Mcm2 and Mcm3, two proteins important for ARS activity, are related in structure and function

Hong Yan, Susan Gibson,¹ and Bik K. Tye

Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853 USA

MCM2 and MCM3 are essential genes believed to play important roles in the initiation of DNA replication in *Saccharomyces cerevisiae*. Mutants defective in Mcm2 or Mcm3 are remarkably similar in phenotype. They both show an autonomously replicating sequence (ARS)-specific minichromosome maintenance defect, although their ARS specificities are not identical. In addition, these mutants exhibit a premitotic cell cycle arrest and an increase in chromosome loss and recombination. Genetic studies suggest that the two MCM gene products play interacting or complementary roles in DNA replication. Double mutants of *mcm2-1* and *mcm3-1* are inviable at the permissive growth temperature (23°C) for each of the single mutants. Furthermore, overproduction of Mcm3 accentuates the deleterious effect of the *mcm2-1* mutation, whereas overproduction of Mcm2 partially complements the *mcm3-1* mutation. MCM2 encodes a protein of 890 amino acids containing a putative zinc-finger domain that is essential for Mcm2 function. Mcm2 shows striking homology to Mcm3 and three other proteins, Cdc46 of *S. cerevisiae*, and Nda4 and Cdc21 of *Schizosaccharomyces pombe*. The phenotypes of mutants defective in these proteins suggest that they belong to a protein family involved in the early steps of DNA replication.

[Key Words: MCM2; MCM3; DNA replication; *Saccharomyces cerevisiae*; ARS]

Received February 7, 1991; accepted March 12, 1991.

The initiation of DNA replication at replication origins is a complex process involving the concerted action of many proteins. In *Escherichia coli*, this sequence of events is carried out by a number of proteins including *dnaA*, which binds and melts the origin region, and the primosome, which unwinds and initiates DNA synthesis (Bramhill and Kornberg 1988). In SV40, the virally encoded T antigen plays an analogous role to the *dnaA* protein in the binding and melting of the origin region (Stillman 1989). The mechanism for the initiation of DNA replication of eukaryotic genomes, which involves initiation at a large number of sites, may be more complex. Even the small genome of the yeast *Saccharomyces cerevisiae* contains at least 250 replication origins (Chan and Tye 1980). The complexity of the enzyme system committed to these initiation events at multiple sites is unknown.

The autonomously replicating sequences (ARSs), which serve as chromosomal replication origins in yeast (Huberman et al. 1988), are AT-rich sequences that contain an 11-bp consensus sequence (Broach et al. 1983). This consensus sequence is essential but not sufficient for ARS function, requiring the presence of flanking sequences that have little in common. To explain how

these seemingly dissimilar flanking sequences might provide similar auxiliary function in different ARSs, it has been suggested that the AT content and, therefore, the local melting properties of the flanking sequences, determine the functionality of an ARS (Umek and Kowalski 1988). Alternatively, the melting of flanking sequences could be facilitated by initiator proteins that bind specifically to these sequences. Interestingly, two ARS-binding proteins, ABF1 (Diffley and Stillman 1988), identified by its affinity to *ARS1*, and OBF1 (Eisenberg et al. 1988), identified by its affinity to *ARS121*, which were first thought to be different proteins based on their affinities to apparently unrelated sequences, turned out to be the same protein with broad binding specificity (Biswas and Biswas 1990). This finding suggests that if ABF1–OBF1 plays the role of an enhancer of DNA replication (Walker et al. 1990), it does so by acting at a large number of ARSs.

Parallel to the studies on ARS-binding proteins, we attempted to identify gene products involved in DNA replication initiation by isolating mutants that fail to activate ARSs. Analysis of the effect of these mutants on a spectrum of ARSs should allow us to determine whether a particular gene product is required for the initiation of DNA replication at all ARSs, at some subset of ARSs, or at only one ARS. Previously, we have identified a number of mutants defective in the maintenance of

¹Present address: MSU–DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 USA.

minichromosomes (Maine et al. 1984). Mutants in five complementation groups were of particular interest because, based on plasmid stability assays, they affected the function of all ARSs tested, but each to a different extent (Gibson et al. 1987). This phenotype of these *mcm* mutants suggests that their gene products are involved in the initiation of DNA replication at ARSs, rather than in other aspects of replication or segregation. In fact, we have confirmed that one of these mutants, *mcm2*, affects the replication, rather than the segregation, of plasmids (Sinha et al. 1986). We have carried out detailed analyses on three of the ARS-specific mutants, *mcm1*, *mcm2*, and *mcm3*, that were isolated in three independent mutant screens, each involving a minichromosome carrying a different ARS (Maine et al. 1984; Sinha et al. 1986; Gibson et al. 1990). Mutations in all of these genes also cause other phenotypes that are characteristic of defects in DNA replication, such as premitotic cell cycle arrest and increased chromosome loss and recombination; this suggests that these gene products may play similar roles in replication initiation at ARSs. Mcm1 is a multifunctional protein that plays important roles in transcriptional regulation in addition to ARS function (Passmore et al. 1988, 1989). In this discussion we examine the structural and functional relationships between the *MCM2* and *MCM3* gene products. We show that Mcm2 and Mcm3 belong to a family of proteins that are likely to be involved in the early steps of DNA replication.

Results

Cloning and mapping of the *MCM2* gene

The *mcm2-1* mutation, although not lethal to the cell, results in a slower growth rate at 37°C. Furthermore, at this temperature, minichromosomes containing *ARS1* are extremely unstable, being lost at a rate of ~0.5/cell per generation. Thus, an *mcm2-1* mutant that relies on *ARS1*-containing plasmids to complement its auxotrophic deficiencies grows extremely poorly on selective medium at 37°C. We cloned the *MCM2* gene by complementation of this poor growth phenotype.

An *mcm2-1* strain, M46-1-3C (*MAT α ura3 leu2 his3*) was transformed to uracil prototrophy using a library of yeast DNA cloned in the vector YCp50 (*ARS1 CEN4 URA3*) (Rose et al. 1987) and to leucine prototrophy using the plasmid YCp101 (*ARS1 CEN5 LEU2*). The transformed culture was plated on complete medium lacking uracil and leucine, grown for 2.5 hr at 23°C and for an additional 70 hr at 37°C. Transformants containing a wild-type copy of the *MCM2* gene will stabilize both plasmids and can be identified by their large colony size. From ~30,000 transformants, 37 gave rise to large colonies and were identified as possibly containing the wild-type *MCM2* gene.

One such transformant, M46-33, was chosen for further study. Stability assays indicate that this transformant maintains minichromosomes at wild-type or almost wild-type levels (Table 1). In addition, its growth defect at 37°C is no longer apparent (data not shown).

Table 1. Minichromosome stability at 35°C in *mcm2-1* strain transformed with plasmid [pM46-33] carrying the putative *MCM2* gene

Strain (genotype)	Stability (%) ^a	Minichromosome
M46-1-3C (<i>mcm2-1</i>)	0	YCp101 (<i>ARS1 CEN5 LEU2</i>)
8534-8C (<i>MCM2</i>)	71	YCp101 (<i>ARS1 CEN5 LEU2</i>)
MY4C/Y4 (<i>mcm2-1/MCM2</i>)	29	pYES3 (<i>ARS1 CEN5 URA3</i>)
M46-1-3C [pM46-33]	55	YCp101 (<i>ARS1 CEN5 LEU2</i>)

^aStability is the final percentage of cells still carrying the test plasmid after 10 generations of growth in nonselective medium at 35°C.

When the plasmid was isolated and retransformed into another *mcm2-1* strain, the strain became Mcm2⁺ in phenotype. Furthermore, we showed that the chromosomal location of the cloned yeast DNA insert is tightly linked to the *mcm2-1* mutation. This was achieved by targeting integration of the *URA3* gene into the chromosomal location of the cloned yeast DNA insert in an *mcm2-1* mutant strain. A transformant, designated tAM46-1-3C, was then crossed with the wild-type strain TD4. The diploid was sporulated, and the Mcm2 and Ura3 phenotypes of the resultant spores were analyzed. The Mcm2⁻ and Ura3⁺ phenotypes cosegregated in all 13 tetrads examined, suggesting that the chromosomal location of the yeast DNA insert is either very close to, or the same as, that of the *mcm2-1* mutation. These results indicate that the cloned yeast DNA insert contains the *MCM2* gene.

We further localized the *MCM2* gene within the 10-kb cloned fragment by transposon mutagenesis (see Materials and methods). As shown in Figure 1a, transposons at positions 6, 20, 31, 52, and 135 destroyed, whereas transposons at positions 1, 12, 14, 19, 21, 32, 108, and 134 preserved the ability of the clone to complement the *mcm2-1* mutation. Therefore, the *MCM2* gene must reside in the region indicated by hatching. A 3.5-kb subclone containing this region (from *Bgl*II to the second *Cla*I; Fig. 1a and b) is fully capable of complementing the *mcm2-1* mutation (data not shown).

We also showed that *MCM2* is located on chromosome II by hybridizing part of the *MCM2* gene to a chromosome blot (kindly supplied by Dr. H. Klein). Tetrad analysis placed *MCM2* between *pet 9* and *AMY 2* on the left arm of chromosome II (Gibson 1989).

MCM2 is essential for cell viability

To investigate whether *MCM2* is essential for growth, we constructed a diploid strain with one of the copies of *MCM2* disrupted by gene replacement (Rothstein 1983). The disrupted copy contained the *URA3* gene as part of the minitransposon Tn3 at position 135 (see Fig. 1a). This diploid was sporulated, and tetrads were dissected. Of the 20 tetrads analyzed, 18 yielded two viable spores and 2 yielded one viable spore. Furthermore, all viable spores were Ura⁻, indicating that they have the wild-

Figure 1. (a) Localization of the *MCM2* gene within the original clone by minitransposon mutagenesis. Numbers and arrows indicate the positions and orientations of the transposons. (+ and -) Complementation and no complementation, respectively, of the temperature-sensitive growth phenotype of *mcm2-1* mutant cells at 37°C. Hatched region indicates the location of the *MCM2* gene. (b) Restriction map of the *BglII*-*Clal* fragment containing the *MCM2* gene. The arrow indicates the ORF and its orientation. (A) *Apal*; (Bg) *BglII*; (C) *Clal*; (M) *MluI*; (P) *PvuII*; (R) *EcoRI*; (S) *SmaI*; (X) *XbaI*.

type copy of the *MCM2* gene. Examination of the dead spores indicated that they arrested after two or three cell divisions with a dumbbell morphology. This result suggests that *Mcm2* is essential for cell-division cycle.

Depletion of Mcm2 results in cell cycle arrest at the end of S phase

The arrest phenotype of the *mcm2* disruption mutant led us to further characterize the phenotype of cells depleted in *Mcm2*. Because we do not have a tight temperature-sensitive mutant allele of *mcm2*, we constructed a conditionally expressed *mcm2* allele. The *MCM2* open reading frame (ORF) was placed downstream of the galactose-inducible *GAL1* promoter in the plasmid pHY12. This plasmid complements the *mcm2::LEU2* disruption in the yeast strain HY101d; therefore, it has a growth rate similar to its parent strain TD4 on galactose medium. Upon shift to glucose medium, however, it stops growing within 6–8 hr, with >95% of the cells arresting at the large bud stage (Fig. 2a). When arrested cells were stained with 4,6-diamidino-2-phenylindole (DAPI), a DNA-specific fluorescent dye, >90% of the cells contained a single nucleus that is not generally localized at the bud neck (Fig 2b).

We also carried out flow cytometric analysis to determine the DNA content of the *Mcm2*-depleted cells (Hutter and Eipel 1978). Cells growing in galactose medium were distributed in two major populations by DNA content with 39% of the cells having 1n DNA and 54% of

Figure 2. Arrest phenotype of *Mcm2*-depleted cells. Strain HY101d (*mcm2::LEU2*) carrying plasmid pHY12 (*GAL1::MCM2*) and wild-type strain TD4 were grown at 30°C to early log phase in complete galactose medium and then switched to complete glucose medium. Samples were taken at 0 or 6 hr after shift and then stained with either DAPI or propidium iodide. (a) DAPI-stained HY101d cells 6 hr after shift to glucose medium. (Top) Phase-contrast field; (Bottom) fluorescence field. (b) Propidium iodide-stained cells analyzed by flow cytometry. The total cellular DNA content is proportional to the level of propidium iodide (in arbitrary units).

the cells having 2n DNA (Fig. 2b; HY101d, 0 hr). In contrast, 6 hr after shift to glucose medium, >85% of the cells contained close to 2n DNA (Fig. 2b; HY101d, 6 hr). However, the peak of this population of cells clearly falls below the 2n DNA content, suggesting that these cells have replicated most, but not all, of their DNA. No further significant change was observed in the distribution of fluorescence in the cell population 12 hr after shift to glucose medium. Similar analysis of wild-type (TD4) cells indicated that changing the carbon source in the growth medium did not affect the DNA content distribution in the population. Minor differences in DNA content distribution between HY101d (39% in 1n, 54% in 2n) and TD4 (48% in 1n, 46% in 2n) growing in galactose may be attributed to the loss of pHY12 in a small fraction of the cells and/or the effects of overproduction of Mcm2 in HY101d.

Mcm2 shows striking homology to Mcm3

The nucleotide sequence of the *MCM2* gene was deter-

mined using the dideoxynucleotide chain-termination method (Sanger et al. 1977) and is available through EMBL data bank (accession number X53539). A long open-reading frame was found within the region corresponding to the *MCM2* gene. This sequence predicts a protein of 890 amino acids assuming that translation begins at the first ATG (Fig 3, upper sequence). When the *GAL1* promoter was placed upstream of the first ATG of the *MCM2* gene, an Mcm2 protein that complemented an *mcm2* disruption was produced on galactose medium but not on glucose medium, indicating that this ORF indeed encodes Mcm2 (see above section). The predicted molecular weight of Mcm2 is 101,351 with a pI value of 6.4.

A search through several sequence banks (GenBank, EMBL, NBRF, and SWISS PROT) failed to identify any known proteins with significant sequence similarity to Mcm2. However, a direct comparison between the ORFs of the three *MCM* genes sequenced in our laboratory uncovered a striking homology between the Mcm2 and

Figure 3. Comparison of the amino acid sequences of the Mcm2 and Mcm3 proteins. The Mcm2 sequence (upper) and the Mcm3 sequence (lower) were aligned using the GAP program of the UWCGC package with the gap weight and length weight set at 5.0 and 0.3, respectively. The zinc-finger motif of Mcm2 is indicated by the boxed region.

Mcm3 sequences (Figs. 3 and 4). These two proteins showed 50% similarity and 30% identity over their entire lengths by the gap program of GCG version 6.1 (Devereux et al. 1984), using a gap weight of 5.0 and length weight of 0.3. The homology concentrates in three regions (Fig. 4): a 200-amino acid stretch from position 493 to 692 in Mcm2 (region II), and two short stretches, amino-terminal (region I) and carboxy-terminal (region III) to region II. Recently, when we submitted these sequences to the protein sequence bank of Dr. Mark Goebel, homologies to three additional proteins, Cdc46 from *S. cerevisiae* (K. Hennessy et al., this issue), and Cdc21 (A. Coxon and S. Kearsey, pers. comm.) and Nda4 (M. Yanagida, pers. comm.) from *Schizosaccharomyces pombe* were identified. Although Cdc46 and Nda4 are clearly homologs, showing >70% identity (Hennessy et al., this issue), the pairwise similarities between Mcm2, Mcm3, Cdc21, and Cdc46/Nda4 are about the same. The phenotypes associated with mutants defective in each of these genes suggest that they are involved in DNA synthesis at the beginning of S phase (Nasmyth and Nurse 1981; Hennessy et al. 1990).

Mcm2 contains a zinc-finger motif that is not conserved in Mcm3

Inspection of the Mcm2 ORF for sequence motifs asso-

ciated with known functions revealed a zinc-finger motif $_{342}\text{Cys-X}_2\text{-Cys-X}_{19}\text{-Cys-X}_2\text{-Cys}_{368}$ (Fig. 3, boxed region) characteristic of DNA-binding domains (Evans and Hollenberg 1988). The Phe, located 2 amino acids upstream from the zinc-finger motif, is a further indication that this motif is likely to function as a DNA-binding zinc finger as it is present at the same position upstream of a number of zinc-finger proteins (Berg 1990). We noted that this zinc-finger motif is not conserved in Mcm3, Cdc46/Nda4, or Cdc21.

To investigate the functional significance of the zinc-finger motif of the Mcm2 protein, we changed the second cysteine pair by site-directed mutagenesis. Each cysteine was changed to one of three different amino acids (Tyr, Phe, and Ser) or both cysteines were changed to His (Fig. 5a; Table 2). In this way, the new *mcm2* mutant alleles, *mcm2-2* to *mcm2-8*, were created. The mutant (*mcm2-1* to *mcm2-8*) and wild-type genes were cloned into vector pSE355 (*TRP1 ARS1 CEN4*) to form plasmids pHY1–pHY9. The resulting plasmids were transformed into HY101d (*mcm2::LEU2*) carrying the resident plasmid pHY10 (*MCM2 URA3*) that provides the essential Mcm2 function for HY101d. Transformants were streaked onto FOA plates, and their growth at 15, 23, 30, and 37°C in the absence of the resident pHY10 plasmid were examined. Transformants of pHY9 (*MCM2*) and pHY1 (*mcm2-1*) were able to grow on 5-fluoro-orotic acid (FOA) plates

Figure 4. A dot plot comparing the Mcm2 and Mcm3 sequences using the program COMPARE of the UWGCG package with a window size of 15 and stringency of 11.

Figure 5. Site-specific mutagenesis of the *MCM2* gene. (a) Oligonucleotides used in the mutagenesis. Nucleotides in parentheses are substituted for the wild-type sequence. (b) Plasmids carrying different *mcm2* alleles were transformed into strain HY101d (*mcm2::LEU2*) containing plasmid pHY10 (*MCM2 URA3*). The resulting transformants were streaked onto either Cm-Leu (left) or Cm-Leu + FOA (right) plates and allowed to grow at 30°C for 5 days.

at all temperatures tested, although transformants of pHY1 showed a smaller colony size than those of pHY9. However, none of the transformants of pHY2-8 grew on FOA plates at any of the temperatures tested, suggesting that both residues of the second cysteine pair in the zinc-finger motif of Mcm2 are crucial to the function of Mcm2 (Fig. 5b). Notably, histidine substitutions at these positions are equally deleterious for Mcm2 function.

The mcm2-1 mutation results in a Glu to Lys substitution 25 amino acids downstream of the putative zinc finger

To gain some insight into the structure–function relationship of the Mcm2 protein, we identified the nature of the *mcm2-1* mutation. The approximate location of the *mcm2-1* mutation was first determined by a series of targeted recombination events (Fig. 6a; Shortle et al. 1984). We constructed three plasmids, each containing a fragment of the *MCM2* gene such that one end extends beyond the *MCM2* gene and the other end lies within the *MCM2* gene (Fig. 6b). The plasmids were cut with the appropriate restriction enzymes and transformed into an *mcm2-1* strain (M46-1-3c). Targeted integration of a plasmid results in a duplication, but only one of the repeats has the entire *MCM2* gene. Recovery of wild-type trans-

formants indicates that the *mcm2-1* mutation must lie between the restriction enzyme cleavage site and the intact end of the *MCM2* gene. Conversely, if only mutant transformants are recovered, then the *mcm2-1* mutation must lie within the other half of the *MCM2* gene.

The result of this experiment is shown in Figure 6b. Plasmids containing the large *Xba*I fragment (pMCM2.X1) digested by *Mlu*I only gave rise to mutant transformants, indicating that the mutation is to the right of the *Mlu*I site. In contrast, plasmids containing the *Bgl*II (pMCM2.Bg) or *Xba*I (pMCM2.X1) fragment cleaved with *Cla*I consistently generated ≥50% wild-type transformants, indicating that the mutation is to the left of the *Cla*I site. The generation of only ~50% wild-type transformants may be due to nuclease degradation from the cleaved site, which would result in a gapped region that could only be repaired from the chromosomal copy. In this way, we were able to localize the *mcm2-1* mutation to the region between the *Mlu*I and *Cla*I sites on the 5' half of the *MCM2* gene.

Plasmids containing the wild-type and *mcm2-1* alleles were recovered from a wild-type transformant derived from the *Cla*I-digested pMCM2.Bg plasmid. Briefly, genomic DNA was isolated, cleaved with *Sst*I or *Hind*III, self-ligated, and transformed into *E. coli*. Plasmids generated from the *Sst*I digestion contain the *mcm2-1* allele, whereas plasmids generated from the *Hind*III digestion contain the wild-type *MCM2* allele (Fig. 6a). Isogenic plasmids containing the wild-type and mutant *MCM2* genes were constructed by substituting the *Mlu*I–*Cla*I fragment from the wild-type *MCM2* gene with that from the mutant. The identities of these alleles were confirmed by their *mcm* and growth phenotypes. When the *Mlu*I–*Cla*I fragments were sequenced, we found a single base difference between the mutant and the wild-type fragments. This G → A transition mutation, which resulted in a Glu → Lys substitution, is therefore responsible for both the *mcm* and temperature-sensitive growth phenotype of *mcm2-1* (Fig. 6c).

This Glu → Lys substitution mutation lies 25 amino

Table 2. Mutations obtained

Mutation	Codon	Position	Amino acid
<i>mcm2-2</i>	TAC	368	Tyr
<i>mcm2-3</i>	TCC	368	Ser
<i>mcm2-4</i>	TTC	368	Phe
<i>mcm2-5</i>	TTT	366	Phe
<i>mcm2-6</i>	TAT	366	Tyr
<i>mcm2-7</i>	TCT	366	Ser
<i>mcm2-8</i>	CAT	366	His
	CAC	368	His

Figure 6. Identification of the *mcm2-1* mutation. (a) Strategy for mapping the *mcm2-1* mutation illustrated by using plasmid pMCM2.Bg cut with *Xba*I. The integration results in an intact copy and a partial copy of the *Mcm2* gene. (b) The *mcm* phenotype of the *mcm2-1* strain transformed with different cleaved plasmids. Arrows indicate the location of the *mcm2-1* mutation relative to the cleavage site. Wild-type and mutant transformants were distinguished by their growth rates at 37°C. Twenty transformants from each transformation were checked. (B) *Bgl*II; (C) *Cla*I; (H) *Hind*III; (M) *Mlu*I; (S) *Sst*I; (X) *Xba*I. (c) Sequences of the wild-type and mutant DNAs in the region containing the *mcm2-1* mutation. The single mutation predicts a substitution of lysine for glutamate at position 392. (d) Alignment of five homologous sequences in this region. Lines indicate identical amino acids. Dots and double dots indicate amino acids of different degrees of similarities. Residues in bold are invariant among all five proteins.

acids downstream of the zinc-finger motif. Interestingly, although the zinc-finger motif is not conserved in Mcm3, Cdc21, Cdc46, or Nda4, this region containing the *mcm2-1* mutation is highly conserved in all five proteins (Fig. 6d). Of 25 amino acids, 7 are invariant in all five proteins. Similarities among all five proteins are obvious even in an expanded region of ~50 amino acids (Fig. 4, region I; K. Hennessy et al., this issue), with the greatest homology concentrated in the vicinity of the *mcm2-1* mutation.

The *mcm2-1 mcm3-1* double mutant is lethal

The similarities in the protein sequences of Mcm2 and Mcm3 suggest that they might perform similar or related functions in the cell. If so, one might expect a double mutant of *mcm2-1 mcm3-1* to have a more severe growth defect than either mutant alone. To determine the phenotype of the *mcm2-1 mcm3-1* double mutant, we constructed a diploid strain that is *mcm2-1* homozygous and *mcm3-1* heterozygous. Briefly, plasmid pMCM2-

1.S, which contains a fragment of the *mcm2-1* mutant gene, was cut with *Bgl*II and transformed into strain R61-4 (*mcm3-1*). The resultant transformants should contain a partial *mcm2-1* gene and the entire *MCM2* gene. One such transformant was then crossed with the *mcm2-1* strain M46-8. The resulting diploid was grown in YEPD to saturation and plated on medium with FOA to select for the excision of the *URA3* gene (Boeke et al. 1984). These excision events should yield an intact copy of either the wild-type or mutant allele of *MCM2*, giving rise to diploids that are either heterozygous or homozygous for *mcm2-1* and that are distinguishable by their mini-chromosome maintenance phenotype.

The *mcm2-1* homozygous, *mcm3-1* heterozygous diploid was sporulated, and the resultant tetrads were dissected (Fig. 7a). Of 35 tetrads dissected, 29 yielded two live and two dead spores, 4 yielded one live spore, and the other 2 yielded no live spores. This result suggests that the *mcm2-1 mcm3-1* double mutant is lethal, although neither mutation alone causes a discernible growth defect at the temperature (23°C) used for spore

Figure 7. (a) The *mcm2-1 mcm3-1* double mutant is lethal. A diploid strain that is *mcm2-1* homozygous and *mcm3-1* heterozygous was sporulated, and the tetrads were dissected on YEPD plates at 23°C. (b) Overproduction of the Mcm3 protein in *mcm2-1* cells is lethal at 37°C. Strain HY101d (*mcm2::LEU2*) containing plasmid pHY1 (*mcm2-1 TRP1*) was used as the host. Transformants carrying different overproducing plasmids were streaked onto Cm-Ura galactose plates and allowed to grow at 37°C for 5 days.

germination. To ensure that the original FOA-resistant diploid did not result from the loss of the chromosome that carries the integrated plasmid, a scenario that would give the same result in tetrad analysis, a minichromosome containing *MCM2 URA3* was transformed into the same diploid. In this strain, of 10 tetrads dissected, 1 yielded four live spores and 2 yielded three live spores. The *mcm3-1* mutation always segregated with the minichromosome (*MCM2 URA3*) in the viable spores. These *mcm3-1* strains were then examined for their ability to grow independently of the plasmid on FOA plates. All of them failed to grow, leading us to conclude that the *mcm2-1 mcm3-1* double mutant is inviable.

Overproduction of Mcm3 is lethal in the *mcm2-1* strain

We have reported previously that overproduction of Mcm3 in a wild-type strain causes an ARS-specific minichromosome maintenance defect (Gibson et al. 1990). An Mcm3-overproducing strain containing pB620.MCM3

was constructed by placing the *MCM3* gene under the control of the *GAL1* promoter in a 2 μ vector, pB620. The stabilities of minichromosomes YCp121 (*ARS121 LEU2*) and YCp131 (*ARS131 LEU2*) in this strain, growing in either glucose or galactose medium, were measured by the rate of plasmid loss per cell per generation. As shown in Table 3, overproduction of Mcm3 has little effect on the stability of YCp121, which has a loss rate of 0.02 in glucose and 0.03 in galactose medium. However, overproduction of Mcm3 has a dramatic effect on the stability of YCp131, which has a loss rate of 0.03 in glucose and 0.25 in galactose medium. In contrast, there is no change in the rates of loss of these plasmids in glucose or galactose medium in the yeast strain containing pB620.MCM2 and pB704, which are similar to pB620.MCM3, except that in these plasmids either *MCM2* or *lacZ* is fused to the *GAL1* promoter instead of *MCM3*.

The lethality of the *mcm2-1 mcm3-1* double mutant suggests that Mcm2 and Mcm3 may be involved in similar or related functions. Because overproduction of Mcm3 has an *Mcm*[−] phenotype similar to that of the *mcm3-1* mutation, we were interested in the effect of overproduction of Mcm3 in the *mcm2-1* mutant strain. Figure 7b shows the effect of overproduction of Mcm3, Mcm2, and β -galactosidase in the *mcm2-1* mutant strain at 37°C. Overproduction of Mcm2, but not β -galactosidase, complements the slow growth phenotype of the *mcm2-1* mutation. In contrast, overproduction of Mcm3 was lethal in the *mcm2-1* strain grown at 37°C.

Overproduction of Mcm2 partially complements the *mcm3-1* mutation

Overproduction of Mcm3 results in an *mcm* defect in the wild-type strain and a lethal phenotype in the *mcm2-1*

Table 3. Effect of Mcm2 or Mcm3 overproduction on minichromosome stability

Overproducing plasmid	Test plasmid	Loss rate in glucose	Loss rate in galactose
pB620.MCM2	YCp121	0.02	0.07
	YCp131	0.06	0.04
pB620.MCM3	YCp121	0.02	0.03
	YCp131	0.03	0.25
pB704	YCp121	0.07	0.05
	YCp131	0.04	0.05

Yeast strain 8534-8C was transformed with one of the overproducing plasmids (pB620.MCM2, pB620.MCM3, or pB704) to uracil prototrophy and one of the test plasmids (YCp121 or YCp131) to leucine prototrophy. The transformed cells were grown in complete medium lacking leucine and uracil, containing 3% glycerol and 2% ethanol as the carbon sources. They were then grown for an additional 10 generations in Cm-Ura medium with either 2% galactose or 2% glucose as the carbon source. Cells were first plated on Cm-Ura and then replica-plated on Cm-Leu-Ura. Loss rate is defined as plasmid loss per cell per generation. Numbers are averages of results from two to three independent experiments.

strain. In contrast, overproduction of Mcm2 has no effect on minichromosome maintenance in the wild-type strain (see Table 3). Furthermore, overproduction of Mcm2 complements the temperature-sensitive growth defect of the *mcm3-1* mutant (Fig. 8). However, this complementation is only partial as colonies resulting from Mcm2 overproduction are smaller than those resulting from Mcm3 overproduction. Also, this partial complementation is only observed when Mcm2 is overproduced (cells grown in galactose medium) but not when Mcm2 is produced at low levels (cells grown in glucose medium). Overproduction of β -galactosidase had no effect on the growth defect of the *mcm3-1* strain.

The *mcm2-1* and *mcm3-1* mutants have different ARS specificities

The structural similarity and functional relationship between the Mcm2 and Mcm3 proteins suggest that these two proteins perform interacting or complementing functions. To gain further insight into their respective roles in ARS function, we felt that a direct comparison of

Figure 8. Overproduction of Mcm2 can partially complement *mcm3-1*. Overproducing plasmids were transformed into R61.4 (*mcm3-1*) strain. The transformants were streaked onto either Cm-Ura glucose (a) or Cm-Ura galactose plates (b) and allowed to grow at 23°C for 6 hr and then at 37°C for 5 days.

Figure 9. The effect of *mcm2-1* and *mcm3-1* mutations on minichromosome maintenance. The loss rates of minichromosomes carrying different ARSs in strains 8534-8C (wild type), M46-1-3C (*mcm2-1*), and R61.4 (*mcm3-1*) are shown. Absolute loss rates in 8534-8C are presented in numbers, and the ratios of loss rates in mutants over those in 8534-8C are presented in a bar graph. (Solid box) *mcm2-1*; (stippled box) *mcm3-1*.

their ARS specificities in minichromosome maintenance might be informative.

We further defined ARS specificity in minichromosome maintenance in the *mcm* mutants by using a larger sample of well-characterized ARSs. *ARS1*, *ARSHML*, *ARSH2B*, and *ARSHO* are ARS elements associated, respectively, with the *TRP1* (Struhl et al. 1979), the silent mating-type locus *HML α* (Feldman et al. 1984), the histone *H2B* (Osley and Hereford 1982), and the endonuclease *HO* (Kearsey 1984) genes. *ARS131*, *ARS131C*, and *ARS120* are three ARSs associated with subtelomeric X sequences (Chan and Tye 1983). *ARS121* is a single-copy ARS element of unknown genomic location (Chan and Tye 1980). The loss rates of eight minichromosomes, each carrying *CEN5* and one of these ARS elements, were measured in wild-type and mutant strains at room temperature. To compare the effects of each mutation on a particular minichromosome, the relative loss rates of each minichromosome in mutant and wild type are presented in a bar graph in Figure 9.

In the wild-type strain, all minichromosomes are stable, although to varying degrees. Four of them, YCpHO, YCpH2B, YCp120, and YCp131C, are extremely stable, with respective loss rates of 0.003, 0.003, 0.007, and 0.003/cell per generation. In contrast, YCp131 and YCpHML are lost at rates of 0.04 and 0.05/cell per generation, ~10-fold higher than the group above. Minichromosomes YCp101 and YCp121 have intermediate loss rates of ~0.02/cell per generation (Fig. 9). A hierarchy for ARSs based on plasmid stabilities in the wild-type strain is, in descending order, *ARSHO*, *ARSH2B*, *ARS131C*, *ARS120* > *ARS1*, *ARS121* > *ARS131*, and *ARSHML*.

We then examined the effect of the *mcm2-1* mutation on the stability of these minichromosomes. We found that there is no correlation between the hierarchy of ARSs in wild-type cells and the stabilities of these ARSs

in *mcm2-1* cells. For example, YCpHO and YCpH2B are comparable in stability in the wild-type strain. In the mutant, YCpH2B is also quite stable (7-fold increase in loss rate over that in the wild-type), yet YCpHO has a 90-fold increase in loss rate in the *mcm2-1* mutant. Similar observations are also made in the *mcm3-1* mutant, suggesting that the *mcm* phenotype of these mutants is not due to an amplification of preexisting differences in ARSs as a result of indirect effects, such as slower growth rates or changes in the length of the phases in the cell cycle.

A comparison of the effects of the *mcm2-1* and *mcm3-1* mutations on minichromosome stabilities reveals that these two mutations can exert quite different effects on the same ARS. For example, YCpH2B, which is among the most stable in wild-type strains, is little affected in *mcm2-1* (7-fold increase in loss rate) but becomes very unstable in *mcm3-1* (110-fold increase in loss rate). Thus, two conclusions can be drawn from this study. First, there is no correlation between the relative strengths of the ARSs in wild-type and *mcm* mutant strains. Second, *mcm2-1* and *mcm3-1* mutants exert their most dramatic effects on different sets of ARSs.

Discussion

Structural relationship between Mcm2 and Mcm3

We used minichromosome maintenance as an assay to screen for mutants that affect the function of ARSs. We chose to study *mcm1*, *mcm2*, and *mcm3* because their ARS-dependent minichromosome maintenance defect suggests that they play important roles in the function of ARSs. These mutants also share other phenotypes characteristic of DNA replication defects, such as a premitotic cell cycle arrest with cells containing almost fully replicated DNA, increased chromosome loss, and hyperrecombination (Gibson et al. 1990).

Although the mutant phenotypes of *mcm1*, *mcm2*, and *mcm3* are remarkably similar, structural similarity is only shared between Mcm2 and Mcm3. While Mcm1 is a relatively small protein (286 amino acids), Mcm2 and Mcm3 are larger proteins of 890 and 971 amino acids, respectively. Extensive homology between the Mcm2 and Mcm3 proteins is localized in three regions (I, II, and III), with the most extensive and best homology found within the 200 amino acids of region II.

Inspection of the Mcm2 ORF revealed that, of nine cysteines in the entire ORF, four are spaced so that they could form a zinc finger similar to those found in certain DNA-binding proteins (Evans and Hollenberg 1988). Three types of zinc-finger motifs, Cys2/His2, Cys5-6, and Cys4, have been described. The zinc-finger motif of Mcm2 is of the Cys4 type. The Cys2/His2 and Cys5-6 zinc fingers have been demonstrated to be functional DNA-binding domains (Berg 1990). Proteins known to contain the Cys4 zinc-finger motifs include the adenovirus protein E1A (Flint and Shenk 1989), the yeast protein HOP1 (Hollingsworth et al. 1990), and eIF2 β (Donahue et al. 1988); all three proteins are implicated in

roles involving DNA or RNA binding. Mutagenesis of the cysteines or the amino acids around them in the zinc-finger motifs either abolished or modified the functions of these proteins. Furthermore, the zinc-finger motif of E1A has been shown to bind zinc (Culp et al. 1988) and constitutes at least part of the DNA-binding domain (Chatterjee et al. 1988). We have mutagenized the second cysteine pair in the putative zinc finger of Mcm2 and found these mutations to be lethal, suggesting that these cysteines are essential for the function of Mcm2.

We have also identified the *mcm2-1* mutation to be a single-base-change mutation that converts a Glu to a Lys, 25 amino acids downstream of the putative zinc-finger motif. The regions carboxyl to zinc fingers have been shown to be important for DNA binding (Pfeifer et al. 1989). Thus, Mcm2 may act by binding to DNA through its zinc-finger motif, a property expected of Mcm2 if it were directly involved in DNA replication at ARSs. Interestingly, although the zinc-finger motif is not conserved in Mcm3, the *mcm2-1* mutation lies within one of the Mcm2–Mcm3 conserved regions, region I, suggesting that this region may also be important for Mcm3 function.

Functional relationship between Mcm2 and Mcm3

Although the ARS-specific minichromosome maintenance phenotype of *mcm2-1* and *mcm3-1* suggests that these two gene products are involved in the initiation of DNA replication at ARSs, cells depleted of or altered in the MCM2 or MCM3 gene products arrest with extensively replicated DNA (Gibson et al. 1990). We believe that this arrest phenotype is the result of selective initiation of DNA replication at only a small number of ARSs under conditions of limiting Mcm2 or Mcm3 activity. Although DNA synthesis can occur under these conditions, replication cannot be completed.

Further genetic analysis suggests that Mcm2 and Mcm3 may play some complementary functions in DNA replication. At room temperature, single mutants of *mcm2-1* or *mcm3-1* are viable, yet the double mutant is lethal. Moreover, overproduction of Mcm2 partially complements the *mcm3-1* mutation. However, it is clear that these two proteins cannot completely substitute for each other as each of them is required for viability. There is some evidence suggesting that Mcm2 and Mcm3 may interact as components of a larger complex because overproduction of Mcm3 in the *mcm2-1* strain exacerbates the *mcm2* defect. The fact that the *mcm2-1* and *mcm3-1* mutations exert their most dramatic effects on different sets of ARSs suggests that if Mcm2 and Mcm3 do function in a complex by binding to ARSs, they both contribute to the binding specificity of the complex as a whole.

Models for action of Mcm2 and Mcm3

One of the most interesting features of the *mcm* mutants is the ARS specificity of the minichromosome maintenance defect. We examined the effects of the *mcm2-1*

and *mcm3-1* mutations on a large number of well-characterized ARSs. At low temperatures, both mutants affect different ARSs to greater or lesser degrees. At less permissive temperatures, all ARSs tested are affected in these mutants. Thus, it is unlikely that the gene products of *MCM2* and *MCM3* act only at a subset of ARSs. Rather, if they do act directly at ARSs, they both contribute to the activation of all ARSs, although each to different extents. It is also unlikely that this ARS specificity of the *mcm* mutants is simply an amplification of pre-existing differences inherent in ARSs, as strong ARSs in wild-type strains are not necessarily strong ARSs in the *mcm* mutants.

Given that Mcm2 and Mcm3 are structurally and functionally related, what might be their specific functions? We speculate that Mcm2 and Mcm3 are proteins directly involved in the activation of most, if not all, ARSs. A plausible model consistent with the data accumulated on Mcm2 and Mcm3 is that these two proteins interact in a multisubunit complex to act at ARSs. We suggest that both Mcm2 and Mcm3 contribute to the binding specificity of the complex as a whole, but contact of the complex with the DNA may be mediated by Mcm2. The *mcm2-1* mutation could thus affect the binding affinity of Mcm2, whereas the *mcm3-1* mutation could affect the interaction between Mcm2 and Mcm3. In this model overproduction of Mcm3 could lead to a stoichiometric imbalance of the complex, resulting in an *mcm* defect; on the other hand, overproduction of Mcm2 could increase the occupancy of the complex on the DNA. An equally plausible model is to suggest that Mcm2 and Mcm3 bind to ARSs independently of each other. In this case, the contribution of each of these proteins to ARS activity depends on the quantity and quality of the binding sites of these proteins at ARSs. This model explains the nonequivalent ARS specificity exhibited by the *mcm2-1* and *mcm3-1* mutants. Again, in this model, we have to invoke Mcm3 acting as a subunit in a complex to account for the *mcm* phenotype that results from overproduction of Mcm3.

Mcm2 and Mcm3 as members of a larger family of proteins

The finding that Mcm2 and Mcm3 are homologous to three other proteins, all of which play important roles at the beginning of S phase, supports the idea that Mcm2 and Mcm3 are proteins involved in the initiation of DNA replication at ARSs. Sequence conservation between Mcm2, Mcm3, Cdc46/Nda4 (*S. pombe*), and Cdc21 (*S. pombe*) is remarkably strong. Each pair of sequences share ~30% identity, with the best homologies localized in three regions. Cdc46 has been shown to be required for DNA synthesis. Interestingly, this protein is mobilized from the cytoplasm to the nucleus in a cell-cycle-dependent manner, such that it appears in the nucleus as mitosis is completed and persists there until the next round of DNA synthesis is initiated (Hennessy et al. 1990). Although tight alleles of *cdc46* arrest at the G₁/S phase with an unreplicated genome, leaky alleles of

cdc46 mutants have an arrest phenotype similar to *mcm2-1* and *mcm3-1* mutants [K. Hennessy and D. Botstein, pers. comm.]. Clearly, Mcm2, Mcm3, and Cdc46 belong to a family of proteins that are likely to serve related functions. Correlation between structure and function in the conserved domains of these proteins will provide information on the involvement of this family of proteins in the early events of DNA replication in yeast.

Materials and methods

Strains and plasmids

Yeast strains used are 8534-8C (*MAT α ura3-52 leu2-3, -112 his4 Δ 34*), M46-1-3C (*MAT α ura3-52 leu2-3, -112 his3-11, -15 mcm2-1*), tAM46-1-3C [M46-1-3C with one copy of the plasmid Y5-M-R-1 [*mcm2::URA3*] integrated at the *mcm2* locus], TD4 (*MAT α ura3-52 leu2-3, -112 his4-519 trp1-289*), MY4C/Y4 (*MAT α /MAT α ura3-52/ura3-52 his3- Δ 200/+ lys2-801/lys2-801 ade2-101/ade2-101 +/tyr1+/trp1 mcm2-1/+*), 8534/R61 (*MAT α /MAT α ura3-52/ura3-52 leu2-3, -112/leu2-3, -112 +/his3-11 his4 Δ 34/+ +/lys2 +/ade2 +/trp1*), HY101d (*MAT α ura3-52 leu2-3, -112 his4-519 trp1-289 mcm2::LEU2*), M46-8 (*MAT α mcm2-1 ura3-52 leu2-3, -112 his3 ade2 ade3*), and R61-4 (*MAT α mcm3-1 ura3-52 leu2-3, -112 his4 Δ 34*).

E. coli HB101 [*hsd20 recA13*] was used for plasmid construction and preparation. *E. coli* JM101 [Δ (*lac-proAB*) *F'*traD36pro-*ABlacI*^q Δ (*lacZ*)M15] was used for preparing single-stranded M13 recombinant DNA. *E. coli* RDP146 [*recA sp'* Δ (*lac-pro*)] and NS2114Sm [*recA sm'* λ -*cre* prophage in chromosome] was used for shuttle mutagenesis (Seifert et al. 1986).

Plasmids used in this study are listed in Table 4.

Media, chemicals, and enzymes.

Rich (YEPD) and complete (Cm) media were prepared as described (Sherman et al. 1974). FOA plates were made according to Boeke et al. (1984). The T7 DNA polymerase sequencing kit was purchased from the United States Biochemical Co., Cleveland, OH. The T4 DNA polymerase deletion kit was from International Biotechnologies, Inc., New Haven, CT. The Mutagenesis M13 in vitro mutagenesis kit is the product of Bio-Rad Laboratories, Richmond, CA.

Yeast genetic techniques and transformation

Mating, sporulation, tetrad dissection, and other yeast genetic manipulations were carried out following the standard procedures described by Sherman et al. (1974). Mitotic plasmid stability assays and protein overproduction in yeast were carried out as described (Gibson 1989). Plasmid loss rate is determined by $1 - (F/I)^{1/N}$, where *I* is the initial percentage of plasmid-containing cells and *F* is the percentage of plasmid-containing cells after *N* generations. Yeast cells were transformed using the lithium acetate method (Ito et al. 1983).

Shuttle mutagenesis

Shuttle mutagenesis using the mini-Tn3 transposon has been described (Seifert et al. 1986). The cloned yeast DNA fragment containing the *MCM2* gene was subcloned into vector pHHS6 and passaged through strain NS2114Sm, which carries a mini-Tn3 transposon with a *URA3* gene. Plasmids containing insertions were isolated, and the insertion sites were mapped using restriction enzymes. Those plasmids with insertions at appro-

Table 4. *Plasmids used in this study*

Plasmid	Description	Source/ reference
YCp50 library	yeast DNA bank in YCp50 (<i>URA3 ARS1 CEN4</i>)	Rose et al. (1987)
YCp101	<i>LEU2 ARS1 CEN5</i>	this lab
YIp56	<i>URA3</i> -containing vector	K. Struhl
YCp86	<i>URA3 ARS1 CEN3</i>	K. Struhl
YCp121	<i>LEU2 ARS121 CEN5</i>	this lab
YCpHML	<i>URA3 ARSHML CEN5</i>	this study
YCpH2B	<i>URA3 LEU2 ARSH2B CEN5</i>	this lab
YCpHO	<i>URA3 LEU2 ARSHO CEN5</i>	this lab
YCp131	<i>LEU2 ARS131 CEN5</i>	this lab
YCp120	<i>URA3 LEU2 ARS120 CEN5</i>	this lab
pYES3	<i>URA3 ARS1 CEN5</i>	this lab
pM46-33	original <i>MCM2</i> clone in YCp50	this study
Y5-M-R-1	<i>URA3</i> , 4.5-kb <i>EcoRI</i> fragment of the yeast clone M46-33	this study
pHSS6	kanamycin ^r , ori	Seifert et al. (1986)
pLB101	chloramphenicol ^r , Tn3 transposase	Seifert et al. (1986)
pOX38 :: m-Tn3	ampicillin ^r , mini-Tn3 (<i>URA3 LACZ</i>)	Seifert et al. (1986)
pHSS6.M46	<i>PvuII</i> fragment of M46-33 cloned into pHSS6	this study
pHSS.M46.n	pHSS6.M46 inserted by mini-Tn3	this study
pHSS.M46.Leu	pHSS6.M46 inserted by mini-Tn3 (<i>LEU2 LACZ</i>) inside <i>MCM2</i>	this study
pMCM2.X1	3.5-kb <i>XbaI</i> fragment of M46-33 (5' part of <i>MCM2</i>) on YIp56	this study
pMCM2.X2	1.8-kb <i>XbaI</i> fragment of M46-33 (3' part of <i>MCM2</i>) on YIp56	this study
pMCM2.Bg	2.6-kb <i>BglII</i> fragment of M46-33 (5' part of <i>MCM2</i>) on YIp56	this study
pMCM2-1.S	<i>SstI</i> to <i>BglII</i> fragment with <i>mcm2-1</i>	this study
pSE355	<i>TRP1 ARS1 CEN3</i>	S. Elledge
pHY1-n	pSE355 with <i>mcm2-1</i> to <i>mcm-2-8</i>	this study
pHY9	pSE355 with <i>MCM2</i>	this study
pHY10	<i>MCM2</i> gene cloned into YCp86	this study
pHY12	pSE355 with <i>GAL1::MCM2</i>	this study
pB620	<i>URA3 GAL1-UAS 2μARS</i>	Goff et al. (1984)
pB620.MCM2	<i>URA3 GAL1::MCM2 2μARS</i>	this study
pB620.MCM3	<i>URA3 GAL1::MCM3 2μARS</i>	this lab
pB704	<i>URA3 GAL1::LACZ 2μARS</i>	Goff et al. (1984)

prate positions were then transformed into the *mcm2-1* strain, M46-1-3C, and Ura⁺ transformants were tested for temperature-sensitive growth.

DAPI staining

Staining of cells with the DNA-specific dye DAPI was carried out according to published procedures (Williamson and Fennell 1975).

Flow cytometry

Cells were stained with propidium iodide as published previously (Hutter and Eipel 1978), except that 0.25% Triton X-100 was added to the solutions and RNase A treatment was extended to 2.5 hr with vigorous shaking at 37°C. The analysis was performed on EPICS Profile.

DNA sequencing

DNA fragments of the *MCM2* gene were cloned into M13mp18 or 19 (Messing 1983). Progressive deletions were constructed by the method of Dale et al. (1985) using the T4 DNA polymerase kit from IBI. Sequences from both strands were determined by the dideoxynucleotide chain-termination method of Sanger et al. (1977) using the T7 Sequenase kit from U.S. Biochemical.

Computer analysis was performed using the program package from the University of Wisconsin Genetics Computer Group (Devereux et al. 1984).

Site-specific mutagenesis

Oligonucleotides used for mutagenesis were synthesized at the Cornell Biotechnology Center. Mutagenesis was carried out using the protocol of Kunkel (Kunkel et al. 1987). Annealing of oligonucleotides and second-strand synthesis were carried out using the Bio-Rad Mutagen kit. Mutagenized clones were screened by direct sequencing. *MluI*–*Clal* restriction fragments containing the mutations were substituted for the corresponding sequence of the wild-type *MCM2* gene on plasmid pHY9.

Acknowledgments

We thank Kevin Hennessy, Mitsuhiro Yanagida, and Stephen Kearsey for communications of their unpublished data. We are grateful to Mark Goebel and Clarence Chan for identifying sequence homologies between Mcm2, Mcm3, Cdc46/Nda4, and Cdc21. This work was supported by National Institutes of Health grant GM34190.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby

marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Berg, J.M. 1990. Zinc finger domains: Hypotheses and current knowledge. *Annu. Rev. Biophys. Biophys. Chem.* **19**: 405–421.
- Biswas, S.B. and E.E. Biswas. 1990. ARS binding factor I of the yeast *Saccharomyces cerevisiae* binds to sequences in telomeric and nontelomeric autonomously replicating sequences. *Mol. Cell. Biol.* **10**: 810–815.
- Boeke, J.D., F. LaCrute, and G. Fink. 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-Fluoro-orotic acid resistance. *Mol. Gen. Genet.* **197**: 345–346.
- Bramhill, D. and A. Kornberg. 1988. A model for initiation at origins of DNA replication. *Cell* **54**: 915–918.
- Broach, J.R., Y.-Y. Li, J. Feldman, M. Jayaram, J. Abraham, K.A. Nasmyth, and J.B. Hicks. 1983. Localization and sequence analysis of yeast origins of DNA replication. *Cold Spring Harbor Symp. Quant. Biol.* **47**: 1165–1173.
- Chan, C.S.M. and B.-K. Tye. 1980. Autonomously replicating sequences in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **77**: 6329–6333.
- . 1983. Organization of DNA sequences and replication origins at yeast telomeres. *Cell* **33**: 563–573.
- Chatterjee, P.K., M. Bruner, and S.J. Flint. 1988. DNA-binding properties of an adenovirus 289R E1A protein. *EMBO J.* **7**: 835–841.
- Culp, J.S., L.C. Webster, D.J. Friedman, C.L. Smith, W.J. Huang, F.Y.-H. Wu, M. Rosenberg, and R.P. Ricciardi. 1988. The 289-amino acid E1A protein of adenovirus binds zinc in a region that is important for *trans*-activation. *Proc. Natl. Acad. Sci.* **85**: 6450–6454.
- Dale, R., B. McClure, and J. Houchins. 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: Application to sequencing the corn mitochondrial 18S rDNA. *Plasmid* **13**: 31–40.
- Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12**: 387–395.
- 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.
- Donahue, T.F., A.M. Cigan, E.K. Pabich, and B.C. Valavicius. 1988. Mutations at a Zn(II) finger motif in the yeast eIF2 β gene alter ribosomal start-site selection during the scanning process. *Cell* **54**: 621–632.
- 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.
- Evans, R.M. and S.M. Hollenberg. 1988. Zinc-fingers: Gilt by association. *Cell* **52**: 1–3.
- Feldman, J.B., J.B. Hicks, and J.R. Broach. 1984. Identification of sites required for repression of a silent mating type locus in yeast. *J. Mol. Biol.* **178**: 815–834.
- Flint, J. and T. Shenk. 1989. Adenovirus E1A protein paradigm viral transactivator. *Annu. Rev. Genet.* **23**: 141–161.
- Gibson, S. 1989. "Analysis of MCM3, a minichromosome maintenance mutant of yeast with a cell division cycle arrest phenotype." Ph.D. thesis. Cornell University, Ithaca, New York.
- Gibson, S., R. 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.* **47**: 341–354.
- Gibson, S., R. 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.
- Goff, C.G., D.T. Moir, T. Kohno, T.C. Gravniss, R.A. Smith, E. Yamasaki, and A. Taunton-Rigby. 1984. Expression of calf prothymosin in *Saccharomyces cerevisiae*. *Gene* **27**: 35–46.
- Hennessey, K.M., C. Clark-Adams, and D. Botstein. 1990. Subcellular localization of yeast CDC46 protein varies with the cell cycle. *Genes & Dev.* **4**: 2252–2263.
- Hollingsworth, N.M., L. Goetsch, and B. Byers. 1990. The HOP1 gene encodes a meiosis-specific component of yeast chromosomes. *Cell* **61**: 73–84.
- Huberman, J.A., J. Zhu, L.R. Davis, and C.S. Newlon. 1988. Close association of a DNA replication origin and an ARS element on chromosome III of the yeast, *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **16**: 6373–6384.
- Hutter, K.J., and H.E. Eipel. 1978. Flow cytometric determinations of cellular substances in algae, bacteria, molds and yeasts. *Antonie Leeuwenhoek J. Microbiol. Serol.* **44**: 269–282.
- Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**: 163–168.
- Kearsey, S. 1984. Structural requirements for the function of a yeast chromosomal replicator. *Cell* **37**: 299–307.
- Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* **154**: 367–382.
- Maine, G.T., P. Sinha, and B.-K. Tye. 1984. Mutants of *S. cerevisiae* defective in the maintenance of minichromosomes. *Genetics* **106**: 365–385.
- Messing, J. 1983. New M13 vectors for cloning. *Methods Enzymol.* **101**: 20–78.
- Nasmyth, K. and P. Nurse. 1981. Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **182**: 119–124.
- Osley, M.A. and L. Hereford. 1982. Identification of a sequence responsible for periodic synthesis of yeast histone 2A mRNA. *Proc. Natl. Acad. Sci.* **79**: 7689–7693.
- 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 MAT α cells. *J. Mol. Biol.* **204**: 593–606.
- 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.
- Pfeifer, K., K.-S. Kim, S. Kogn, and L. Guarente. 1989. Functional dissection and sequence of yeast HAP1 activator. *Cell* **56**: 291–301.
- Rose, M.D., P. Novick, J.H. Thomas, D. Botstein, and G.R. Fink. 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. *Gene* **60**: 237–243.
- Rothstein, R.J. 1983. One-step gene disruption in yeast. *Methods Enzymol.* **101**: 202–211.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Seifert, H.S., E.Y. Chen, M. So, and F. Heffron. 1986. Shuttle

- mutagenesis: A method of transposon mutagenesis for *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **83**: 735–739.
- Sherman, F., G.R. Fink, and C.W. Lawrence. 1974. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Shortle, D., P. Novick, and D. Botstein. 1984. Construction and genetic characterization of temperature-sensitive mutant alleles of the yeast actin gene. *Proc. Natl. Acad. Sci.* **81**: 4889–4893.
- Sinha, P., V. Chan, and B. Tye. 1986. A mutant that affects the function of autonomously replicating sequences in yeast. *J. Mol. Biol.* **192**: 805–814.
- Stillman, B. 1989. Initiation of eukaryotic DNA replication *in vitro*. *Annu. Rev. Cell Biol.* **5**: 197–245.
- Struhl, K., D.T. Stinchcomb, S. Scherer, and R.W. Davis. 1979. High-frequency transformation of yeast: Autonomous replication of hybrid molecules. *Proc. Natl. Acad. Sci.* **76**: 1035–1039.
- Umek, R.M. and D. Kowalski. 1988. The ease of DNA unwinding as a determinant of initiation at yeast replication origins. *Cell* **52**: 559–567.
- Walker, S.S., S.C. Francesconi, and S. Eisenberg. 1990. A DNA replication enhancer in *Saccharomyces cerevisiae*. *Proc. Nat. Acad. Sci.* **87**: 4665–4669.
- Williamson, D.H. and D.I. Fennell. 1975. The use of fluorescent DNA-binding agent for detecting and separating yeast mitochondrial DNA. *Methods Cell Biol.* **12**: 335–351.

Mcm2 and Mcm3, two proteins important for ARS activity, are related in structure and function.

H Yan, S Gibson and B K Tye

Genes Dev. 1991, **5**:

Access the most recent version at doi:[10.1101/gad.5.6.944](https://doi.org/10.1101/gad.5.6.944)

References

This article cites 45 articles, 17 of which can be accessed free at:
<http://genesdev.cshlp.org/content/5/6/944.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).