Scheduled Conversion of Replication Complex Architecture at Replication Origins of *Saccharomyces cerevisiae* during the Cell Cycle*

Replication of DNA within *Saccharomyces cerevisiae* chromosomes is initiated from multiple origins, whose activation follow their own inherent time schedules during the S phase of the cell cycle. It has been demonstrated that a characteristic replicative complex (RC) that includes an origin recognition complex is formed at each origin and shifts between post- and pre-replicative states during the cell cycle. We wanted to determine whether there was an association between this shift in the state of the RC and firing events at replication origins. Time course analyses of RC architecture using UV-footprinting with synchronously growing cells revealed that pre-replicative states at both early and late firing origins appeared simultaneously during late M phase, remained in this state during G1 phase, and converted to the post-replicative state at various times during S phase. Because the conversion of the origin footprinting profiles and origin firing, as assessed by two-dimensional gel electrophoresis, occurred concomitantly at each origin, then these two events must be closely related. However, conversion of the late firing origin occurred without actual firing. This was observed when the late origin was suppressed in *clb5*-deficient cells and a replication fork originating from an outside origin replicated the late origin passively. This mechanism ensures that replication at each chromosomal locus occurs only once per cell cycle by shifting existing pre-RCs to the post-RC state, when it is replicating without firing.

Replication of DNA within eukaryotic chromosomes is initiated from multiple sites termed origins of replication. In somatic cells, initiation at origins of replication occurs in a fixed order during a limited period called the S phase (1, 2). Precise replication of chromosomal DNA once per cell cycle requires suppression of each origin of replication after it has fired until the beginning of next S phase. In addition, if a region served by one replication origin is replicated after initiation at a flanking origin, then this unused origin must also be suppressed. Although such a phenomenon has been generally accepted, its precise mechanism has not yet been elucidated.

In the budding yeast, *Saccharomyces cerevisiae*, short distinct chromosomal fragments, identified as autonomously replicating sequences act as replication origins in chromosomes (3, 4). Several lines of evidence obtained from *in vivo* footprinting, chromatin immunoprecipitation, and chromatin fractionation demonstrate that the initiation factors are assembled into replication complexes (RC)\(^1\) at replication origins (5–10). The origin recognition complex (ORC) binds to an essential sequence (autonomously replicating sequence (ARS) consensus sequence (ACS)) within replication origins throughout the cell cycle (5–7). In G1 phase, Cdc6p and minichromosome maintenance (MCM) proteins are sequentially recruited to the ACS-bound ORC to form the pre-replicative complex (pre-RC) (6, 7, 10, 11). Its formation can be monitored by *in vivo* footprinting with DNase I or UV, which yields footprints that are distinct from those observed at other cell cycle phases, referred to as post-replicative complex (post-RC) footprints (5, 10). In the post-RC state, the protection pattern is consistent with that of a simple ORC-ACS complex, resembling the one obtained when only purified ORC proteins are used in *in vitro* footprinting experiments (5, 10). Thus, it is held that the pre-RC is formed from the existing ORC-ACS complex and is a prerequisite for the initiation of DNA replication. Conversion of the pre-RC to the post-RC in S phase is thought to be closely associated with origin firing. For example, dissociation of MCM proteins from the ACS and dissociation of polymerase a and e, which are loaded just before the dissociation of the MCM proteins, occur with the same kinetics (6). Furthermore, stalling of replication forks by the addition of hydroxyurea concomitantly blocks both the conversion and firing at late replicating origins (12).

Several S-phase-specific protein kinases, for example cyclin-dependent kinase and Cdc7-Dbf4 protein kinase are believed to regulate the RC status. They phosphorylate one or more factors in the RC to directly trigger initiation of DNA replication. Cdc7-Dbf4 kinase activity increases at G1/S and remains high throughout S phase (13–16). Inactivation of Cdc7p in G1 phase prevents initiation of S phase, and inactivation at early S phase results in inactivation of the late origins. Thus, Cdc7 kinase activity is necessary for both early and late replication origin firing throughout S phase (15, 16). Among six closely related

---

* This work was supported in part by a grant-in-aid for Scientific Research on Priority Areas (C) Genome Biology and Cancer Cell biology and on Priority Areas (B) and Basic Research Area (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to C. O. and T. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: BF Research Institute, Inc., National Cardiovascular Center, 7-1, 5-Chome, Fujishiro-dai, Suita, Osaka 565-0873, Japan.

‡ Present address: BF Research Institute, Inc., National Cardiovascular Center, 7-1, 5-Chome, Fujishiro-dai, Suita, Osaka 565-0873, Japan.

§ Present address: RIKEN Yokohama Institute, 1-7-22, Suehiro, Tsurumi-ku, Yokohama, Kanagawa 220-0045, Japan.

¶ Present address: BF Research Institute, Inc., National Cardiovascular Center, 7-1, 5-Chome, Fujishiro-dai, Suita, Osaka 565-0873, Japan.

‖ To whom correspondence should be addressed. Tel.: 81-7437-2-5512; Fax: 81-7437-2-5519; E-mail: c-obuse@bs.aist-nara.ac.jp.

---

1 The abbreviations used are: RC, replicative complex; ORC, origin recognition complex; ACS, autonomously replicating sequence (ARS) consensus sequence; MCM, minichromosome maintenance; FACS, fluorescence-activated cell sorter.
B-type cyclins (Clb1–6), it appears that Clb5p and -6p are involved mainly in S phase events (17, 18). Interestingly, Clb5p and Clb6p can cause early origins to fire, whereas only Clb5p can fire late origins (19). These results strongly suggest that these kinases are involved in origin activation and may determine the order of origin firing.

We have attempted to elucidate the molecular mechanism of how the timing of replication origin firing is regulated through the characterization of the protein-DNA complex architecture at replication origins using UV footprinting in budding yeast. This method allows us to monitor the dynamic conversion of the RC architecture at origins of replication in living cells. Comparable studies of early and late replication origins using two-dimensional gel electrophoresis show a close association in the timing between RC conversion and origin firing. We also observed conversion without firing when late origin activation was suppressed by a Clb5 mutation. Based on these results, we propose mechanisms that ensure that replication occurs once and only once per cell cycle through assembly and disassembly of the pre-RCs at chromosomal replication origins.

EXPERIMENTAL PROCEDURES

Yeast Strains—Genotypes for W303-1A and RM14-3A have been described previously (10). The genotype for the Cdc15 temperature-sensitive mutant strain (DK329-10a) is (MATa cdc15-1 his3-11 leu2-3112 ura3-1) (29). To obtain the clb5-deficient strain (SKY009), the clb5 gene in W303-1A was disrupted by transformation with a CLB5 (5′-Trp1-CLB53′) fragment that had been amplified by PCR from Yiplac204 (30) using a primer containing 18 nucleotides of the Yiplac204 vector sequence and 100 nucleotides of the sequence flanking the CLB5 open reading frame.

UV Photofootprinting and Two-dimensional Gel Electrophoresis Analyses—Methods used for UV photofootprinting in vivo and in vitro and quantification of the band intensities have been described previously (10). Construction of plasmids carrying ori602 or ori607 are described in Shirahige et al. (31). Primers to extent the sequences at ori602 and ori607 are 5′-AAGGGCAGTCCACACTGCAAAGCTTCCG-3′ and 5′-GATTCTATGTTTTCTAGTACCTACTGCGC-3′, respectively. Band intensities obtained from time course experiments using ori1, ori602, and ori607 were calculated as the relative amounts using the highest and lowest values as 100 and 0%, respectively. Two-dimensional gel analyses were performed as described previously (21).

Cell Cycle Synchronization—Yeast cells were mainly cultured in YPDA medium at 23 °C (10). Synchronization of the cdc15-1 strain (DK329-10a) from late metaphase was done by shifting the temperature down to 23 °C after the cells had been incubated at 37 °C for 2 h. RM14-3a cells were synchronized at the G1/S boundary following the methods described previously (18, 21). W303-1A and SKY009 cells were synchronized at G1 phase by arresting cell cycle progression at this stage with a-factor peptide (2 μg/ml for 2.5 h) followed by exchange of medium at 23 °C to that without α-factor but containing 50 μg/ml Pronase (Calbiochem-Novabiochem). Cell cycle distribution was monitored by measuring the DNA content of cells using FACS analyses as described previously (17).

RESULTS

UV Footprinting Profiles of ORC-ACS Complexes at ori1, ori602, and ori607—We have analyzed UV footprinting profiles of ORC-ACS complexes at various replication origins that fire at different times during S phase. Chromosomal DNA was purified from exponentially growing haploid yeast cells immediately after UV irradiation. Using the DNA as a template, we have performed primer extensions to identify sites of pyrimidine dimer formation at these ACS regions. As shown previously, the intensity of a band located within the T cluster of the A element of ori1 was significantly reduced (Ref. 10; Fig. 1A, left panel, arrowhead). This reduction was not observed in DNA produced from cells exposed to UV light at the non-permissive temperature (10). Furthermore, the addition of purified ORC to the naked ori1 DNA produced a band profile that was identical to that of the in vivo footprint (Ref. 10; Fig. 1A, right panel). Therefore, the in vivo footprinting patterns demonstrate pro-
FIG. 2. Transitions between pre- and post-RC at the three origins after release from the cdc15-1 arrest point. DK329-10a (cdc15-1) cells were arrested at anaphase (cdc15; cdc15-1 arrest point) and released as described under “Experimental Procedures.” A, FACS analyses of the DNA content in cells withdrawn for analyses at the indicated time points. B–D, the time course footprints of ori1, ori602, and ori607. ND, naked DNA; log, DNA from asynchronous growing cells. E, relative intensities of the bands indicated with arrowheads at the indicated time points calculated as described under “Experimental Procedures.”
tection against pyrimidine dimer formation by ORC binding to ori1 as described previously (10).

We also examined whether the same ORC footprint could be obtained at other replication origins by this method. We chose ori602 and ori607 as typical late and early origins on chromosome VI. As with ori1, we found there was a significant reduction in intensity of bands located at the 3'-ends of the T clusters within the ACS of ori602 and ori607 in asynchronous growing cells (Fig. 1, B and C, left panels, with arrowheads). For ori602, a band corresponding to the 5'-end of the T cluster was also protected region to the same extent as that of the 3'-end (Fig. 1B). To address whether the protection of ori602 and ori607 was because of ORC binding, we performed UV footprinting in vitro with purified ORC proteins and plasmids carrying the various origins (Fig. 1, B and C, right panels). The same ATP-dependent protection profiles were observed, indicating that ORC binds specifically to ori602 and ori607 in vivo to produce the characteristic protection patterns in the UV-footprinting experiments as observed for ori1.

Time Course Footprinting of Various Origins in Synchronously Growing Cells—We have reported that the UV-footprinting pattern of ori1 switches between pre- and post-replicative states in a cell cycle-dependent manner (10). We wanted to determine whether other replication origins had similar patterns of cell cycle-dependent switching. Yeast cells growing synchronously after release from three different cell cycle arrest points (late M, G1, and G1/S transition) were collected at defined time intervals, washed quickly once in phosphate-buffered saline, and immediately irradiated with UV light (Figs. 2–4). Because UV footprinting does not require the preparation of spheroplasts, we were able to study accurately the formation of protein-DNA complexes in cells without any time lags. We took advantage of this to perform time course footprinting for ori1, ori602, and ori607. Although only one representative result from each experiment is shown, it should be noted that the time course experiments were performed at least twice and that essentially the same results were obtained each time.

Transition of the Footprinting Profiles at ACSs in Synchronously Growing Cells Released at Late M Phase—cdc15-1 (DK329-10a) temperature-sensitive cell cycle mutant cells were incubated at the non-permissive temperature for 120 min to arrest the cells in late M phase. The cells started to grow synchronously after a temperature shift-down (release) as shown by FACS analysis (Fig. 2A). DNA samples extracted

![Image](http://www.jbc.org/)

**Fig. 3. Conversion between pre- and post-RCs after release from the cdc7-1 arrest point.** RM14-3a (bar1, cdc7-1) cells were arrested at the G1/S boundary (Cdc7; cdc7-1 arrest point) and released for the time course footprints as described under "Experimental Procedures." A, FACS analyses of the DNA content in cells withdrawn at the indicated time points. B–D, the time course footprints of ori1, ori602, and ori607. ND, naked DNA; log, DNA from asynchronous growing cells. E, relative intensities of the bands indicated with arrowheads at the indicated time points are shown as in Fig. 2E.
from the cells at the indicated time points after the release were purified and analyzed in footprint experiments using ori1-, ori602-, and ori607-specific primers as shown in Fig. 2, B–D. The intensity of a band within the A element of ori1 (indicated by an arrowhead in Fig. 2B) at the cdc15-1 arrest point was weaker compared with the one obtained using naked DNA. This indicates that there was strong protection against thymine dimer formation at this site through ORC binding during this phase of the cell cycle. The same decrease in intensity of this band in the ACS regions of ori602 and ori607 was also observed (Fig. 2, C and D). Time course footprints of these replication origins show clear increases followed by decreases in the intensities of the bands associated with cell cycle progression. Band intensities were quantified, and transitions in their relative intensities during cell cycle progression were calculated. The weakest values (0%) were assigned to the band intensities at the arrest points for ori602 and ori607 and 20 min after release from arrest for ori1. The strongest values (100%) were assigned to the band intensities at 55 min for ori1 and ori607 and 85 min for ori602 (Fig. 2E). We have previously shown that the increased band intensity at the A element represents the pre-RC state at the ACS region (10). Thus, increases in band intensities indicate a shift in the state of the ACS in the cell population from post-RC to pre-RC.

The increase in band intensities at the three origins started almost simultaneously between 20 and 35 min after release.

**Fig. 4.** Transitions between pre- and post-RCs and two-dimensional gel analyses of the three origins after release from the a-factor arrest point. W303-1A (wild type) cells were arrested at G1 phase (α; a-factor arrest point) and released as described under “Experimental Procedures.” A, FACS analyses of the cells used for time course analyses. Peak transitions of cells between 1N and 2N are shown in the lower panel of F. 1N and 2N positions were determined on asynchronously growing cells (log). B–D, the time course footprints of ori1, ori602, and ori607. Relative intensities of the bands indicated with arrowheads at the indicated time points are shown in the upper panel of F as in Fig. 2E. log, DNA from asynchronous growing cells; α, DNA from cells arrested by α-factor. E, two-dimensional gel analyses of replicative intermediates at ori601/2 and ori607 regions with DNA obtained from the same samples used in the time course footprints. The potential initiation and replication time zone on ori601/2 and ori607 judged by two-dimensional gel analyses are graphically represented with red and blue slanted bars in the middle of F, respectively (see “Results”).
Fig. 5. Effect of clb5 deficiency on firing and transition between pre- and post-RCs at early and late origins. A, two-dimensional gel analyses of replicative intermediates at ori601/2 and ori607 regions in asynchronously growing clb5-deficient cells (Δclb5; SKY009) and wild type cells (wild; W303-1A). Asynchronously growing SKY009 cells (log) were arrested at G1 phase (α, α-factor arrest point) and released as for W303-1A (Fig. 4). B, FACS analyses of cells used for analyses. Their peak positions between 1N and 2N are plotted in the lower panel of G as described in.
and before cytokinesis as shown by FACS analysis (Fig. 2, A and E). The transition profiles of the bands at the early replicating origins, ori1 and ori607, were almost the same and showed sharp peaks at ~50 min after release, just before entry into G1 phase (Fig. 2, B, D, and E). The band intensities decreased quickly before the start of bulk DNA replication (65 min) and reached levels at early S phase (75 min) that were similar to those at the start of the experiment. The profile of the late replicating origin, ori602, was slightly different (Fig. 2, C and E). The intensities of two bands in the ACS started to increase at the same time and rate as those for ori1 and ori607 but remained high for up to 75–85 min after release. This period corresponds to the middle of S phase, when both ACS bands for ori1 and ori607 had decreased to their initial levels. The bands for ori602 gradually decreased in intensity by G2 phase but never reached their initial level during the time course of the experiment.

Transitions in the Footprinting Profiles at ACSs in Synchronously Growing Cells Released at G1/S—To study the different footprint transitions at individual origins more precisely and especially to gain more information about the transition from pre- to post-RC during progression from the G1/S boundary to S phase, time course footprints were done with cells synchronized at the G1/S boundary (Fig. 3). Temperature-sensitive cdc7-1 mutant cells were first arrested in G1 phase by treatment with α-factor and then allowed to progress to the G1/S boundary where they were arrested by incubating the cells at the non-permissive temperature for 90 min after having treated them in parallel with Pronase. After a shift-down of the temperature (release), the arrested cells progressed from the G1/S boundary to G2 phase synchronously by as shown by FACS analysis (Fig. 3A). The time course footprints for ori1, ori602, and ori607 were carried out as described above with cells withdrawn at the indicated time points after release (Fig. 3, B–D). The relative intensities of bands across the time course were calculated using the lowest intensities (35 min for ori1, 45 min for ori602, and ori607) as 0% and the highest intensities (0 min for ori1 and ori602 and 5 min for ori607) as 100% (Fig. 3E).

At the G1/S boundary arrest point (Cdc7), the bands of interest within the A element of ori1 and within the ACS of ori602 and ori607 (arrowheads) had stronger intensities than those of asynchronously growing cells (log phase) (Fig. 3, B–E). This indicates that these origins in the cells arrested at the G1/S boundary are in the pre-RC state as suggested by experimental results described above (Fig. 2). Upon release from the G1/S boundary, the band intensities decreased within 5 min for ori1 and ori607 (Fig. 3, B, D, and E). For ori602, the decrease in band intensity was delayed slightly and started between 5 and 15 min (Fig. 3, C and E). Band intensities of all origins reached their lowest levels during S phase. This corresponded to 25 min after release for ori607, 35 min for ori1, and 45 min for ori602 (Fig. 3E). The transition from pre- to post-RC for ori607 was slightly earlier than that for ori1. The transition time for ori602 was the latest, which occurred in the middle of S phase. The transition time for each origin was consistent with its order of replication as determined by two-dimensional gel electrophoresis or density transfer experiments in the same synchronization background (20, 21).

Comparison of Origin Firing Times in Synchronously Growing Cells Released at G1, with the Transition Times of Their ACS-footprinting Profiles—Next, we studied the relationship between the transition from pre- to post-RC and actual firing at individual replication origins in W303-1A (wild type) cells, synchronized by release at G1 phase after α-factor arrest (Fig. 4). We also investigated whether the transitions in the footprinting patterns were because of the effect of mutations used in the above experiment. The arrested cells passed through G1 phase synchronously and entered S phase 30–40 min after release as shown using FACS analysis (Fig. 4, A and F). Time course footprints for ori1, ori602, and ori607 were obtained as above (Fig. 4, B–D), and the relative intensities of their specific bands were plotted as in Figs. 2E and 3E (Fig. 4F).

Bands intensities for these origins were stronger in G1-arrested cells (0 min) than in asynchronously growing cells (log). This indicates that the origins in early G1 phase cells are present in the pre-RC state as observed above. Upon release, the band intensities for the early origins stayed at the same level through G1 phase and then started to weaken between 30 and 40 min after release and declined to the level of asynchronously growing cells within a further 20 min (Fig. 4, A, B, D, and F). The band intensity for the late origin, ori602, was still high even after 50 min, which corresponds to the middle of S phase (Fig. 4, C and F). At this time, the early origins had already entered the post-RC state. A gradual decrease in band intensity of ori602 started between 50 and 60 min. The band intensity reached its lowest level at 80 min, which corresponded to the end of S phase as judged by FACS analysis (Fig. 4, A, B, and F). We also observed a synchronized second increase in band intensities at all origins at 120 min, just before cytokinesis (Fig. 4). These results are consistent with the previous two time course foot printing experiments using different synchronization procedures (Figs. 2 and 3) and confirm that the early and late origins are intrinsically different with respect to the timing of the switch from the pre-RC state to the post-RC state.

To measure the time window of origin firing and fork passage, replication intermediates from the ori602 and ori607 regions were analyzed by two-dimensional gel electrophoresis using the same synchronized cells prepared for the time course footprints (Fig. 4E). Because the distance between the ACSs of ori601 and ori602 is only 240 base pairs and their firing events cannot be distinguished by two-dimensional gel analysis, they have been considered as a single origin (20, 21). As shown in Fig. 4E, both Y- and bubble-arcs appeared at 60 and 80 min, respectively, in the ori602 region. This indicates that this region replicates autonomously through firing or passively through fork progression from 40 to later than 80 min at most or between 60 and 80 min at least (a red bar in Fig. 4F). On the other hand, only a bubble arc appeared at ori607 at 40 min (Fig. 4E), suggesting that firing at this origin occurs at most between 20 and 60 min or at least at 40 min (a blue bar in Fig. 4F). These time windows for origin firing are consistent with the period required to convert the cell population from the pre-RC to the post-RC state for ori602 and ori607 (Fig. 4F). Therefore, the pre- to post-RC transition times as determined by UV-footprinting at these replication origins are entirely consistent with the timing of firing at these origins.

The Transition at the Late Origin, ori602, Occurs Independently of Its Firing in clb5-deficient Cells—It has been reported that late-replicating origins are inactivated in clb5-deficient cells (19). Indeed, bubble arcs were undetectable, and only Y-areas were detectable around the ori602 region as determined using two-dimensional gel electrophoresis analysis in clb5-de-
Replication Complex Architecture on Yeast Chromosomes

We have shown by time course footprinting of ori1, ori602, and ori607 that replication complex architecture at these sites shifts between that of a pre-RC and a post-RC in a cell cycle-dependent manner. This is the first report showing transition of the architecture directly in synchronously growing budding yeast cells. Previous comprehensive studies using chromatin immunoprecipitation assays show that replication complexes undergo scheduled protein assembly at budding yeast origins (6–8). However, in contrast to the former experiments, our UV-probing based method allows a more direct analysis of the DNA structural changes associated with replication complex transitions at origins and facilitates the study of the dynamic changes that occur in these complexes during cell cycle progression. Furthermore, the method allowed us to correlate the timing of the shift in the replicative complex architecture with the appearance of replicative intermediates.

Strong protection against thymine dimer formation at the T-cluster in the ACS, which appeared from the middle of S phase to late M phase, indicated persistent ORC binding and the establishment of the post-RC state. From M to G1 phase, there was decreased protection, indicating that the binding shifts to the G1 mode. Because our previous data indicate that MCM proteins and Cdc6p are required to maintain the G1 mode, their association with the ORC-ACS complex probably induces this shift (10). Our results showed that the shift occurs after the cdc15-1 arrest point and before the start of cytokinesis. This corresponds to the time of MCM protein-loading onto replication origins, a process that depends on the accumulation of Cdc6p and the inactivation of cyclin-dependent kinase (7–9). This means that a shift in the UV-footprinting profile may represent a topological change in the origin DNA induced by the assembly of proteins at these sites as has already been observed by chromatin immunoprecipitation or chromatin fractionation assays. Therefore, the accumulation of Cdc6p and inactivation of cyclin-dependent kinase apparently induces the transitions within the RCs. The finding that transitions start almost simultaneously and proceed at the same rate at both early and late origins suggests that the signals that initiate the structural changes (pre-RC formation signals) are distributed over the whole chromosome and that they have even access to all origins at the same time once the cell cycle reaches the appropriate time point.

The timing of transition from a pre- to a post-RC differed between early and late origins. After formation of the pre-RC through the loading of MCM proteins onto the existing ORC-ACS complex, other factors, for example, Cdc45p, Cdc7-Dbf4 protein kinase, S phase cyclin-dependent kinase, replication protein A, polymerase α and ε are predicted to act on the origins to initiate DNA synthesis (Refs. 8 and 22–25 and reviewed by Kelly and Brown (26)). Because the firing of the origin is linked tightly to the transition from the pre- to post-RC state, it is possible that the association of these factors with the pre-RC triggers the architectural shift as well as origin firing. Once the early origins, ori1 and ori607, begin to shift to the post-RC state, the transition at these origins in the whole cell population is completed within a short time span (10–20 min) at the beginning of S phase. In contrast, the late origin, ori602, was still in the pre-RC state at this time, and its transition started in the middle of S phase and took more than 30 min. This slow start and slow shift indicates that the triggering signal has slow access the late origin probably caused by some masking mechanism. It has been shown that late origins, including ori602, can essentially behave as early origins if they are harbored on yeast plasmids (21, 27). Therefore, it is believed that the delay in late origin firing may depend on the origin location within the nucleus, its chromatin structure, or its association with the nuclear matrix. To elucidate the exact mechanism, it will be necessary to compare the effects of these factors on early and late origin firing.

In clb5-deficient cells, firing of ori602 was suppressed, as reported for other late origins (19). This suppression was not because of failure to form an ORC-ACS complex or pre-RC without Clb5p at the late origin, because we observed a normal footprinting profile at ori602 in the mutant Clb5p cells. ORC binding and pre-RC formation at a known dormant origin, ori301, have been reported (28). Thus, irrespective of their firing activity, all potential origins create pre-RCs at their ORC-ACS complexes in response to the pre-RC formation signal. What is the mechanism that suppresses late origin firing in Clb5p-deficient cells? Loading of Cdc45p onto chromatin has been shown to be delayed in the absence of Clb5p and Clb6p activity (9). This means that the ordered assembly of the triggering signal, including that of Cdc45p, may depend on S phase-specific cyclin-dependent kinase activities. Thus, deficiency in clb5 may cause a selective delay in the assembly of triggering signals at late origins. Loci associated with late origins may thus replicate through fork passage from distantly located origins if delays occur in the firing of local origins.

Even in the clb5-deficient cells with an inactive ori602, the shift from a pre- to a post-RC still occurred at the origin. This suggests that in addition to direct firing, there exists a secondary mechanism controlling the shift from the pre-RC state at a late origin. The timing of the shift at ori602 correlated exactly with the appearance of the Y-arc in this region. Thus, we propose that passage of replication fork from an extraneous origin into a late origin region could work as a secondary mechanism to change a pre-RC into a post-RC. This hypothesis...
leads to a plausible mechanism about why replication of all chromosomal regions occurs only once in a cell cycle. If a pre-RC at a late origin were maintained even after the passage of a replication fork, the origin could fire again within the same S phase, leading to more than one replication cycle of the origin region. Our observations suggest that this risk is averted at late origins by the conversion of pre-RCs into post-RCs through replication fork passage. This idea is supported by an experiment that examined the transition from a pre- to a post-RC at ori602 in hydroxyurea-treated cells. As expected, without replication fork progression from early origins, no transitions were observed at ori602, as reported previously for other late origins (12).

We have shown that the transition of replication origins from the pre- to post-RC state is tightly linked to either direct origin firing or passive replication of the origin by a replication fork from a distantly located origin. We assume in both cases that replication fork movement at or around the origins directly induces association or dissociation of some factor(s), which then trigger the change in the replication complex architecture at these sites. Future studies to reconstitute pre-RCs and reproduce their transition in a cell free system will be necessary to elucidate the exact mechanism of origin triggering.

Acknowledgments—We thank Drs. H. Araki, B. Stillman, and B. Brewer for plasmids and strains.

REFERENCES

1. Brewer, B. J., Diller, J. D., Friedman, K. L., Kolor, K. M., Raghuraman, M. K., and Fangman, W. L. (1993) Cold Spring Harbor Symp. Quant. Biol. 58, 435–434

2. R. Tadokoro and C. Obuse, unpublished results.
Scheduled Conversion of Replication Complex Architecture at Replication Origins of *Saccharomyces cerevisiae* during the Cell Cycle

Ryusuke Tadokoro, Masako Fujita, Hitoshi Miura, Katsuhiko Shirahige, Hiroshi Yoshikawa, Toshiki Tsurimoto and Chikashi Obuse

*J. Biol. Chem.* 2002, 277:15881-15889.  
doi: 10.1074/jbc.M200322200 originally published online February 12, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200322200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 31 references, 14 of which can be accessed free at http://www.jbc.org/content/277/18/15881.full.html#ref-list-1