Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10459–10463, November 1992
Biochemistry

CDC46/MCM5, a yeast protein whose subcellular localization is cell cycle-regulated, is involved in DNA replication at autonomously replicating sequences

(minichromosome instability/cell cycle-dependent nuclear localization/protein family involved in DNA replication)

YANRU CHEN*, KEVIN M. HENNESSY†, DAVID BOTSTEIN†, AND BIK-KWOON TYE*

*Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853; and †Department of Genetics, Stanford University, Stanford, CA 94305

Contributed by David Botstein, July 31, 1992

ABSTRACT ***Saccharomyces cerevisiae*** **cells containing mutations in the cell-division-cycle gene** ***CDC46*** **arrest with a large bud and a single nucleus with unreplicated DNA at the nonpermissive temperature. This G_1/S arrest, together with the increased rates of mitotic chromosome loss and recombination phenotype, suggests that these mutants are defective in DNA replication. The subcellular localization of the CDC46 protein changes with the cell cycle; it is nuclear between the end of M phase and the G_1/S transition but is cytoplasmic in other phases of the cell cycle. Here we show that** ***CDC46*** **is identical to** ***MCM5*****, based on complementation analysis of the** ***mcm5-1*** **and** ***cdc46-1*** **alleles, complementation of the minichromosome maintenance defect of** ***mcm5-1*** **by** ***CDC46*****, and the genetic linkage of these two genes. Like** ***mcm5-1, cdc46-1*** **and** ***cdc46-5*** **also show a minichromosome maintenance defect thought to be associated with DNA replication initiation at autonomously replicating sequences. Taken together, these observations suggest that CDC46/MCM5 acts during a very narrow window at the G_1/S transition or the beginning of S phase by virtue of its nuclear localization to effect the initiation of DNA replication at autonomously replicating sequences.**

The eukaryotic genome is replicated once and only once within a defined period of the cell cycle. Replication requires the coordinated initiation of DNA synthesis at multiple sites. These initiation events are under cell cycle control such that DNA synthesis occurs only during S phase and reinitiation is prevented until the next cell cycle. Molecular events that lead to replication initiations in response to cell cycle signals are largely unknown.

The genome of the budding yeast *Saccharomyces cerevisiae* is estimated to contain 200–400 replication origins that can be cloned on plasmids as autonomously replicating sequences (ARSs) (1, 2). The cloning of ARSs on plasmids provides a convenient means to identify gene products involved in ARS function by screening for mutants that destabilize minichromosomes containing one of these ARSs (3). The most attractive feature of this approach is that it allows one to isolate viable mutants with a defect in a specific function without relying on general phenotypes, such as cell cycle arrest, which often give no information on the execution point of the lesion. This feature is particularly important if replication origins are coordinately regulated in groups via shared initiator proteins. Thus, elimination of a particular initiator protein that is responsible for the initiation of only a subset of ARSs may not necessarily be lethal to the cell since replication of inactive replicons by merging neighboring replicons would be expected.

With this approach, a number of minichromosome maintenance mutants were isolated. Mutants in several complementation groups were of particular interest because they show an ARS-specific minichromosome maintenance defect. These mutants have an effect on the stability of all minichromosomes tested, but those carrying certain ARSs are much more affected than others (3). Although minichromosome instability seems to be a general property of replication-defective mutants, this ARS specificity is unique to the *mcm* mutants, suggesting that they harbor defects in DNA replication targeted at ARSs (4). Three of these *MCM* genes, *MCM1*, *MCM2*, and *MCM3*, have been cloned and further characterized. Mutants in *MCM1* (4), *MCM2* (5), and *MCM3* (6) all show increased chromosome loss and recombination, characteristic of mutants defective in DNA replication (7). Furthermore, instability of minichromosomes in the *mcm2-1* mutant has been shown to be due to plasmid loss or nonreplication rather than missegregation (5).

MCM1 encodes a DNA-binding protein that acts both as a transcription factor (8, 9) and a replication initiator (10) by binding to regulatory sequences upstream of a number of mating type-specific genes (11–13) and to ARSs (C. Christ, V. Chang, and B.-K.T., unpublished results). Genetic analysis indicates that *MCM2* and *MCM3* carry out related functions (14). Double mutants of *mcm2* and *mcm3* result in a synthetic lethal phenotype. Furthermore, overproduction of MCM2 complements the *mcm3-1* mutation while overproduction of MCM3 is lethal to the *mcm2-1* mutant. Interestingly, MCM2 and MCM3 show striking similarity in their protein sequences (14). This homology extends to other known proteins, CDC46 of *S. cerevisiae* and CDC21 of *Schizosaccharomyces pombe* (A. Coxon and S. Kearsey, personal communication). Phenotypes of *cdc46* and Cdc21⁻ mutant strains are consistent with defects in DNA replication. This finding led us to postulate that these proteins constitute a family of at least four members, all of which play important roles in the early steps of DNA replication (14, 15).

Conditional mutations that completely block the initiation step of all DNA synthesis are expected to have a G_1-arrest phenotype. The *cdc46* mutations were originally identified as allele-specific suppressors of *cdc45* and *cdc54* mutations (16). These *cdc* mutants arrest at the G_1/S transition or the beginning of the S phase of the cell cycle. The subcellular localization of CDC46 has been shown to be under cell cycle control: it appears in the nucleus at late M phase and persists in the nucleus until the G_1/S transition (17). In addition to the striking homology of CDC46 to the MCM2 and MCM3 proteins, the *cdc46-1* mutant exhibits phenotypes similar to those of the *mcm2* and *mcm3* mutants, showing high rates of mitotic chromosome loss and recombination (15). If MCM2,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ARS, autonomously replicating sequence; FOA, 5-fluoroorotic acid; Cm−ura, complete medium lacking urocil.

MCM3, and CDC46 are members of a protein family related in structure and function, it seems likely that *cdc46* mutants should also show an ARS-specific minichromosome maintenance defect. In fact, we surmised that *CDC46* may correspond to one of the original ARS-specific *MCM* genes identified by Maine *et al.* (3).

In this paper, we show that *CDC46* is identical to *MCM5*. We further present data to show that CDC46 is required for minichromosome maintenance in an ARS-specific manner.

MATERIALS AND METHODS

Strains and Plasmids. *Escherichia coli* strain DH5α (BRL) was used for plasmid construction and amplification. Yeast strain 8534-8C (*MATα leu2-3,112 ura3-52 his4-Δ34*) is the parent strain of the *mcm* mutants (3, 18). The *mcm5-1* strain is strain 1-11 described by Maine *et al.* (3). Two strains of *mcm5-1* that resulted from backcross were used in this study: RM11-2A (*MATa leu2-3,112 ura3-52 mcm5-1*) and RM11-2B (*MATα leu2-3,112 his4-Δ34 mcm5-1*). R61-2B (*MATa, leu2-3, 112 ura3-52 his3-11,15 mcm3-1*) has been described (6). The double mutant *mcm2-1 mcm4-1* (*MATa leu2-3,112 ura3-52 his4Δ34 mcm2 mcm4*) was constructed by H. Yan (Cornell University, Ithaca, NY). DBY2028 (*MATa ura3-52 ade2-1 lys2-801 leu2-3,112 cdc46-1*) and YRC1 (*MATα lys2-801 ade2-101 his3-Δ200 leu2-Δ1 ura3-52 cdc46-5*) have two different *cdc46* alleles that suppress *cdc45-1* and *cdc54-1*, respectively (17). The strain YPH500 (*MATα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3Δ200 leu2-Δ1*) used for genetic mapping was obtained from the Yeast Genetic Stock Center (Berkeley, CA).

The plasmids used in this study are listed in Table 1. YCp50CDC46 has a 15.6-kilobase (kb) fragment containing the wild-type *CDC46* gene on the YCp50 vector (15), and YCpLC46 is the same as YCp50CDC46 except that the *URA3* marker is replaced by *LEU2*. pRS306CDC46 and pRS306CDC46F were constructed by subcloning either the 5-kb *Sca* I–*Sal* I fragment containing the *CDC46* gene or a 4.4-kb *Sca* I–*Sal* I fragment containing the flanking sequence of *CDC46*, respectively, into the polylinker site of pRS306 (20).

Media. Rich medium (YEPD) and complete medium (Cm) were made as described by Sherman *et al.* (21). FOA (5-fluoroorotic acid) plates were made according to Boeke *et al.* (22).

Table 1. Plasmids used in this study

Plasmid	Description	Source/ reference
YCp50	*URA3 ARS1 CEN4*	19
YCp50CDC46	15.6-kb fragment containing *CDC46* on YCp50	17
YCp50MCM3	*MCM3* cloned in YCp50	H. Yan
YCpLC46	*CDC46 LEU2 ARS1 CEN4*	K. Hennessy
pRS306	*URA3* Amp^R, KS polylinker from pbluescript	20
pRS306CDC46	*CDC46* on pRS306	This study
pRS306CDC46F	The flanking sequence of *CDC46* on pRS306	This study
YCp101	*LEU2 ARS1 CEN5*	14
YCpUM2	*URA3 ARS1 CEN3 MCM2*	14
YCpLM2	*LEU2 ARS1 CEN5 MCM2*	H. Yan
YCp1	*URA3 LEU2 ARS1 CEN5*	3
YCpHML	*URA3 ARSHML CEN5*	14
YCpH2B	*URA3 LEU2 ARSH2B CEN5*	14
YCpHO	*URA3 LEU2 ARSHO CEN5*	14
YCp120	*URA3 LEU2 ARS120 CEN5*	14
YCp131	*LEU2 ARS131 CEN5*	3
YCp131C	*URA3 LEU2 ARS131C CEN5*	3
YCp131A	*URA3 LEU2 ARS131A CEN5*	3
YCp121	*LEU2 ARS121 CEN5*	3

Construction of Heterozygous Diploids from Haploids with Similar Genetic Markers. To cross strains, such as RM11-2A and 8534-8C, that contain many of the same selectable markers, 8534-8C was transformed with YCp101 (carrying *LEU2*). Diploids were selected on complete medium lacking histidine and leucine (Cm−his−leu). Diploids were confirmed by their ability to sporulate and their inability to mate.

Genetic Techniques and DNA Transformation. Mating, sporulation, and meiotic tetrad analysis were carried out as described (21). Transformation of yeast cells with plasmids was carried out by a modified lithium acetate method (23).

Mitotic Stability Assay for Plasmids. The stability assay measured the cumulative loss of minichromosomes from a yeast culture after 10–12 generations of nonselective growth at specified temperatures (3). Complete medium lacking uracil (Cm−ura) served as the selective medium for retaining YCp50CDC46 and YCp50 in the cells, while Cm−ura−leu additionally selected for the test plasmid. The final stability (*F*) of the test plasmid was calculated as the percentage of cells growing on Cm−ura−leu after 10–12 generations of nonselective growth on Cm−ura plates. The minichromosome loss rate is defined as the rate of plasmid loss per cell division. It is expressed as $1-(F/I)^{1/N}$, where *I* is the initial percentage of plasmid-containing cells and *F* is the percentage of plasmid-containing cells after *N* generations of nonselective growth.

RESULTS

***CDC46* Complements *mcm5* but Not *mcm2, mcm3, mcm4*, or *mcm7*.** Yeast cells containing mutations in *MCM2, MCM3, MCM4, MCM5*, and *MCM7* are defective in the stable maintenance of minichromosomes in an ARS-specific manner. Plasmid carrying *CDC46* (YCp50CDC46) and the control vector plasmid (YCp50) were transformed independently into each of the mutant strains, *mcm5-1, mcm4-1*, and *mcm7-1*, containing the test plasmid, YCp101 or YCp131. The stabilities of the test plasmids in the mutant strains were measured (Table 2). YCp101 is unstable in *mcm4-1* whether carrying YCp50, the vector control, or YCp50CDC46. Similarly, YCp131 is unstable in *mcm7-1* whether carrying YCp50 or YCp50CDC46. In contrast, although YCp101 has a stability of 0.2% in *mcm5-1* carrying YCp50, this Mcm$^-$ defect is complemented by the presence of *CDC46* on the YCp50CDC46 plasmid, as evident by the increased stability of the YCp101 plasmid to 51%. These results indicate that *CDC46* complements *mcm5-1* but not *mcm4-1* or *mcm7-1*.

The *mcm3-1* mutant is temperature sensitive for growth at 37°C (6). Plasmids YCp50, YCp50CDC46, and YCp50MCM3

Table 2. *CDC46* complements the Mcm-defective phenotype of *mcm5-1*

Strain	Final stability, % [(Cm−ura−leu/Cm−ura) × 100]
mcm4-1[YCp50 + YCp101]	<1
mcm4-1[YCp50CDC46 + YCp101]	<0.6
mcm5-1[YCp50 + YCp101]	<0.2
mcm5-1[YCp50CDC46 + YCp101]	51
mcm7-1[YCp50 + YCp131]*	2.0
mcm7-1[YCp50CDC46 + YCp131]*	2.5
mcm5-1::CDC46[YCp101]	50
mcm5-1::CDC46F[YCp101]	<0.5

The final stability was measured as the percentage of cells able to grow on Cm−ura−leu after they had been grown on Cm−ura for 10–12 generations.

*YCp131 was used instead of YCp101 as the test plasmid because *mcm7-1* has little effect on *ARS1* activity.

were transformed into the R61-2B strain to check for complementation of growth at 37°C. The *mcm3-1*[YCp50MCM3] transformants grew but the *mcm3-1*[YCp50] and *mcm3-1* [YCp50CDC46] transformants failed to grow at 37°C. This result indicates that *CDC46* does not complement the *mcm3-1* mutation.

Single mutants of *mcm2-1* and *mcm4-1* are viable at all temperatures, but the double mutant is lethal. This lethal phenotype can be used as an assay for complementation of either the *mcm2* or the *mcm4* mutation. In fact, *MCM2* carried on plasmid YCpUM2 complements this lethal phenotype. YCpLC46 was transformed into the double mutant containing YCpUM2, and the transformants were streaked on Cm−ura−leu plates. Twelve of these transformants were restreaked onto Cm−leu+FOA plates to select against cells carrying the YCpUM2 plasmid. Cells can grow on Cm−leu+FOA plates only if *CDC46* complements *mcm2* or *mcm4*. The result was that no colonies grew up on Cm−leu+FOA plates (data not shown). In the control experiment, YCpLM2 was transformed into *mcm2-1mcm4-1* [YCpUM2]. These transformants grew well on Cm−leu+ FOA plates because of the presence of *MCM2* on YCpLM2. These experiments indicate that *CDC46* on a centromere-bearing (*CEN*) plasmid fails to complement the minichromosome maintenance defect of *mcm2-1* or *mcm4-1*. Thus, *CDC46* specifically complements the minichromosome maintenance defect of *mcm5-1*, suggesting that *CDC46* may be the same as *MCM5*.

***cdc46-1* and *mcm5-1* Belong to the Same Complementation Group.** The *cdc46-1* and *mcm5-1* mutant strains were crossed with each other and with the wild-type strain 8534-8C. The stabilities of YCp101 in the resulting diploids were examined (Table 3). The *cdc46-1*/*mcm5-1* diploid shows an obvious minichromosome maintenance defect when compared with each of the heterozygotes and the wild-type diploid. These results indicate that *cdc46-1* and *mcm5-1* belong to the same complementation group.

***CDC46* Is Tightly Linked to *MCM5*.** The genetic linkage of *CDC46* to the *mcm5-1* mutation was determined by two parallel tetrad analyses. In the first analysis, the pRS306CDC46 plasmid was target-integrated into the genome of the *mcm5-1* strain at the genomic *CDC46* sequence by cutting with the restriction enzyme *Cla* I. The structure of the integrated plasmid at the genomic *CDC46* location was confirmed by Southern analysis (data not shown). The stability of YCp101 in this strain (Table 2) confirms that *CDC46* complements the minichromosome maintenance defect of *mcm5-1*. The strain *mcm5-1*::*CDC46* was then crossed to the wild-type strain YPH500. The diploid was sporulated and tetrads were dissected to determine linkage of *mcm5-1* and *CDC46*. If *CDC46* is identical to *MCM5*, integration of the plasmid should occur at *mcm5-1*, resulting in the cosegregation of *CDC46* with *mcm5-1*. In other words, all spores from these tetrads should be Mcm^+. In contrast, if *CDC46* and *mcm5-1* are unlinked, then Mcm^- spores would be expected. YCp101 was transformed into each of the spores and its stability was examined. All spores from seven tetrads were

Table 3. Complementation tests based on minichromosome stability in various diploid strains

Strain	Final stability, % [(Cm−leu/YEPD) × 100]
cdc46-1/*mcm5-1*	0.5
mcm5-1/*MCM5*	43
cdc46-1/*CDC46*	64
Wild-type diploid	60

The final stability of YCp101 was measured as the percentage of cells able to grow on Cm−leu after they had been grown in YEPD for 10–12 generations.

wild-type for minichromosome maintenance with plasmid stability at values of about 50% (Table 4, cross A). The absence of Mcm^- spores indicates that *CDC46* and *MCM5* are tightly linked.

In a parallel tetrad analysis, the plasmid pRS306CDC46F, containing the flanking sequence of *CDC46*, was transformed into an *mcm5-1* strain by targeting integration at the *Mlu* I site at the genomic *CDC46* flanking sequence. The precise location of the integrated plasmid was confirmed by Southern blot analysis (data not shown). Since pRS306 carries the *URA3* gene, the integration site is marked by *URA3*. This strain, *mcm5-1*::*CDC46F*, which remains Mcm-defective (see Table 2), was crossed to the wild-type strain YPH500. Ten tetrads were analyzed to test for linkage of *mcm5-1* to the *CDC46* flanking sequence (Table 4, cross B). All *URA3* prototrophic spores were defective in minichromosome maintenance. The stability of YCp101 in these spores was <0.5% after 10–12 generations of nonselective growth. However, the *ura3* auxotrophic spores were all wild-type for minichromosome maintenance, with the stability of YCp101 ranging from 55% to 65%. This result indicates that the *CDC46* flanking sequence was integrated close to *mcm5-1*. Combined data from these two crosses place *CDC46* and *MCM5* within 6 centimorgans of each other.

The *cdc46* and *mcm5* Mutants Have Similar ARS Specificity for Minichromosome Maintenance. *mcm5-1* was originally isolated as a mutant that affected the stability of minichromosomes in an ARS-dependent manner but otherwise had no detectable growth defect. In contrast, *cdc46-1* and *cdc46-5* are cell-division-cycle mutants that are temperature sensitive for growth at 37°C, resulting in an arrest at the G_1/S transition of the cell cycle. *cdc46-1* is an allele-specific suppressor of *cdc45*, whereas *cdc46-5* is an allele-specific suppressor of *cdc54*. If *cdc46* and *mcm5* represent different mutant alleles of the same gene, then one may also expect *cdc46-1* and *cdc46-5* to have an ARS-specific minichromosome maintenance defect. Nine well-characterized minichromosomes, each carrying a different ARS, were individually transformed into the *mcm5-1*, *cdc46-1*, and *cdc46-5* mutant strains. *ARS1*, *ARSHML*, *ARSH2B*, and *ARSHO* are ARS elements associated, respectively, with *TRP1* (24), the silent mating-type locus *HMLα* (25), the histone *H2B* gene (26), and the endonuclease *HO* gene (27). *ARS120*, *ARS131*, *ARS131C*, and *ARS131A* are four ARSs associated with subtelomeric X sequences (28). *ARS121* is a single-copy ARS element on chromosome X (29). The loss rates of these different ARS plasmids in the *mcm5* and *cdc46* mutants were measured at both room temperature and 30°C (Table 5).

In all three mutant strains, all ARS plasmids tested are equally or more stable at room temperature than at 30°C. In fact, all ARS plasmids are fairly stable at room temperature, so that the specificity for plasmid stability in these strains is not obvious. However, at 30°C, some ARSs become much more affected than others, revealing a significant difference in the stability of minichromosomes carrying specific ARSs in all three *mcm5* and *cdc46* strains. For example, plasmids carrying *ARS1*, *ARS131*, *ARS131A*, and *ARSHO* are unstable in all three mutants. In contrast, the stability of plasmids carrying *ARS121* and *ARS131C* is only minimally affected in any of these mutants. Most ARS plasmids tested are relatively less stable in *cdc46-1* and *cdc46-5* than in *mcm5-1*; this difference is greatest for plasmids containing *ARSHML*, *ARSH2B*, or *ARS120* (Table 5). This result reflects the greater severity of the *cdc46* alleles that also exhibit a temperature-sensitive growth defect.

DISCUSSION

The *mcm* mutants are putative replication initiation mutants identified by their inability to efficiently propagate autonomously replicating plasmids. This defect shows a great de-

Table 4. Genetic mapping of CDC46 to mcm5-1

Cross A. *MCM5* × *mcm5-1::URA3CDC46*

Tetrad	Spore [YCp101]	Growth Cm−ura	Cm−trp	Final stability, %
1	a	+	−	52
	b	−	+	55
	c	−	−	56
	d	+	+	48
2	a	+	−	57
	b	−	−	56
	c	+	+	47
	d	−	+	53
3	a	−	+	58
	b	+	−	42
	c	−	+	56
	d	+	−	54
4	a	+	+	41
	b	+	−	40
	c	−	−	48
	d	−	+	54
5	a	−	+	59
	b	+	+	48
	c	+	−	51
	d	−	−	41
6	a	−	−	57
	b	+	−	42
	c	+	+	40
	d	−	+	55
7	a	−	−	54
	b	−	+	47
	c	+	−	52
	d	+	+	47

Cross B. *MCM5* × *mcm5-1::URA3CDC46F*

Tetrad	Spore [YCp101]	Growth Cm−ura	Cm−trp	Final stability, %
1	a	+	−	<0.4
	b	−	−	55
	c	−	+	50
	d	+	+	<0.3
2	a	+	−	<0.5
	b	−	−	58
	c	+	+	<0.4
	d	−	+	49
3	a	+	−	<0.5
	b	−	+	57
	c	−	−	64
	d	+	+	<0.5
4	a	+	−	<0.4
	b	−	+	68
	c	+	+	<0.2
	d	−	−	64
5	a	−	−	66
	b	−	−	55
	c	+	+	<0.3
	d	+	+	<0.3
6	a	+	+	<0.6
	b	+	−	<0.3
	c	−	−	62
	d	−	+	65
7	a	+	+	0.38
	b	+	+	<0.4
	c	−	−	67
	d	−	−	62
8	a	−	−	67
	b	−	+	52
	c	+	+	<0.4
	d	+	−	<0.5
9	a	−	−	61
	b	−	+	58
	c	+	+	<0.5
	d	+	−	<0.4
10	a	−	−	63
	b	−	−	56
	c	+	+	<0.3
	d	+	+	<0.4

In each tetrad, both uracil and tryptophan markers segregate 2:2. The final stability of YCp101 in each spore was measured as the percentage of cells growing on Cm−leu after they had been growing in YEPD for 10–12 generations.

pendency on the type of ARS present on the minichromosome, suggesting that the lesion is specific to the initiation of DNA replication at ARSs. Since most ARSs have been shown to serve as efficient initiation sites for DNA replication at their chromosomal locations (30), the products of the *MCM* genes are likely candidates for proteins that lead to initiation events at chromosomal replication origins. The fact that these *MCM* gene products also exert a discernible effect on the stability of plasmids carrying *ARSHML*, which is silent in its natural chromosomal location (31), suggests that this ARS has many of the same features of other chromosomally active ARSs. This context-dependent activity of *AR-*

Table 5. Loss rates of minichromosomes in *mcm5* and *cdc46* mutants

Strain	Temp.	Minichromosomes (YCp) *1*	*HML*	*H2B*	*HO*	*120*	*131*	*131C*	*131A*	*121*
mcm5-1	30°C	0.22	0.07	0.03	0.13	0.04	0.14	0.02	0.14	0.02
	RT	0.04	0.05	0.03	0.04	0.01	0.06	0.01	0.05	0.02
cdc46-1	30°C	0.21	0.15	0.12	0.20	0.07	0.13	0.07	0.20	0.03
	RT	0.08	0.09	0.03	0.06	0.02	0.08	0.04	0.07	0.03
cdc46-5	30°C	0.23	0.11	0.08	0.23	0.10	0.20	0.06	0.19	0.05
	RT	0.10	0.12	0.04	0.09	0.06	0.13	0.03	0.12	0.03
Wild type*	30°C	0.05	0.05	<0.01	0.01	0.01	0.03	0.01	ND	0.01
	RT	0.02	0.05	<0.01	<0.01	0.01	0.04	<0.01	ND	0.02

RT, room temperature. ND, not determined.

*Values for wild-type strain were obtained under similar conditions by Yan *et al.* (14) and are included for comparison.

SHML may be explained by its inaccessibility to replication proteins or the presence of negative elements at its chromosomal location. Previous work showed that the gene products of *MCM2* and *MCM3* are related in structure and function (14). They share homologous domains common to a family of proteins that include CDC46 of *S. cerevisiae* (15) and CDC21 of *Sch. pombe* (A. Coxon and S. Kearsey, personal communication). This homology is prominent in three regions, with region II being the most extensive and most conserved (Fig. 1). The homologous domains shared by these proteins suggest that they may interact with similar targets or interact with one another to form a complex. Here, we show that MCM5 is identical to CDC46. The identification of three members in this protein family by using the same mutant screen confirms the efficacy and directedness of this genetic approach.

Moir *et al.* (16) originally searched for mutants that cannot proceed beyond the G_1/S boundary as a criterion for replication initiation mutants. *cdc46* mutants were identified as allele-specific suppressors of *cdc45* and *cdc54* mutants that arrest at the G_1/S transition of the cell cycle. CDC46, mutants of which also exhibit growth arrest at the G_1/S boundary, gained credence as an important factor in the initiation of DNA replication because of its cell cycle-regulated subcellular localization. It is nuclear between late M phase and the G_1/S transition but predominantly cytoplasmic at other phases of the cell cycle. Its pattern of nuclear localization is consistent with that postulated for the replication control factor, or "licensing factor," whose presence in the nucleus signals the initiation of DNA replication and whose subsequent absence prevents the reinitiation of DNA synthesis at replication origins (32). Our finding that *CDC46* is identical to *MCM5* has two important ramifications. (*i*) The ARS-specific minichromosome maintenance defect of *cdc46* supports the idea that CDC46 plays an important role in the initiation of DNA replication and that it probably effects initiation by interacting directly with replication origins and not through a signal-transduction relay mechanism. The fact that two different *cdc46* alleles exhibit similar ARS-specific minichromosome maintenance defects is particularly interesting because each of these alleles suppresses a different *cdc* mutation in an allele-specific manner. If allele-specific suppression indicates physical interactions between two proteins, then this observation suggests that the interaction of CDC46 with both CDC45 and CDC54 may be important for the activation of the same replication origins. (*ii*) The identification of *CDC46* with *MCM5* links the network of interacting *CDC* genes (15) with the genetically interacting *MCM* genes (14). The merging of these two independently isolated families of genes bodes well for our eventual understanding of the complex regulatory mechanism that controls DNA replication in eukaryotes.

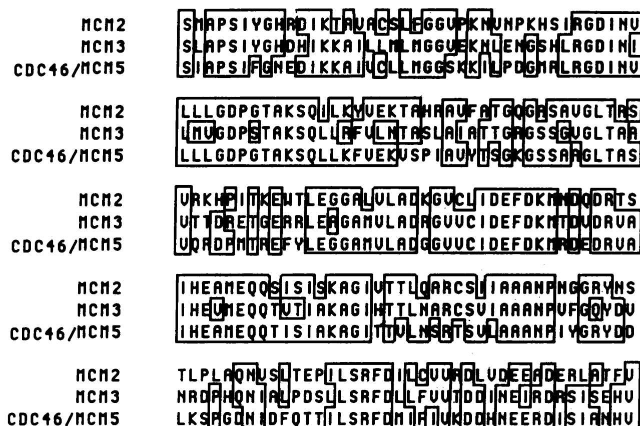

FIG. 1. Comparison of the amino acid sequences in region II of MCM2 (residues 500–699), MCM3 (366–565), and CDC46/MCM5 (373–572). Position with identities between at least two of the three proteins are boxed.

This work was supported by National Institutes of Health Grant GM34190, awarded to B.-K.T.

1. Brewer, B. & Fangman, W. L. (1987) *Cell* **51**, 463–471.
2. Huberman, J. A., Zhu, J., Davis, L. R. & Newlon, C. S. (1988) *Nucleic Acids Res.* **16**, 6373–6384.
3. Maine, G. T., Sinha, P. & Tye, B. K. (1984) *Genetics* **106**, 365–385.
4. Elble, R. & Tye, B. K. (1992) *Mol. Cell. Biol.*, in press.
5. Sinha, P., Chang, V. & Tye, B. K. (1986) *J. Mol. Biol.* **192**, 805–814.
6. Gibson, S., Surosky, R. & Tye, B. K. (1990) *Mol. Cell. Biol.* **10**, 5707–5720.
7. Hartwell, L. H. & Smith, D. (1985) *Genetics* **110**, 381–395.
8. Passmore, S., Maine, G. T., Elble, R., Christ, C. & Tye, B. K. (1988) *J. Mol. Biol.* **204**, 593–606.
9. Elble, R. & Tye, B. K. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10966–10970.
10. Christ, C. & Tye, B. K. (1991) *Genes Dev.* **5**, 751–763.
11. Passmore, S., Elble, R. & Tye, B. K. (1989) *Genes Dev.* **3**, 921–935.
12. Jarvis, E. E., Clark, K. L. & Sprague, G. F., Jr. (1989) *Genes Dev.* **3**, 936–945.
13. Keleher, C. A., Passmore, S. & Johnson, A. D. (1989) *Mol. Cell. Biol.* **9**, 5228–5230.
14. Yan, H., Gibson, S. & Tye, B. K. (1991) *Genes Dev.* **5**, 944–957.
15. Hennessy, K. M., Lee, A., Chen, E. & Botstein, D. (1991) *Genes Dev.* **5**, 958–969.
16. Moir, D., Stewart, S. E., Osmond, B. C. & Botstein, D. (1982) *Genetics* **100**, 565–577.
17. Hennessy, K. M., Clark, C. D. & Botstein, D. (1990) *Genes Dev.* **4**, 2252–2263.
18. Gibson, S., Surosky, R., Sinha, P., Maine, G. & Tye, B. K. (1987) *UCLA Symp. Mol. Cell. Biol.* **47**, 341–354.
19. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. & Fink, G. R. (1987) *Gene* **60**, 237–243.
20. Sikorski, R. & Hieter, P. (1989) *Genetics* **122**, 19–27.
21. Sherman, F., Fink, G. R. & Lawrence, C. W. (1974) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
22. Boeke, J. D., LaCroute, F. & Fink, G. (1984) *Mol. Gen. Genet.* **197**, 345–346.
23. Elble, R. (1992) *Biotechniques* **13**, 18–20.
24. Struhl, K., Stinchcomb, D. T., Scherer, S. & Davis, R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1035–1039.
25. Feldman, J. B., Hicks, J. B. & Broach, J. R. (1984) *J. Mol. Biol.* **178**, 815–834.
26. Osley, M. A. & Hereford, L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7689–7693.
27. Kearsey, S. (1984) *Cell* **37**, 299–307.
28. Chan, C. S. M. & Tye, B. K. (1983) *Cell* **33**, 563–573.
29. Walker, S. S., Malik, A. K. & Eisenberg, S. (1991) *Nucleic Acids Res.* **19**, 6255–6262.
30. Fangman, W. L. & Brewer, B. (1991) *Annu. Rev. Cell Biol.* **7**, 375–402.
31. Dubey, D. D., Davis, L. R., Greenfeder, S. A., Ong, L. Y., Zhu, J., Broach, J., Newlon, C. & Huberman, J. (1991) *Mol. Cell. Biol.* **11**, 5346–5355.
32. Blow, J. J. & Laskey, R. A. (1988) *Nature (London)* **332**, 546–548.