Chromosome Loss, Hyperrecombination, and Cell Cycle Arrest in a Yeast *mcm1* Mutant

Randolph Elble and Bik-Kwoon Tye

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

Submitted April 1, 1992; Accepted June 30, 1992

The original *mcm1-1* mutant was identified by its inability to propagate minichromosomes in an ARS-specific manner, suggesting that it is defective in the initiation of DNA synthesis at ARSs. This mutant is also defective in expression of α -mating-type-specific genes. Further genetic and biochemical studies confirmed that Mcm1 is a transcription factor that mediates the transcriptional regulation of a number of genes, including genes outside of the mating type complement, by interacting with different cofactors. Although *MCM1* is an essential gene, none of the previously characterized *mcm1* mutants exhibits significant growth defects. To assess which of the many roles of Mcm1 is essential for growth, we constructed and characterized a temperature-sensitive conditional mutant of *mcm1*, *mcm1-110L*. This mutant exhibits a temperature-dependent cell-cycle arrest, with a large, elongated bud and a single, undivided nucleus that has a DNA content of close to 2n. In addition, it shows elevated levels of chromosome loss and recombination. In spite of the severity of the *mcm1-110L* mutation, this mutant still retains an ARS-specific pattern of minichromosome instability. All of these phenotypes are precisely those exhibited by mutants in three *MCM* genes, *MCM2*, *MCM3*, and *MCM5/CDC46*, that have been shown to play interacting roles in the early steps of DNA replication.

INTRODUCTION

Mcm1 is a versatile transcription factor that regulates the expression of diverse genes by interacting with multiple cofactors. It acts as a positive regulator of α -specific genes using $\alpha 1$ as a cofactor (Bender and Sprague, 1987; Passmore *et al.*, 1988, 1989). It acts as a negative regulator of α -specific genes by binding cooperatively with $\alpha 2$ (Keleher *et al.*, 1988; Passmore *et al.*, 1989; Elble and Tye, 1991). Recently, it has been shown to regulate the cell-cycle dependent expression of the *SW15* gene, which encodes a transcription factor for the *HO* endonuclease, by interacting with SFF, or SW15 factor (Lydall *et al.*, 1991). It is also involved in the regulation of arginine metabolic genes by interacting with the ARGR proteins (Dubois and Messenguy, 1991). Additional genes under the regulation of Mcm1 are likely to be identified. It appears that through combinatorial action with multiple cofactors, Mcm1 may play a pivotal role in coordinating cell growth in yeast.

MCM1 was originally identified as a gene involved in the propagation of minichromosomes in an ARS-dependent manner. This phenotype suggests that Mcm1 may be involved in the initiation of DNA replication at

ARSs either indirectly, as a transcription factor that regulates the expression of replication initiator proteins, or directly, as a replication initiator protein that binds to ARSs. Recently, its role as a replication initiator has been shown by the direct binding of Mcm1 to multiple sites at ARSs (Christ, Chang, and Tye, unpublished results). Because initiation of DNA synthesis commits a cell to enter and to complete the next cell cycle, Mcm1 may also play a key role in coordinating cell division.

Functional dissection of Mcm1 indicates that the 80 amino acid DNA binding domain (amino acids 18–97) of Mcm1 is sufficient for viability, minichromosome maintenance, and general transcriptional activation (Christ and Tye, 1991). Proteins containing homology to this 80 amino acid domain have been identified in diverse organisms including yeast, plants, and humans. Among these, the human serum response factor (SRF) and the plant homeotic genes, *AG* and *DEF*, are particularly interesting because of their regulatory role in cell growth and cell division. SRF is a cell proliferation factor that mediates the transcription of growth-stimulated genes in response to growth factors in serum (Norman *et al.*, 1988). The *AG* gene in *Arabidopsis* (Yanofsky *et al.*, 1990) and the *DEF* gene in *Antirrhinum* (Sommer *et*

al., 1990) are homeotic genes that are important in flower development. Organ development in plants requires the coordination of gene expression and DNA replication to achieve cell differentiation and cell division.

Although *MCM1* is an essential gene that appears to play a key role in the regulation of gene expression and DNA replication, none of the currently available *mcm1* mutants exhibits significant growth defects. To learn more about the essential role of *Mcm1* in the cell, we constructed a conditional mutant by truncating the *MCM1* gene after amino acid codon 110 (out of 286 total). This *mcm1-110L* mutation results in an extremely unstable *MCM1* mRNA as well as a truncated protein (Elble and Tye, 1991). The effect of this mutation on transcriptional regulation of mating type-specific genes is also more dramatic than any of the known *mcm1* mutant alleles; it affects not only the expression of α -specific genes, but also both the repression and activation of α -specific genes (Elble and Tye, 1991). In this paper, we characterize the physiological defects of this *mcm1-110L* mutant strain which has depleted *Mcm1* activity. Properties of this mutant indicate that it suffers from multiple defects characteristic of mutants that have lesions in DNA replication, such as cell cycle arrest before nuclear division, increased chromosome loss, hyperrecombination, and UV sensitivity (Hartwell and Smith, 1985). In fact, these properties of the *mcm1-110L* mutant are remarkably similar to those exhibited by mutants in three interacting *MCM* genes, *MCM2* (Sinha *et al.*, 1986) *MCM3* (Gibson *et al.*, 1990), and *MCM5/CDC46* (Hennessy *et al.*, 1991). This family of structurally related *MCM* genes, like *MCM1*, is required for the stable maintenance of minichromosomes in an ARS-specific manner (Yan *et al.*, 1991; Chen *et al.*, in press).

MATERIALS AND METHODS

Strain Constructions

Construction of α strains C2-286L and C2-110L and strains 14D-286L and 14D-110L has been described (Elble and Tye, 1991). The diploid strains 14D-286L/UH5 and 14D-110L/UH5 were constructed by crossing 14D-286L or 14D-110L with strain UH5-MT (*MAT α leu2::URA3 mcm1 Δ Xho I/BamHI [MCM1 ARS1 CEN5 TRP1]*) and selecting *Trp*⁻ segregants. Strain UH5-MT contains the *URA3* gene inserted by gene replacement at the *LEU2* locus on chromosome III. This was accomplished by cleaving the plasmid pLURA with *Hpa* I and *Eco*RI, transforming, and selecting *Ura*⁺*Leu*⁻ transformants. The replacement was verified by Southern analysis. pLURA was constructed by replacing the *Clal*/EcoRV fragment of Ylp351 containing *LEU2* with the *Clal*/Sma I fragment of Ylp5 containing *URA3*. To determine the recessiveness of the *mcm1-110L* allele, the C2-110L strain was transformed with either pMCM1-TRP or Ylp5-XB286 (Elble and Tye, 1991). pMCM1-TRP was constructed by inserting the 3.3 kb *Sph* I/*Bam*HI fragment containing *MCM1* into pSE358.

Other Strains

The *cdc* strains used were K72-1 (*MAT α cdc7-4 ura3*), 6433-4C (*MAT α cdc4-1 leu2-3*), F391 (*MAT α cdc14 leu2 ura3 ade2*), 4837-5-2 (*MAT α*

cdc17-2 his7 leu2 trp1 ura3), and H17C1B1 (*MAT α cdc17-1 his7 ura1*). The *cdc17-2* strain was used in plasmid stability experiments, whereas the *cdc17-1* allele was used in the ultraviolet light (UV) sensitivity experiment. The *tub2* allele used was *tub2-401*, a gift from T. Huffaker (Cornell University). The *mcm3-1* strain R61 has been described (Gibson *et al.*, 1990). The *rad9* strain 7859-7-4 and the isogenic wild-type strain 7859-10-2 were a gift from T. Weinert (University of Arizona).

Determination of Cell Doubling Times

Doubling times were determined by plotting the optical densities of cultures growing logarithmically at 30°C in yeast extract, peptone, dextrose (YPD) medium.

Microscopic Techniques

Cell staining with the DNA-specific dye 4,6-diamidino-2-phenylindole (DAPI) was carried out according to published procedures (Williamson and Fennell, 1975). Cells were stained with Calcofluor (Sigma, St. Louis, MO) as described by Pringle *et al.* (1989). Cells were photographed using a Zeiss (Thornwood, NY) microscope equipped with a 100 $\times$ objective.

Flow Cytometry

Cells were stained with propidium iodide according to the method of Hutter and Eipel (1978) and analyzed using an Ortho Diagnostics System 2151 machine (Coulter Electronics, Hialeah, FL).

Chromosome Loss and Recombination Assay

Loss frequencies were determined essentially as described by Gibson *et al.* (1990). Single colonies were picked from plates containing complete medium minus uracil, suspended in water, and spread on YPD plates. The plates were incubated at 30°C for ~25 generations of growth. Colonies were then picked, resuspended in water, and spread onto plates containing 5-fluoroorotic acid (FOA) or YPD to determine the fraction of cells from which the *URA3* marker had been lost.

Mitotic Plasmid Stability Assay

Plasmid stability was determined as described by Christ and Tye (1991). Cells were grown at 30°C except for the *tub2* strain, which was grown at 20°C.

RESULTS

The *mcm1-110L* Mutation Affects Different ARSs Differently

We have previously described the phenotype of the *mcm1-1* allele (Passmore *et al.*, 1988; Christ and Tye, 1991). The *mcm1-1* allele had an ARS-specific minichromosome-maintenance defect as well as a reduced ability to activate α -specific genes. Several observations suggested that the *mcm1-110L* allele represents a more severe mutation than *mcm1-1*. For instance, the doubling time of *mcm1-110L* cells in YPD medium at 30°C is ~4 h compared with the 1.5- to 2-h doubling time of wild-type or *mcm1-1* cells. The effects of *mcm1-110L* on transcriptional activation are also more extreme (Elble and Tye, 1991). Thus, we expected the *mcm1-110L* allele to have greater effects on minichromosome stability and we sought to determine whether the *mcm1-110L* mutant maintains the ARS-specificity observed for *mcm1-1*. To this end, we transformed plasmids containing ARS1 or

ARS120 into *mcm1-110L* or isogenic wild-type strains and measured the plasmid loss rates. *ARS1* is representative of a subset of ARSs, including *ARS131* and several telomeric ARSs that are unstable in *mcm1-1* and in all of the other ARS-specific *mcm* mutants (Maine *et al.*, 1984). Similarly, *ARS120* represents a subset of ARSs, including *ARS121*, *ARS131C*, and *ARS206*, that are much more stable in these mutants. Figure 1 shows that, in the *mcm1-110L* mutant strain, the *ARS1* plasmid was dramatically less stable than the *ARS120* plasmid, approaching the theoretical maximum of 50% loss per cell division. The specificity of the *mcm1* defect for *ARS1* is not absolute. Although the *ARS120* plasmid is much less affected by the *mcm1-110L* mutation than is the *ARS1* plasmid, it is nonetheless 10 times less stable in the mutant than in the wild-type strain. These defects in ARS stability are partially complemented by a plasmid containing the wild-type *MCM1* gene, a centromere, and a *TRP1* marker (*MCM1/mcm1*; Figure 1). Incomplete complementation may be due to instability of the *MCM1*-containing plasmid. Derivatives of the 2μ plasmid are also affected by the *mcm1-110L* mutation. The plasmid *YE24* had a loss rate of 3% per cell division in the wild-type strain and 15% in the mutant strain, a fivefold difference. This instability is significant because the 2μ plasmid shares little in common with natural chromosomes other than the presence of an ARS; its segregation fidelity is apparently based on maintenance of high copy number rather than association with microtubules (Som *et al.*, 1988).

To assess whether ARS-specificity for minichromosome maintenance is a general property of replication-defective mutants, we also measured the loss rates of these plasmids in a *cdc17* strain, defective in DNA polymerase α (Budd and Campbell, 1987) and several other mutant strains with defects in S phase initiation or chromosome segregation. Figure 1 shows that the *cdc17* mutant had a severe *mcm* defect when grown at 30°C, a semipermissive temperature for this allele, but both *ARS1*- and *ARS120*-containing plasmids were equally affected. We tested several other mutants that have been reported to be defective in maintenance of chromosomes or minichromosomes, namely, *cdc4*, *cdc7*, *cdc14*, and *tub2* (Hartwell and Smith, 1985; Huffaker *et al.*, 1988; Palmer *et al.*, 1990). *cdc4* and *cdc7* are thought to be defective in late G1 events leading to initiation of DNA synthesis, whereas *cdc14* and *tub2* are defective in chromosomal segregation (Pringle and Hartwell, 1981; Huffaker *et al.*, 1988). These mutants all had moderate *mcm* defects when grown at semipermissive temperatures (30°C for *cdc4*, *cdc7*, and *cdc14*; 20°C for *tub2*) but showed no ARS specificity (Figure 1). Loss rates for both plasmids in these four mutants ranged from 7 to 13% per cell division, compared with loss rates of 1–2% in wild-type controls. Thus, origin-specific plasmid-maintenance defects are not a general feature of repli-

Figure 1. Mitotic stability of minichromosomes containing different ARSs in wild-type and *mcm1-110L* mutant cells. The minichromosomes used were YCp120, containing *ARS120*, *CEN5*, *LEU2*, and *URA3*; and pCD4, containing *ARS1*, *CEN5*, *LEU2*, and *URA3*. Error bars represent deviation from the mean of two independent assays. The theoretical maximum is 50% loss per cell division. *MCM1/mcm1* denotes a strain in which the *mcm1-110L* mutation was complemented by a plasmid bearing the *MCM1* gene, a centromere, and a *TRP1* marker. Loss rate for this strain is based on the ratio of *Trp*⁺*Ura*⁺ to *Trp*⁺ cells.

cation-defective or other *cdc* mutants and point to a specific lesion in initiation at ARSs.

The mcm1-110L Mutation Causes Elevated Frequencies of Chromosome Loss and Recombination

A number of mutations that arrest nuclear division in yeast also enhance chromosome loss when cells are grown at a semipermissive temperature (Hartwell and Smith, 1985). However, only mutations that interfere with DNA replication or repair lead to higher levels of recombination in addition to chromosome loss (Hartwell and Smith, 1985). For example, mutations in DNA polymerases α (*cdc17*; Budd and Campbell, 1987) and δ (*cdc2*; Sitney *et al.*, 1989) lead to increased levels of both chromosome loss and recombination, whereas a mutation in the segregation machinery, *tub2*, affects only chromosome stability (Huffaker *et al.*, 1988). To determine whether the truncation allele had either of these properties, we constructed wild-type and mutant diploid strains and monitored stability of the *MAT α* -containing copy of chromosome III. This chromosome also contained a copy of the *URA3* gene integrated by gene-replacement at the *leu2* locus on the left arm (Figure 2B). This modification allowed the use of FOA to monitor loss of the chromosome since only *ura3* cells can grow on FOA-containing media (Boeke *et al.*, 1984). However, some *Ura*[−] cells might result from recombination events between the centromere and *leu2::URA3* resulting in loss of only the left arm rather than the entire chromosome. These events can be distinguished by testing *Ura*[−] cells for the ability to mate as if they

Figure 2. Frequencies of chromosome loss and recombination in wild-type and *mcm1-110L* mutant strains. (A) Frequencies of loss of chromosome III and of recombination between *leu2* and *CEN3* were determined. Each bar represents the median of 5 independent trials for wild-type and 10 independent trials for mutant cells. (B) Schematic representation of the *MATα*- and *MATa*-containing copies of chromosome III. Sites of integration of the *URA3* marker and the wild-type and mutant *Mcm1* alleles are shown. *CEN3* is represented by the circles within the bars.

were a cells. Mating would indicate that *MATα*, on the right arm of the chromosome, had also been lost. In contrast, cells that retain *MATα* are unable to mate and must have lost *URA3* by a recombination event.

Wild-type and mutant diploid strains were grown at 30°C on YPD medium for ~25 generations to allow chromosome loss. The cells were then plated on YPD or FOA-containing medium to determine the percentage of cells that had lost the *URA3* marker, and *Ura⁻* cells were then screened for ability to mate. The frequencies of chromosome loss and recombination were both much higher in the *mcm1* mutant strain than in the wild-type control (Figure 2A). Chromosome loss was ~40 times more frequent and recombination ~90 times more frequent. Elevated chromosome loss is not restricted to chromosome III; OFAGE analysis of a clone of cells that had lost chromosome III revealed that two other chromosomes had also been lost. The hyper-recombination phenotype of *mcm1-110L* is consistent with a defect in DNA replication in the mutant strain but not with a segregation defect.

mcm1-110L Arrests at Nuclear Division

We established previously that the *MCM1* gene is essential for yeast cell growth (Passmore *et al.*, 1988). The

mcm1-110L allele causes a growth defect; its doubling time on YPD at 30°C is twice that of an isogenic wild-type strain. Furthermore, mutant cells shifted to 37°C arrest growth. To determine whether the cells arrested at a particular point in the cell cycle, cells were grown up initially at 16°C, a semipermissive temperature for the mutant, and then shifted to 37°C for various times, treated with a DNA-specific stain (DAPI), and examined microscopically. At 16°C, both wild-type and mutant populations contained mostly unbudded cells of normal shape, although the mutant cells were somewhat larger than wild type (Figures 3, A and B, and 4). However, after 4 h at 37°C, 89% of the mutant cells were large budded with a single nucleus in or near the neck, whereas the wild-type population contained mostly unbudded G1 cells (Figures 3, C-F, and 4). This phenotype indicates that the *mcm1* truncation mutant is blocked in nuclear division, consistent with defects in DNA replication or mitosis. This defect is recessive. Mutant cells in which a wild-type copy of *MCM1* was integrated at *URA3* were not delayed in nuclear division but are indistinguishable from wild-type cells in cell cycle distribution and morphology. In a logarithmic culture grown at 30°C, ~40% of the cells were unbudded and 60% were budded, the same as a wild-type control.

In a separate experiment, the DNA content of the cells was measured by flow cytometry. Cells that have defects in DNA replication arrest with DNA contents from 1n to 2n, depending on where in S phase the mutant gene product is supposed to act and the tightness of the allele; leaky mutants arrest near 2n because of the presence of a checkpoint for DNA integrity at the G2/M boundary (Weinert and Hartwell, 1988; Hartwell and Weinert, 1989; Hennessy *et al.*, 1991). Both wild-type and mutant cells grown at the semi-permissive temperature for the mutant, 16°C, had mostly 1n DNA contents, suggesting that G1 is the limiting part of the cell cycle at this temperature (Figure 5). The DNA peaks for the mutant cells were shifted slightly to the right, possibly due to accumulation of mitochondrial DNA. Mutant cells shifted to 37°C displayed a 2n peak with a large component of less-than-2n material but no discrete 1n peak, suggesting the presence of partially replicated DNA or even DNA degradation.

Examination of cells growing logarithmically at 30°C revealed that *mcm1-110L* cells often develop multiple, elongated buds (Figure 6, B and D). (Cells bearing the *mcm1-1* allele also exhibit a large bud morphology at 37°C but do not arrest growth or develop additional buds.) Most of the buds appear to be nucleated; however, the nuclei have irregular stretched-out shapes, are usually near the neck, and often appear to have connections with nuclei in adjacent buds (Figure 6F). To ascertain that the elongated structures were true buds, we treated the cells with Calcofluor (Sigma), which stains the chitin rings that occur at the neck between

Figure 3. Photomicrographs of wild-type (A, C, and E) and *mcm1-110L* mutant (B, D, and F) cells. Cells were grown at 16°C (A and B), shifted to 37°C for 4 h (C–F), stained with DAPI, and photographed. Nomarski optics (A–D) reveals cell morphology, and DAPI fluorescence (E and F) reveals DNA content. Bar, 10 μm.

Figure 4. Quantitation of arrest morphologies. Samples of DAPI-stained cells examined in Figure 3 were analyzed. At least 200 cells were counted per sample. Percentages of cells that were unbudded, small-budded, or large-budded are indicated. Large-budded cells contained either a single nucleus in one cell body, an undivided nucleus in the bud neck, or divided nuclei segregated into each cell body. Cells were considered large-budded if the bud was at least three-fourths the size of the mother cell.

mother and bud. Figure 6H shows that the constricted regions between cellular compartments stain heavily, indicating that each compartment does represent a mother cell or bud; however, the chitin rings are often elongated like the rest of the cell body. The sites of bud emergence also appear to be abnormal in most of the cells examined. For example, one of the buds in Figure 6H occurs at the end of a row of bud scars (arrowhead),

while another bud has emerged from the opposite end of the mother cell. The irregular shape of the cells is reminiscent of shmoo cells arrested in G1 by mating factor or by mutations in *CDC28* or G1 cyclin genes (Reed, 1980; Richardson *et al.*, 1989). We considered the possibility that the morphology might be related to the role of Mcm1 as a mating type regulator. It was conceivable, e.g., that derepression of the α -specific receptor for α factor in α cells (Elble and Tye, 1991) could lead to an autocrine response to endogenous α factor and, consequently, to partial arrest. However, the same aberrant morphology is observed in *mcm1-110L* mutant cells of all three mating types. Moreover, *mcm1-110L* cells arrest with a G2, not G1, DNA content (Figure 5). These morphological aberrations may be due to some as yet unknown Mcm1 function or functions disrupted in the mutant.

Sensitivity to UV in an *mcm1* Mutant

Inhibition of DNA replication leads to the accumulation of damaged DNA (Hennessy *et al.*, 1991). The elevated levels of recombination observed in the mutant are consistent with this possibility. We reasoned that if DNA damage were present, the mutant cells might be hypersensitive to additional DNA damage caused by mutagens such as ultraviolet light. We found that the *mcm1-110L* strain is moderately sensitive to UV, at about the same level as a *rad9* deletion mutant (Figure 7; Schiestl *et al.*, 1989). The UV sensitivity of the *mcm1* mutant might be related to its apparent replication defect or to some other function controlled by Mcm1. To help distinguish between these possibilities, we also examined

Figure 5. DNA content of arrested cells. Wild-type and *mcm1-110L* mutant cells were grown in YPD medium at 16°C and then shifted to 37°C for 4 h. Aliquots were removed and analyzed by flow cytometry.

Figure 6. Abnormal morphology of *mcm1-110L* cells growing at semipermissive temperatures. Wild-type (A, C, E, and G) and *mcm1-110L* mutant (B, D, F, and H) cells were grown at 30°C in YPD medium and stained with DAPI alone (E and F) or DAPI plus Calcofluor (G and H). (A–D) Nomarski optics. The arrowheads point to chitin rings and bud scars. Calcofluor quenches DAPI fluorescence, making it difficult to observe chitin rings and DNA simultaneously, especially in the mutant. Bar, 10 μ m.

another *mcm* mutant, *mcm3-1*, and a known replication-defective mutant, *cdc17-1*, for sensitivity to UV. *mcm3-1* was previously reported not to be hypersensitive when cells were grown at a permissive temperature, 23°C. However, we found that both strains were moderately hypersensitive when shifted to the restrictive temperature briefly before and after UV exposure (Figure 7). This temperature shift had little effect on the survival of a wild-type strain relative to the same strain grown continuously at 30°C (Figure 7). Thus it appears that hypersensitivity to UV may be implicit to the *mcm* phenotype. The moderate nature of the defects, compared with a *rad3* mutant, e.g., suggests that the Mcm proteins may not be directly involved in DNA repair (Haynes and Kunz, 1981). Inspection of *mcm1* mutant cells 1 d after irradiation revealed that UV treatment greatly exacerbates the elongated, irregular bud morphology; even *mcm1-1* cells, which normally have wild-type morphology at 30°C, display this aberrant phenotype after irradiation whereas isogenic wild-type cells do not. These observations provide additional evidence that the elongated bud phenotype is linked to DNA damage in the *mcm1* mutants.

DISCUSSION

We have shown that a mutation in the *MCM1* gene results in a conditional arrest at nuclear division. This

mutation also greatly increases the levels of chromosome loss and recombination, as well as severely destabilizing minichromosomes with an extreme bias toward certain ARSs, represented here by *ARS1* (Maine *et al.*, 1984). These are just the phenotypes expected if Mcm1 is involved in DNA replication, especially at the level of

Figure 7. Ultraviolet-light sensitivity of wild-type and mutant cells. Dilutions of logarithmically growing cultures were plated on YPD and then exposed to an ultraviolet germicidal lamp for various times. Cells were grown at 30°C except for the *mcm3* and *cdc17* strains which, together with a wild-type control, were shifted to 37°C for 1.5 h before and after exposure and then incubated at room temperature until colonies appeared.

initiation. The mostly 2n DNA content of the arrested cells seems at first inconsistent with an initiation defect. However, Hennessy *et al.* (1991) have demonstrated that different alleles of *cdc46*, which are allelic to the ARS-specific mutant *mcm5-1* (Chen, unpublished results), arrest with DNA contents from 1n to 2n, depending on the tightness of the particular allele. Other workers have found that apparently leaky mutations in the genes for DNA polymerases α and δ lead unexpectedly to cell cycle arrests with 2n, or nearly 2n, DNA contents (Conrad and Newlon, 1983; Hartwell, 1973). Thus, DNA content at arrest is not a reliable indicator of the execution point of an allele. We expect that tighter conditional alleles of *mcm1* may arrest with a 1n DNA content.

Several transcription factors in mammalian cells appear to be involved in modulating progress from G1 to S phase or coordinating cell growth and cell division. Some act by promoting the transcription of other proliferation-related genes, e.g., SRF, AP1, and E2F (Angel *et al.*, 1987; Norman *et al.*, 1988; Mudryj *et al.*, 1990; Chittenden *et al.*, 1991); E2F has also been shown to interact directly with the cell cycle regulator Cyclin A (Mudryj *et al.*, 1991). Others appear to play direct roles in replication initiation, especially at viral origins (DePamphilis, 1988). NF1 promotes SV40 replication by binding adjacent to the origin (Jones *et al.*, 1987; Cheng and Kelly, 1989), whereas p53 may act negatively on the SV40 origin (Bargonetti *et al.*, 1991). The one mammalian origin that has been well characterized, the DHFR origin, contains potential binding sites for several transcription factors, among them AP1, and NFIII (Daily *et al.*, 1990). Mutations that abolish function of these transcription factors are not available; however, other methods have been employed to demonstrate that some of these factors are required for the G1 to S transition. For example, antibodies to Fos and Jun proteins have been shown to inhibit DNA synthesis in cultured cells (Kovary and Bravo, 1991). Similarly, interference with Myc function by antisense oligomers causes cells to arrest in G1 (Heikkilä *et al.*, 1987).

In contrast, the *mcm1* mutation described here causes cells to arrest at a later stage of the cell cycle, nuclear division. To our knowledge, this is the first reported instance of a mutation in a transcription factor causing a cell cycle arrest at nuclear division. Mutations in other essential yeast transcription factors such as Rap1 (Shore and Nasmyth, 1987) or Abf1 (Rhode *et al.*, 1992) do not cause arrest at a specific point in the cell cycle. However, in the case of Abf1, which is also an ARS-binding protein, prior synchronization of *abf1* mutant cells by α factor will arrest cells in S phase at the non-permissive temperature (Rhode *et al.*, 1992), suggesting that the first essential function performed by Abf1 after the G1/S transition may be DNA replication. Like Abf1, the fact that Mcm1 is a transcription factor means that the arrest phenotype may represent a composite of

many different effects. However we believe that the arrest phenotype results largely from the inability of the mutant cells to complete DNA replication. The mutant cells have many of the hallmarks of replication-defective mutants, including elevated levels of chromosome loss and recombination, sensitivity to ultraviolet light, and an appropriate arrest morphology. However the principal characteristic of the *mcm1* mutant that ties it to initiation of replication is its defect in ARS function, a phenotype shared only with other *mcm* mutants, including *mcm2* (Sinha *et al.*, 1986), *mcm3* (Gibson *et al.*, 1990), and *mcm5/cdc46* (Chen *et al.*, *in press*) and the *abf1* mutants (Rhode *et al.*, 1992). Mcm2, Mcm3, and Mcm5 are a family of structurally and functionally related proteins (Hennessy *et al.*, 1991; Yan *et al.*, 1991; Chen *et al.*, *in press*). Their subcellular localization is cell-cycle-regulated such that it is nuclear between late M phase and the G1/S transition, but cytoplasmic at other phases of the cell cycle (Yan, unpublished results). The properties of these proteins suggest that they act during a very narrow window at the G1/S transition or the beginning of S phase by virtue of their nuclear localization to effect the initiation of DNA replication at ARSs. In addition to their similarities in phenotypes, Mcm1 has been tied to these *mcm* mutants genetically. An *mcm1-1 mcm2-1* double mutant forms a synthetic lethal (Yan, unpublished results), implying that the Mcm1 and Mcm2 proteins may interact functionally.

Does Mcm1 play a direct role in activation of ARSs by direct binding, or is it involved indirectly by activating expression of some replication initiation factor? We believe that Mcm1 may act at both levels. We have observed potential Mcm1 binding sites upstream of two genes thought to be involved in DNA replication, *MCM3* and *RPA1*, centered at about -180 and -250, respectively, from the translation start codons. *RPA1* encodes the yeast homologue of a single-stranded-DNA binding protein required for SV40 replication *in vitro* (Heyer *et al.*, 1990). The possibility that Mcm1 might regulate *RPA1* is especially interesting because the terminal morphology of *rpa1* mutants resembles that of *mcm1* mutants. On the other hand, we have also observed Mcm1 binding sites in ARSs such as *ARS1* and found that disruption of the sites reduces ARS function (Chang, unpublished data). The difference in behavior between *ARS1* and *ARS120* in *mcm* mutants may be explained by differences in quality and abundance of Mcm1 binding sites at these ARSs. We have found by inspection and by *in vitro* binding experiments that *ARS120* contains several Mcm1 binding sites, whereas *ARS1* contains fewer sites, of lower affinity (Chang and Christ, unpublished data). In a hypomorphic allele such as *mcm1-110L*, ARSs such as *ARS1* may be less likely to bind Mcm1 and therefore less likely to initiate replication. Residual initiation events at ARSs such as *ARS120* may result in the incomplete replication of the yeast genome.

ACKNOWLEDGMENTS

We thank Tim Huffaker for advice, use of his microscope, and critical reading of the manuscript. This work was supported by NIH Grant GM34190.

REFERENCES

- Angel, P., Imagawa, M., Chui, R., Stein, B., Imbra, R., Rahmsdorf, H., Jonat, C., Herrlich, P., and Karin, M. (1987). Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. *Cell* 49, 729–739.
- Bargonetti, J., Friedman, P., Kern, S., Vogelstein, B., and Prives, C. (1991). Wild-type but not mutant p53 immunopurified proteins bind to sequences adjacent to the SV40 origin of replication. *Cell* 65, 1083–1091.
- Bender, A., and Sprague, G. F. (1987). MAT α 1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. *Cell* 50, 681–691.
- Boeke, J. D., LaCroute, F., and Fink, G. R. (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.
- Budd, M., and Campbell, J. L. (1987). Temperature-sensitive mutations in the yeast DNA polymerase I gene. *Proc. Natl. Acad. Sci. USA* 84, 2838–2842.
- Chen, Y., Hennessy, K., Botstein, D., and Tye, B. K. (1991). CDC46/MCM5, a yeast protein whose subcellular localization is cell-cycle regulated, is involved in DNA replication at ARSs. *Proc. Natl. Acad. Sci. (in press)*
- Cheng, L., and Kelly, T. J. (1989). Transcriptional activator nuclear factor I stimulates the replication of SV40 minichromosomes in vivo and in vitro. *Cell* 59, 541–551.
- Chittenden, T., Livingston, D., and Kaelin, W. (1991). The T/E1A-binding domain of the retinoblastoma product can interact selectively with a sequence-specific DNA-binding protein. *Cell* 65, 1073–1082.
- Christ, C., and Tye, B. (1991). Functional domains of the yeast transcription/replication factor MCM1. *Genes Dev.* 5, 751–763.
- Conrad, M., and Newlon, C. (1983). *Saccharomyces cerevisiae* *cdc2* mutants fail to replicate approximately one-third of their nuclear genome. *Mol. Cell. Biol.* 3, 1000–1012.
- Daily, L., Caddle, M. S., Heintz, N., and Heintz, N. H. (1990). Purification of RIP60 and RIP100, mammalian proteins with origin-specific DNA-binding and ATP-dependent DNA helicase activities. *Mol. Cell. Biol.* 10, 6225–6235.
- DePamphilis, M. L. (1988). Transcriptional elements as components of eukaryotic origins of DNA replication. *Cell* 52, 635–638.
- Dubois, E., and Messenguy, F. (1991). In vitro studies of the binding of the ARGR proteins to the ARG5,6 promoter. *Mol. Cell. Biol.* 11, 2169–2179.
- Elble, R., and Tye, B. K. (1991). Both activation and repression of mating-type-specific genes in yeast require transcription factor Mcm1. *Proc. Natl. Acad. Sci. USA* 88, 10966–10970.
- Gibson, S. I., Surosky, R. T., and Tye, B. (1990). The phenotype of the minichromosome-maintenance mutant *mcm3* is characteristic of mutants defective in DNA replication. *Mol. Cell. Biol.* 10, 5707–5720.
- Hartwell, L. H. (1973). Three additional genes required for DNA synthesis in *S. cerevisiae*. *J. Bacteriol.* 115, 966–974.
- Hartwell, L. H., and Smith, D. (1985). Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. *Genetics* 110, 381–395.
- Hartwell, L. H., and Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science* 246, 629–634.
- Haynes, R. H., and Kunz, B. A. (1981). DNA repair and mutagenesis in yeast. In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, ed. J. N. Strathern, E. W. Jones, and J. R. Broach. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 371–414.
- Heikkila, R., Schwab, G., Wickstrom, E., Loke, S. L., Pluznik, D., Watt, R., and Neckers, L. (1987). A c-myc antisense oligodeoxynucleotide inhibits entry into S phase but not progress from G0 to G1. *Nature* 328, 445–449.
- Hennessy, K. M., Lee, A., Chen, E., and Botstein, D. (1991). A group of interacting yeast DNA replication genes. *Genes Dev.* 5, 958–969.
- Heyer, W., Rao, M., Erdile, L., Kelly, T., and Kolodner, R. (1990). An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. *EMBO J.* 9, 2321–2329.
- Huffaker, T. C., Thomas, J. H., and Botstein, D. (1988). Diverse effects of β -tubulin mutations on microtubule formation and function. *J. Cell Biol.* 106, 1997–2010.
- Hutter, K. J., and Eipel, H. E. (1978). Flow cytometric determinations of cellular substances in algae, bacteria, molds and yeasts. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 44, 269–282.
- Jones, K. A., Kadonaga, J. T., Rosenfeld, P. J., Kelly, T. J., and Tjian, R. (1987). A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. *Cell* 48, 79–89.
- Keleher, C., Goutte, C., and Johnson, A. (1988). The yeast cell-type-specific repressor $\alpha 2$ acts cooperatively with a non-cell-type-specific protein. *Cell* 53, 927–936.
- Kovary, K., and Bravo, R. (1991). The Jun and Fos protein families are both required for cell cycle progression in fibroblasts. *Mol. Cell. Biol.* 11, 4466–4472.
- Lydall, D., Ammerer, G., and Nasmyth, K. (1992). A new role for MCM1 in yeast: cell cycle regulation of *SW15* transcription. *Genes Dev.* 5, 2405–2419.
- Maine, G. T., Sinha, P., and Tye, B. K. (1984). Mutants of *S. cerevisiae* defective in the maintenance of minichromosomes. *Genetics* 106, 365–385.
- Mudryj, M., Heibert, S., and Nevins, J. (1990). A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. *EMBO J.* 9, 2179–2184.
- Mudryj, M., Devoto, S., Heibert, S., Hunter, T., Pines, J., and Nevins, J. R. (1991). Cell cycle regulation of the E2F transcription factor involves an interaction with Cyclin A. *Cell* 65, 1243–1253.
- Norman, C., Runswick, M., Pollock, R., and Treisman, R. (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.
- Palmer, R. E., Hogan, E., and Koshland, D. (1990). Mitotic transmission of artificial chromosomes in *cdc* mutants of the yeast, *Saccharomyces cerevisiae*. *Genetics* 125, 763–774.
- Passmore, S., Maine, G. T., Elble, R., Christ, C., and Tye, B. K. (1988). *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of MAT α cells. *J. Mol. Biol.* 204, 593–606.
- Passmore, S., Elble, R., and Tye, B. K. (1989). A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes Dev.* 3, 921–935.
- Pringle, J. R., and Hartwell, L. H. (1981). The *Saccharomyces cerevisiae* cell cycle. In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, ed. J. N. Strathern, E. W. Jones, and J. R. Broach. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 97–142.
- Pringle, J., Preston, R., Adams, A., Stearns, T., Drubin, D., Haarer, B., and Jones, E. (1989). Fluorescence microscopy methods for yeast. *Methods Cell Biol.* 31, 357–435.

- Reed, S. (1980). The selection of *S. cerevisiae* mutants defective in the start event of cell division. *Genetics* 95, 561–577.
- Rhode, P. R., Elsasser, S., and Campbell, J. L. (1992). Role of multifunctional autonomously replicating sequence binding factor 1 in the initiation of DNA replication and transcriptional control of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12, 1064–1077.
- Richardson, H., Wittenberg, C., Cross, F., and Reed, S. (1989). An essential G1 function for cyclin-like proteins in yeast. *Cell* 59, 1127–1133.
- Schiestl, R. H., Reynolds, P., Prakash, S., and Prakash, L. (1989). Cloning and sequence analysis of the *Saccharomyces cerevisiae* RAD9 gene and further evidence that its product is required for cell cycle arrest induced by DNA damage. *Mol. Cell. Biol.* 9, 1882–1896.
- Shore, D., and Nasmyth, K. (1987). Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* 51, 721–732.
- Sinha, P., Chang, V., and Tye, B. K. (1986). A mutant that affects the function of autonomously replicating sequences in yeast. *J. Mol. Biol.* 192, 805–814.
- Sitney, K. C., Budd, M. E., and Campbell, J. L. (1989). DNA polymerase III, a second essential DNA polymerase, is encoded by the *S. cerevisiae* CDC2 gene. *Cell* 56, 599–605.
- Som, T., Armstrong, K., Volkert, F., and Broach, J. (1988). Autoregulation of 2 μ m circle gene expression provides a model for maintenance of stable plasmid copy levels. *Cell* 52, 27–37.
- Sommer, H., Beltran, J.-P., Huijser, P., Pape, H., Lonnig, W.-E., Saeidler, H., and Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homology to transcription factors. *EMBO J.* 9, 605–613.
- Weinert, T. A., and Hartwell, L. H. (1988). The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* 241, 317–322.
- Williamson, D. H., and Fennell, D. J. (1975). The use of fluorescent DNA-binding agent for detecting and separating yeast mitochondrial DNA. *Methods Cell Biol.* 12, 1985–1998.
- Yan, H., Gibson, S., and Tye, B. K. (1991). Mcm2 and Mcm3, two proteins important for ARS activity, are related in structure and function. *Genes Dev.* 5, 944–957.
- Yanofsky, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldman, K. A., and Meyerowitz, E. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* 346, 35–39.