Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 268, No. 34, Issue of December 5, pp. 25261–25264, 1993

Yeast DNA Replication*

Judith L. Campbell

From the Braun Laboratories, California Institute of Technology, Pasadena, California 91125

The tractability of both genetic and biochemical analysis in yeast has led ever increasing numbers of investigators to choose *Saccharomyces cerevisiae* for their studies of the replication of eukaryotic chromosomes. The availability of small DNA sequences that correspond to chromosomal origins of replication and that function as origins of replication on extrachromosomal plasmids expands even further the versatility of the yeast system for studies of DNA replication. This review summarizes recent results on the roles of various replication proteins and the organization and utilization of origins of replication. Since the topic was reviewed at length in 1991 (1), results published since that time are emphasized here, and earlier results are included only for context. Mitochondrial DNA replication, telomere replication, and integration of DNA replication into the cell cycle are not included here because of space limitation, although there is considerable progress in these areas as well.

DNA Polymerases

One of the most surprising outcomes of research on the yeast DNA polymerases is that at least three DNA polymerases appear to be essential for DNA replication, DNA polymerases α, δ, and ϵ. Another outcome, surprising to some, is the extremely high degree of conservation between these yeast proteins and their homologs in other eukaryotes. Human and yeast DNA polymerase αs are 31% identical, and bovine and yeast polymerase δs are 44% homologous (2, 3). The question of the hour is what does each polymerase do *in vivo*? Do they interact and how are their activities coordinated?

DNA polymerase α appears to be required for the initiation of both the leading and the lagging strands during replication of simian virus 40 *in vitro* (*e.g.* Refs. 4–6). This leads one to expect DNA polymerase α to be a target for regulatory mechanisms that control the initiation of DNA synthesis in S phase, and much attention has focused on polymerase α for this reason. DNA polymerase α consists of four subunits having molecular masses of 180, 86, 58, and 48 kDa. The respective yeast genes, *POL1, POL12, PRI2*, and *PRI1*, have been cloned and sequenced, and the gene products have been overproduced (1, 7–12).[1] p180 encodes the DNA polymerase activity. Temperature-sensitive mutants in this subunit show defects in initiation or elongation, depending on the site of the mutation. Elongation mutants fail to incorporate precursors into DNA at the nonpermissive temperature. This suggests that the replication fork completely falls apart in this mutant and that the other polymerases cannot continue synthesis in the absence of DNA polymerase α. *pol1* mutants are also deficient in premeiotic DNA synthesis, meiotic recombination, and in sporulation, but they are not deficient in repair of x-ray damage (1). The precise role of p86 is unknown. However, the phenotype of $pol12_{ts}$ mutants indicates that p86 is required at an early stage of replication. In addition, p86 is phosphorylated in a cell cycle-dependent manner. The mutant phenotype, along with the S phase-specific phosphorylation, suggests an important regulatory role for this subunit.[1] p58 and p48 are tightly associated with each other and can be purified in a complex free of p180 and p86. It was originally thought that both were part of the primase that is intrinsic to DNA polymerase α. p48 alone, however, is sufficient for synthesizing primers *in vitro*, and p58 may serve to bind p48 to p180 (13, 14). DNA polymerase α is thought to synthesize an RNA primer 8–10 nucleotides in length and then to extend that primer for less than 50 nucleotides. Some mechanism, yet to be understood, then causes a switch to another polymerase (15), either δ or ϵ, both of which are unlike DNA polymerase α in that they do not copurify with primase activities.

DNA polymerase δ from various sources, including yeast, purifies as a protein with two subunits of 124 and 55 kDa (16). The *CDC2* gene of yeast, which is now known as *POL3*, was shown to encode DNA polymerase δ, establishing that this polymerase is essential in yeast (17, 18). *pol3* temperature-sensitive mutants were originally found to synthesize DNA at the nonpermissive temperature. However, recent work shows an immediate shutoff of DNA synthesis, suggesting a role in replication elongation (19). Furthermore, *pol3* mutants are deficient in DNA synthesis in Brij-treated yeast cells, a system in which synthesis at replication forks formed *in vivo* continues *in vitro* (20). Mutations in the 3′- to 5′-exonuclease domain cause a mutator phenotype, suggesting that this domain is involved in accuracy of replication (21). The product of the *POL3* gene has been overproduced in active form in *Escherichia coli*, allowing one to assess the interaction of the core catalytic subunit with polymerase accessory proteins (22, 23). Yeast PCNA[2] stimulates the processivity of the core subunit in the absence of the 55-kDa subunit, suggesting a direct interaction between the subunits (24). The gene encoding the 55-kDa protein has not yet been described, and an important goal is to delineate the function of this subunit.

The finding of a second essential DNA polymerase that used PCNA as a cofactor confirmed the SV40 studies that suggested that both DNA polymerase α and a second, PCNA-dependent DNA polymerase, namely DNA polymerase δ, were involved in replication (25–27). However, the story may be even more complicated, since a third DNA polymerase is required for yeast viability. Yeast DNA polymerase ϵ has been purified and consists of five subunits of 255, 80, 34, 30, and 29 kDa (28, 29). The genes corresponding to p255, p80, and p30 and 34 are called *POL2*, *DPB2*, and *DPB3*, respectively, and have been cloned and sequenced (30–32). As in other DNA polymerases the largest subunit has DNA polymerase catalytic activity. Disruption of the yeast *POL2* gene is lethal, as is disruption of *DPB2* (30, 31). Thus, neither DNA polymerase α nor δ can substitute for DNA polymerase ϵ. Temperature-sensitive *pol2* mutants are deficient in DNA synthesis at the nonpermissive temperature, consistent with an essential role in DNA replication (19, 33). The question is whether pol ϵ participates on the leading or lagging strand as a component of the standard replication fork or if it is involved in some sort of repair function that is essen-

* This minireview will be reprinted in the Minireview Compendium, which will be available in December, 1993.

[1] M. Foiani and P. Plevani, unpublished observations.

[2] The abbreviations used are: PCNA, proliferating cell nuclear antigen; ARS, autonomously replicating sequence(s); ORC, origin recognition complex.

tial during replication, such as bypass of damage. There is evidence that DNA polymerase ϵ is also involved in repair. Extracts prepared from *pol2* mutants are unable to carry out base excision repair synthesis *in vitro* (34). Furthermore, in double mutants carrying temperature-sensitive mutations in both DNA polymerases ϵ and δ, there is a deficiency, though not a complete absence, of repair *in vivo*.[3]

To recapitulate, DNA polymerases δ and ϵ, which apparently function during elongation, are each encoded by an essential gene in yeast, showing that one cannot compensate for the loss of the other and thus that they do not carry out completely overlapping functions *in vivo*. The finding that DNA polymerase ϵ is essential warrants a careful reinvestigation of the DNA polymerases involved in reconstituting the various steps of SV40 DNA replication *in vitro*. However, the results may be very difficult to interpret. The polymerases are very complex biochemically, making them hard to purify in physiologically relevant forms. They are also very similar, so that thus far it appears that while DNA polymerase δ can itself carry out complete *in vitro* replication, DNA polymerase ϵ, where it has been tried, can also work (35). Thus, biochemically the two polymerases appear to be interchangeable. An intelligent use of yeast genetics will be required to solve the apparent paradox between the genetic and biochemical results and to decide if both DNA polymerase δ and ϵ are at the replication fork and, if so, where. In the meantime, studies of interaction with other replication proteins may give some clue.

Proteins at the Replication Fork

Fractionation of the crude SV40 *in vitro* replication system and careful reconstitution have identified a number of additional proteins that participate in replication. Counterparts of all of these proteins have now been purified from yeast, providing a strong case that replication mechanisms in yeast are typical of all eukaryotes. One of these proteins is a single-stranded DNA binding protein, and the others are accessory proteins of DNA polymerase δ and perhaps ϵ.

As in higher cells, the yeast single-stranded DNA binding protein consists of three subunits of 69, 36, and 13 kDa (36, 37). The largest subunit binds to single-stranded DNA with a 1000-fold greater affinity than to RNA (37, 38). The 36-kDa subunit is phosphorylated periodically in the cell cycle, at the G_1/S transition (39). The yeast protein will substitute for the human counterpart in origin-unwinding reactions mediated by SV40 T antigen consistent with conservation of replication function in yeast. Yeast RP-A will not substitute for human RP-A in reconstituting SV40 DNA replication *in vitro*, however, which can be explained if RP-A participates in species-specific protein/protein interactions (37). In addition to its role as a sequence-independent, single-stranded DNA binding protein, RP-A has recently been identified as a sequence-specific, double-stranded DNA binding protein involved in both positive and negative regulation of a large number of yeast genes (40). Thus, yeast RP-A may participate in both transcription and DNA replication. The genes for all three subunits have been identified. They reveal amino acid sequence similarity with human analogs, and they are essential for viability of yeast (41, 42). Conditional mutants are not yet available, but the dual role predicted by RP-A's biochemical properties may lead to pleiotropic defects that are difficult to interpret.

The first DNA polymerase δ accessory factor identified in yeast was the processivity factor PCNA. Demonstration that yeast PCNA stimulated mammalian DNA polymerase δ revealed for the first time the striking functional conservation between the yeast and mammalian DNA polymerases (43). Yeast PCNA is a 26-kDa protein that is a trimer in active form. The PCNA multimer is postulated to bind topologically to DNA analogous to the β subunit of *E. coli* DNA polymerase III. The DNA is thought to be threaded through the center of a ring made by PCNA around the DNA (44). Yeast PCNA has been crystallized and although the structure has not been completely solved, PCNA forms a donut-shaped homotrimer that, despite less than 5% primary sequence conservation, is structurally superimposable on the *E. coli* β subunit of the polymerase III holoenzyme.[4] Thus, PCNA is thought to function as a sliding clamp in yeast, as it does in *E. coli*. Its biochemical properties are indistinguishable from those of the bacterial β subunit. The *POL30* gene, which encodes PCNA, is essential for yeast viability (45). Under certain conditions PCNA also stimulates DNA polymerase ϵ, although its interaction with DNA polymerase ϵ appears to be weaker (46).

While PCNA stimulates DNA polymerase δ on linear, duplex templates, PCNA is inactive on circular templates unless another protein, RF-C, is added. RF-C binds to primer template and then recruits PCNA and positions it at the primer terminus in a process requiring ATP hydrolysis for formation of a stable complex. DNA polymerase binds through PCNA, and DNA synthesis is initiated (46–48). ATP hydrolysis may be required to open the PCNA ring and close it around the DNA. These same proteins also stimulate DNA polymerase ϵ (46). However, the affinity of DNA polymerase ϵ for the RF-C, PCNA, primer-template complex is lower than that of DNA polymerase δ (46). RF-C was purified from yeast by assaying for stimulation of polymerase δ/PCNA on primed single-stranded DNA templates (47, 48). RF-C appears to consist of four subunits of 120, 40, 37, and 36 kDa. The genes for all four subunits are highly homologous, especially in their nucleotide binding sites, and are homologous to their counterparts in human cells (49, 92).[5] The large subunit also contains motifs common to DNA ligase and poly(ADP-ribose) polymerase (49). The 120-kDa subunit is encoded by *CDC44*, an essential gene required for cell cycle progression (49, 50). The *CTF18* gene also encodes a homolog of an RF-C subunit. Null mutants are viable, lose chromosomes at high rates, and have shortened telomeres.[6] This genetic redundancy is not yet understood. There is accumulating evidence that RF-C and PCNA mimic the γ·δ complex and the β subunit of the *E. coli* DNA polymerase III holoenzyme, respectively. However, no τ subunit, which interacts with the γ-δ complex, has yet been found in eukaryotes (51).

Origins of Replication

Bacterial and viral paradigms define a pattern of initiation in which a specific DNA sequence, the origin of replication, is recognized by a specific DNA binding protein. This protein unwinds the DNA and recruits the components of the replication fork to the origin. Studies in yeast suggest that this mode of initiating DNA replication will be conserved in eukaryotic chromosomes. Yeast origins of replication were discovered in 1979 and have received constant attention since then because it has been impossible to identify specific sequences involved in initiation of DNA replication in other eukaryotes. Yeast origins of replication are known as autonomously replicating sequences (ARSs), because they can confer on nonreplicating DNA sequences the ability to replicate extrachromosomally. There are 400 such sequences in the yeast genome (1). That a subset of ARSs also function as origins of replication in the chromosome was first demonstrated by two-dimensional gel electrophoretic techniques that have proven to be invaluable for analyzing

[3] M. E. Budd and J. L. Campbell, unpublished observations.

[4] P. M. J. Burgers, unpublished observations.

[5] K. Fien and B. Stillman, unpublished observations.

[6] F. Spencer, P. Hieter, and V. Lundblad, unpublished observations.

origin utilization in chromosomes (for review see Refs. 1 and 52–55). First, all of the active origins on chromosome III have been identified; their efficiency of firing has been determined; and the direction of movement of replication forks through particular sections of the chromosome has been delineated, providing an accurate replication map of the chromosome (56–60). Second, the timing of origin activations has been assessed. At least two classes of origins have been identified, those that cause adjacent sequences to replicate early in S phase and those that cause late replication (52, 61). Late replicating regions are often found near the ends of chromosomes but are also found internally (62). Third, many sequences that function as ARSs on plasmids are not utilized as origins in the chromosome (57, 60). Active research is going on to find out what sequences either within or without ARSs contribute to the observed spatial and temporal origin activation patterns. Fourth, the factors governing origin spacing in chromosomes are being determined by constructing chromsomes with altered origin configurations and measuring the effect on origin usage. Finally, the technique is being used to study termination of replication as well (56, 63).

The minimal sequence capable of autonomous replication has been studied for ARS1, ARS307, and several other ARSs in less detail. Based on plasmid stability assays, the ARS can be grossly divided into three domains, A, B, and C, the most important being A and B (1). The A domain contains an 11–15-base pair conserved DNA sequence that is essential for autonomous replication, as measured in plasmid stability assays, at all ARSs tested. At ARS1, linker scanning analysis has identified three important subsites in domain B, called B1, B2, and B3 (64). B1 is immediately adjacent to domain A and is the subsite most important for ARS activity. B2 is a 9/11 match to the core consensus sequence in domain A, and B3 is the recognition site for the transcription factor ABF1. The *ABF1* gene is essential, and conditional lethal *abf1* mutants are defective in plasmid maintenance and DNA synthesis (65). A DNA sequence that provides a binding site for a protein with a transcriptional activation domain can replace the B3 site, suggesting that the role of this site may be to bring an activation domain in contact with the origin (64). It remains to be seen whether the result indicates that the transcription and replication machinery share components that interact with such "transcriptional" activation domains. At other ARSs, the B3 site can be located up to 1000 bases away and still activate replication, leading to its designation as a replication enhancer (66).

Initiation of DNA Replication, Genes and Proteins

The presence of the features just described suggested that multiple proteins might bind to the ARS. Consistent with this premise, a complex of six proteins (120, 72, 62, 57, 53, and 50 kDa) that binds in an ATP-dependent manner to ARSs has been purified from yeast (67). The complex is known as origin recognition complex (ORC). Since ORC gives a characteristic DNase I protection pattern over the ARS that coincides closely with *in vivo* footprints and since the DNase I protection pattern is altered in origins containing mutations in domain A that inactivate the ARS, these proteins are candidates for chromosomal initiators (67, 68). It has not been possible, however, to demonstrate any of the other activities associated with known initiator proteins, such as ATPase, helicase, or DNA unwinding. The genes for all six subunits have been cloned and sequenced,[7–9] greatly facilitating further functional analysis. All six genes are essential.[7–9] The 120-kDa subunit, *ORC1*, is homologous to a chromatin component known as *SIR3* that is required for silencing/repression at both the mating-type loci and telomeres.[7] The 72-kDa subunit, *ORC2*,[7] is directly implicated in transcriptional silencing, since *orc2* strains were first identified as mutants that derepress the silent mating-type loci.[8] A role in replication is also suggested since $orc2_{ts}$ mutants arrest at a unique point in the cell cycle, with a dumbbell morphology and nucleus stuck between the mother and the bud, a morphology typical of DNA replication mutants. *orc2* mutants also show a severe defect in plasmid maintenance.[8] The 53-kDa subunit, *ORC 5*,[7] may also be involved in silencing and DNA replication since *orc5* mutants have a silencing defect similar to *orc2*.[8] Since the same proteins that bind to origins of replication are required for transcriptional silencing, an important function of ORC may be to link DNA replication with the determination of transcriptional state. The *ORC6* gene, encoding the 50-kDa subunit,[7] was identified in a screen for proteins that bind to tandem copies of the domain A consensus sequence.[9] Genetic studies suggest that the *ORC6* gene product may interact with the products of *CDC6*, *CDC46*, *CDC47*, and *CDC54*,[9] which are required for the G_1/S transition (1, 50). Several other domain A consensus-binding proteins have been described but are less well characterized (69–71).

How any of these proteins leads to the initiation of replication remains to be shown. The process may not be a simple one, however, as there is strong genetic evidence that additional gene products are involved. The *CDC46* (*MCM5*), *CDC47*, *CDC54*, *MCM2*, and *MCM3* genes encode a family of homologous proteins that are required for cell cycle progression. Conditional mutants have an arrest point consistent with a defect in DNA replication, though most of them make considerable DNA at the nonpermissive temperature, perhaps because they are not protein nulls (50, 72–76). The *mcm* mutants were isolated as strains that were deficient in minichromosome maintenance and that showed ARS specificity, in that different ARSs were affected in different *mcm* mutants. Thus, they appear to have a replication defect. The proteins show the provocative property of being translocated into the nucleus at the end of mitosis and disappearing from the nucleus at the beginning of S phase. This is a property predicted for the initiator protein in the so-called licensing factor model of regulation of replication initiation (77, 78). Little is known about the Cdc46/Mcm proteins biochemically. They each contain ATP binding sites. It has been proposed that they may bind to ARS DNA in conjunction with the product of another gene that gives an Mcm^- mutant phenotype, *MCM1*, and that they may be the actual initiator proteins. *MCM1* encodes a transcription factor involved in control of cell-type specific genes (79, 80). In any case, their importance is underscored by their identification on the basis of amino acid sequence conservation in other yeasts as well as in mouse cells (72).

The *CDC6* gene, though not part of this family, also appears to be required for the initiation of replication (1, 81, 82). In addition, both *CDC6* and its homolog in *Schizosaccharomyces pombe*, $cdc18^+$, appear to function in preventing cells from entering mitosis when DNA synthesis is incomplete (81, 83). *CDC6* and $cdc18^+$ are 28% identical and share nucleotide binding motifs reminiscent of DNA-dependent ATPases and DNA helicases (83, 84). A number of DNA helicases and DNA-dependent ATPases have been purified from yeast (85–91), but none has yet been shown to be essential for DNA replication. The large number of helicases makes the process of reverse genetics time consuming, but the assumption is that at least one helicase will be required for replication, just as the helicase of T antigen is required for elongation in the SV40 *in vitro* system.

[7] S. Bell and B. Stillman, unpublished observations.
[8] M. Foss, S. Loo, F. J. McNally, P. Laurenson, and J. Rine, unpublished observations.
[9] J. Li and I. Herskowitz, unpublished observations.

Conclusions

While the SV40 *in vitro* replication system guided the identification of many yeast replication proteins, an important goal of the yeast field is to develop a yeast *in vitro* replication system in which synthesis is ARS-dependent. This will be the quickest path to an understanding of the organization of the polymerases at the replication fork, protein interactions during initiation of DNA replication at chromsomal origins of replication, and to identification of additional replication proteins whose existence is indicated by a number of yeast mutants that appear to be replication-deficient but for which no specific assay is available.

REFERENCES

1. Campbell, J. L., and Newlon, C. S. (1991) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis and Energetics* (Broach, J. R., Pringle, J. R., and Jones, E. R., eds) pp. 41–146, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. Zhang, J., Chung, D. W., Tan, C.-K., Downey, K. M., Davie, E. W., and So, A. G. (1991) *Biochemistry* **30,** 11742–11750
3. Wang, T. S.-F., Wong, S. W., and Korn, D. (1989) *FASEB J.* **3,** 14–20
4. Nethanel, T., and Kaufman, G. (1990) *J. Virol.* **64,** 5912–5918
5. Bullock, P. A., Seo, Y. S., and Hurwitz, J. (1991) *Mol. Cell. Biol.* **11,** 2350–2361
6. Tsurimoto, T., Melendy, T., and Stillman, B. (1990) *Nature* **346,** 534–539
7. Johnson, L. M., Snyder, M., Chang, L. M. S., Davis, R. W., and Campbell, J. L. (1985) *Cell* **43,** 369–377
8. Pizzagalli, A., Valsasnini, P., Plevani, P., and Lucchini, G. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 3772–3776
9. Lucchini, G., Mazza, C., Scacheri, E., and Plevani, P. (1988) *Mol. Gen. Genet.* **212,** 459–465
10. Badaracco, G., Capucci, L., Plevani, P., and Chang, L. M. S. (1983) *J. Biol. Chem.* **258,** 10720–10726
11. Plevani, P., Francesconi, S., and Lucchini, G. (1987) *Nucleic Acids Res.* **15,** 7975–7989
12. Foiani, M., Santocanale, C., Plevani, P., and Lucchini, G. A. (1989) *Mol. Cell. Biol.* **9,** 3081–3087
13. Santocanale, C., Foiani, M., Lucchini, G., and Plevani, P. (1993) *J. Biol. Chem.* **268,** 1343–1348
14. Longhese, M. P., Jovine, L., Plevani, P., and Lucchini, G. (1993) *Genetics* **133,** 183–191
15. Tsurimoto, T., and Stillman, B. (1991b) *J. Biol. Chem.* **266,** 1961–1968
16. Bauer, G. A., Heller, H. M., and Burgers, P. M. J. (1988) *J. Biol. Chem.* **263,** 917–924
17. Sitney, K. C., Budd, M. E., and Campbell, J. L. (1989) *Cell* **56,** 599–605
18. Boulet, A., Simon, M., Faye, G., Bauer, G. A., and Burgers, P. M. J. (1989) *EMBO J.* **8,** 1849–1854
19. Budd, M. E., and Campbell, J. L. (1993) *Mol. Cell. Biol.* **13,** 496–505
20. Kuo, C.-L., Huang, C.-H., and Campbell, J. L. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 6465–6469
21. Simon, M., Giot, L., and Faye, G. (1991) *EMBO J.* **10,** 2166–2170
22. Brown, W. C., and Campbell, J. L. (1993) *Gene (Amst.)* **127,** 99–103
23. Brown, W. C., Duncan, J. A., and Campbell, J. L. (1993) *J. Biol. Chem.* **268,** 982–990
24. Brown, W. C., and Campbell, J. L. (1993) *J. Biol. Chem.* **268,** 21706–21710
25. Prelich, G., Kostura, M., Marshak, D. R., Mathews, M. B., and Stillman, B. (1987) *Nature* **326,** 471–475
26. Prelich, G., Tan, C.-K., Kostura, M., Mathews, M. B., So, A. G., Downey, K. M., and Stillman, G. (1987) *Nature* **326,** 517–520
27. Prelich, G., and Stillman, B. (1988) *Cell* **53,** 117–126
28. Budd, M. E., Sitney, K., and Campbell, J. L. (1989) *J. Biol. Chem.* **264,** 6557–6565
29. Hamatake, R. K., Hasegawa, H., Clark, A. B., Bebenek, K., Kunkel, T. A., and Sugino, A. (1990) *J. Biol. Chem.* **265,** 4072–4083
30. Morrison, A., Araki, H., Clark, A. B., Hamatake, R. K., and Sugino, A. (1990) *Cell* **62,** 1143–1151
31. Araki, H., Hamatake, R. K., Johnston, L. H., and Sugino, A. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 4601–4605
32. Araki, H., Hamatake, R. K., Morrison, A., Johnston, L. H., and Sugino, A. (1991) *Nucleic Acids Res.* **19,** 4867–4872
33. Araki, H., Ropp, P. A., Johnson, A. L., Johnston, L. H., Morrison, A., and Sugino, A. (1992) *EMBO J.* **11,** 733–740
34. Wang, Z., Wu, X., and Friedberg, E. C. (1993) *Mol. Cell. Biol.* **12,** 1051–1058
35. Lee, S.-H., Pan, Z.-Q., and Kwong, A. D. (1991) *J. Biol. Chem* **266,** 22707–22717
36. Heyer, W.-D., and Kolodner, R. D. (1989) *Biochemistry* **28,** 2856–2862
37. Brill, S. J., and Stillman, B. (1989) *Nature* **342,** 92–95
38. Alani, E., Thresher, R., Griffith, J. D., and Kolodner, R. D. (1992) *J. Mol. Biol.* **227,** 54–71
39. Din, S., Brill, S. J., Fairman, M. P., and Stillman, B. (1990) *Genes & Dev.* **4,** 968–977
40. Luche, R. M., Smart, W. C., Marion, T., Tillman, M., Sumrada, R. A., and Cooper, T. G. (1993) *Mol. Cell. Biol.* **13,** 5749–5761
41. Heyer, W. D., Rao, M. R. S., Erdile, L. F., Kelly, T. J., and Kolodner, R. D. (1990) *EMBO J.* **9,** 2321–2329
42. Brill, S. J., and Stillman, B. (1991) *Gene (Amst.)* **5,** 1589–1600
43. Bauer, G. A., and Burgers, P. M. J. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 7506–7510
44. Kong, X.-P., Onrust, R., O'Donnell, M., and Kuriyan, J. (1992) *Cell* **69,** 425–437
45. Bauer, G. A., and Burgers, P. M. J. (1990) *Nucleic Acids Res.* **18,** 261–265
46. Burgers, P. M. J. (1991) *J. Biol Chem.* **266,** 22698–22706
47. Yoder, B. L., and Burgers, P. M. J. (1991) *J. Biol. Chem.* **266,** 22689–22697
48. Fien, K., and Stillman, B. (1992) *Mol. Cell. Biol.* **12,** 155–163
49. Howell, E. A., McAlear, M. A., Rose, D., and Holm, C. (1993) *Mol. Cell. Biol.*, in press
50. Moir, D., Stewart, S. E., Osmond, B. C., and Botstein, D. (1982) *Genetics* **100,** 547–563
51. O'Donnell, M. (1992) *Bioessays* **14,** 105–111
52. Ferguson, B. M., Brewer, B. J., and Fangman, W. L. (1991b) *Cold Spring Harbor Symp. Quant. Biol.* **56,** 293–302
53. Fangman, W. L., and Brewer, B. J. (1992) *Cell* **71,** 363–366
54. Fangman, W. L. (1991) *Annu. Rev. Cell Biol.* **7,** 375–402
55. Brewer, B. J., and Fangman, W. L. (1991) *Bioessays* **13,** 317–322
56. Zhu, J. G., Newlon, C. S., and Huberman, J. A. (1992) *Mol. Cell. Biol.* **12,** 4733–4741
57. Greenfeder, S. A., and Newlon, C. S. (1992a) *Mol. Biol. Cell* **12,** 999–1013
58. Greenfeder, S. A., and Newlon, C. S. (1992b) *Mol. Cell. Biol.* **12,** 4056–4066
59. Newlon, C. S., Lipschitz, L. R., Collins, I., Deshpande, A., and Devenish, R. J. (1991) *Genetics* **129,** 343–357
60. Dubey, D. D., Davis, L. R., Greenfeder, S. A., Ong, L. Y., Zhu, J. G., and Newlon, C. (1991) *Mol. Cell. Biol.* **11,** 5346–5355
61. Ferguson, B. M., Brewer, B. J., Reynolds, A. E., and Fangman, W. L. (1991a) *Cell* **65,** 507–515
62. Ferguson, B. M., and Fangman, W. L. (1992) *Cell* **68,** 333–339
63. Brewer, B. J., Lockshon, D., and Fangman, W. L. (1992) *Cell* **71,** 267–276
64. Marahrens, Y., and Stillman, B. (1992) *Science* **255,** 817–823
65. Rhode, P. R., Elsasser, S., and Campbell, J. L. (1992) *Mol. Cell. Biol.* **12,** 1064–1077
66. Walker, S. S., Francesconi, S. C., and Eisenberg, S. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 4665–4669
67. Bell, S. P., and Stillman, B. (1992) *Nature* **357,** 128–133
68. Diffley, J. F. X., and Cocker, J. H. (1992) *Nature* **357,** 169–172
69. Hofman, J. F. X., and Gasser, S. M. (1991) *Cell* **64,** 951–960
70. Schmidt, A. M. A., Herterich, S. U., and Krauss, G. (1991) *EMBO J.* **10,** 981–985
71. Estes, H. G., Robinson, B. S., and Eisenberg, S. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11156–11160
72. Hennessy, K. M., Lee, A., Chen, E., and Botstein, D. (1991) *Genes & Dev.* **6,** 958–969
73. Hennessy, K. M., and Botstein, D. (1991) *Cold Spring Harbor Symp. Quant. Biol.* **56,** 279–284
74. Hennessy, K. M., Clark, C. D., and Botstein, D. (1990) *Genes & Dev.* **4,** 2252–2263
75. Chen, Y. R., Hennessy, K. M., Botstein, D., and Tye, B. K. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 10459–10463
76. Yan, H., Gibson, S., and Tye, B. K. (1991) *Genes & Dev.* **5,** 944–957
77. Blow, J. J., and Laskey, R. A. (1988) *Nature* **332,** 546–548
78. Blow, J. J., Sheehan, M. A., Watson, J. V., and Laskey, R. A. (1989) *J. Cell Sci.* 183–195
79. Elble, R., and Tye, B. K. (1992) *Mol. Cell. Biol.* **3,** 971–980
80. Elble, R., and Tye, B. K. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 966–970
81. Bueno, A., and Russell, P. (1992) *EMBO J.* **11,** 2167–2176
82. Hogan, E., and Koshland, D. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 3098–3102
83. Kelly, T. J., Martin, G. S., Forsburg, S. L., Stephen, R. J., Russo, A., and Nurse, P. (1993) *Cell* **74,** 371–382
84. Zhou, C., Huang, S.-H., and Jong, A. Y. (1989) *J. Biol. Chem.* **264,** 9022–9029
85. Shimizu, K., and Sugino, A. (1993) *J. Biol. Chem.* **268,** 9578–9584
86. Sugino, A., Ryu, B. H., Sugino, T., Naumovski, L., and Friedberg, E. C. (1986) *J. Biol. Chem.* **261,** 11744–11750
87. Rong, L., and Klein, H. L. (1993) *J. Biol. Chem.* **268,** 1252–1259
88. Biswas, E. E., Ewing, C. M., and Biswis, S. B. (1993) *Biochemistry* **32,** 3020–3026
89. Li, X. Y., Yoder, B. L., and Burgers, P. M. J. (1992) *Chromosoma (Berl.)* **102,** 93–99
90. Li, X. Y., Yoder, B. L., and Burgers, P. M. J. (1993) *J. Biol. Chem.* **267,** 25321–25327
91. Lahaye, A., Stahl, H., Thinessempoux, D., and Foury, F. (1991) *EMBO J.* **10,** 997–1007
92. Li, X., and Burgers, P. M. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.*, in press