (Fig. 5, *C–E*). Based on these results, we propose that pre-RC assembly in yeast is widely perturbed by sporadic transcription; however, when the transcription bubble has passed the loci of replication origins, pre-RCs can quickly reassemble, ensuring that sufficient amounts of functional replication initiation loci will be available to carry out S phase.

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