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DNA Replication in Yeast

Carol S. Newlon
Department of Microbiology and Molecular Genetics
UMDNJ-New Jersey Medical School
Newark, New Jersey 07103

The details of chromosome replication are better understood in the budding yeast, *Saccharomyces cerevisiae*, than in any other eukaryotic organism. *cis*-Acting replicator sequences required for chromosomal replication origin function were identified on the basis of their ability to promote the extrachromosomal maintenance of plasmids. These autonomously replicating sequence (ARS) elements have been dissected using the plasmid assay. The ease with which chromosomal sequences can be replaced by homologous recombination in this yeast has facilitated analysis of the effects of ARS mutations on chromosomal origin activity, which can be assayed by two-dimensional (2D) gel analysis. Both the isolation and analysis of mutants defective in DNA replication and the use of reverse genetics to identify and mutate genes encoding proteins thought to participate in DNA replication have yielded insights into proteins required for the initiation and elongation steps of DNA replication.

The fission yeast, *Schizosaccharomyces pombe*, offers many of the same advantages for the study of chromosomal DNA replication as *S. cerevisiae*. A similar plasmid assay has been used to identify ARS elements that appear to be associated with chromosomal replication origins. The sequences required for ARS activity in the plasmid assay have not been dissected thoroughly, but preliminary indications suggest that *S. pombe* ARS elements, like *S. pombe* centromeres, are larger than those of *S. cerevisiae*. In addition, the genes encoding a number of *S. pombe* replication proteins have been isolated and characterized.

Despite the enormous progress in characterizing the replicators and proteins required for yeast chromosomal DNA replication, there is still not an in vitro DNA replication system that depends on bona fide replicators for the initiation of replication. The SV40 in vitro replication system has partially filled the gap caused by the lack of a yeast in vitro

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system. As required proteins have been identified in the SV40 system, yeast structural and functional homologs have been identified and the genes encoding them have been cloned. The construction and analysis of mutations in the yeast genes has allowed assessment of the in vivo roles of these proteins.

PROTEINS AT THE REPLICATION FORK

The biochemical properties and structural features of yeast proteins that function at the replication fork and are highly homologous to proteins in other eukaryotes are covered in the section on replication proteins. New insights about the roles of these proteins in DNA metabolism, gained largely through the analysis of conditional mutations in the genes encoding these enzymes in *S. cerevisiae*, are emphasized in this section. Table 1 provides a list of *S. cerevisiae* proteins implicated to function at the replication fork.

DNA Polymerases

A major contribution of the yeast system was the unexpected finding that three DNA polymerases, pol-α, pol-δ, and pol-ε, are all essential for life. Much ongoing research is directed at understanding the essential role(s) of each of these polymerases.

pol-α, which contains an intrinsic primase activity, is required for the initiation of both leading-strand and lagging-strand synthesis in the SV40 in vitro system. This polymerase is thought to synthesize the RNA primer for both the leading and lagging strands and to extend the RNA primer by synthesizing a short initiator DNA (Waga and Stillman 1994). As has been found for other pol-α:primase enzymes, *S. cerevisiae* DNA primase activity copurifies with the tightly associated 58-kD and 48-kD subunit complex, which can be separated from the two larger subunits. Although it has not been possible to separate it in active form from the heterodimeric complex with the 58-kD subunit, free 48-kD subunit present in yeast extracts is capable of RNA primer synthesis (Santocanale et al. 1993). Consistent with this observation, the properties of pol-α immunopurified from strains carrying ts mutations in *PRI1* or *PRI2* suggest that the 48-kD subunit has a major role in primase activity. The same immunopurification experiments suggest that the 58-kD subunit mediates or stabilizes the interaction of the 48-kD subunit with the four-subunit pol-α complex (see Table 1).

DNA polymerase activity is associated with the 167-kD subunit of

pol-α. Some ts mutations in the gene encoding this subunit cause an immediate cessation of DNA synthesis at the nonpermissive temperature, suggesting that other DNA polymerases cannot continue synthesis in the absence of pol-α. This "quick-stop" phenotype may reflect strong coupling between the activities of replicative polymerases. Alternatively, these mutations may, at the nonpermissive temperature, cause a conformation change in, or instability of, pol-α that causes the replication fork to fall apart. The gene encoding the catalytic subunit of *S. pombe* pol-α has been cloned and sequenced (Damagnez et al. 1991; Park et al. 1993) and shows substantial similarity to pol-α subunits from other organisms (see Wang, this volume).

No enzymatic activity has been associated with the fourth subunit of pol-α, encoded by the *POL12* gene of *S. cerevisiae*. This subunit appears to mediate the interaction of pol-α:primase with T antigen in the SV40 in vitro system, suggesting a possible role in the initiation of both leading and lagging strands (Collins et al. 1993). The characterization of a strain carrying a ts mutation in *POL12* has revealed that this subunit plays an essential role in an initial stage of DNA replication that is complete before the hydroxyurea-sensitive step, presumably chain elongation (Foiani et al. 1994). This subunit shows a cell-cycle-regulated phosphorylation, becoming dephosphorylated as cells complete mitosis and rephosphorylated at the G_1/S boundary (Foiani et al. 1995). The ts mutant phenotype and the cell-cycle-dependent phosphorylation pattern suggest a regulatory role for this subunit. A speculative model is that the nonphosphorylated form of this subunit is required for loading pol-α:primase at replication origins by direct or indirect interactions with the origin recognition complex (Foiani et al. 1994).

Additional proteins that interact with the pol-α catalytic subunit have been sought by protein affinity chromatography. Six polypeptides were identified that bound to a matrix carrying immobilized pol-α but not to a control matrix (Miles and Formosa 1992a). One of these polypeptides, called POB1 (for *p*olymerase *o*ne *b*inding), is encoded by a gene that was identified in a screen for mutants that cause an increased rate of mitotic loss of chromosome III, *CTF4/CHL15* (Table 1) (Kouprina et al. 1992; Miles and Formosa 1992b). Although *CTF4* is not essential, null mutants have phenotypes suggesting a role in DNA metabolism. These phenotypes include elevated rates of chromosome loss and genetic recombination, enhanced temperature sensitivity of strains carrying mutations in the pol-α catalytic subunit, and accumulation in the population of cells with large buds and undivided nuclei. The other five pol-α-binding polypeptides have not been characterized further.

Table 1 Proteins at the yeast replication fork

Protein	Gene	Gene product	Remarks	References
	POL1 (*CDC17*, *HPR3*)	167-kD catalytic subunit	essential; some ts alleles cause "quick stop" phenotype; some alleles isolated in screen for hyperrecombination	reviewed by Campbell and Newlon (1991)
	POL12	86-kD (apparent) subunit	essential; predicted 79-kD protein; ts mutations cause defect in replication initiation; phosphorylated in cell-cycle-dependent manner	Foiani et al. (1994, 1995)
pol-α	*PRI1*	48-kD subunit	essential; ts mutants defective in DNA synthesis; has primase activity in vitro	Lucchini et al. (1987); Francesconi et al. (1991); Santocanale et al. (1993)
	PRI2	58-kD subunit	essential; tightly associated with 48-kD subunit; ts mutants defective in DNA synthesis; mediates or stabilizes binding of 48-kD subunit to catalytic subunit	Foiani et al. (1989); Francesconi et al. (1991); Longhese et al. (1993)
POB1	*CTF4* (*POB1*, *CHL15*)	105 kD	not essential; interacts in vitro with catalytic subunit of pol-α; mutants show increased rates of chromosome loss and mitotic recombination	Kouprina et al. (1992); Miles and Formosa (1992a,b)
pol-δ	*POL3* (*CDC2*, *HPR6*)	124-kD catalytic subunit	essential; interacts with and processivity stimulated by PCNA; one ts mutant deficient in synthesis of high molecular weight daughter strands; mutations in 3′→5′ proofreading exonuclease domain cause mutator phenotype	Boulet et al. (1989); Sitney et al. (1989); Burgers (1991); Budd and Campbell (1993); Simon et al. (1991); Brown et al. (1993); Morrison et al. (1993)

	?	55-kD subunit	function unknown	Bauer et al. (1988)
PCNA	*POL30*	29-kD monomer	essential; processivity factor for pol-δ and -ε; crystal structure of homotrimer reveals closed ring capable of encircling duplex DNA with striking similarity to *E. coli* pol III β subunit; *pol30* mutations suppress ts *cdc44* (*rfc1*) mutations	Bauer and Burgers (1990); Burgers (1991); Krishna et al. (1994); McAlear et al. (1994)
pol-ε	*POL2*	255-kD catalytic subunit	essential; ts mutants deficient in DNA synthesis; stimulated by PCNA; extracts from ts mutants deficient in excision repair synthesis in vitro; mutations in C-terminus cause deficiency in S-phase checkpoint; mutations in 3′→5′ exonuclease domain cause mutator phenotype	Morrison et al. (1990, 1991); Burgers (1991); Araki et al. (1992); Budd and Campbell (1993); Wang et al. (1993); Navas et al. (1995)
	DPB2	80-kD subunit	essential; ts mutants partially defective in DNA synthesis	Araki et al. (1991a)
	DPB3	30-kD and 34-kD subunits	deletion mutant viable but exhibits increased spontaneous mutation rate	Araki et al. (1991b)
	?	29-kD subunit	function unknown	
RP-A	*RFA1*	70.4-kD subunit	essential; binds DNA independently of other two subunits; ts mutations cause defects in DNA replication and repair; also involved in recombination	Heyer et al. (1990); Brill and Stillman (1989, 1991); Longhese et al. (1994); Smith and Rothstein (1995); Firmenich et al. (1995)

(Continued on following pages.)

Table 1 (continued)

Protein	Gene	Gene product	Remarks	References
RP-A	*RFA2*	29.9-kD subunit	essential; cell-cycle-regulated phosphorylation at G_1/S and dephosphorylation at M	Brill and Stillman (1991); Din et al. (1990)
	RFA3	13.8-kD subunit	essential	Brill and Stillman (1991)
RF-C	*RFC1* (*CDC44*)	95-kD Rfc1p	essential; contains DNA-binding domain and shows significant homology with other four RF-C subunits; in combination with other subunits functions to load PCNA at RNA primer terminus	Burgers (1991); Howell et al. (1994); Cullmann et al. (1995)
	RFC2	37.9-kD Rfc3p	essential; significant homology with other yeast and human RF-C subunits	Noskov et al. (1994); Cullmann et al. (1995)
	RFC3	38.2-kD Rfc3p	essential; homology with other RF-C subunits; purified protein has ATPase activity stimulated by ssDNA	Li and Burgers (1994b); Cullmann et al. (1995)
	RFC4	36.2-kD Rfc4p	essential; homology with other RF-C subunits; no ATPase activity found	Li and Burgers (1994a); Cullmann et al. (1995)
	RFC5	39.9-kD Rfc5p	essential; homology with other RF-C subunits	Cullmann et al. (1995)
Chl12p	*CHL12* (*CTF18*)	84-kD	not essential; predicted protein contains NTP-binding domain and shows homology with RF-C subunits; null mutations cause chromosome loss, increased rates of mitotic recombination, slow growth, cold-sensitivity	Kouprina et al. (1994)

Topoisomerase I	*TOP1* (*MAK1*)	90-kD	not essential; deletion mutations cause reduction in growth rate, show transient accumulation of ~5-kb daughter strands during DNA replication; *top1 top2* double mutants defective in DNA elongation	Goto and Wang (1985); Thrash et al. (1984); Brill et al. (1987); Kim and Wang (1989)
Topoisomerase II	*TOP2*	150-kD homodimer	essential; required for decatenation of chromosomes at mitosis; either *TOP1* or *TOP2* required for DNA chain elongation	DiNardo et al. (1984); Goto and Wang (1984); Holm et al. (1985, 1989); Brill et al. (1987); Spell and Holm (1994)
DNA ligase	*CDC9*	87-kD protein	essential; required for completion of DNA replication and DNA repair	Johnston and Nasmyth (1978); Johnston (1979, 1983)
5′→3′-exo-nuclease (flap endonuclease)	*YKL510* (*RTH1*, *RAD27*, *ERC11*)	45-kD protein	not essential, but null mutations cause temperature-sensitive lethality; protein has structure-specific endonuclease and 5′→3′-exonuclease activity; null mutants exhibit lengthened S phase, accumulation of large-budded cells with a single nucleus, and increased plasmid loss rates, MMS sensitivity; mutant allele isolated as synthetic lethal with null mutations of *cln1*, *cln2*	Harrington and Lieber (1994); Sommers et al. (1995); Reagan et al. (1995); Vallen and Cross (1995)
DNA2 helicase	*DNA2* (*DNA154*)	171-kD protein	essential; quick-stop replication defect in permeabilized cells; no high molecular weight DNA synthesized in vivo at nonpermissive temperature; DNA-stimulated ATPase and 3′→5′-helicase activity	Kuo et al. (1983); Budd and Campbell (1995a)

The other two essential DNA polymerases differ from pol-α in several ways. Both pol-δ and pol-ε have intrinsic "proofreading" 3′→5′ exonuclease activities, the processivity of both polymerases is stimulated by PCNA, and neither has DNA primase activity. pol-δ appears to be a two-subunit enzyme (Table 1), but the gene encoding the smaller subunit has not been cloned, and little is known of its function. The *S. pombe* gene encoding the catalytic subunit of pol-δ has been cloned and sequenced (Pignede et al. 1991; Park et al. 1993). Purified pol-ε has five subunits (Table 1); the two largest subunits are encoded by essential genes. The third and fourth subunits are encoded by a single nonessential gene. These two subunits may represent different posttranslational modifications of the primary gene product, or one may be a proteolytic degradation product of the other. The gene encoding the smallest subunit has not been identified.

A critical, as yet unanswered, question is what is the essential, nonoverlapping function of each of these PCNA-dependent polymerases. One possibility is that both polymerases function as part of the replication fork, one responsible for leading-strand synthesis and the second responsible for completing Okazaki fragments on the lagging strand. Consistent with this idea, strains carrying some ts mutations in the catalytic subunit of pol-ε exhibit a "quick stop" elongation defect when shifted to the nonpermissive temperature, and under some conditions ts mutations in the catalytic subunit of pol-δ cause a similar defect (Budd and Campbell 1993). However, pol-δ is clearly able to complete both leading- and lagging-strand replication in the SV40 system (Waga and Stillman 1994). A second possibility is that pol-ε plays an essential role in DNA repair, perhaps in correcting errors made by the replicative polymerase. Consistent with a role for pol-ε in repair, extracts prepared from *pol2* mutants are inactive in base excision repair synthesis in vitro (Wang et al. 1993). However, either pol-δ or pol-ε is sufficient for the in vivo repair of UV damage (Budd and Campbell 1995b).

Whatever the essential role(s) of its catalytic activity, the catalytic subunit of pol-ε also has been implicated strongly as a component of the cell-cycle checkpoint that prevents entry into mitosis in the presence of incompletely replicated or damaged DNA, the S-phase checkpoint (Navas et al. 1995). Mutations causing defects in this checkpoint map to the carboxyl terminus of the protein, in a domain distinct from the catalytic domain. The checkpoint-defective mutants are temperature-sensitive for growth and have defects in DNA replication, suggesting that the carboxyl terminus is also required for DNA synthesis. It has been proposed that the role of pol-ε in the checkpoint is to act as a sensor of

DNA replication that coordinates the transcriptional and cell-cycle responses to replication blocks.

Proliferating Cell Nuclear Antigen

PCNA, encoded by the essential *POL30* gene of *S. cerevisiae* (Table 1) and the *pcn1*$^+$ gene of *S. pombe* (Waseem et al. 1992), is a processivity factor for both pol-δ and pol-ε (see Table 1). Although PCNA shows no amino acid sequence similarity to the β subunit of *Escherichia coli* DNA polymerase III, and is a homotrimer rather than a homodimer, the crystal structures of these two proteins are strikingly similar. The trimer is a ring-shaped complex with a central hole large enough to accommodate a DNA duplex. These proteins, along with the bacteriophage T4 gene 45 protein, thus function as sliding clamps that anchor their interacting polymerases to DNA (for review, see Stillman 1994). These sliding clamps normally require assistance from additional factors for loading onto DNA, which likely requires opening of the ring. Both biochemical and genetic analysis implicates replication factor C (RF-C) as the protein required to load PCNA (for review, see Krishna et al. 1994). The genetic evidence is that mutations in *POL30* suppress the phenotype of ts mutations in the gene encoding the large subunit of RF-C, *CDC44* (McAlear et al. 1994; Ayyagari et al. 1995). These mutations change amino acids that lie throughout the entire protein and fail to identify a small domain of interaction between PCNA and RF-C. The suppression of phenotypes resulting from defective RF-C complexes by these PCNA mutations is likely to result from weakened interactions between PCNA subunits that make the circular clamp easier to open for loading onto DNA.

PCNA also interacts with proteins that are not part of the replication apparatus. The human cyclin-dependent protein kinase inhibitor, p21, binds to PCNA and inhibits its DNA replication functions but not its DNA repair functions (Flores-Rozas et al. 1994; Li et al. 1994; Waga et al. 1994). The identification of mutant derivatives of PCNA that support normal growth rates and interact normally with RF-C and pol-δ and pol-ε, but show defects in one or more DNA repair processes, strongly suggests that PCNA interacts with repair-specific protein(s) (Ayyagari et al. 1995).

RF-C

Like the bacteriophage T4 accessory protein complex gp44/gp62 and the *E. coli* γτ complex, RF-C appears to bind to primer/template structures

and load the ring-shaped sliding clamp, PCNA. The five RF-C subunits show considerable amino acid sequence similarity, including a putative purine nucleotide-binding region (see Table 1 for references). Moreover, the five RF-C subunits show significant homology with the functionally related proteins of bacteriophage T4 (gp44) and *E. coli* (the γτ subunits of the DNA polymerase III holoenzyme), and the large subunit, encoded by the *RFC1* gene, contains a region of significant homology with prokaryotic DNA ligases and poly(ADP-ribose)-polymerases of eukaryotes (Cullmann et al. 1995). It is not clear whether all five subunits of RF-C associate in a single complex, or whether several complexes with different subunit compositions act in different aspects of DNA replication or repair. Another unresolved issue is what is (are) the specific function(s) of each RF-C subunit. Finally, the observation that loss of the *CHL12* gene product, which shows significant homology with subunits of RF-C, results in a cold-sensitive phenotype consistent with a leaky defect in DNA replication raises the possibility that additional RF-C-like proteins function in some aspect of DNA replication (Kouprina et al. 1994).

Replication Protein A

RP-A is a heterotrimeric single-stranded DNA-binding protein required for DNA replication, recombination, and repair (see Table 1). It appears to be the functional analog of T4 gene 32 protein and *E. coli* SSB. Although each of the three subunits is encoded by an essential gene, the activities of the subunits are poorly understood. The large subunit is capable of binding DNA independently of the other two subunits (Brill and Stillman 1989), and specific mutant alleles of *RPA1* implicate this subunit in interactions with recombination and repair enzymes (Firmenich et al. 1995; Smith and Rothstein 1995). The 34-kD subunit is phosphorylated in a cell-cycle-dependent manner (Din et al. 1990), suggesting a regulatory activity. The inability of yeast RP-A to efficiently substitute for human RP-A in the complete SV40 replication system suggests that RP-A makes specific protein contacts with one or more subunits of the replication complex (Brill and Stillman 1989).

Topoisomerases

The unwinding of DNA in advance of replication forks introduces positive supercoils, which must be removed by a swivel. The phenotypes of

topoisomerase mutants suggest that either a type I or a type II topoisomerase can provide the essential function (see Table 1). The observation that *top1* mutants of *S. cerevisiae* transiently accumulate relatively short daughter strands during DNA replication suggests that topoisomerase I normally provides the replication swivel (Kim and Wang 1989).

A second topological problem resulting from DNA replication occurs near the end of the elongation step, when replication forks converge. The sister duplexes either end up as a pair of catenated helices that must be resolved by a type II topoisomerase, or as a pair of gapped molecules that can be resolved by either a type I or a type II topoisomerase (for review, see Wang 1991). Recent analysis of a *top3* mutant of budding yeast, deficient in a type I topoisomerase, and an extragenic suppressor of the mutant, *sgs1*, which encodes a putative helicase, is consistent with the notion that this pair of gene products has a role in the resolution of daughter helices following the completion of replication (Gangloff et al. 1994).

In both fission and budding yeast, the essential function of topoisomerase II appears to be in the untangling of sister chromatids at mitosis, where it plays a role in resolving tangles during both chromosome condensation and segregation (Holm et al. 1985, 1989; Uemura and Yanagida 1986; Funabiki et al. 1993; Spell and Holm 1994). It is interesting that Sgs1p also interacts with topo II, and that *sgs1* null mutants have a chromosome segregation defect, implicating this putative helicase in chromosome segregation as well as in the completion of DNA replicons (Watt et al. 1995).

Other Proteins

Several other proteins, including an enzyme that removes RNA primers, a DNA polymerase to fill in the gaps left by removal of primers, a DNA ligase to close single-strand nicks, and one or more helicases to drive movement of the replication fork, have a role at the replication fork. In *E. coli*, the 5′→3′ exonuclease activity of DNA polymerase I appears to remove the RNA primers attached to the 5′ ends of newly replicated DNA (Konrad and Lehman 1974). In the SV40 in vitro system, both an RNase H and a 45-kD 5′→3′ exonuclease, probably the FEN-1 nuclease, are required for primer removal (Ishimi et al. 1988; Goulian et al. 1990; Turchi and Bambara 1993; Turchi et al. 1994). Deletion of the yeast homolog of FEN-1 nuclease, encoded by the *YKL510* gene, causes phenotypes consistent with defects in both DNA replication and DNA repair (Table 1). The observation that *ykl510* null mutations cause

temperature-sensitive lethality suggests that another protein can substitute for the *YKL510* gene product at 30°C but not at 37°C.

The genes encoding DNA ligase, *CDC9* of *S. cerevisiae* and *cdc17*$^+$ of *S. pombe*, were among the first cell-cycle genes to be associated with a protein product (Nasmyth 1977; Johnston and Nasmyth 1978). The yeast DNA ligases show significant homology with each other and with human DNA ligase I (Barker et al. 1987) and also have catalytic properties very similar to the mammalian DNA ligase I enzyme (Tompkinson et al. 1992). Temperature-sensitive DNA ligase mutants are defective in DNA replication, failing to join Okazaki fragments and completed replicons (Johnston and Nasmyth 1978; Johnston 1983), and are also defective in DNA repair and mitotic recombination (for review, see Johnston 1983). No activities corresponding to mammalian DNA ligases II and III have been reported in yeast.

A strong candidate for a replicative helicase has recently been identified as the product of the *DNA2* gene (Table 1) (Budd and Campbell 1995a). This protein has both a DNA-stimulated ATPase activity and 3′→5′ helicase activity that depends on the ATPase. Interestingly, it copurifies with a nuclease that shows a substrate specificity similar to the nuclease encoded by the *YKL510* gene, and the temperature sensitivity of the *dna2* mutant is suppressed by a high-copy-number plasmid carrying *YKL510*, suggesting that the copurification may reflect a physiologically significant interaction between these two proteins. A number of other helicases have been identified in yeast, but their specific roles in DNA replication have not yet been identified (Li et al. 1992; Bean et al. 1993; Shimizu and Sugino 1993).

ORIGINS OF REPLICATION

It has been possible to define chromosomal origins of replication more precisely in yeasts than in any other eukaryotic organism. The replicator sequences necessary for replication origin activity were identified by their ability to promote the extrachromosomal maintenance of plasmids in *S. cerevisiae* and called autonomously replicating sequence (ARS) elements (for review, see Campbell and Newlon 1991). *S. pombe* sequences with similar properties were identified soon thereafter (Beach and Nurse 1981). The development of 2D gel methods for the analysis of replication intermediates made it possible to demonstrate that replication origins coincide with ARS elements in plasmids (Brewer and Fangman 1987; Huberman et al. 1987) and in chromosomes (for review, see Newlon and Theis 1993).

ARS Structure

Our current view of ARS structure, gained from the detailed analysis of a small number of ARS elements, is summarized in Figure 1. An essential region of approximately 17 bp, called domain A, contains a match to an 11-bp sequence (5′-[A/T]TTTA[T/C][A/G]TTT[A/T]-3′), the ARS consensus sequence (ACS). This ACS serves as a point of reference for other essential or stimulatory sequences, with domain B on the 3′ side of the T-rich strand of the ACS and domain C on the 5′ side. Deletion analysis has revealed that the minimal sequences required for ARS activity, defined by the ability to promote high-frequency transformation and extrachromosomal maintenance of plasmids, include domain A and a variable number of nucleotides in domain B. Additional sequences in domain B, and sometimes in domain C, increase the efficiency of ARS function, measured by plasmid stability assays.

The ACS is the only highly conserved DNA sequence in ARS elements, and the sequence conservation reflects its functional significance. Point mutations in the ACS either abolish or reduce ARS activity (Van Houten and Newlon 1990; Rivier and Rine 1992; Li and Herskowitz 1993). Although ARS elements almost always contain multiple matches to the ACS, usually a single ACS is essential, with mutations in the other matches having little or no effect on ARS activity (Marahrens and Stillman 1992; Huang and Kowalski 1993; Miller and Kowalski 1993; Shirahige et al. 1993; Rao et al. 1994; Theis and Newlon 1994). However, in 3 of the 21 ARS elements studied, either of 2 overlapping or closely spaced ACSs can provide the essential function (Van Houten and Newlon 1990; Shirahige et al. 1993; J.F. Theis and C.S. Newlon, in prep.). In about half of the ARS elements studied, the essential ACS is an exact match to the consensus; most others match at 10 of 11 positions (for review, see Newlon and Theis 1993). The extreme cases are *ARS121* and *ARS309*, which have essential 9 of 11 matches to the ACS (Walker et al. 1990; J.F. Theis and C.S. Newlon, in prep.). Domain A is the core of the recognition region of a multiprotein complex, origin recognition complex (ORC) (see below).

In contrast to domain A, there is no highly conserved DNA sequence within domain B. However, linker scan analysis of several ARS elements suggests that domain B is modular. *ARS1* contains 3 domain B elements, B1, B2 and B3, in which linker substitutions reduce ARS activity (Marahrens and Stillman 1992). The presence of any 2 of these 3 elements is sufficient for ARS activity. *ARS307* contains 2 domain B elements (Rao et al. 1994; Theis and Newlon 1994), and a similar pattern of elements is apparent in the linker scan analysis of 3 ARS elements from

chromosome VI, *ARS605*, *ARS607*, and *ARS609* (Rashid et al. 1994). Although the B elements of *ARS307* share little or no sequence homology with the B elements of *ARS1*, the B1 elements of the 2 ARSs are functionally interchangeable, as are the B2 elements (Rao et al. 1994). As described below, the B1 and B3 elements function as protein-binding sites, but the function of the B2 element is unclear.

A saturation mutagenesis of the B1 element of *ARS1* revealed that point mutations in either of two adjacent base pairs caused dramatic decreases in plasmid stability (Rao et al. 1994). The B1 element of *ARS307* contains a pair of identical base pairs at a similar distance from the ACS which are important for B1 function (Rao et al. 1994; Theis and Newlon 1994). This region of the B1 element is important for ORC binding, both in vitro and in vivo (Rao and Stillman 1995; Rowley et al. 1995). Interestingly, the analysis of point mutations in the B1 element of

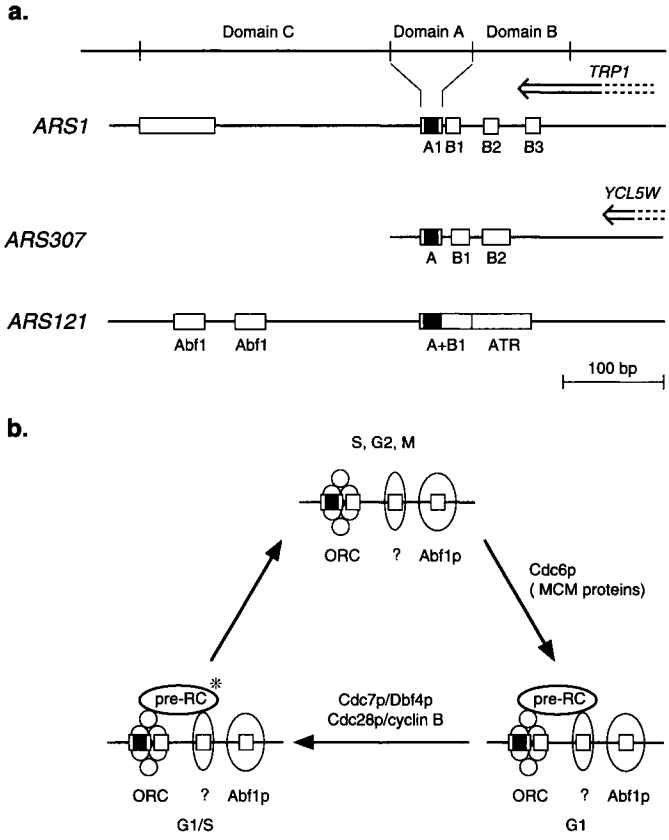

Figure 1 (*See facing page for legend.*)

ARS1 revealed that mutations in only one of the two nucleotides with significant effects on B1 function reduced ORC binding, suggesting that this element has other functions in replication initiation beyond its role in ORC-DNA interactions (Rao and Stillman 1995).

The B3 element of *ARS1* is the binding site for the multifunctional transcription and replication protein, ARS-binding factor 1 (Abf1p, also called OBF1; see ARS-binding factors below). Abf1p binding sites are found in some but not all ARS elements, where they function in a position- and orientation-independent fashion as replication enhancers (Walker et al. 1990, 1991). The B3 element of *ARS1* can be replaced by binding sites for other transcription factors, Gal4p and Rap1p, suggesting that other transcriptional activators might serve as replication enhancers (Marahrens and Stillman 1992).

The role(s) of the B2 element is not yet clearly defined. Although 8-bp linker substitutions in the B2 elements of *ARS1* and *ARS307* affected ARS activity, single base-pair changes in the B2 element of *ARS1* showed no phenotype (Marahrens and Stillman 1992; Rao et al. 1994;

Figure 1 ARS structure. (*a*) Schematic drawings of three *S. cerevisiae* ARS elements. Boxes represent functional elements identified by mutational analysis. The essential ACS of each ARS element is represented by the filled box within domain A. The domain A and domain B elements of *ARS1* were defined by Marahrens and Stillman (1992) and domain C by Strich et al. (1986). *ARS307* was dissected by Palzkill and Newlon (1988), Van Houten and Newlon (1990), Theis and Newlon (1994), and Rao et al. (1994). The *ARS121* diagram is based on data from Walker et al. (1991). The core region of *ARS121*, identified by deletion analysis, contains the essential ACS and, presumably, the B1 element. The AT-rich region (ATR) stimulates the activity of the core, and by analogy with *ARS1* and *ARS307*, is likely to contain the B2 element. (*b*) Cell-cycle-regulated protein interactions with *ARS1*. Diagram is based on data from Diffley et al. (1994) and Cocker et al. (1996). ORC, shown as a six-subunit protein, and Abf1p, shown as a monomer, are bound throughout the cell cycle. Origin activation appears to be a two-step process. In the first step, at the end of mitosis, additional factors are recruited to *ARS1* to form the prereplicative complex (pre-RC). The assembly and maintenance of the pre-RC depends on Cdc6p, which is likely to be a component of the pre-RC. In the second step, at the transition from G_1 to S phase, the pre-RC is activated (indicated by an asterisk) and replication initiates. Activation of the pre-RC requires the activity of two protein kinases (Cdc7p and Cdc28p) and their regulatory subunits (Dbf4p and a B-type cyclin). During S phase, the additional factors that form the pre-RC are dissociated and/or degraded. The factor shown interacting with the B2 element is hypothetical (see text).

Theis and Newlon 1994). These observations are consistent with a role for B2 as a structural element or as a protein-binding site with loose sequence specificity. One attractive hypothesis is that B2 functions as a DNA unwinding element (DUE). In well-studied replication origins, replication initiation is facilitated by the unwinding of a small region adjacent to the initiator protein-binding site where the remainder of the replication apparatus assembles (for review, see Kornberg and Baker 1992). The observed correlation between the progressive loss of ARS activity and increases in helical stability caused by deletions into domain B, and the demonstration that domain B can be functionally substituted by heterologous, easily unwound sequences, support the idea that a DUE is contained within domain B (Umek and Kowalski 1988; Natale et al. 1992). However, two lines of evidence suggest that B2 has a function beyond acting as a DUE. First, some linker scan mutations in B2 that reduce plasmid stability are not predicted to affect the helical stability of domain B (Theis and Newlon 1994). Second, mutations in B2 reduce the ability of *ARS1* to recruit Dbf4p (see below), suggesting that Dbf4p interacts directly or indirectly with B2 (Dowell et al. 1994).

Fewer than half of the ARS elements characterized have stimulatory sequences in domain C. The Abf1p binding sites in *ARS121* are the only well-studied domain C elements (Walker et al. 1990, 1991). The domain C sequences of *ARS1* are located approximately 200 bp from domain A and are not well characterized (Strich et al. 1986). Five of the nine chromosome VI ARS elements have been reported to have stimulatory sequences in domain C, but only in *ARS605* has the position of these sequences been defined (Shirahige et al. 1993; Rashid et al. 1994).

S. pombe ARS elements have not been studied as extensively as those of *S. cerevisiae*. An 11-bp consensus sequence, the PACS, was found by the comparison of the DNA sequences of nine ARS-containing fragments (Maundrell et al. 1988). However, unlike the *S. cerevisiae* ACS, this sequence does not appear to play an essential role in ARS function (Maundrell et al. 1988; Zhu et al. 1994). Both the sizes of *S. pombe* ARS-containing fragments and deletion analysis of two *S. pombe* ARS elements, *ARS3002* and *ARS3003*, suggest that the sequences required for full ARS activity are approximately ten times the length of *S. cerevisiae* ARS elements (Maundrell at al. 1988; Zhu et al. 1994). As in *S. cerevisiae*, *S. pombe* ARS elements clearly function as replication origins on plasmids (Caddle and Calos 1994; Wohlgemuth et al. 1994).

ARS elements have also been identified in chromosomal DNA of *Kluyveromyces lactis* (Fabiani et al. 1990). The detailed analysis of one of these ARSs revealed an essential core of approximately 40 bp, con-

taining a sequence related to the ACS of *S. cerevisiae*, and flanked by sequences that stimulate activity (Fabiani et al. 1996).

Chromosomal Replication Origins

All *S. cerevisiae* chromosomal replication origins, identified by 2D gel analysis, coincide with known ARS elements. Moreover, no additional origins were found in systematic searches for origins of replication not associated with ARS elements on chromosome III (Greenfeder and Newlon 1992; Huberman et al. 1992; Collins and Newlon 1994). The well-documented finding that the effects of mutations that reduce or abolish ARS activity on plasmids have corresponding effects on chromosomal origin activity demonstrates directly that ARS elements are the *cis*-acting sequences required for chromosomal replication origin activity (Deshpande and Newlon 1992; Rivier and Rine 1992; Huang and Kowalski 1993; Marahrens and Stillman 1994; Theis and Newlon 1994). Similarly, the deletion of ARS elements from near the *ura4* locus of *S. pombe* was found to inactivate replication initiation events in their vicinity (Dubey et al. 1994).

The issue of the exact location relative to ARS elements of the actual replication initiation sites, i.e., the positions of leading-strand and lagging-strand primers, is not clearly resolved. If the B2 element is actually a DUE, then initiations would be expected to occur in and around B2. The accuracy of 2D gel origin mapping techniques is sufficient only to place initiation sites within a few hundred base pairs of ARS elements. Preliminary data suggest that the 5′ ends of leading strands map primarily to domain B of *ARS307* (S. Jenab and C.S. Newlon, in prep.).

ARS-binding Proteins

The activity of ARS elements as chromosomal replicators is almost certainly mediated by proteins that interact with them. Consistent with this idea, positioning a nucleosome over the ACS of *ARS1* reduces plasmid stability, presumably by blocking access of other proteins to the ARS element (Simpson 1990). The extreme sensitivity of the ACS to point mutations makes it the best candidate binding site for an initiator protein. After more than 10 years of effort in several laboratories, the multisubunit ORC that binds to the ACS was identified 3 years ago (Bell and Stillman 1992). The second DNA-binding protein that interacts with ARS elements, Abf1p, binds to the B3 element.

ORC

Initially identified by DNA footprinting assays on fractionated *S. cerevisiae* nuclear extracts, this protein with subunits of 120, 72, 62, 56, 53, and 50 kD requires ATP for DNA binding (Bell and Stillman 1992). It has been estimated that there are approximately 600 copies of Orc2p per cell (Rowley et al. 1995), which translates to approximately one ORC per replication origin. The ORC footprint includes domain A and extends into domain B of several ARS elements. The features of the footprint include protection of the domain A sequence and a strong hypersensitive site in element B1, with additional hypersensitive sites at approximately 10-bp intervals extending toward the B2 element (Bell and Stillman 1992; Bell et al. 1993; Micklem et al. 1993). The observed disruption of ORC binding by mutations in the ACS that abolish replicator activity and the reduction in the efficiency of ORC binding by mutations in B1 demonstrate the biological significance of the ORC/DNA interaction (Bell and Stillman 1992; Rao and Stillman 1995; Rowley et al. 1995). The similarity of the genomic footprint of proteins bound to *ARS1* and the 2 μm ARS in permeabilized cells to the in vitro footprint of purified ORC adds further support to the idea that ORC binds to replication origins in vivo (Diffley and Cocker 1992; Diffley et al. 1994). The pattern of DNase I hypersensitive sites and the sensitivity of domain B to copper-phenanthroline cleavage suggest that the DNA of domain B is wrapped on the ORC protein surface and is under torsional stress (Diffley and Cocker 1992).

Genetic analysis of ORC mutants has provided both additional support for the function of ORC in the initiation of DNA replication and evidence that ORC is involved in the transcriptional silencing of the silent mating loci. *ORC6* was identified in a screen for proteins that interact with the ACS and shows genetic interactions with several proteins implicated in the initiation of replication (Li and Herskowitz 1993; see below). Mutations in *ORC2* (Foss et al. 1993; Micklem et al. 1993) and *ORC5* (Loo et al. 1995) cause a plasmid maintenance defect that can be suppressed by additional replication origins, and ts *orc2* mutants are defective in the G_1/S transition at the nonpermissive temperature (Bell et al. 1993). Moreover, the efficiency of initiation at chromosomal replication origins, determined by 2D gel analysis, is reduced in *orc2* and *orc5* mutants at the permissive temperature and is further reduced at the nonpermissive temperature (Fox et al. 1995; Liang et al. 1995).

A role for ORC in transcriptional silencing was made obvious by the isolation of the *orc2* and *orc5* mutants discussed above in screens that made use of the *HMR E* silencer element that participates in the tran-

scriptional repression (silencing) of the *HMR* locus (Foss et al. 1993; Micklem et al. 1993; Loo et al. 1995). One of the three *cis*-acting sequences required for the function of this silencer is an ACS, and the silencer is active as a chromosomal replication origin (Rivier and Rine 1992). In addition to their defects in DNA replication, these Orc$^-$ mutants are defective in transcriptional silencing. Two lines of evidence suggest that the roles of ORC in DNA replication and silencing are independent. First, the *HMR E* silencer can be functionally replaced by Gal4p DNA-binding sites when a hybrid Sir1p-Gal4p DNA-binding domain protein is expressed (Chien et al. 1993). This observation suggests that the role of ORC and other silencer-binding proteins may be to recruit Sir1p to the silencer. Second, alleles of *ORC5* have been isolated that are proficient for DNA replication but defective in silencing (Fox et al. 1995).

Aside from its DNA-binding activity, no biochemical activities have been attributed to ORC. ATP or an ATP analog with a hydrolyzable β-γ bond is required for DNA binding by ORC, suggesting that ATP hydrolysis might be required (Bell and Stillman 1992). However, no ATPase activity has been identified. SV40 T antigen and *E. coli* DnaA protein both require ATP for untwisting DNA (Bramhill and Kornberg 1988; Borowiec et al. 1990). Orc5p has a predicted guanine nucleotide-binding site, but mutation of a highly conserved lysine that alters ATP binding or hydrolysis by other proteins with a similar binding site was not lethal (Loo et al. 1995). The mutant strain did exhibit slow growth at elevated temperatures, suggesting that the putative ATP-binding motif plays a role in Orc5p function.

ARS-binding Factor 1

Abf1p was identified in several laboratories on the basis of its binding to ARS elements, transcriptional silencers, or promoters (for review, see Campbell and Newlon 1991). In *ARS1* it interacts with the B3 element to stimulate replicator activity (Diffley and Stillman 1988; Marahrens and Stillman 1992), and it has been shown to function as a replication enhancer at *ARS121* (Walker et al. 1990). Not all ARS elements have Abf1p binding sites. Binding sites for this protein have also been demonstrated to function in transcriptional activation, transcriptional repression, and plasmid segregation (for review, see Campbell and Newlon 1991). The observations that *abf1* mutants show defects in plasmid maintenance (Rhode et al. 1992) and that genomic footprints of the B3 element of *ARS1* look similar to footprints with purified Abf1p in vitro (Diffley and

Cocker 1992; Rowley et al. 1995) strongly suggest that Abf1p interacts with ARS elements in vivo.

It is not clear how Abf1p enhances replicator activity. Its binding could have a direct role, for example, by inducing a conformational change in the ARS element or by interacting with another component of the replication apparatus. The observation that the mutations in element B3 of *ARS1* have no effect on ORC binding in vitro or in vivo indicates that Abf1p is unlikely to interact with ORC (Rao and Stillman 1995; Rowley et al. 1995). The possibility that RP-A is a target is suggested by the observation that the Gal4p and VP16 transcription factors interact with RP-A in vitro (He et al. 1993; Li and Botchan 1993). Consistent with this idea, mutations in the B3 element of *ARS1* appear to affect a late step in replicator activation, acting after the assembly of the prereplicative complex discussed below (Rowley et al. 1995). Alternatively, Abf1p could play an indirect role.

Other Proteins?

Using *ARS121* as a target, Eisenberg and colleagues have sought ARS-binding proteins. In addition to Abf1p (Eisenberg et al. 1988; Francesconi and Eisenberg 1991), two additional factors have been identified that are required for the formation of a maximally retarded *ARS121* complex in gel shift assays (Estes et al. 1992). OBF2 interacts with Abf1p and DNA to form a complex of intermediate mobility. In the presence of ATP, this complex is then competent to bind the third factor, core binding factor (CBF). The requirement of ATP and an ACS for the binding of CBF to *ARS121* suggests that CBF may be the same as ORC. However, in contrast to the reported properties of CBF, the binding of ORC to DNA does not require Abf1p (Rao and Stillman 1995; Rowley et al. 1995).

The B2 element is also a potential protein-binding site. The lack of DNA sequence conservation in this region of ARS elements, and the insensitivity of the B2 element of *ARS1* to point mutations, suggest that any proteins binding to B2 should have little sequence specificity. However, interaction with ORC could provide the specificity needed to recruit such a protein to replicators.

Other Proteins Required for Replication Initiation

The clarity of the ORC genomic footprint in cells from asynchronous cultures indicated that ORC is bound to replicators throughout most of

the cell cycle (Diffley and Cocker 1992), suggesting that replication initiation is unlikely to be mediated by the cell-cycle-regulated assembly of ORC at replication origins. It is possible that ORC activity is regulated by a cell-cycle-dependent modification. The presence of potential cyclin-dependent kinase phosphorylation sites in Orc2p and Orc6p (Li and Herskowitz 1993; Micklem et al. 1993) and the recent demonstration that B-type cyclins encoded by *CLB5* and *CLB6* normally trigger S phase (Epstein and Cross 1992; Schwob and Nasmyth 1993; Schwob et al. 1994; see Nasmyth, this volume) suggest one attractive model for the regulation of ORC activity.

Alternatively, one or more additional proteins may interact with ORC to regulate its activity. Evidence in support of this possibility is provided by the observation that the genomic footprints at *ARS1* and the 2 μm ARS change as a function of the cell cycle (Diffley et al. 1994). The footprints during S, G_2, and early M resemble the footprints generated by purified ORC and Abf1p in vitro. Beginning at anaphase and continuing through G_1, an additional region of protection appears that overlaps the ORC footprint and extends well into the B2 element, suggesting that additional protein(s) assembles on replicators at mitosis and remains there until S phase is triggered. The kinetics of appearance and disappearance of this prereplicative complex fit very well with observed properties of "licensing factor" in *Xenopus* and the presence of an S-phase-promoting factor deduced from mammalian cell fusion experiments (for review, see Su et al. 1995).

Excellent candidates for proteins that regulate the initiation of replication and models for how they work have recently emerged from studies of gene products identified by several genetic screens designed to target replication proteins.

CDC7 *and* DBF4

CDC7 encodes a 58-kD protein kinase having homology with cyclin-dependent protein kinases (Patterson et al. 1986). Although its target(s) has not been identified, Cdc7p is capable of phosphorylating both itself and histone H1 in vitro. It is required for several aspects of DNA metabolism, including mitotic DNA replication, meiotic DNA recombination, and replication-dependent DNA repair (for review, see Sclafani and Jackson 1994). Cdc7p appears to act just at the G_1/S boundary in mitotic cells, after completion of the protein synthesis required for DNA replication but before replication intermediates are produced (Hereford and Hartwell 1974; Petes and Newlon 1974). Although the level of Cdc7p is

constant, its kinase activity is regulated during the cell cycle and peaks at the G_1/S boundary (Jackson et al. 1993; Yoon et al. 1993).

Both genetic and biochemical data suggest that Cdc7p interacts with the product of the *DBF4/DNA52* gene, mutations in which cause a cell-cycle arrest similar to *cdc7* (Johnston and Thomas 1982a,b; Soloman et al. 1992). *DBF4* acts as a multicopy suppressor of *cdc7*, and vice versa, and the *cdc7dbf4* double mutant is inviable (Kitada et al. 1992). Cdc7p kinase activity is reduced in *dbf4* mutants, and Dbf4p and Cdc7p physically interact (Kitada et al. 1992; Jackson et al. 1993; Dowell et al. 1994). In contrast to *CDC7*, *DBF4* is periodically expressed, with mRNA levels peaking at the G_1/S boundary (Chapman and Johnston 1989). These observations suggest that Cdc7p kinase activity could be activated by association with Dbf4p in a manner similar to the activation of the Cdc28p kinase by association with cyclins.

The interaction of the Cdc7p kinase with Dbf4p is of particular interest because Dbf4p has recently been shown to interact with ARS elements (Dowell et al. 1994). The latter interaction was found in a "one-hybrid" genetic screen for hybrid proteins fused to the Gal4p activation domain capable of activating transcription of a reporter gene placed downstream from an ARS element. The recruitment of Dbf4p to *ARS1* requires domain A and is reduced by mutations in B1 and B2 but not B3, suggesting that Dbf4p interacts either directly or indirectly with ORC. The domain of Dbf4p that interacts with ARS elements is separable from the domain that interacts with Cdc7p, indicating that Dbf4p is likely to recruit Cdc7p to replication origins. Taken together, these observations suggest that the target of the Cdc7p kinase is a protein in prereplicative complexes whose phosphorylation may trigger the initiation of replication.

A *CDC7* homolog has recently been identified in *S. pombe*, and named *hsk1*$^+$ (Masai et al. 1995). Disruption of the gene is lethal, and analysis of germinating spores carrying the disruption suggests that DNA replication is inhibited. Moreover, a fraction of these spores undergo an aberrant mitosis, suggesting either that Hsk1p is required for inhibiting mitosis until S phase is completed or that it has an additional role in the proper execution of mitosis. The isolation of conditional alleles of *hsk1* should help to resolve this issue.

CDC6

Cdc6p was initially implicated in the initiation of DNA replication by two observations. First, early reciprocal shift experiments suggested that

its function was required for an early event in DNA replication, prior to the hydroxyurea-sensitive step (Hartwell 1976). Second, *cdc6* mutants exhibit a plasmid maintenance defect that can be suppressed by the inclusion of multiple ARS elements on the plasmid (Hogan and Koshland 1992). Recently, 2D gel analysis has been used to demonstrate directly that replication initiation at chromosomal origins is inefficient in *cdc6* mutants at the permissive temperature, and that the defect is more pronounced after a short time at the nonpermissive temperature (Liang et al. 1995). The inefficient initiation of replication observed in a ts *cdc6* strain is likely to result from partial function of the mutant protein, because the depletion of Cdc6p results in a strikingly different phenotype. In this case, both *S. pombe* strains depleted of Cdc18p and *S. cerevisiae* strains depleted of Cdc6p undergo a transient arrest at the G_1/S boundary, and then undergo an aberrant mitosis in the absence of DNA replication that results in cell death (Kelly et al. 1993; Piatti et al. 1995). Therefore, it is likely that Cdc6p and its *S. pombe* homolog Cdc18p are required both for the initiation of DNA replication and to delay mitosis until S phase is completed.

Genetic interactions between *CDC6* and *ORC6* (Li and Herskowitz 1993), the synthetic lethality of *cdc6* and *orc5* or *orc6*, and the identification of *CDC6* as a multicopy suppressor of *orc5-1* (Liang et al. 1995) suggested that Cdc6p might interact with ORC. Although the physical interaction of ORC with Cdc6p has not been directly demonstrated in yeast cells, the observation that Cdc6p expressed in insect cells could be immunoprecipitated by monoclonal antibodies against ORC following addition of purified ORC to the insect cell extracts provides biochemical evidence that the proteins interact (Liang et al. 1995).

CDC6 is expressed periodically during the cell cycle, primarily during late mitosis, but with a second burst of synthesis late during G_1 (Zhou and Jong 1990; Bueno and Russell 1992; Zwerschke et al. 1994; Piatti et al. 1995). Constitutive expression of *CDC6* at high levels causes a delay in entry into mitosis, indicating that the periodic expression pattern is important for normal function (Bueno and Russell 1992). The mitotic delay is not seen if *CDC6* is overexpressed by placing the gene under the control of its own promoter on a high copy plasmid, implying that Cdc6p either is unstable or is specifically degraded at some point in S or G_2 phase. Recent analysis using epitope-tagged Cdc6p has demonstrated that Cdc6p is unstable and disappears soon after the beginning of S phase (Piatti et al. 1995).

The timing of its expression and its interaction with ORC suggest that Cdc6p could be a component of, or required for, the assembly of the pre-

replicative complex assembled at replication origins. The finding that a *cdc6* mutant gives a postreplicative footprint at the nonpermissive temperature is consistent with this idea (Diffley et al. 1994), which is further supported by the observation that depletion of wild-type Cdc6p causes a defect in the formation and maintenance of the prereplicative complex (Cocker et al. 1996). The biochemical function of Cdc6p is unclear. It is predicted to be a 58-kD protein with a purine nucleotide-binding site (Zhou et al. 1989), and it has been reported to bind and hydrolyze ATP and GTP in a DNA-independent reaction (Zwerschke et al. 1994).

MCM Proteins

Genetic screens for mutations that influence plasmid stability have identified a number of candidates for proteins that function in the initiation of DNA replication (for review, see Campbell and Newlon 1991). Those most clearly implicated are a subset of the minichromosome maintenance (*mcm*) mutants that decrease the stability of plasmids carrying different ARS elements to varying extents (Maine et al. 1984; Gibson et al. 1987). These ARS-specific mutants identified a family of genes encoding related proteins and a gene encoding a transcription factor, Mcm1p.

The family of related proteins now includes the products of five *S. cerevisiae* genes, *MCM2*, *MCM3*, *CDC46/MCM5*, *CDC47*, and *CDC54* (Hennessy et al. 1990; Yan et al. 1991; Chen et al. 1992; Dalton and Whitbread 1995; Whitbread and Dalton 1995), as well as homologs from *S. pombe* (Coxon et al. 1992; Miyake et al. 1993), *Xenopus* (Coxon et al. 1992; Kubota et al. 1995), and a number of other eukaryotes (for review, see Su et al. 1995). In *S. cerevisiae* each of the five genes encoding members of this family is essential, suggesting that their functions are not completely overlapping. However, genetic interactions, both high copy suppression and synthetic lethality, suggest that the gene products interact with each other (Gibson et al. 1990; Hennessy et al. 1991; Yan et al. 1991) and with ORC (Li and Herskowitz 1993; Liang et al. 1995; Loo et al. 1995).

Several lines of evidence implicate these gene products in DNA replication. In addition to the ARS-specific plasmid maintenance defect, the mutants show hyperrecombination and chromosome loss phenotypes characteristic of DNA replication mutants (Sinha et al. 1986; Yan et al. 1991; Chen et al. 1992). More direct evidence includes the observations that *ARS1* is used inefficiently as a replication origin even at the permissive temperature in *mcm2* and *mcm3* mutants (Yan et al. 1993) and that *cdc46* mutants accumulate chromosomal DNA molecules that are unable

to enter pulsed-field gels, almost certainly because they are partially replicated (Hennessy et al. 1991). The intracellular localization of these proteins changes in an interesting fashion as well. They accumulate in the cytoplasm during S and G_2 and enter the nucleus at mitosis, remaining there until the G_1/S boundary, when they are either degraded or become cytoplasmic again (Hennessy et al. 1990; Yan et al. 1993; Dalton and Whitbread 1995).

The predicted amino acid sequence of these proteins places them in a superfamily of ATPases that includes the replication initiator proteins *E. coli* DnaA and SV40 T antigen (Koonin 1993). However, none of these proteins has yet been demonstrated to have ATPase or any other biochemical activity. These proteins are also relatively abundant, making it unlikely that they interact only with replicators.

MCM1 is an essential gene that encodes a transcription factor having homology with human serum response factor (Norman et al. 1988; Passmore et al. 1988, 1989). Mcm1p interacts with at least four other regulatory proteins and possibly also independently to modulate the transcription of diverse genes including cell-type-specific genes and Ty elements (for review, see Kuo and Grayhack 1994). Mcm1p is also implicated in DNA replication by its plasmid maintenance defect and its chromosome loss and hyperrecombination phenotypes (Elble and Tye 1992). Moreover, a ts *mcm1* mutant causes the temperature-dependent accumulation of large budded cells with single nuclei and partially replicated genomes, a phenotype shared with many DNA replication mutants. How the effects of Mcm1p on DNA replication are mediated is not known. One possibility is that it binds to ARS elements, perhaps by interaction with other ARS-binding proteins. Alternatively, it may be required for the expression of one or more proteins required for DNA replication.

Determinants of Origin Use

Whereas ARS elements clearly function as chromosomal replicators, their efficiency of use is highly variable, ranging from initiating replication in every cell cycle to initiating at an undetectable frequency (Brewer and Fangman 1988; Linskens and Huberman 1988; Dubey et al. 1991; Greenfeder and Newlon 1992; Newlon et al. 1993). As described above, mutations in ARS elements and the *trans*-acting factors that interact with them reduce the efficiency with which chromosomal replicators are used. However, the observation that chromosomal replicator efficiency does not correlate with ARS efficiency, measured by plasmid stability, suggests that some aspect of the chromosomal environment beyond the *cis*-

acting sequences intrinsic to ARS elements influences replicator activity. These "chromosomal context" effects can increase or decrease chromosomal replicator efficiency relative to the efficiency of the same replicator in plasmids. For example, *ARS307* functions relatively inefficiently in plasmids (Palzkill and Newlon 1988; Van Houten and Newlon 1990; Theis and Newlon 1994) but initiates replication in virtually every cell cycle in the chromosome (Deshpande and Newlon 1992; Greenfeder and Newlon 1992). In contrast, *ARS301* is an efficient plasmid replicator, but is inactive as a chromosomal replicator (Dubey et al. 1991).

One clear example of the influence of chromosomal context on replicator activity is the phenomenon called origin interference. The basic observation is that placement of efficient replicators close to each other in a chromosome or in a plasmid creates a situation in which replication initiates at only one of the closely spaced replicators in any particular cell cycle. Thus, when *ARS1* was inserted 6.5 kb away from *ARS501* on chromosome V, replication initiated at either *ARS1* or *ARS501* in any given cell, but not at both. A similar result was found for three copies of *ARS1* at the normal position of *ARS1* on chromosome IV; again, replication initiated at only one copy of *ARS1* in any particular cell, but all three copies were used in the population (Brewer and Fangman 1993). A strikingly different result was obtained with a construct containing two copies of *ARS1* at its normal position on chromosome IV (Marahrens and Stillman 1994). In this case, only one of the copies of *ARS1* was used in the population, and the second copy remained silent. An inactivating point mutation in the ACS of the dominant copy of *ARS1* caused the second copy to become active. Origin interference has also been observed in plasmids. From the pattern of plasmid replication intermediates, it had been inferred that only a single replicator was active in multimeric plasmids from yeast (Brewer and Fangman 1987), mammalian cells (Schvartzman et al. 1990), and *E. coli* (Martin-Parras et al. 1992). However, only recently has a plasmid in which the two ARS elements could be distinguished been studied (Brewer and Fangman 1994). In this case, only one of the two equally spaced copies of *ARS1* was used on any given plasmid, with one copy used approximately four times more frequently than the other. The sequences that establish replicator preference were mapped to the *URA3* gene on the plasmid. Thus, it appears that an active replicator can somehow suppress the activity of nearby replicators and that the selection of the active replicator is influenced by local context.

The mechanism of origin interference has not been established. It is possible that an active replicator actually inhibits initiation at a nearby

replicator, perhaps by steric hindrance of the formation of a second initiation complex by the first or by transmission of some sort of topological signal along the DNA molecule. Alternatively, the apparent inhibition could simply reflect slight differences in the timing of initiation. In this case, a fork from the origin that was activated first would replicate the second replicator before it was activated. Once the fork had passed, activation of the second replicator would be prevented by the mechanism that ensures that each chromosome is replicated once and only once per S phase.

The extent to which origin interference can account for the inefficient use of some chromosomal replicators and the apparent inactivation of others is not certain. However, the available information suggests that other mechanisms must exist. *ARS308*, which functions inefficiently in the chromosome, initiating replication in only 10–15% of cell cycles, is not activated by deletion of an efficient replicator (*ARS307*) located approximately 5 kb away (Greenfeder and Newlon 1992). Moreover, five inactive ARS elements on the left end of chromosome III are not activated by deletion of all active replicators from the adjacent 200-kb region of the chromosome, demonstrating that they must be inactivated by a mechanism other than origin interference (A. Dershowitz and C.S. Newlon, in prep.). Another important question is the distance over which origin interference can act. The average distance between active replicators in *S. cerevisiae* chromosomes is approximately 40 kb. The distances over which origin interference has been observed do not exceed 6.5 kb.

The active and inactive replicators on chromosome III are not interspersed but, rather, are present in blocks. The left 40 kb of the chromosome contains five inactive replicators, and two other inactive replicators are present in a 20- to 30-kb region in the middle of the right arm (Dubey et al. 1991; Newlon et al. 1993). This arrangement suggests that the chromosome might be organized into domains, some of which are permissive for replicator activity and some not. An attractive explanation for the inactivity of replicators at the left end of the chromosome was based on studies of transcriptional silencing by telomeres and by the silencer elements associated with the silent mating-type loci in *S. cerevisiae*. Mutations in a common set of approximately ten genes, including the genes encoding the core histones and *SIR2*, *SIR3*, and *SIR4*, are known to abrogate silencing by both telomeres and silencer elements (for review, see Laurenson and Rine 1992). The *SIR1* gene product functions at silencer elements but not at telomeres. The observation that mutations in the genes encoding histones abrogate silencing suggests that

silencing is mediated through the assembly of a repressive chromatin structure. The presence of both a telomere and a silent mating-type locus, *HML*, at or near the left end of chromosome III suggested that the putative repressive chromatin structure inactivated the replicators. Finding that the replicators on the left end of chromosome III are not active in a *sir1* or a *sir4* mutant or in a circular derivative of the chromosome that lacks telomeres (Dubey et al. 1991) demonstrates that it is unlikely that replicator inactivation is mediated by telomeres or transcriptional silencers. The observation that an active replicator is associated with the *HMR* locus on the right arm of chromosome III (Rivier and Rine 1992) is also inconsistent with inactivation of replicators by silenced chromatin.

Another possibility is that the activity of replicators is influenced by the physiological state of the cell. For example, it is clear that *S. cerevisiae* diploids respond to nitrogen deprivation by undergoing meiosis and sporulation. Several lines of evidence suggested that mitotic S phase and premeiotic S phase were regulated differently, leading to the idea that replicator use might be different in meiotic cells. However, examination of replication origin use on chromosome III revealed that the same origins are used in meiotic S phase and mitotic S phase, demonstrating that the inactive ARS elements at the left end of the chromosome are not meiotic origins (Collins and Newlon 1994).

In the case of *S. pombe*, it is not yet clear to what extent chromosomal replicator use varies. Replication bubble-containing replication intermediates have been found in chromosomal DNA fragments containing each of several different ARS elements (Caddle and Calos 1994; Dubey et al. 1994; Wohlgemuth et al. 1994). In every case examined, the replication intermediates included both Y-shaped molecules and bubble-containing molecules of all sizes, suggesting that replication initiates inefficiently at these replicators. In the single region that has been well-studied, it has been shown that at least three separable ARS elements contribute to the initiation events and that origin interference certainly contributes to the inefficiency of use of individual replicators (Dubey et al. 1994).

In summary, it seems likely that some aspect of structure or context influences chromosomal replicator activity in *S. cerevisiae*. The level at which these effects are mediated is presently unclear. One possible model suggests that *cis*-acting elements recruit activators or repressors that interact with or prevent the formation of prereplicative complexes at replicators. Other alternatives are that chromatin structure or chromosome organization within the nucleus functions as a mediator of these effects. A clear challenge for the future is to understand the mechanism(s) of these controls.

Temporal Control of Replication

It had been known for many years that there is a reproducible temporal pattern of replication of mammalian chromosomes (see Simon and Cedar, this volume). Because intact *S. cerevisiae* chromosomal DNAs are about the size of domains of mammalian chromosomes whose replication timing varies, and S phase is short, it was not clear whether there would be a reproducible temporal pattern of replication in yeast chromosomes. The earliest indication of a specific temporal order of replication was given by experiments that made use of nitrosoguanidine, a mutagen thought to act at the replication fork, to induce mutations in synchronous cultures. The finding that different genes were susceptible to mutation at different times suggested that they were replicated at different times (Burke and Fangman 1975; Sim and Haber 1975). Direct measures of replication timing, either by the incorporation of radioactive precursors into particular DNA molecules or by the use of density-shift experiments, demonstrated that particular DNA fragments replicate at reproducibly different times (Zakian et al. 1979; Brewer et al. 1980; Fangman et al. 1983; McCarroll and Fangman 1988).

Do replicators initiate at different times, or do all replicators initiate early and does replication timing simply reflect distance from an active replicator? In a systematic examination of chromosome III, it was found that fragments containing ARS elements are replicated at different times (Reynolds et al. 1989), and direct analysis of replication intermediates isolated from synchronous cultures has confirmed that replication initiates at *ARS305* and *ARS306* 5–10 minutes earlier than at *ARS307* and *ARS309* (S. Jenab and C.S. Newlon, unpubl.). There is a 28-minute difference between the times of initiation at the earliest and latest origins identified so far, suggesting that initiation events occur throughout most of S phase (Brewer et al. 1993).

Initiation timing appears to be determined by sequences extrinsic to ARS elements. This conclusion is based on experiments that made use of *ARS1*, which initiates early in its usual location on chromosome IV, and *ARS501*, which initiates late in its usual position on chromosome V. When a fragment containing *ARS1* was placed near *ARS501* on chromosome V, *ARS1* initiated late. In contrast, when a 14-kb fragment containing *ARS501* was cloned in a circular plasmid, *ARS501* initiated early (Ferguson et al. 1991; Ferguson and Fangman 1992). These observations rule out models in which different replicators are activated at different times because they have different affinities for an initiation factor or because they are activated by different initiation factors that are synthesized sequentially.

Several observations suggest that late timing may be conferred by telomere proximity. The early-replicating circular plasmid containing *ARS501* was turned into a late-replicating linear plasmid by the addition of telomeres (Ferguson and Fangman 1992). The 2 μm plasmid ARS also initiates 10–15 minutes later on a linear plasmid that in its normal context in a circular plasmid (Wellinger et al. 1993). The presence of telomeric repeats rather than linearity per se appears to be the determinant of late replication, because a linear plasmid produced by the introduction of a specific double-stranded break in vivo still replicates early (Raghuraman et al. 1994).

Telomeres do not appear to be the only late-replication determinant. The *KEX2 ARS*, which is the latest-initiating chromosomal replicator discovered so far, is more than 200 kb from the nearest telomere. Moreover, a 16-kb fragment containing this ARS causes a circular plasmid to replicate late. Therefore, a late-timing determinant must reside within this fragment, perhaps within the ARS itself (Brewer et al. 1993).

The studies described above demonstrate that late-replicating regions of yeast chromosomes arise in two ways. The region can be far from an early replicator, as is the case for the latest-replicating region of chromosome III, the left telomere (Reynolds et al. 1989). The left telomere is replicated by a fork that initiates at *ARS305*, which is the earliest-initiating replicator identified so far and is approximately 40 kb away (Newlon et al. 1993). Alternatively, the region can contain its own late-activated replicators, e.g., *ARS501* and the *KEX2 ARS*.

Replication Termination

Replication intermediates containing converging replication forks characteristic of replication termination can be distinguished by 2D gel analysis from replication intermediates containing replication bubbles or single forks. The available evidence suggests that replication termination in *S. cerevisiae* occurs throughout broad regions rather than at specific sites. Analysis of a 61-kb circular derivative of chromosome III that contains three ARS elements revealed three replication termination zones located approximately midway between the three pairs of replicators (Greenfeder and Newlon 1992). Altering the sites of replication initiation by deletion of *ARS307* from the circular chromosome created a new termination region, demonstrating that termination is not controlled by specific *cis*-acting sequences, but rather that termination regions are determined by the positions of active replicators. Two other termination zones have been examined. Replication termination intermediates were

found over a broad region midway between *ARS305* and *ARS306* on chromosome III (Zhu et al. 1992). The termination zone centromere-proximal to *ARS501* is so broad that termination intermediates associated with any particular restriction fragment were too faint to be seen in standard 2D gel analysis. Instead, the termination zone was identified by a gradual shift in the direction of replication fork movement through the region (Brewer et al. 1993). The excellent stability of chromosomes from which active replicators were deleted provides additional evidence against the existence of specific replication termini (Dershowitz and Newlon 1993; Huang and Kowalski 1993; Newlon et al. 1993). The existence of specific replication termini should prevent the replication of a region from which all origins were deleted and cause decreased chromosome stability.

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REFERENCES

Araki, H., R.K. Hamatake, L.H. Johnston, and A. Sugino. 1991a. *DPB2*, the gene encoding DNA polymerase II subunit B, is required for chromosomal replication in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **88:** 4601–4605.

Araki, H., R.K. Hamatake, A. Morrison, A.L. Johnson, L.H. Johnston, and A. Sugino. 1991b. Cloning *DPB3*, the gene encoding the third subunit of DNA polymerase II of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **19:** 4867–4872.

Araki, H., P.A. Ropp, A.L. Johnson, L.H. Johnston, A. Morrison, and A. Sugino. 1992. DNA polymerase II, the probable homolog of mammalian DNA polymerase ε, replicates chromosomal DNA in *Saccharomyces cerevisiae*. *EMBO J.* **11:** 733–740.

Ayyagari, R., K.J. Impellizzeri, B.L. Yoder, S.L. Gary, and P.M.J. Burgers. 1995. A mutational analysis of the yeast proliferating cell nuclear antigen indicates distinct roles in DNA replication and DNA repair. *Mol. Cell. Biol.* **15:** 4420–4429.

Bauer, G.A. and P.M.J. Burgers. 1990. Molecular cloning, structure, and expression of the yeast proliferating cell nuclear antigen. *Nucleic Acids Res.* **18:** 261–265.

Bauer, G.A., H.M. Heller, and P.M.J. Burgers. 1988. DNA polymerase III from *Saccharomyces cerevisiae*. I. Purification and characterization. *J. Biol. Chem.* **263:** 917–924.

Barker, D.G., J.H.M. White, and L.H. Johnston. 1987. Molecular characterisation of the DNA ligase gene, *CDC17*, from the fission yeast *Schizosaccharomyces pombe*. *Eur. J. Biochem.* **162:** 659–667.

Beach, D.H. and P. Nurse. 1981. High frequency transformation of the fission yeast *Schizosaccharomyces pombe*. *Nature* **290:** 140–142.

Bean, D.W., W.E. Kallam, Jr., and S.W. Matson. 1993. Purification and characterization of a DNA helicase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 21783–21790.

Bell, S.P. and B. Stillman. 1992. ATP-dependent recognition of eukaryotic origins of replication by a multiprotein complex. *Nature* **357:** 128–134.

Bell, S.P., R. Kobayashi, and B. Stillman. 1993. Yeast origin recognition complex functions in transcription silencing and DNA replication. *Science* **262:** 1844–1849.

Borowiec, J.A., F.B. Dean, P.A. Bullock, and J. Hurwitz. 1990. Binding and unwinding—How T antigen engages the SV40 origin of DNA replication. *Cell* **60:** 181–184.

Boulet, A., M. Simon, G. Faye, G.A. Bauer, and P.M.J. Burgers. 1989. Function of the *S. cerevisiae CDC2* gene encoding the large subunit of DNA polymerase III. *EMBO J.* **8:** 1849–1854.

Bramhill, D. and A. Kornberg. 1988. A model for initiation at origins of DNA replication. *Cell* **54:** 915–918.

Brewer, B.J. and W.L. Fangman. 1987. The localization of replication origins on ARS plasmids in *S. cerevisiae*. *Cell* **51:** 463–471.

———. 1988. A replication fork barrier at the 3′ end of yeast ribosomal RNA genes. *Cell* **55:** 637–643.

———. 1993. Initiation at closely spaced replication origins in a yeast chromosome. *Science* **262:** 1728–1731.

———. 1994. Initiation preference at a yeast origin of replication. *Proc. Natl. Acad. Sci.* **91:** 3418–3422.

Brewer, B.J., V.A. Zakian, and W.L. Fangman. 1980. Replication and meiotic transmission of yeast ribosomal RNA genes. *Proc. Natl. Acad. Sci.* **77:** 6739–6743.

Brewer, B.J., J.D. Diller, K.L. Friedman, K.M. Kolor, M.K. Raghuraman, and W.L. Fangman. 1993. The topography of chromosome replication in yeast. *Cold Spring Harbor Symp. Quant. Biol.* **58:** 425–434.

Brill, S.J. and B. Stillman. 1989. Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication. *Nature* **342:** 92–95.

———. 1991. Replication factor-A from *Saccharomyces cerevisiae* is encoded by three essential genes coordinately expressed at S phase. *Genes Dev.* **5:** 1589–1600.

Brill, S.J., S. DiNardo, K. Voekel-Meiman, and R. Sternglanz. 1987. Need for DNA topoisomerase activity as a swivel for DNA replication and for transcription of ribosomal RNA. *Nature* **326:** 414–416.

Brown, W.C., J.A. Duncan, and J.L. Campbell. 1993. Purification and characterization of the *Saccharomyces cerevisiae* DNA polymerase δ overproduced in *E. coli*. *J. Biol. Chem.* **268:** 982–990.

Budd, M.E. and J.L. Campbell. 1993. DNA polymerases δ and ε are required for chromosomal replication in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13:** 496–505.

———. 1995a. A yeast gene required for DNA replication encodes a protein with homology to DNA helicases. *Proc. Natl. Acad. Sci.* **92:** 7642–7646.

———. 1995b. DNA polymerases required for repair of UV-induced damage in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15:** 2173–2179.

Bueno, A. and P. Russell. 1992. Dual functions of *CDC6*: A yeast protein required for DNA replication also inhibits nuclear division. *EMBO J.* **11:** 2167–2176.

Burgers, P.M.J. 1991. *Saccharomyces cerevisiae* replication factor C. II. Formation and activity of complexes with proliferating cell nuclear antigen and with DNA

polymerases δ and ε. *J. Biol. Chem.* **266:** 22698–22706.

Burke, W. and W.L. Fangman. 1975. Temporal order in yeast chromosome replication. *Cell* **5:** 263–269.

Caddle, M.S. and M.P. Calos. 1994. Specific initiation at an origin of replication from *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **14:** 1796–1805.

Campbell, J.L. and C.S. Newlon. 1991. Chromosomal DNA replication. In *The Molecular and Cellular Biology of the Yeast* Saccharomyces: *Genome Dynamics, Protein Synthesis, and Energetics* (ed. J.R. Broach et al.), pp. 41–146. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Chapman, J.W. and L.H. Johnston. 1989. The yeast gene, *DBF4*, essential for entry into S phase, is cell cycle regulated. *Exp. Cell Res.* **180:** 419–428.

Chen, Y., K.M. Hennessy, D. Botstein, and B.-K. Tye. 1992. CDC46/MCM5, a yeast protein whose subcellular localization is cell cycle-regulated, is involved in DNA replication at autonomously replicating sequences. *Proc. Natl. Acad. Sci.* **89:** 10459–10463.

Chien, C.-T., S. Buck, R. Sternglanz, and D. Shore. 1993. Targeting of SIR1 protein establishes transcriptional silencing. *Cell* **75:** 531–541.

Cocker, J.L., S. Piatti, C. Santocanale, K. Nasmyth, and J.F.X. Diffley. 1996. An essential role for the Cdc6 protein in forming the pre-replicative complexes of budding yeast. *Nature* **379:** 180–182.

Collins, I. and C.S. Newlon. 1994. Chromosomal DNA replication initiates at the same origins in meiosis and mitosis. *Mol. Cell. Biol.* **14:** 3524–3534.

Collins, K.L., A.A.R. Russo, B.Y. Tseng, and T.J. Kelly. 1993. The role of the 70 kDa subunit of human DNA polymerase α in DNA replication. *EMBO J.* **12:** 4555–4566.

Coxon, A., K. Maundrell, and S.E. Kearsey. 1992. Fission yeast *cdc21*$^+$ belongs to a family of proteins involved in an early step of chromosome replication. *Nucleic Acids Res.* **20:** 5571–5577.

Cullmann, G., K. Fein, R. Kobayashi, and B. Stillman. 1995. Characterization of the five replication factor C genes from *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15:** 4661–4671.

Dalton, S. and L. Whitbread. 1995. Cell cycle-regulated nuclear import and export of Cdc47, a protein essential for initiation of DNA replication in budding yeast. *Proc. Natl. Acad. Sci.* **92:** 2514–2518.

Damagnez, V., J. Tillit, A.-M. Recondo, and G. Baldacci. 1991. The *POL1* gene from fission yeast, *Schizosaccharomyces pombe*, shows conserved amino acid blocks specific for eukaryotic DNA polymerase alpha. *Mol. Gen. Genet.* **266:** 182–189.

Dershowitz, A. and C.S. Newlon. 1993. The effect on chromosome stability of deleting replication origins. *Mol. Cell. Biol.* **13:** 391–398.

Deshpande, A.M. and C.S. Newlon. 1992. The ARS consensus sequence is required for chromosomal origin function in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 4305–4313.

Diffley, J.F.X. and J.H. Cocker. 1992. Protein-DNA interactions at a yeast replication origin. *Nature* **357:** 169–172.

Diffley, J.F.X. and B. Stillman. 1988. Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. *Proc. Natl. Acad. Sci.* **85:** 2120–2124.

Diffley, J.F.X., J. Cocker, S.J. Dowell, and A. Rowley. 1994. Two steps in the assembly of complexes at yeast replication origins in vivo. *Cell* **78:** 303–316.

Din, S., S.J. Brill, M.P. Fairman, and B. Stillman. 1990. Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. *Genes Dev.* **4:** 968–977.

DiNardo, S., K. Voelkel, and R. Sternglanz. 1984. DNA topoisomerase II mutant of *Saccharomyces cerevisiae*: Topoisomerase II is required for segregation of daughter molecules at termination of DNA replication. *Proc. Natl. Acad. Sci.* **81:** 2616–2620.

Dowell, S.J., P. Romanowski, and J.F.X. Diffley. 1994. Interaction of DBF4, the Cdc7 protein kinase regulatory subunit, with yeast replication origins in vivo. *Science* **265:** 1243–1246.

Dubey, D.D., J. Zhu, D.L. Carlson, K. Sharma, and J.A. Huberman. 1994. Three ARS elements contribute to the *ura4* replication origin region in the fission yeast, *Schizosaccharomyces pombe*. *EMBO J.* **13:** 3638–3647.

Dubey, D.D., L.R. Davis, S.A. Greenfeder, L.Y. Ong, J. Zhu, J.R. Broach, C.S. Newlon, and J.A. Huberman. 1991. Evidence suggesting that the ARS elements associated with silencers of the yeast mating type locus, *HML*, do not function as chromosomal DNA replication origins. *Mol. Cell. Biol.* **11:** 5346–5355.

Eisenberg, S., C. Civalier, and B.-K. Tye. 1988. Specific interaction between a *Saccharomyces cerevisiae* protein and a DNA element associated with certain autonomously replicating sequences. *Proc. Natl. Acad. Sci.* **85:** 743–746.

Elble, R. and B.-K. Tye. 1992. Chromosome loss, hyperrecombination, and cell cycle arrest in a yeast *mcm1* mutant. *Mol. Biol. Cell* **3:** 971–980.

Epstein, C.B. and F. Cross. 1992. CLB5: A novel B cyclin from budding yeast with a role in S phase. *Genes Dev.* **6:** 1695–1706.

Estes, H.G., B.S. Robinson, and S. Eisenberg. 1992. At least three distinct proteins are necessary for the reconstitution of a specific multiprotein complex at a eukaryotic chromosomal origin of replication. *Proc. Natl. Acad. Sci.* **89:** 11156–11160.

Fabiani, L., M. Aragona, and L. Frontali. 1990. Isolation and sequence analysis of a *K. lactis* chromosomal element able to autonomously replicate in *S. cerevisiae* and *K. lactis*. *Yeast* **6:** 69–76.

Fabiani, L., L. Frontali, and C.S. Newlon. 1996. Identification of an essential core element and stimulatory sequences in a *Kluyveromyces lactis* ARS element, KARS101. *Mol. Microbiol.* **19:** 757–766.

Fangman, W.L., R.H. Hice, and E. Chlebowicz-Sledziewska. 1983. ARS replication during the yeast S phase. *Cell* **32:** 831–838.

Ferguson, B.M. and W.L. Fangman. 1992. A position effect on the time of replication origin activation in yeast. *Cell* **68:** 333–339.

Ferguson, B.M., B.J. Brewer, A.E. Reynolds, and W.L. Fangman. 1991. A yeast origin of replication is activated late in S phase. *Cell* **65:** 507–515.

Firmenich, A., E.-A. Montserrat, and P. Berg. 1995. A novel allele of *Saccharomyces cerevisiae RFA1* that is deficient in recombination and repair and suppressible by *RAD52*. *Mol. Cell. Biol.* **15:** 1620–1631.

Flores-Rozas, H., Z. Kelman, F.B. Dean, Z.-Q. Pan, J.W. Harper, S.J. Elledge, M. O'Donnell, and J. Hurwitz. 1994. Cdk-interacting protein 1 directly binds with proliferating cell nuclear antigen and inhibits DNA replication catalyzed by the DNA polymerase δ holoenzyme. *Proc. Natl. Acad. Sci.* **91:** 8655–8659.

Foiani, M., G. Liberi, G. Lucchini, and P. Plevani. 1995. Cell cycle-dependent phosphorylation and dephosphorylation of the yeast DNA polymerase α-primase B subunit. *Mol. Cell. Biol.* **15:** 883–891.

Foiani, M., C. Santocanale, P. Plevani, and G. Lucchini. 1989. A single essential gene, *PRI2*, encodes the large subunit of DNA primase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **9:** 3081–3087.

Foiani, M., F. Marini, D. Gamba, G. Lucchini, and P. Plevani. 1994. The B subunit of the DNA polymerase α-primase complex in *Saccharomyces cerevisiae* executes an essential function at the initial stage of DNA replication. *Mol. Cell. Biol.* **14:** 923–933.

Foss, M., F.J. McNally, P. Laurenson, and J. Rine. 1993. Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae*. *Science* **262:** 1838–1844.

Fox, C.A., S. Loo, A. Dillin, and J. Rine. 1995. The origin recognition complex has essential functions in transcriptional silencing and chromosomal replication. *Genes Dev.* **9:** 991–924.

Francesconi, S.C. and S. Eisenberg. 1991. The multifunctional protein OBF1 is phosphorylated at serine and threonine residues in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **88:** 4089–4093.

Francesconi, S., M.P. Longhese, A. Piseri, C. Santocanale, G. Lucchini, and P. Plevani. 1991. Mutations in conserved yeast DNA primase domains impair DNA replication in vivo. *Proc. Natl. Acad. Sci.* **88:** 3877–3881.

Funabiki, H., I. Hagan, S. Uzawa, and M. Yanagida. 1993. Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. *J. Cell Biol.* **121:** 961–976.

Gangloff, S., J.P. McDonald, C. Bendixen, L. Arthur, and R. Rothstein. 1994. The yeast type topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: A potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* **14:** 8391–8398.

Gibson, S.I., R.T. Surosky, and B.-K. Tye. 1990. The phenotype of the minichromosome maintenance mutant *mcm3* is characteristic of mutants defective in DNA replication. *Mol. Cell. Biol.* **10:** 5707–5720.

Gibson, S., F. Surosky, P. Sinha, G. Maine, and B.-K. Tye. 1987. Complexity of the enzyme system for the initiation of DNA replication in yeast. *UCLA Symp. Mol. Cell. Biol. New Ser.* **47:** 341–354.

Goto, T. and J.C. Wang. 1984. Yeast DNA topoisomerase II is encoded by a single-copy, essential gene. *Cell* **36:** 1073–1080.

———. 1985. Cloning of yeast *TOP1*, the gene encoding topoisomerase I, and construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II. *Proc. Natl. Acad. Sci.* **82:** 7178–7182.

Goulian, M., S.H. Richards, C.J. Heard, and B.M. Bigsby. 1990. Discontinuous DNA synthesis by purified mammalian proteins. *J. Biol. Chem.* **265:** 18461–18471.

Greenfeder, S.A. and C.S. Newlon. 1992. A replication map of a 61kb circular derivative of *Saccharomyces cerevisiae* chromosome III. *Mol. Biol. Cell* **3:** 999–1013.

Harrington, J.J. and M.J. Lieber. 1994. Functional domains within FEN-1 and RAD2 define a family of structure-specific endonucleases: Implications for nucleotide excision repair. *Genes Dev.* **8:** 1344–1355.

Hartwell, L.H. 1976. Sequential function of gene products relative to DNA synthesis in the cell cycle. *J. Mol. Biol.* **104:** 803–817.

He, Z., B.T. Brinton, J. Greenblatt, J.A. Hassell, and C.J. Ingles. 1993. The transactivator proteins VP16 and GAL4 bind replication factor A. *Cell* **73:** 1223–1232.

Hennessy, K.M., C.D. Clark, and D. Botstein. 1990. Subcellular localization of yeast CDC46 varies with the cell cycle. *Genes Dev.* **4:** 2252–2263.

Hennessy, K.M., A. Lee, E. Chen, and D. Botstein. 1991. A group of interacting yeast DNA replication genes. *Genes Dev.* **5:** 958–969.

Hereford, L.M. and L.H. Hartwell. 1974. Sequential gene function in the initiation of *S. cerevisiae* DNA synthesis. *J. Mol. Biol.* **84:** 445–481.

Heyer, W.-D., M.R.S. Rao, L.F. Erdile, T.J. Kelly, and R.D. Kolodner. 1990. An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. *EMBO J.* **7:** 2321–2329.

Hogan, E. and D. Koshland. 1992. Addition of extra origins of replication to a minichromosome suppresses its mitotic loss in *cdc6* and *cdc14* mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **89:** 3098–3102.

Holm, C., T. Stearns, and D. Botstein. 1989. DNA topoisomerase II must act at mitosis to prevent nondisjunction and chromosome breakage. *Mol. Cell. Biol.* **9:** 159–168.

Holm, C., T. Goto, J.C. Wang, and D. Botstein. 1985. DNA topoisomerase II is required at the time of mitosis in yeast. *Cell* **41:** 553–563.

Howell, E.A., M.A. McAlear, D. Rose, and C. Holm. 1994. CDC44: A putative nucleotide-binding protein required for cell cycle progression that has homology to subunits of replication factor C. *Mol. Cell. Biol.* **14:** 255–267.

Huang, R-Y. and D. Kowalski. 1993. A DNA unwinding element and an ARS consensus comprise a replication origin within a yeast chromosome. *EMBO J.* **12:** 4521–4531.

Huberman, J.A., L.D. Spotila, K.A. Nawotka, S.M. El-Assouli, and L.R. Davis. 1987. The in vivo replication origin of the yeast 2 μm plasmid. *Cell* **51:** 473–481.

Huberman, J.A., D.D. Dubey, K.A. Nawotka, G. Russev, J.A. Sanchez, Y. Yoon, and M.H.K. Linskens. 1992. Directions of DNA replication in yeast and mammalian cells. In *DNA replication: The regulatory mechanisms* (ed. P. Hughes et al.), pp. 83–95. Springer-Verlag, Paris.

Ishimi, Y., A. Claude, P. Bullock, and J. Hurwitz. 1988. Complete enzymatic synthesis of DNA containing the SV40 origin of replication. *J. Biol. Chem.* **263:** 19723–19733.

Jackson, A.L., P.M. Pahl, K. Harrison, J. Rosamond, and R.A. Sclafani. 1993. Cell cycle regulation of the yeast Cdc7 protein kinase by association with Dbf4 protein. *Mol. Cell. Biol.* **13:** 2899–2908.

Johnston, L.H. 1979. The repair capability of *cdc9*, the *Saccharomyces cerevisiae* mutant defective in DNA ligase. *Mol. Gen. Genet.* **170:** 89–92.

———. 1983. The *cdc9* ligase joins completed replicons in baker's yeast. *Mol. Gen. Genet.* **190:** 315–317.

Johnston, L.H. and K.A. Nasmyth. 1978. *Saccharomyces cerevisiae* cell cycle mutant *cdc9* is defective in DNA ligase. *Nature* **274:** 891–893.

Johnston, L.H. and A.P. Thomas. 1982a. Isolation of new DNA synthesis mutants in the yeast *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **186:** 439–444.

———. 1982b. A further two mutants defective in initiation of the S phase in the yeast *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **186:** 445–448.

Kelly, T.J., G.S. Martin, S.L. Forsburg, R.J. Stephen, A. Russo, and P. Nurse. 1993. The fission yeast *cdc18*$^{+}$ gene product couples S phase to START and mitosis. *Cell* **74:** 371–382.

Kim, R.A. and J.C. Wang. 1989. The function of DNA topoisomerases as replication swivels in *Saccharomyces cerevisiae*. *J. Mol. Biol.* **208:** 257–267.

Kitada, K., L.H. Johnston, T. Sugino, and A. Sugino. 1992. Temperature-sensitive *cdc7* mutations of *Saccharomyces cerevisiae* are suppressed by the *DBF4* gene, which is required for the G1/S cell cycle transition. *Genetics* **131:** 21–29.

Konrad, E.B. and I.R. Lehman. 1974. A conditional lethal mutant of *Escherichia coli* K12 defective in the 5′-3′ exonuclease associated with DNA polymerase I. *Proc. Natl. Acad. Sci.* **71:** 2048–2051.

Koonin, E.V. 1993. A common set of conserved motifs in a vast variety of putative nucleic acid-dependent ATPases including MCM proteins involved in the initiation of eukaryotic DNA replication. *Nucleic Acids Res.* **21:** 2541–2547.

Kornberg, A. and T.A. Baker. 1992. *DNA replication, 2nd edition.* W.H. Freeman, New York.

Kouprina, N., E. Kroll, A. Kirillov, V. Bannikov, V. Zakharyev, and V. Larionov. 1994. *CHL12*, a gene essential for the fidelity of chromosome transmission in the yeast *Saccharomyces cerevisiae*. *Genetics* **138:** 1067–1069.

Kouprina, N., E. Kroll, V. Bannikov, V. Bliskovsky, R. Gizatullin, A. Kirillov, B. Shestopalov, V. Zakharyev, P. Hieter, F. Spencer, and V. Larionov. 1992. *CTF4* (*CHL15*) mutants exhibit defective DNA metabolism in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 5736–5747.

Krishna, T.S., X.P. Kong, S. Gary, P.M. Burgers, and J. Kuriyan. 1994. Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell* **79:** 1233–1243.

Kubota, Y., S. Mimura, S. Nishimoto, H. Takisawa, and H. Nojima. 1995. Identification of the yeast MCM3-related protein as a component of *Xenopus* licensing factor. *Cell* **81:** 601–609.

Kuo, C.-L., N.-H. Huang, and J.L. Campbell. 1983. Isolation of DNA replication mutants in permeabilized cells. *Proc. Natl. Acad. Sci.* **80:** 6465–6469.

Kuo, M.-H. and E. Grayhack. 1994. A library of yeast genomic MCM1 binding sites contains genes involved in cell cycle control, cell wall and membrane structure, and metabolism. *Mol. Cell. Biol.* **14:** 348–359.

Laurenson, P. and J. Rine. 1992. Silencers, silencing, and heritable transcriptional states. *Microbiol. Rev.* **56:** 543–560.

Li, J.J. and I. Herskowitz. 1993. Isolation of *ORC6*, a component of the yeast origin recognition complex, by a one-hybrid system. *Science* **262:** 1870–1874.

Li, R. and M.R. Botchan. 1993. The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate in vitro BPV-1 DNA replication. *Cell* **73:** 1207–1221.

Li, R., S. Waga, G.J. Hannon, D. Beach, and B. Stillman. 1994. Differential effects by the p21 CDK inhibitor on PCNA-dependent DNA replication and repair. *Nature* **371:** 534–537.

Li, X. and P.M.J. Burgers. 1994a. Cloning and characterization of the essential *Saccharomyces cerevisiae RFC4* gene encoding the 37 kDa subunit of replication factor C. *J. Biol. Chem.* **269:** 21880–21884.

———. 1994b. Molecular cloning and expression of the *Saccharomyces cerevisiae RFC3* gene, an essential component of replication factor C. *Proc. Natl. Acad. Sci.* **91:** 868–872.

Li, X., B.L. Yoder, and P.M.J. Burgers. 1992. A *Saccharomyces cerevisiae* DNA helicase associated with replication factor C. *J. Biol. Chem.* **267:** 25321–25327.

Liang, C., M. Weinreich, and B. Stillman. 1995. ORC and Cdc6p interact and determine the frequency of initiation of DNA replication in the genome. *Cell* **81:** 667–676.

Linskens, M.H.K. and J.A. Huberman. 1988. Organization of replication of ribosomal DNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **8:** 4927–4935.

Longhese, M.P., P. Plevani, and G. Lucchini. 1994. Replication factor A is required in

vivo for DNA replication, repair, and recombination. *Mol. Cell. Biol.* **14:** 7884–7890.

Longhese, M.P., L. Jovine, P. Plevani, and G. Lucchini. 1993. Conditional mutations in the yeast DNA primase genes affect different aspects of DNA metabolism and interactions in the DNA polymerase α-primase complex. *Genetics* **133:** 183–191.

Loo, S., C.A. Fox, J. Rine, R. Kobayashi, B. Stillman, and S. Bell. 1995. The origin recognition complex in silencing, cell cycle progression, and DNA replication. *Mol. Biol. Cell* **6:** 741–756.

Lucchini, G., S. Francesconi, M. Foiani, G. Badaracco, and P. Plevani. 1987. The yeast DNA polymerase-primase complex: Cloning of *PRI1*, a single essential gene related to DNA primase activity. *EMBO J.* **6:** 737–742.

Maine, G.T., P. Sinha, and B.-K. Tye. 1984. Mutants of *S. cerevisiae* defective in the maintenance of minichromosomes. *Genetics* **106:** 365–385.

Marahrens, Y. and B. Stillman. 1992. A yeast chromosomal origin of DNA replication defined by multiple functional elements. *Science* **255:** 817–823.

———. 1994. Replicator dominance in a eukaryotic chromosome. *EMBO J.* **13:** 3395–3400.

Martin-Parras, L., P. Hernandez, M.L. Martinez-Robles, and J.B. Schvartzman. 1992. Initiation of DNA replication in ColE1 plasmids containing multiple potential origins of replication. *J. Biol. Chem.* **267:** 22496–22505.

Masai, H., T. Miyake, and K. Arai. 1995. *hsk1*$^+$, a *Schizosaccharomyces pombe* gene related to *Saccharomyces cerevisiae CDC7*, is required for chromosomal replication. *EMBO J.* **14:** 3094–3104.

Maundrell, K., A. Hutchison, and S. Shall. 1988. Sequence analysis of ARS elements in fission yeast. *EMBO J.* **7:** 2203–2209.

McAlear, M.A., E.A. Howell, K.K. Espenshade, and C. Holm. 1994. Proliferating cell nuclear antigen (*pol30*) mutations suppress *cdc44* mutations and identify potential regions of interaction between the two encoded proteins. *Mol. Cell. Biol.* **14:** 4390–4397.

McCarroll, R.M. and W.L. Fangman. 1988. Time of replication of yeast centromeres and telomeres. *Cell* **54:** 505–513.

Micklem, G., A. Rowley, J. Harwood, K. Nasmyth, and J.F.X. Diffley. 1993. Yeast origin recognition complex is involved in DNA replication and transcriptional silencing. *Nature* **366:** 87–89.

Miles, J. and T. Formosa. 1992a. Protein affinity chromatography with purified yeast DNA polymerase α detects proteins that bind to yeast DNA polymerase. *Proc. Natl. Acad. Sci.* **89:** 1276–1280.

———. 1992b. Evidence that POB1, a *Saccharomyces cerevisiae* protein that binds to DNA polymerase α, acts in DNA metabolism in vivo. *Mol. Cell. Biol.* **12:** 5724–5735.

Miller, C.A. and D. Kowalski. 1993. *cis*-Acting components in the replication origin from ribosomal DNA of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13:** 5360–5369.

Miyake, S., N. Okishio, I. Samejima, Y. Hiraoka, T. Toda, I. Saitoh, and M. Yanagida. 1993. Fission yeast genes *nda1*$^+$ and *nda4*$^+$, mutations of which lead to S-phase block, chromatin alteration, and Ca^{2+} suppression, are members of the *CDC46/MCM2* family. *Mol. Biol. Cell* **4:** 1003–1015.

Morrison, A., J.B. Bell, T.A. Kunkel, and A. Sugino. 1991. Eukaryotic DNA polymerase amino acid sequence required for 3′→5′ exonuclease activity. *Proc. Natl. Acad. Sci.* **88:** 9473–9477.

Morrison, A., A.L. Johnson, L.H. Johnston, and A. Sugino. 1993. Pathway correcting

DNA replication errors in *Saccharomyces cerevisiae*. *EMBO J.* **12:** 1467–1473.

Morrison, A., H. Araki, A.B. Clark, R.K. Hamatake, and A. Sugino. 1990. A third essential DNA polymerase in *S. cerevisiae*. *Cell* **62:** 1143–1151.

Nasmyth, K.A. 1977. Temperature-sensitive lethal mutants in the structural gene for DNA ligase in the yeast *Schizosaccharomyces pombe*. *Cell* **12:** 1109–1120.

Natale, D.A., A.E. Schubert, and D. Kowalski. 1992. DNA helical stability accounts for mutational defects in a yeast replication origin. *Proc. Natl. Acad. Sci.* **89:** 2654–2658.

Navas, T.A., Z. Zhou, and S.J. Elledge. 1995. DNA polymerase ε links the DNA replication machinery to the S phase checkpoint. *Cell* **80:** 29–39.

Newlon, C.S. and J.F. Theis. 1993. The structure and function of yeast ARS elements. *Curr. Opin. Genet. Dev.* **3:** 752–758.

Newlon, C.S., I. Collins, A.M. Dershowitz, S.A. Greenfeder, L.Y. Ong, and J.F. Theis. 1993. Analysis of replication origin function on chromosome III of *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp. Quant. Biol.* **58:** 415–423.

Norman, C., M. Runswick, R. Pollock, and R. Treisman. 1988. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-*fos* serum response element. *Cell* **55:** 989–1003.

Noskov, V., S. Maki, Y. Kawasaki, S.H. Leem, B. Ono, H. Araki, Y. Pavlov, and A. Sugino. 1994. The *RFC2* gene encoding a subunit of replication factor C of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **22:** 1527–1535.

Palzkill, T.G. and C.S. Newlon. 1988. A yeast replication origin consists of multiple copies of a small conserved sequence. *Cell* **53:** 441–450.

Park, H., S. Francesconi, and T.S.-F. Wang. 1993. Cell cycle expression of two replicative polymerases α and δ from *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **4:** 145–157.

Passmore, S., R. Elble, and B.-K. Tye. 1989. A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes Dev.* **3:** 921–935.

Passmore, S., G.T. Maine, R. Elble, C. Christ, and B.-K. Tye. 1988. *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of MATα cells. *J. Mol. Biol.* **204:** 593–606.

Patterson, M., R.A. Sclafani, W.L. Fangman, and J. Rosamond. 1986. Molecular characterization of cell cycle gene *CDC7* from *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **6:** 1590–1598.

Petes, T.D. and C.S. Newlon. 1974. Structure of DNA in DNA replication mutants of yeast. *Nature* **251:** 637–639.

Piatti, S., C. Lengauer, and K. Nasmyth. 1995. Cdc6 is an unstable protein whose de novo synthesis in G1 is important for the onset of S phase and for preventing a "reductional" anaphase in the budding yeast *Saccharomyces cerevisiae*. *EMBO J.* **14:** 3788–3799.

Pignede, G., D. Bouvier, A.-M. Recondo, and G. Baldacci. 1991. Characterization of the POL3 gene product from *S. pombe* indicates interspecies conservation of the catalytic subunit of DNA polymerase δ. *J. Mol. Biol.* **222:** 209–218.

Raghuraman, M.K., B.J. Brewer, and W.L. Fangman. 1994. Activation of a yeast replication origin near a double-stranded DNA break. *Genes Dev.* **8:** 554–562.

Rao, H. and B. Stillman. 1995. The origin recognition complex interacts with a bipartite DNA binding site within yeast replicators. *Proc. Natl. Acad. Sci.* **92:** 2224–2228.

Rao, H., Y. Marahrens, and B. Stillman. 1994. Functional conservation of multiple ele-

ments in yeast chromosomal replicators. *Mol. Cell. Biol.* **14:** 7643–7651.

Rashid, M.B., K. Shirahige, N. Ogasawara, and H. Yoshikawa. 1994. Anatomy of the stimulative sequences flanking the ARS consensus sequence of chromosome VI in *Saccharomyces cerevisiae*. *Gene* **150:** 213–220.

Reagan, M.S., C. Pittenger, W. Siede, and E.C. Friedberg. 1995. Characterization of a mutant strain of *Saccharomyces cerevisiae* with a deletion of the *RAD27* gene, a structural homolog of the *RAD2* nucleotide excision repair gene. *J. Bacteriol.* **177:** 364–371.

Reynolds, A.E., R.M. McCarroll, C.S. Newlon, and W.L. Fangman. 1989. Time of replication of ARS elements along yeast chromosome III. *Mol. Cell. Biol.* **10:** 4488–4494.

Rhode, P.R., S. Elsasser, and J.L. Campbell. 1992. Role of multifunctional autonomously replicating sequence binding factor 1 in the initiation of DNA replication and transcriptional control in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 1064–1077.

Rivier, D.H. and J. Rine. 1992. An origin of DNA replication and a transcriptional silencer require a common element. *Science* **256:** 659–663.

Rowley, A., J.H. Cocker, J. Harwood, and J.F.X. Diffley. 1995. Initiation complex assembly at budding yeast replication origins begins with the recognition of a bipartite sequence by limiting amounts of the initiator, ORC. *EMBO J.* **14:** 2631–2641.

Santocanale, C., M. Foiani, G. Lucchini, and P. Plevani. 1993. The isolated 48,000-dalton subunit of yeast DNA primase is sufficient for RNA primer synthesis. *J. Biol. Chem.* **268:** 1343–1348.

Schvartzman, J.B., S. Adolph, L. Martin-Parras, and C.L. Schildkraut. 1990. Evidence that replication initiates at only some of the potential origins in each oligomeric form of bovine papillomavirus type 1 DNA. *Mol. Cell. Biol.* **10:** 3078–3086.

Schwob, E. and K. Nasmyth. 1993. *CLB5* and *CLB6*, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*. *Genes Dev.* **7:** 1160–1175.

Schwob, E., T. Boehm, M.D. Mendenhall, and K. Nasmyth. 1994. The B-type cyclin kinase inhibitor $p40^{sic1}$ controls the G1 to S transition in *S. cerevisiae*. *Cell* **79:** 233–244.

Sclafani, R.A. and A.L. Jackson. 1994. Cdc7: A protein kinase for DNA metabolism comes of age. *Mol. Microbiol.* **11:** 805–810.

Shimizu, K. and A. Sugino. 1993. Purification and characterization of DNA helicase III from the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 9578–9584.

Shirahige, K., T. Iwasaki, M.B. Rashid, N. Ogasawara, and H. Yoshikawa. 1993. Localization and characterization of autonomously replicating sequences (ARSs) from chromosome VI of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13:** 5043–5056.

Sim, G.K. and J.E. Haber. 1975. Cell cycle-dependent induction of mutations along a yeast chromosome. *Proc. Natl. Acad. Sci.* **72:** 1179–1183.

Simon, M., L. Giot, and G. Faye. 1991. The 3′ to 5′ exonuclease activity located in the DNA polymerase δ subunit of *Saccharomyces cerevisiae* is required for accurate DNA replication. *EMBO J.* **10:** 2165–2170.

Simpson, R.T. 1990. Nucleosome positioning can affect the function of a *cis*-acting DNA element in vivo. *Nature* **343:** 387–390.

Sinha, P., V. Chang, and B.-K. Tye. 1986. A mutant that affects the function of autonomously replicating sequences in yeast. *J. Mol. Biol.* **192:** 805–814.

Sitney, K.C., M.E. Budd, and J.L. Campbell. 1989. DNA polymerase III, a second essential DNA polymerase, is encoded by the *Saccharomyces cerevisiae CDC2* gene. *Cell* **56:** 599–605.

Smith, J. and R. Rothstein. 1995. A mutation in the gene encoding the *Saccharomyces cerevisiae* single-stranded DNA-binding protein Rfa1 stimulates a *RAD52*-independent pathway for direct-repeat recombination. *Mol. Cell. Biol.* **15:** 1632–1641.

Soloman, N.A., M.B. Wright, S. Chang, A.M. Buckley, L.B. Dumas, and R.F. Gaber. 1992. Genetic and molecular analysis of *DNA43* and *DNA52*: Two new cell-cycle genes in *Saccharomyces cerevisiae*. *Yeast* **8:** 273–289.

Sommers, C.H., E.J. Miller, B. Dujon, S. Prakash, and L. Prakash. 1995. Conditional lethality of null mutations in *RTH1* that encodes the yeast counterpart of a mammalian 5′- to 3′-exonuclease required for lagging strand DNA synthesis in reconstituted systems. *J. Biol. Chem.* **270:** 4193–4196.

Spell, R.M. and C. Holm. 1994. Nature and distribution of chromosomal intertwinings in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **14:** 1465–1476.

Stillman, B. 1994. Initiation of chromosomal DNA replication in eukaryotes. *J. Biol. Chem.* **269:** 7047–7050.

Strich, R., M. Woontner, and J.F. Scott. 1986. Mutations in *ARS1* increase the rate of simple loss of plasmids in *Saccharomyces cerevisiae*. *Yeast* **2:** 169–178.

Su, T.T., P.J. Follette, and P.H. O'Farrell. 1995. Qualifying for the license to replicate. *Cell* **81:** 825–828.

Theis, J.F. and C.S. Newlon. 1994. Domain B of *ARS307* contains two functional elements and contributes to chromosomal replication origin function. *Mol. Cell. Biol.* **14:** 7652–7659.

Thrash, C., K. Voelkel, S. DiNardo, and R. Sternglanz. 1984. Identification of *Saccharomyces cerevisiae* mutants deficient in DNA topoisomerase I activity. *J. Biol. Chem.* **259:** 1375–1377.

Tompkinson, A.E., N.J. Tappe, and E.C. Friedberg. 1992. DNA ligase I from *Saccharomyces cerevisiae*: Physical and biochemical characterization of the *CDC9* gene product. *Biochemistry* **31:** 11762–11771.

Turchi, J.J. and R.A. Bambara. 1993. Completion of mammalian lagging strand DNA replication using purified proteins. *J. Biol. Chem.* **268:** 15136–15141.

Turchi, J.J., L. Huang, R.S. Murante, Y. Kim, and R.A. Bambera. 1994. Enzymatic completion of mammalian lagging-strand DNA replication. *Proc. Natl. Acad. Sci.* **91:** 9803–9807.

Uemura, T. and M. Yanagida. 1986. Mitotic spindle pulls but fails to separate chromosomes in type II DNA topoisomerase mutants: Uncoordinated mitosis. *EMBO J.* **5:** 1003–1010.

Umek, R.M. and D. Kowalski. 1988. The ease of DNA unwinding as a determinant of initiation of yeast replication origins. *Cell* **52:** 559–567.

Vallen, E.A. and F.R. Cross. 1995. Mutations in *RAD27* define a potential link between G1 cyclins and DNA replication. *Mol. Cell. Biol.* **15:** 4291–4302.

Van Houten, J.V. and C.S. Newlon. 1990. Mutational analysis of the consensus sequence of a replication origin from yeast chromosome III. *Mol. Cell. Biol.* **10:** 3917–3925.

Waga, S. and B. Stillman. 1994. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication in vitro. *Nature* **369:** 207–212.

Waga, S., G.J. Hannon, D. Beach, and B. Stillman. 1994. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* **369:** 574–578.

Walker, S.S., S.C. Francesconi, and S. Eisenberg. 1990. A DNA replication enhancer in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **87:** 4665–4669.

Walker, S.S., A.K. Malik, and S. Eisenberg. 1991. Analysis of the interactions of functional domains of a nuclear origin of replication from *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **19:** 6255–6262.

Wang, J.C. 1991. DNA topoisomerases: Why so many? *J. Biol. Chem.* **266:** 6659–6662.

Wang, Z., X. Wu, and E.C. Friedberg. 1993. DNA repair synthesis during base excision repair in vitro is catalyzed by DNA polymerase ε and is influenced by DNA polymerases α and δ in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13:** 1051–1058.

Waseem, N.H., K. Labib, P. Nurse, and D.P. Lane. 1992. Isolation and analysis of the fission yeast gene encoding polymerase δ accessory protein PCNA. *EMBO J.* **11:** 5111–5120.

Watt, P.M., E.J. Louis, R.H. Borts, and I.D. Hickson. 1995. Sgs1: A eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. *Cell* **81:** 253–260.

Wellinger, R.J., A.J. Wolf, and V.A. Zakian. 1993. Origin activation and formation of single-strand TG_{1-3} tails occur sequentially in late S phase on a yeast linear plasmid. *Mol. Cell. Biol.* **13:** 4057–4065.

Whitbread, L.A. and S. Dalton. 1995. Cdc54 belongs to the Cdc46/Mcm3 family of proteins which are essential for initiation of eukaryotic DNA replication. *Gene* **155:** 113–117.

Wohlgemuth, J.G., G.H. Bulboaca, M. Moghadam, M.S. Caddle, and M.P. Calos. 1994. Physical mapping of origins of replication in the fission yeast *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **5:** 839–849.

Yan, H., S. Gibson, and B.-K. Tye. 1991. Mcm2 and Mcm3, two proteins important for ARS activity, are related in structure and function. *Genes Dev.* **5:** 944–957.

Yan, H., A.M. Merchant, and B.-K. Tye. 1993. Cell cycle-regulated nuclear localization of MCM2 and MCM3 which are required for the initiation of DNA synthesis at chromosomal replication origins in yeast. *Genes Dev.* **7:** 2149–2160.

Yoon, H.J., S. Loo, and J.L. Campbell. 1993. Regulation of *Saccharomyces cerevisiae CDC7* function during the cell cycle. *Mol. Biol. Cell* **4:** 195–208.

Zakian, V.A., B.J. Brewer, and W.L. Fangman. 1979. Replication of each copy of the 2 μm plasmid occurs during the S phase. *Cell* **17:** 923–934.

Zhou, C. and A. Jong. 1990. *CDC6* mRNA fluctuates periodically in the yeast cell cycle. *J. Biol. Chem.* **265:** 19904–19909.

Zhou, C., S.-H. Huang, and A.Y. Jong. 1989. Molecular cloning of *Saccharomyces cerevisiae CDC6* gene. *J. Biol. Chem.* **264:** 9022–9029.

Zhu, J., C.S. Newlon, and J.A. Huberman. 1992. Localization of a DNA replication origin on chromosome III of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 4733–4741.

Zhu, J., D.L. Carlson, D.D. Dubey, K. Sharma, and J.A. Huberman. 1994. Comparison of the two major ARS elements of the *ura4* replication origin region with other ARS elements in the fission yeast, *Schizosaccharomyces pombe*. *Chromosoma* **103:** 414–422.

Zwerschke, W., H.-W. Rottakob, and H. Kuntzel. 1994. The *Saccharomyces cerevisiae CDC6* gene is transcribed at late mitosis and encodes a ATP/GTPase controlling S phase initiation. *J. Biol. Chem.* **269:** 23351–23356.