The Cdc7p protein kinase in the budding yeast \textit{Saccharomyces cerevisiae} is thought to help trigger DNA replication by modifying one or more of the factors that assemble at replication origins (ARSs). To investigate events catalyzed by Cdc7p, we compared the structure of replication origins in cells containing conditional mutations in Cdc7p and Cdc8p, a thymidylate kinase that is required for DNA synthesis. High resolution genomic fingerprinting indicated that the presumptive lagging strand template in ARS1 became highly sensitive to KMnO$_4$ modification after the \textit{CDC7} execution point. These results suggested that Cdc7p triggers DNA unwinding. The transition from late G$_1$ phase to the \textit{CDC7} execution point and from the \textit{CDC7} to the \textit{CDC8} execution points was accompanied by small but ARS-dependent changes in DNA topology. These results suggested that DNA unwinding before the \textit{CDC8} execution point either is highly localized or that the torsional stress associated with initial DNA unwinding is minimized by compensatory protein-DNA structural changes. The ARS DNA structural attributes evident in cells blocked at the \textit{CDC8} execution point were also evident in \alpha-factor-blocked, G$_1$ phase cells containing the \textit{CDC7} bypass mutant mem5\textit{cdc}46-bo\textit{b}1. This result strongly suggests that the structural changes during the transition from the \textit{CDC7} to \textit{CDC8} execution points depend on the Cdc7p protein kinase and involve alteration of the minichromosome maintenance protein complex.

Faithful transmission of genetic material from one cell generation to the next requires that DNA be precisely duplicated each cell cycle. Consequently, cells possess mechanisms that trigger DNA replication at a discrete point in the cell cycle and delay the onset of mitosis until replication is complete. Cells also possess mechanisms that coordinate the firing of multiple discrete replication origins with respect to one another during S phase and inhibit re-replication before mitosis. Some of these regulatory pathways directly affect the assembly or activation of the multisubunit replication initiation complexes that form with replication origins. Replication origins in the budding yeast \textit{Saccharomyces cerevisiae} were first identified by their ability to promote replication of episomal DNA (1). Many of these autonomously replicating sequences (ARSs)$^1$ were shown subsequently to act as replicators in their native chromosomal loci (1), and recent replication initiation mapping studies indicate that DNA synthesis begins at one or a few discrete sites within ARSs (2, 3).

Early analyses indicated that ARSs are composed of an essential A domain containing the conserved ARS consensus sequence (ACS; Ref. 4) and a B domain. The archetypal ARS1 also contains a C domain (5, 6), which is packaged in a uniquely positioned nucleosome. This nucleosome may reduce the probability that the adjacent ACS will be packaged into a nucleosome capable of suppressing ARS activity (cf. Ref. 7). Marahrens and Stillman (8) found later that the B domain of the ARS1 could be subdivided into a B3 element that is bound by the transcription factor Abf1p (cf. Ref. 9), a B2 element thought to serve as a DNA-unwinding element (10, 11), and a B1 element. The B1 element acts in conjunction with the ACS to bind a six-polypeptide origin recognition complex (ORC; Ref. 12). ORC is required for the initiation of replication (13, 14) and possesses an ATPase activity that is modulated by origin binding (15). Although the functional significance of this ATPase activity is unknown, one role of ORC is to help recruit additional components of the replication initiation complex. Specifically, ORC-ARS complexes recruit Cdc6p during late mitosis or early G$_1$ phase; this is followed by recruitment of a 6-subunit complex consisting of MCM proteins 2 through 7 (16). These proteins make up the “pre-replicative complex,” which protects a large segment of ARS DNA from DNase I (17). Another essential factor, Cdc45p, joins the already-formed pre-replicative complex later in G$_1$ phase to form the “pre-initiation complex” (18, 19). The single-stranded DNA-binding protein RPA, DNA polymerase $\epsilon$, and presumably other replicative enzymes as well join the pre-initiation complex at about the time of origin firing (18, 20) to form the replicative complex. Origin firing appears to involve loss of the MCM2-7 complex and Cdc45p from the pre-initiation complex and redistribution of Cdc45p and some of the MCM proteins to the nascent replication forks (18). This last finding together with the discovery that an MCM subcomplex possesses helicase activity in vitro suggested that an MCM subcomplex serves as the replicative helicase in yeast (21). The recent discovery that an MCM-like protein in \textit{Methanobacterium thermoautotrophicum} not only possesses helicase activity but also assembles into a double hexamer (22) suggests that MCM complexes are highly functionally conserved and serve as replicative helicases in all eukaryotes. After origin firing, a “post-replicative complex” remains asso-

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$^1$ The abbreviations used are: ARS, autonomously replicating sequences; ACS, ARS consensus sequence; ORC, origin recognition complex; HU, hydroxyurea; MNase, micrococcal nuclease; bp, base pair(s); MCM, minichromosome maintenance; RPA, replication protein A.
ciliated with ARS DNA through the remainder of S phase and into the G2 and M phases; the post-replicative complex has an altered DNase I profile and may consist solely of ORC and Abf1p bound to ARS1 DNA (17).

The sequence of events described above is tightly regulated by Cdc28p, a cyclin-dependent kinase (CDK) protein kinase required for commitment of cells to a program of replication and cell division (reviewed in Ref. 23) and by cyclin-dependent kinase (CDK)-like protein kinases, notably Cdc7p in association with its cyclin-like partner Dbf4p (24–27). During late G1 phase, the Cdc28p kinase in association with the Cln cyclins phosphorylates the B-type (Cib) cyclin inhibitor Sic1p, which renders it susceptible to ubiquitin-mediated proteolysis (28, 29). Cyclin-dependent kinase phosphorylation of Cdc6p during late G1 renders it susceptible to proteolysis as well (30, 31). Loss of Cdc6p and phosphorylation of MCM proteins prevents their re-association with ARS-ORC complexes until late in mitosis, when the Clb cyclins are degraded. This sequence of phosphorylation and proteolysis events makes activation of replication complexes a unidirectional event and helps prevent re-replication before mitosis. The discovery that the Cdc7p-Dbf4p kinase complex physically associates with individual replication initiation complexes (32, 33) and acts throughout S phase to trigger the firing of individual origins (34, 35) suggests that Cdc7p directly modifies one or more factors in the replication initiation complex. This inference is supported by in vitro evidence that the Cdc7p-Dbf4p kinase modifies Mcm 2, 3, 4, 6, and 7 as well as the largest subunit of DNA polymerase α-primase (33, 36, 37). Cdc7p-Dbf4p homologs in Schizosaccharomyces pombe, Xenopus laevis, and humans appear to have similar in vitro substrate preferences (38–43). It is noteworthy, however, that a specific Cdc7p bypass mutation in S. cerevisiae, mcm5/cdc46-bob1, resides in Mcm5p (44), a subunit of the MCM complex that is not efficiently phosphorylated in vitro by Cdc7p (33). Thus, it is not entirely clear if the in vitro targets of Cdc7p are identical to its in vivo targets. Therefore, the consequences of the action of Cdc7p at replication origins are still unclear. What is clear is that Cdc7p plays a critical role in activation of replication, and in this paper we have investigated structural changes at replication origins that can be attributed to the action of Cdc7p.

Previous in vitro studies of cell cycle-regulated events at ARSs have used cdc mutants, the phenome α-factor, the replication elongation inhibitor hydroxyurea (HU), and the microtubule-destabilizing agent nocodazole to block cells at selected points in the cell cycle (e.g. Refs. 17 and 45). Cells blocked with α-factor or at the Cdc7p execution point exhibit a post-replicative complex footprint, whereas cells blocked with HU or nocodazole exhibit a post-replicative complex footprint (17). To further dissect events that occur after the Cdc7p execution point but before the HU-sensitive step, we investigated the use of additional cdc mutants whose execution points have been mapped to within the Cdc7p-HU interval. One such mutant is cdc8-1, which encodes a thymidylate kinase required for DNA synthesis (46, 47). cdc8-1 mutants display a quick stop replication arrest when shifted to the restrictive temperature and resume DNA synthesis after their return to the permissive temperature. This rapid and reversible phenotype and the fact that Cdc8p is required shortly after Cdc7p (48) suggested that the study of cdc8-1-blocked cells might reveal structural events that could be attributed directly to the action of Cdc7p. The ARS1 footprints and DNA topology in cdc8-1-blocked cells proved to be distinct from those in cdc7-1-blocked cells and suggest that localized DNA unwinding occurs before the CDC8 execution point. The cdc8-1 block is fully reversible, suggesting that the structure evident in cdc8-1-blocked cells reflects a bona fide replication intermediate. The DNA topology as well as micrococcal nuclease (MNase) cleavage and Kmnt4 modification patterns characteristic of cdc8-1-blocked cells were also evident in both mcm5/cdc46-bob1 single mutants and cdc7Δ mcm5/cdc46-bob1 double mutants that had been blocked in G1 phase by a-factor. Since mcm5/cdc46-bob1 is a CDC7- and DBF4-specific bypass mutant, this result strongly suggests that the structural changes evident in cdc8-1-blocked cells are normally induced by the Cdc7p kinase and involve changes in the MCM complex.

EXPERIMENTAL PROCEDURES

Plasmids and Cell Strains—Plasmid pJL347 (provided by J. Li, University of California at San Francisco) was used in epifocal footprinting and toposomer studies and contains a centromere (CEN6), a selectable marker (URA3), and the A and B domains of ARS1. Plasmid pMD-2 was used as a footprinting control and was constructed by inserting into the Nhel site of pJL347 a second copy of ARS1 rendered non-functional by a G to T substitution in the ACS. Plasmid pKW50N (provided by M. Gartenberg, University of Medicine and Dentistry of New Jersey) contains the 2-μm ARS, URA3, and an excision cassette that consists of a 2480-bp BglII-XhoI fragment from the yeast LYS2 gene flanked by direct repeats of a 58-bp Euglenochromosomes rousii recombinase recognition sequence (49). Co-transfection of pKW50N and the recombinase expression vector pHM153 (50) followed by induction of recombinase expression leads to excision and recircularization of the excision cassette to form an ARS-less plasmid that was used as a control in the toposomeroid experiments. Plasmids were transfected by the method of Ito et al. (51) into S. cerevisiae strain TCK1 (W3031A; bar1::HIS3) (provided by J. Kurjan, University of Vermont), strains Y236 (MATa bar1 his6 ura3 trpl leu2 cdc7-1), Y728 (MATa bar1 his6 trp1 ura3 leu2 can1 cdc8-1) (provided by R. Sclafani, University of Colorado Health Science Center), and strain MYY19 (MATa lys2 ura3-52 his3 112 leu1-2, 112 cyh2 cdc7-1::HIS3 mcm5/cdc46-bob1 gal1 bar1::LEU2). MYY19 was derived from strain P211 (from R. Sclafani) by disruption of the BAR1 gene.

Genomic Footprinting—The procedures used for genomic footprinting were shown previously to faithfully reflect protein-DNA interactions in cells (52–54), and the results for each cell strain or cell cycle point examined in this paper are based on at least three independent experiments. Briefly, cells were grown to early to mid-log phase (1–2 × 10⁷ cells/ml) in synthetic complete medium that lacked nutrients required for plasmid selection and contained 25 mM phthalate (pH 5.5 with NH4OH). Nuclei were isolated and treated with varying amounts of MNase, and DNA was isolated. The overall extent of digestion of chromatin and naked DNA control samples was estimated by agarose gel electrophoresis (not shown). Samples digested to approximately equal extents were selected for footprint analyses and cleaved with NolI and EcoRV. The footprint of template molecules that support primer extension to either the Nhel or EcoRV sites provided a direct measure of the extent of nucleosome cleavage within the ARS1-DNA-containing region. Cleavage of templates with NdeI and EcoRV also provided a pre-replicative control for the specificity of the primers used for mapping nucleosome positions. Nucleosome positions in plasmid chromatin DNA control samples were then mapped by polymerase chain reaction-mediated, reiterative extension of end-labeled oligonucleotide primers (5′-GCAAATTTCAGGTGAAATACCTACCGCATTAAAGCTTATGC-3′, 5′-AAATGATGAATGTTAAAGGATCGTGTGGGCT-3′, 5′-GCGAGACATCAGGGCCAGGAAGTGCTGCCGCTGC-3′, and 5′-GCCGATAGTTGACATCAAGGGCATATC-3′), as described (55). Genomic footprints of ARS1 in chromosome IV were mapped using chromosome-specific primers 5′-AAATGGCCTATTTGCTTGATGTAAGGCGAGGT-3′ and 5′-TGCGGCGGTTAAATGTTAAGGACCCCTCGGATGT-3′. Extension products were resolved by electrophoresis on sequencing gels and visualized by autoradiography with Kodak X-Omat film. Cleavage sites were mapped by comparison with sequence ladders generated in parallel polymerase chain reactions using the same end-labeled primers and linearized plasmid DNA templates.

For cell cycle experiments, strains were grown at 23 °C to 1–10⁷ cells/ml and blocked in G1 phase by the addition of α-factor (Sigma) to a concentration of 20 nm. After 3 h, unblocked cells were collected by centrifugation and resuspended in fresh 37 °C media containing 0.1–1 mg/ml protease (Sigma). After 1 h at 37 °C, at least 90% of cells had reached their characteristic terminal phenotype, as judged by microcopy (56, 57) and were harvested for footprint analyses as above.
that elevated temperatures were used to sustain the blocked state until the moment of cell lysis.

KMN0 footprinting was carried out essentially as described by Park et al. (58). Briefly, 50-ml aliquots of yeast cells, grown as described above, were treated with 2–6 mM KMN0 (Sigma) for 1 min at 42 °C. The reaction was stopped by the addition of an equal volume (50 ml) of ice-cold STE buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) containing freshly added 5 mM dithiothreitol. The resulting mixture was chilled on ice, and cells were pelleted at 3600 rpm in RT6000B centrifuge (Sorvall) at 4 °C for 4–6 min, washed with 1 ml of 5 mM Tris-HCl, pH 8.0, and resuspended in 700 mM EDTA, 100 mM Tris-HCl, pH 8.0) containing freshly added β-mercaptoethanol (Sigma). Cells were lysed using glass beads, and DNA was isolated and cleaved with EcoRV. Samples were then treated with a final concentration of 0.1 mM NaOH for 5 min at room temperature and neutralized by the addition of HCl to a final concentration of 0.1 mM. Modification sites in chromatin and naked DNA control samples were visualized and mapped by reiterative extension of end-labeled primers, as described above.

Topoisomer Analyses—Initial studies of pJL347 and TRP1ARS1 topoisomers were performed using DNA isolated from cells grown in glucose-containing media and blocked at selected points in the cell cycle, as described above. However, since galactose is required to induce expression of the Z. rouxii recombinase (50), we also examined pJL347 topoisomer distribution from cells that had been shifted to galactose, as described below, and found that the different carbon sources had no detectable effect on the topoisomer distributions (data not shown).

To examine the topology of plasmids lacking an ARS, cells containing both pKD50N and pHM153 were grown to mid-log phase at 23 °C in synthetic medium containing 2% raffinose. Cells were blocked in G1 phase by the addition of α-factor to 20 nM. After 2–3 h α-factor, recombinase expression was induced by the addition of galactose to a final concentration of 2%. After 1 h in the presence of both galactose and α-factor, α-factor was removed, and half the culture was shifted to 37 °C, whereas the other half was maintained at 23 °C. At appropriate times, cells were lysed with glass beads in the presence of 10 mM N-ethylmaleimide (Sigma), and plasmid DNA was isolated. Plasmid topoisomer distribution by gel electrophoresis for 15 h at 2 V/cm in 0.9–1.4% agarose gels containing chloroquine diphosphate (Sigma). Chloroquine concentrations were varied in trial experiments to ensure that amounts used were sufficient to induce a positive writhe in the topoisomers to be examined (59). After transfer to Protran nitrocellulose membranes (Schleicher & Schuell), the excision cassette, pJL347, pKD50N, and TRP1ARS1 topoisomers were visualized by hybridization with 32P-labeled LYS2, pBR322, and TRP1ARS1 probes, respectively. For each plasmid, individual topoisomers were assigned an integral value, and the amount of each topoisomer was quantified (and corrected for background) using a Bio-Rad phosphoimager. The topoisomer amounts were summed, and the number average distribution was calculated. The number average linking number for plasmids isolated from α-factor-blocked cells at 23 °C was used as a reference value. Thus, the ΔLk reported in Table I for plasmids in other cell types or at other points in the cell cycle refers to the linking number change relative to the linking number in α-factor-blocked cells. Each ΔLk reported is based on the average value of at least three separate experiments.

RESULTS

Alterations in the ARS1 Genomic Footprint during the Cell Cycle—As outlined in the Introduction, DNA replication requires the assembly and activation of initiation complexes followed by DNA unwinding by helicases, stabilization of unwound DNA by single-stranded DNA-binding proteins, and synthesis of an RNA primer by DNA primase. To link these events to factors that control cell cycle progression, we examined the chromatin structure of ARS1 in both its native location in chromosome IV and in an episomal plasmid, pLS47, at specific points in the cell cycle. Cells were blocked either in G1 phase by α-factor (a) or in S phase with hydroxyurea (HU). We also blocked cells at selected points in the cell cycle using cell strains containing temperature-sensitive mutations in either Cdc28p, Cdc7p, or Cdc8p. To ensure maximal viability of mutants used in this study and thereby avoid possible artifacts associated with cell death, cells were first blocked in G1 phase with α-factor and then released to restrictive temperature for just 1 h. The viability of both cdc7-1 and cdc8-1 mutants subjected to this regimen exceeded 85%. We also compared nuclease cleavage patterns in wild type cells arrested with α-factor at both permissive and restrictive temperatures (23 °C and 37 °C, respectively). No temperature-dependent differences were evident (data not shown). Thus, any alterations in the chromatin structure evident in cdc7-1 and cdc8-1-blocked cells could be ascribed to loss of Cdc7p or Cdc8p function rather than to exposure of cells to elevated temperatures before isolation of nuclei. Finally, the cleavage pattern in wild type cells arrested in α-factor was also evident in cdc28 mutants blocked at START and in cdc7-1 and cdc8-1 mutants arrested in G1 phase with α-factor (not shown). These observations ruled out the possibility that structural changes in cdc7-1 and cdc8-1-blocked cells described below are due to defects evident at earlier points in the cell cycle.

Earlier studies of ARS1 chromatin using DNase I as a probe indicated that the characteristic pre-replicative complex footprint in α-factor-blocked cells is also evident in cdc7-1-blocked cells (17), and this proved true for MNase as well (compare cleavages in lanes 5–7 with those in lanes 8–9 in Fig. 1A). Consequently, the footprinting results for these two points in the cell cycle were used as a reference point for later comparisons and are described together here. As expected, comparison of MNase cleavages in nuclei (Fig. 1A, lanes 5–9) with those in naked DNA controls (Fig. 1A, lane 4) revealed evidence of previously documented protein-DNA interactions. On the A-rich strand, for example, the B3 element is flanked by hypersensitive cleavage sites (Fig. 1A, filled circles, lanes 5–9), which, along with the suppression of cleavages within the B3 element, probably reflect the binding of Abf1p. On the same strand, two closely spaced naked DNA cleavage sites within the B2 element also occur in chromatin, but a third is suppressed; there is also a chromatin-specific cleavage site within B2 (Fig. 1A, filled circle, lanes 5–9). The chromatin-specific cleavages in B2 DNA might reflect interactions with Orc2p, as have been observed in vitro (60), or an altered DNA conformation in vivo, reflecting the probable role of B2 as a DNA-unwinding element. Finally, we observed enhanced cleavage of phosphodiester bonds between the B1 element and the ACS on the A-rich strand (Fig. 1A, filled circles, lanes 5–9) as well as partial suppression of cleavages within the ACS and B1 elements. In the T-rich strand, certain phosphodiester bonds that are sensitive to cleavage in naked DNA are also protected in nuclei, whereas two sites within the ACS and two others just 3′ of the B1 element show enhanced sensitivity to cleavage in nuclei (Fig. 2A, compare lanes 7–9 with lanes 5–6). Control experiments indicate that mutations that reduce B1 function or abolish ACS function alter the corresponding B1 and ACS cleavage patterns. For example, two prominent chromatin-specific cleavage sites within and near the wild type ACS (denoted by filled circles in Fig. 1A, lanes 5–9) are protected in an ACS mutant (open circles, Fig. 1B), suggesting that the cleavage patterns observed in the B1 and ACS region of the wild type ARS1 reflect the binding of ORC.

We next compared MNase cleavages of both the A-rich and T-rich strands of ARS1 in cdc8-1-blocked cells with those in cdc7-1-blocked cells. The cleavage pattern on the A-rich strand changed only slightly as cells progressed from the CDC7 to the CDC8 execution point (Fig. 1A, compare lanes 8–9 with lanes 10–11). These changes included the suppression of cleavage at a site within the ACS and enhanced cleavage at a site near the edge of B1 (denoted by stars in Fig. 1A, lanes 10–11). On the T-rich strand of ARS1, the frequency of cleavage at sites within the B2 element on the T-rich strand (indicated by stars adjacent to lanes 12, 15, and 17 in Fig. 2A) increased relative to...
Fig. 1. Genomic footprinting of the A-rich strand of ARS1 at selected points in the cell cycle. A, nuclei containing pJL347 were isolated from wild type cells blocked in G1 phase with α-factor or in S phase with HU, from cdc7-1 and cdc8-1 mutants blocked with α-factor and then released to the restrictive temperature, and from cdc7Δ mcm5-bob1 mutants blocked with α-factor. Isolated nuclei were treated with varying amounts of MNase, and DNA was isolated. Single-strand cleavage sites in the A-rich strand in chromatin and in naked DNA controls (lane N) were mapped by polymerase chain reaction-mediated primer extension, as described under “Experimental Procedures.” Cleavage sites were mapped using sequencing ladders (lane T). Primer extension reactions performed with intact and mock-digested DNA templates (lanes N0 and C0) showed only minimal pausing by Taq polymerase. In the diagram to the right of each panel, open rectangles refer to the ACS, B1, B2, and B3 elements in ARS1. Filled circles mark a subset of the phosphodiester bonds that are hypersensitive to cleavage in nuclei. Stars mark chromatin-specific cleavage sites in cdc8-1- and HU-blocked cells and in α-factor-blocked cdc7Δ mcm5-bob1 cells that differ from those in cdc7-1- and α-factor-blocked cells. B, cleavage sites in a version of ARS1 rendered non-functional by a G to T mutation in the ACS (asterisk) were mapped by genomic
cleavage at a site at the edge of the B2 element (indicated by a filled circle adjacent to lanes 9, 12, 15, and 17 in Fig. 2A) as cells progressed from the CDC7 to the CDC8 execution point. Modest changes in the pattern and relative intensity of cleavages flanking the B1 element also were evident (compare bands adjacent to the vertical bar in lanes 7–9 with those in lanes 10–12 in Fig. 2A). Together, these changes suggested an alteration either in protein-DNA contacts or in DNA structure, particularly at the B2 element, following the action of Cdc7p.

To directly relate the footprinting results to the DNA topology analyses described below, most of the footprint analyses in this paper were done using low copy number plasmids. However, differences similar to those described above for the episomal ARS1 also occurred at ARS1 in its native locus in chromosome IV (Fig. 2B). This result strongly suggests that the alterations in the ARS1 footprints following the CDC7 execution point reflect bona fide structural changes associated with the initiation of replication.

Although previous studies have also documented changes in ARS1 chromatin following the CDC7 execution point, those studies typically compared cleavage patterns seen in cdc7-1-arrested cells with those in HU-blocked cells (e.g., 17). The transition from the CDC8 execution point to the HU-sensitive step led to a pronounced asymmetry between the A- and T-rich strands of ARS1 in their sensitivity to MNase. This can be seen by comparing the intensity of “parental” bands at the tops of lane 13 in Fig. 1A and lane 15 in Fig. 2A (parental bands reflect the fraction of template molecules that have escaped MNase cleavage within the region of interest and can thus be used to estimate local nuclease cleavage rates. The same DNA sample was used in these two lanes to visualize cleavage sites in, respectively, the A- and T-rich strands of ARS1 in their sensitivity to MNase. This can be seen by comparing the intensity of “parental” bands at the tops of lane 13 in Fig. 1A and lane 15 in Fig. 2A (parental bands reflect the fraction of template molecules that have escaped MNase cleavage within the region of interest and can thus be used to estimate local nuclease cleavage rates. The same DNA sample was used in these two lanes to visualize cleavage sites in, respectively, the A- and T-rich strands of ARS1, yet at this point in digestion, virtually all of the T-rich strand molecules have been cleaved, whereas a large fraction of the A-rich strands remain intact. This asymmetry in MNase sensitivity can be attributed to an increase in sensitivity of the T-rich footprinting and compared to cleavage sites in naked DNA controls (lane N1). Filled circles mark four cleavage sites identical to those marked in Fig. 1A; open circles mark two cleavage sites that are prominent in the wild type ARS1 but absent in the ace mutant. C, cells were collected at selected points in the cell cycle and prepared for FACS analysis as described (83).

Fig. 2. Genomic footprinting of the T-rich strand of ARS1 at selected points in the cell cycle. A, single-strand MNase cleavage sites in the T-rich strand of ARS1 in pJL347 were mapped as described in Fig. 1. In the diagram to the right of each panel, open rectangles refer to the ACS, B1, and B2 elements. Filled circles mark a subset of cleavage sites that are hypersensitive in nuclei as compared with naked DNA. Stars indicate DNA cleavage sites that displayed increased MNase sensitivity in cdc8-1-arrested cells, HU-blocked cells, and α-factor-blocked cdc7Δ mem5-bob1 cells as compared with that observed in cdc7-1-arrested cells (nuclease sensitivity was estimated by comparison with a site at the edge of the B2 element, marked with a filled circle: cf. “Results”). A vertical bar marks a segment of DNA that also is cleaved differently at different points in the cell cycle. B, MNase cleavage sites of the T-rich strand in the chromosomal copy of ARS1 were mapped as described above. Filled circles and stars mark the same cleavage sites marked in A.
Structural Changes at ARS1 Catalyzed by the Cdc7p Kinase

Evidence That DNA Unwinding Occurs after the Cdc7p Execution Point—Fig. 3 summarizes the previously identified DNA synthesis start sites (2, 3) as well as sites sensitive to DNase I (12, 17), MNase (this study), and KMnO4 (this study, see below). Interestingly, the major changes in the cleavage pattern of ARS1 DNA after the Cdc7p execution point involve the T-rich bottom strand, whereas only minor differences are evident in the A-rich top strand (Fig. 1A and 2A). One possible explanation for this asymmetric change in cleavage pattern is suggested by the finding that the T-rich bottom strand in this segment of ARS1 serves as a template for lagging strand synthesis, whereas the A-rich top strand serves as a template for leading strand synthesis (2, 3). At an early step in replication, the lagging strand template may have more single-strand character than the leading strand, and unlike DNase I, MNase cleaves single-strand DNA efficiently (61). This line of reasoning suggested that Cdc7p might trigger local DNA unwinding at ARSs.

To directly test whether DNA unwinding occurs after the Cdc7p execution point, we used KMnO4 to probe for single-stranded regions within and near ARS1 at selected points in the cell cycle. KMnO4 preferentially modifies unpaired thymidine (T) residues and, at higher concentrations, unpaired adenine (A) residues. Certain distortions in double-stranded DNA can also lead to enhanced KMnO4 sensitivity (62, 63). In either case, modification sites can be mapped by treating modified residues with NaOH, which creates a lesion that cannot be bypassed by Taq DNA polymerase (64). In exponentially growing, wild type cells, ARS1 DNA was only minimally reactive to KMnO4 (data not shown). This was as expected, since only a small fraction of ARS1 molecules in an asynchronous population of cells is undergoing replication during the 1-min exposure of cells to KMnO4. Likewise, in cells blocked with α-factor in G1 phase and in cdc7-1 mutants blocked at the CDC7 execution point, ARS1 DNA displayed little or no KMnO4 sensitivity (Fig. 4A, lanes 5–9). By contrast, thymidine residues within and flanking ARS1 DNA in HU-blocked wild type cells showed an increased level of KMnO4 sensitivity, most likely due to DNA unwinding (Fig. 4A, lanes 13–14). Sequences within and near ARS1 in cdc8-1-blocked cells were similarly KMnO4-sensitive (Fig. 4A, lanes 10–12). Modified residues in both cdc8-1- and HU-blocked cells included a thymidine that lies within the ORC binding site on the T-rich strand (Fig. 4A, asterisk). KMnO4 modification of thymidine residues outside ARS1 in cdc8-1- and HU-blocked cells suggest fairly extensive DNA unwinding at both of these points in the cell cycle. The KMnO4 modification pattern outside ARS1 in cdc8-1-blocked cells differed somewhat from that in HU-blocked cells (compare lanes 10–12 with 13–14, Fig. 4A), and we were able to generate the HU pattern by returning cdc8-1-blocked cells to the permissive temperature in the presence of HU (data not shown). This result indicated that the cdc8-1 block is fully reversible and occurs before the HU-sensitive step in DNA replication.

To determine if the enhanced sensitivity of ARS1 DNA to KMnO4 following the Cdc7p execution point depends on a functional ARS, we conducted parallel studies with a version of ARS1 rendered non-functional by a point mutation in the ACS (Fig. 1B) (65). In all cell cycle stages tested, including cdc8-1- and HU-blocked cells, this mutation abolished the ARS-specific sensitivity to KMnO4. Sequences 3′ of the non-functional ARS still exhibited moderate KMnO4 sensitivity in HU-arrested cells (Fig. 4B, lanes 12–13), most likely due to a stalled replication intermediate initiated from a nearby, functional ARS. Together, these observations strongly suggest that DNA unwinding at replication origins is CDC7-dependent and requires a functional ARS.

Analysis of DNA Topoisomers at Selected Points in the Cell Cycle—Unwinding of circular DNA produces compensatory positive superhelical stress. This stress may be relieved by the action of DNA topoisomerases, resulting in a change in linking
number or ΔLk (Ref. 66 and references therein). However, particularly if the DNA unwinding in cdc8-1-blocked cells is localized and limited in extent, the resulting superhelical stress might be relieved through changes in local protein-DNA interactions, with little or no ΔLk. Loss of a single nucleosome, for example, would release enough negative superhelical density to compensate for the unwinding of ~10 bp DNA (52, 67–69). To determine if the CDC7-dependent structural changes documented above are accompanied by a linking number change, we examined the distribution of pJL347 topoisomers at selected stages in the cell cycle (Fig. 5 and Table I). DNA topoisomers were resolved by gel electrophoresis in buffer containing chloroquine diphosphate such that the least negative supercoiled DNA topoisomers migrated most rapidly. We first examined the topoisomer distribution in wild type cells and in cdc7-1 and cdc8-1 mutants blocked with α-factor at both 23 °C and 37 °C. Cells were monitored to ensure that the block was efficient at both temperatures. The DNA linking number distribution in cdc7-1 and cdc8-1 mutants blocked with α-factor was virtually identical to that in α-factor blocked wild type cells. This result is in accord with our footprinting results, which suggested that mutations in CDC7 and CDC8 have no effect on replication initiation complexes prior to START. To block cells at the CDC7 and CDC8 execution points, it was necessary to shift mutant cells from 23 °C to 37 °C. Because DNA untwists with increasing temperature (70), DNA isolated from cells incubated at 37 °C will be more negatively supercoiled than DNA isolated from cells grown at 23 °C. The magnitude of this change for DNA in yeast chromatin is approximately −0.008 degree twist/°C/bp (71). Therefore, the predicted in vivo ΔLk for the 6027-bp pJL347 following a shift from 23 °C to 37 °C is −0.008 × (37 °C − 23 °C = 14 °C) × 6027/360° = −1.80 turns. The measured ΔLk associated with a shift from 23 °C to 37 °C in α-factor-blocked cells was −1.68, −1.69, and −1.81 for wild type cells, cdc7-1 mutants, and cdc8-1 mutants, respectively (Table I). These results indicated that cdc7-1 and cdc8-1 mutants respond to temperature-induced superhelical stress in the same fashion as wild type cells. Thus, any ΔLk that accompanied the transition from START to the CDC7 and CDC8 execution points could be attributed to structural changes that occur at those points in the cell cycle.

Between START and the Cdc7p execution point, there was a small but statistically significant decrease in negative superhelicly of approximately +1.6 (Fig. 5A and Table I). KMNO₄ footprint analyses (Fig. 4A) showed no evidence of DNA unwinding during this interval and indeed, had unwinding occurred, we might expect the Lk to remain the same or become more negative. If the observed ΔLk were due to pre-replicative events at ARS1, the ΔLk for different sized plasmids might be similar. To test this prediction, we measured ΔLk values at the
same points in the cell cycle for TRP1ARS1, a multicopy plasmid that is just one-quarter the size of pJL347, and for pKWD50N, a larger plasmid (8278 bp) used in studies to be described below. The TRP1ARS1 and pKWD50N topoisomer distributions both changed between the α-factor and Cdc7p steps in the same manner as for pJL347, but the change was proportional to plasmid size (Table I). This result suggested that the observed ΔLk might be the consequence of cell cycle-specific alterations in chromatin structure rather than structural changes associated specifically with ARS DNA.

The transition from the CDC7 to the CDC8 execution point was accompanied by a small, negative ΔLk for pJL347 (Fig. 5A, Table I). This result coupled with evidence of DNA unwinding from the KMNO₄ studies suggested that the helical stress associated with initial DNA unwinding is either largely inaccessible to topoisomerase or balanced by changes in protein-DNA architecture. By contrast, there was a substantial ΔLk of -4.1 during the transition between the cdc8-1-and HU-sensitive steps (Fig. 5, lane 7, Table I). The change in DNA topology evident in HU-blocked cells was also seen in cdc7-1 and cdc8-1 mutants that first were released from an α-factor block and shifted to the restrictive temperature for 1 h and then returned to 23 °C in the presence of HU (data not shown). These results further demonstrated that the cdc7-1- and cdc8-1-blocked states are fully reversible. The increased linking number in HU-blocked cells was surprising since the predicted further DNA unwinding would be expected to lead to a reduction in linking number (66). Events that may underlie this ΔLk are addressed under “Discussion.” Here, we note only that the substantial ΔLk associated with the transition from the CDC8 execution point to the HU-sensitive step clearly demonstrates that our methods for isolating DNA are capable of capturing changes in DNA topology and that the post-replicative complex defined by the HU block differs from the cdc8-1-blocked state.

Changes in DNA Topology in Late G₁ and Early S Phase Are ARS-dependent—It was possible that the observed cell cycle-specific changes in DNA topology are unrelated to DNA replication. To address this possibility, we transfected cells with a plasmid encoding the Z. rouxii R site-specific recombinase under control of the GAL promoter (50) together with a second plasmid containing recombinase target sites. Expression of the recombinase during G₁ phase results in the formation of a circular, ARS-less plasmid (Ref. 49 and “Experimental Procedures”), cdc7-1 and cdc8-1 mutants and wild type cells containing these plasmids were grown to mid-log phase and blocked in G₁ phase by the addition of α-factor. Recombinase expression was then induced for 1 h in the presence of α-factor, after which α-factor was removed, and cells were either shifted to the restrictive temperature or blocked by addition of HU. Topological analyses of ARS-less circular DNA revealed no changes in linking number between the α-factor-, Cdc7p-, Cdc8p-, and HU-sensitive steps (Fig. 5B, Table I). These results indicate that the topological changes we observed in ARS-containing plasmids are in fact ARS-dependent.

The Structural Attributes in ARS1 DNA Evident in cdc8-1-blocked Cells Are CDC7-dependent—As described in the Introduction, Cdc7p appears to act directly on origin complexes to trigger the initiation of DNA replication. Thus, we wanted to determine if the structural and topological changes evident in cdc8-1-blocked cells were due to the direct action of Cdc7p. Because CDC7 is essential, we took advantage of a strain that can bypass the requirement for Cdc7p by virtue of a mutation in MCM5 known as mcm5/cdc46-bob1 (25, 44). We reasoned that if Cdc7p triggers a structural change by modifying a component of the pre-initiation complex, that change may be evident earlier in the cell cycle in cdc7Δ mcm5/cdc46-bob1 bar1 mutant (strain MVY19). To test this prediction, mid-log phase MVY19 cells were arrested in G₁ phase with α-factor at both 23 °C (Figs. 1A and 2A) and 37 °C (not shown), and ARS1 chromatin structure was examined using genomic footprinting and DNA topology assays. Even though FACS analyses show no indication of premature replication in the α-factor-arrested cdc7Δ mcm5/cdc46-bob1 cells (Fig. 1C; Refs. 33 and 44), the MNase-derived cleavage pattern for ARS1 was distinct from that evident in α-factor-blocked wild type cells and in cdc7Δ-blocked cells and strikingly similar to the pattern seen in cdc8-1-blocked cells (Fig. 1A, compare lanes 14–16 with 10–11 and Fig. 2A, compare lanes 16–17 with 10–12). Thus, the cleavage pattern normally evident only in early S phase is already present in G₁ phase cdc7Δmcm5/cdc46-bob1 cells.

As with ARS1 DNA in cdc8-1- and HU-blocked cells, ARS1 DNA in α-factor-arrested, cdc7Δ mcm5/cdc46-bob1 cells was also sensitive to KMnO₄ (Fig. 4A, compare lanes 15–16 with lanes 5–6). In particular, the same thymidine residue within the ACS that is sensitive to KMnO₄ in cdc8-1 and HU-blocked cells but not in α-factor-blocked wild type cells or in cdc7Δ-blocked cells was KMnO₄-sensitive in α-factor-blocked cdc7Δ mcm5/cdc46-bob1 cells (Fig. 4A, asterisk). It was important as well to ensure that the premature KMnO₄ sensitivity of ARS1 and surrounding sequences in MVY19 cells was due to the mcm5/cdc46-bob1 mutation and not the absence of Cdc7p. We therefore treated α-factor-blocked mcm5/cdc46-bob1 cells with KMnO₄ and observed the same pattern of modification within
Structural Changes at ARS1 Catalyzed by the Cdc7p Kinase

Table I

| Treatment | Relative average Lk No. | ΔLk from previous cell cycle step | Relative average Lk No. | ΔLk from previous cell cycle step | Relative average Lk No. | ΔLk from previous cell cycle step | Relative average Lk No. | ΔLk from previous cell cycle step | Relative average Lk No. | ΔLk from previous cell cycle step |
|-----------|-------------------------|----------------------------------|-------------------------|----------------------------------|-------------------------|----------------------------------|-------------------------|----------------------------------|-------------------------|----------------------------------|
| α-Factor-blocked, 23 °C | 0.0 +/- 0.50 | -0.90 | 0.0 +/- 0.08 | +0.40 | 0.0 +/- 0.13 | -2.17 | 0.0 +/- 0.10 | NA |
| α-Factor-blocked, 23 °C > 37 °C | -1.73 +/- 0.53 | -1.73 (-1.80 predicted) | -0.30 (WT only) | -0.30 (-0.62 predicted) | -2.29 +/- 0.16 | -2.29 (-2.48 predicted) | -0.54 +/- 0.06 | -0.54 (-0.74 predicted) |
| α-Factor-blocked, 23 °C; remove block > Cdc7p step, 37 °C | -0.11 +/- 0.15 | +1.62 | -0.20 | +0.10 | -0.34 | +1.95 | -0.46 | +0.08 |
| α-Factor-blocked, 23 °C; remove block > Cdc8p step, 37 °C | -0.96 +/- 0.19 | -0.85 | -0.40 | -0.20 | -0.15 | +0.17 | -0.46 | 0 |
| α-Factor-blocked, 23 °C; remove block > HU block, 23 °C | 4.83 (WT only) | +4.06 | ND | ND | ND | ND | ~0 | ~0 |

and surrounding ARS1 as before (Fig. 4A). As before, the KMnO₄ sensitivity was dependent on a functional ARS (Fig. 4B). These results suggested that the increased sensitivity to KMnO₄ modification reflects a change in the structure of the MCM complex that ordinarily is induced by Cdc7p.

Because the MNase and KMnO₄ profiles in cdc7Δ mem5Δ cdc46-bob1 cells arrested in G₁ phase with α-factor were similar to those in cdc8-1-blocked cells, we predicted that cdc7Δ mem5Δ cdc46-bob1 mutants might also exhibit premature changes in DNA topology. Although it must be emphasized that the magnitude of the predicted ΔLk was small, plJ347 DNA isolated from cdc7Δ mem5Δ cdc46-bob1 cells arrested with α-factor had a linking number similar to that seen in cdc8-1-blocked cells (Table I). plJ347 DNA isolated from cdc7Δ mem5Δ cdc46-bob1 cells that were released from α-factor and subsequently arrested with HU showed a change in linking number comparable with that in wild type, cdc7-1, and cdc8-1 mutant cells arrested with HU (Table I). Taken together, these results argue that both the DNA structural and topological changes evident in cdc8-1-blocked cells are due to the action of the Cdc7p protein kinase at replication origins.

DISCUSSION

Structure of Replication Complexes in cdc8-1-blocked Cells—In this paper, we have investigated structural events that occur at ARS1 at discrete points in the cell cycle. Following the CDC7 execution point, we observed increased MNase sensitivity of phosphodiester bonds within the probable DNA-unwinding element (B2) and increased sensitivity of ARS1 and surrounding DNA to modification by permanganate (Figs. 1, 2, and 4). Given that permanganate preferentially modifies unpaired thymidine residues and that MNase efficiently cleaves single-strand DNA, our results strongly suggest that ARS1 DNA unwinds following the action of Cdc7p. This inference is consistent with cross-linking experiments that indicate that replication protein A, the eukaryotic replicative single-strand DNA-binding protein, becomes associated with ARSs soon after the Cdc7C execution point (20). As cells progress from the CDC8 execution point to the HU-sensitive step, ARS1 DNA becomes substantially more MNase-sensitive, due principally to an increased rate of cleavage of the presumptive template for lagging strand synthesis (2, 3). These results might reflect arrest before the onset of lagging strand synthesis or at a point during replication when the lagging strand has more single-strand character than does the leading strand. Alternatively, the asymmetric nuclelease sensitivity might reflect an asymmetric distribution of initiation factors or replicative enzymes.

The pattern of nuclelease sensitivity and distribution of topoisomerors in cdc8-1-blocked cells differs from that in both cdc7-1- and HU-blocked cells. Additionally, the topoisomer distribution and nuclease sensitivity characteristic of HU-blocked cells becomes evident after releasing cdc8-1-blocked cells into HU-containing media. These observations suggest that the CDC8-dependent step in replication precedes the HU-sensitive step, an inference consistent with the temporal ordering of the CDC8 execution point by Hartwell (48). It is unclear, however, if the ARS1 structure in cdc8-1-arrested cells reflects a distinct, previously uncharacterized replication intermediate. Specifically, the cdc8-1-mediated arrest appears stringent, whereas HU-arrested cells eventually overcome the HU block (i.e. adapt) and resume replication. The observed structural differences between HU- and cdc8-1-arrested cells might therefore reflect more extensive DNA synthesis in HU-treated cells.

The known function of Cdc8p is to supply cells with dTTP. Thus, it is not immediately clear why cdc8-1 mutants would arrest at what appears to be a very early step in replication. One possible explanation is that Cdc8p is also important for the structural integrity of the multienzyme replication apparatus. Such a role could help explain the “quick stop” replication arrest that cdc8-1 mutants display when shifted to the restrictive temperature (46) as well as the limited ΔLk that accompanies the transition from the CDC7 to the CDC8 execution point (Fig. 5 and Table I). Although there do not appear to be any genetic data that support the hypothesis that Cdc8p contributes to the structural integrity of the multienzyme replication complex (cf. Ref. 9), several other observations are at least consistent with this idea. For example, DNA synthesis in permeabilized cdc8-1 mutants halts when they are shifted to the restrictive temperature, despite the presence of exogenous dTTP and other dNTPs (72, 73). Additionally, a multienzyme complex of DNA precursor-synthesizing enzymes, which includes thymidylate kinase, forms in both T4 phage and Chinese hamster embryo model replication systems and appears to directly participate in DNA replication (74, 75). Finally, kinases responsible for the sequential phosphorylation of thymi-
dine to dTTP appear to be localized at discrete sites in nuclei (76).

Role of Cdc7p in Replication Initiation—The permutagenic sensitivity of ARS1 and surrounding sequences that we observed in mcm5/cdc46-bob1 and cdc7Δ mcm5/cdc46-bob1 mutants arrested in G1 phase with α-factor. The fact that a replication intermediate that normally forms in early S phase is also evident in G1 phase in an mcm mutant that bypasses the requirement for Cdc7p strongly suggests that the action of Cdc7p leads to structural changes in the MCM complex. The Cdc7p-Dbh4p complex physically associates with individual replication initiation complexes and acts throughout S phase to trigger the firing of individual origins. Cdc7p-Dbh4p modifies Mcm2p, -3p, -4p, -6p, -7p in vitro (33, 36), and a specific Cdc7p bypass mutation, mcm5/cdc46-bob1, resides in Mcm5p (44). This strongly suggests that one target of Cdc7p-Dbh4p in vivo is the MCM2-7 complex. Possibly, phosphorylation of Mcm proteins and the bob1 lesion in Mcm5p both alter the MCM2-7 complex in the same manner. This alteration might then activate the MCM helicase (if in fact an MCM subcomplex serves as the replicative helicase). Although this scenario is speculative, it could account for the apparent unwinding of ARS DNA in both α-factor-blocked mcm5/cdc46-bob1 mutants and cdc8-1 cells blocked in early S phase. Despite what appears to be premature DNA unwinding in these mutants, there is no evidence of premature DNA synthesis (Fig. 1C; Refs. 33 and 44). Thus, although the MCM2-7 complex in mcm5/cdc46-bob1 mutants may exist in a replication-competent state, this is not sufficient to trigger DNA replication. As noted in the Introduction, DNA replication also requires Cdc28-Cib kinase, an activity that is absent in early G1 phase and in α-factor-arrested cells.

Changes in DNA Topology during the Initiation of DNA Replication in Yeast—Our study has revealed small, ARS-decorated DNA structures early in G1 phase that normally forms in early S phase is also evident in G1 phase in an mcm mutant that bypasses the requirement for Cdc7p may “diffuse” into the unwound region (66); right-handed wrapping of the presumptive DNA template strands on a “virtual” surface within this region might even facilitate interactions between enzyme complexes that form on leading and lagging strands (cf. Refs. 79 and 80).

The transition from the CDC8 execution point to the HU-sensitive step was accompanied by a ΔLk of +4.1 in pJL347 (Table I). This result was surprising since the linking number of replicating plasmid DNA must eventually go to zero (cf. Ref. 66). There are two possible explanations for this result. First, release of cells from an α-factor block directly into HU-containing media, as was done in our experiments, may activate a checkpoint that inhibits ARS1 firing or permits only limited DNA synthesis from ARS1. In this case, the positive ΔLk would most likely reflect pre-replicative chromatin remodeling events. This explanation would seem to be ruled out by the finding that other early-firing ARSs support synthesis of long nascent DNA strands in the presence of HU (81), although such synthesis might have occurred in only a small fraction of cells or from early firing ARSs that escaped checkpoint inhibition. A second explanation for the positive ΔLk observed in HU-blocked cells is that the plasmids used in this study are fully replicated during the 1-h HU treatment but that chromatin maturation is incomplete. The packaging of newly replicated DNA into nucleosomes not only requires specific acetylated forms of histone H3 and H4 but also assembly factors that couple deposition of H3-H4 tetramers to DNA replication (Ref. 82 and references therein). HU treatment might slow the final steps in nucleosome assembly and thereby delay restoration of a linking number close to that in α-factor-blocked cells. The available data do not distinguish between these two explanations, but each explanation makes specific, testable predictions.

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REFERENCES
1. Fangman, W. L., and Brewer, B. J. (1991) Annu. Rev. Cell Biol. 7, 375–402
2. Bielinsky, A. K., and Gerbi, S. A. (1998) Science 279, 85–88
3. Bielinsky, A. K., and Gerbi, S. A. (1999) Mol. Cell. Biol. 19, 477–486
4. Broach, J. R., Li, Y. Y., Feldman, J., Jayaram, M., Abraham, J., Nasmyth, K. A., and Hicks, J. B. (1983) Cold Spring Harbor Symp. Quant. Biol. 48, 1165–1173
5. Celniker, S. E., Sweder, K. S., Krici, F., Bailey, J. E., and Campbell, J. L. (1984) Mol. Cell. Biol. 4, 2455–2466
6. Kosmid, D. R., Kent, J. C., and Hartwell, L. H. (1985) Cell 40, 393–403
7. Simpson, R. T. (1990) Nature 343, 387–390
8. Marahrens, Y., and Stillman, B. (1992) Science 255, 817–823
9. Campbell, J. L., and Newton, C. S. (1991) The Molecular and Cellular Biology of the Yeast S-phase, Vol. 1, pp. 41–146, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
10. Natale, D. A., Schubert, A. E., and Kowalski, D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2654–2658
11. Umek, R. M., and Kowalski, D. (1990) Nucleic Acids Res. 18, 6601–6605
12. Bell, S. P., and Stillman, B. (1992) Nature 357, 128–134
13. Dutta, A., and Bell, S. P. (1997) Annu. Rev. Cell Dev. Biol. 13, 293–332
14. Newton, C. S. (1997) Cell 88, 717–729
15. Klemm, R. D., Austin, R. J., and Bell, S. P. (1997) Cell 88, 493–502
16. Tye, B. K. (1999) Annu. Rev. Biochem. 68, 649–666
17. Deyrup, J. P., Cocker, J. H., Dowell, S. J., and Bowley, A. (1994) Cell 78, 303–316
18. Aparicio, O. M., Weinstein, D. M., and Bell, S. P. (1997) Cell 91, 59–69
19. Zou, L., and Stillman, B. (1998) Science 280, 593–596
20. Tanaka, T., and Nasmyth, K. (1998) EMBO J. 17, 5182–5191
21. You, Z., Komamura, Y., and Ishimi, Y. (1999) Mol. Cell. Biol. 19, 8003–8015
22. Chong, J. P. J., Hayashi, M. K., Simon, M. N., Xu, R. M., and Stillman, B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1530–1535
23. Nasmyth, K. (1993) Curr. Top. Cell Biol. 5, 165–179
24. Chapman, J. W., and Johnston, L. H. (1989) Exp. Cell Res. 177, 389–401
25. Jackson, A. L., Pahl, P. M., Harrison, K., Rosomand, J., and Scalfani, R. A. (1993) Mol. Cell. Biol. 13, 2899–2908
26. Youn, H. J., and Campbell, J. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3574–3578
27. Youn, H. J., Lou, S., and Campbell, J. L. (1993) Mol. Biol. Cell 4, 195–208
28. Feldman, R. M., Correll, C. C., Kaplan K. B., and Deshaies, R. J. (1997) Cell 91, 221–230
29. Schwab, E., Bohm, T., Mendenhall, M. D., and Nasmyth, K. (1994) Cell 79,
Premature Structural Changes at Replication Origins in a Yeast Minichromosome Maintenance (MCM) Mutant

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