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The Role of the Carboxyterminal Domain of RNA Polymerase II in Regulating Origins of DNA Replication in *Saccharomyces cerevisiae*

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Manuscript received May 15, 2002
Accepted for publication August 29, 2002

ABSTRACT

MCM (minichromosome maintenance) proteins function as a replication licensing factor (RLF-M), which contributes to limiting initiation of DNA replication to once per cell cycle. In the present study we show that a truncation of the pol II CTD in a *S. cerevisiae* strain harboring a mutation in *mcm5* partially reverses its *ts* phenotype and improves maintenance of CEN/ARS minichromosomes. We correlate this phenotype to effects on DNA replication rather than to effects on transcription or specific gene expression. We also demonstrate that a similar truncation of the CTD reduces minichromosome stability and impairs stimulation of DNA replication by *trans*-activators and that tethering of recombinant pol II CTD to an origin of replication has a significant stimulatory effect on minichromosome stability. Furthermore, we show that pol II is recruited to ARS1. We propose that in *S. cerevisiae* a mechanism of coordinating pol II transcription and DNA replication is mediated by the CTD of pol II.

DNA replication in eukaryotes initiates at DNA locations referred to as origins of replication. In *Saccharomyces cerevisiae* origins behave as autonomously replicating sequences (ARS) when placed on an extrachromosomal DNA. These contain one essential (A) and three auxiliary (B1, B2, and B3) elements (Marahrens and Stillman 1992). The A element is a core sequence that binds a protein complex called origin recognition complex (ORC; Bell and Stillman 1992), which in turn recruits *CDC6* and six minichromosome maintenance (MCM) proteins to form the prereplicative complex (Cocker *et al.* 1996). Activation of these prereplicative complexes by protein kinases is absolutely required for initiation of replication. It is believed that disruption of the ORC-MCM interaction at the time of initiation is responsible for limiting origin firing to once per cell cycle (Tye 1999a; Labib and Diffley 2001; Lei and Tye 2001). It has been shown that MCMs are associated with moving replication forks in yeast (Aparicio *et al.* 1997; Labib *et al.* 2000; Labib and Diffley 2001), suggesting a postinitiation function in DNA replication. The precise biochemical role of the MCM proteins remains unclear. They do possess helicase activity (Ishimi 1997), which could be involved in facilitating replication fork movement (Labib and Diffley 2001). Mammalian MCM2 binds to histones H3/H4 (Ishimi *et al.* 1988, 1996) and to HBO1, a putative histone acetyl transferase (Burke *et al.* 2001), indicating a potential role for MCMs in chromatin remodeling.

The function of the auxiliary (B1, B2, and B3) elements in yeast origins is not completely understood. The B1 element provides an additional binding site for ORC (Rao and Stillman 1995; Rowley *et al.* 1995). The role of the B2 element is not clear. The B3 element in ARS1 contains a binding site for the Abf1 protein, which also operates as a transcriptional activator or a transcriptional repressor in other contexts. The function of the Abf1-binding site in ARS1 can be replaced by binding sites for other transcriptional activators (Marahrens and Stillman 1992; Li *et al.* 1998; Hu *et al.* 1999) or by tethering viral or mammalian transcriptional activation domains (Bennett-Cook and Hassell 1991; Li *et al.* 1998; Hu *et al.* 1999; Stagljar *et al.* 1999) to the B3 element. Extensive evidence shows that transcriptional activators also stimulate viral origins of replication (DePamphilis 1988). It is obvious that transcriptional activators are positive regulators of origins of DNA replication; however, their mechanism of action in these contexts is largely unknown. Several reports correlate these positive effects to the binding of the *trans*-activator to the replication factor RPA (He *et al.* 1993; Li and Botchan 1993). Other reports show that the ability of *trans*-activators to stimulate replication is tightly linked to their ability to stimulate pol II transcription (Bennett-Cook and Hassell 1991; He *et al.* 1993; Li *et al.* 1998) and chromatin

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remodeling (Hu *et al.* 1999). Recently it was shown that recruitment of pol II or pol III complexes to ARS1 is sufficient to enhance replication from a minichromosome origin (Stagljar *et al.* 1999; Bodmer-Glavas *et al.* 2001), presumably by remodeling chromatin.

Large pol II complexes, which contain some or all of the pol II general transcription factors, have been purified from a variety of sources and designated RNA polymerase II holoenzyme (Kim *et al.* 1994; Koleske and Young 1994; Hengartner *et al.* 1995; Ossipow *et al.* 1995; Chao *et al.* 1996; Maldonado *et al.* 1996; Pan *et al.* 1997). Pol II holoenzyme complexes from *S. cerevisiae* contain the SRB and MED family of proteins, the SWI/SNF complex, RGR1, and GAL11. Temperature-sensitive (*ts*) mutations in genes for holoenzyme proteins show that some (SRB4 and SRB6) are essential for mRNA synthesis (Thompson and Young 1995), whereas others contribute to the response to *trans*-activators (Kim *et al.* 1994; Hengartner *et al.* 1995; Li *et al.* 1995; Wilson *et al.* 1996; Myers *et al.* 1998; Han *et al.* 1999). Mammalian pol II holoenzyme complexes contain homologs of SRB, MED, and SWI/SNF proteins (Ossipow *et al.* 1995; Chao *et al.* 1996; Maldonado *et al.* 1996; Pan *et al.* 1997) but also DNA repair factors (Maldonado *et al.* 1996; Scully *et al.* 1997; Anderson *et al.* 1998), replication factors (Maldonado *et al.* 1996; Yankulov *et al.* 1999), and cleavage/polyadenylation factors (McCracken *et al.* 1997). A direct involvement in mRNA synthesis for some of these factors is not evident. Similar factors are not detected in the yeast holoenzyme. These dissimilarities could result from different strategies of purification or might reflect some fundamental differences between yeast and higher eukaryotes.

Most of the yeast pol II holoenzyme components join pol II via interactions with the highly conserved carboxyterminal domain (CTD) of its largest subunit. The CTD is composed of heptapeptide repeats (26 in *S. cerevisiae* and 52 in higher eukaryotes) with a consensus YSPTSPT. Antibodies against the CTD dissociate the yeast holoenzyme into core pol II and another complex named the "mediator" (Kim *et al.* 1994; Hengartner *et al.* 1995; Myers *et al.* 1997). The CTD, therefore, plays a key role in assembly of the pol II holoenzyme. As expected, the function of different mediator components is dependent on the CTD (Nonet and Young 1989; Myers *et al.* 1997).

Recently we reported that human pol II holoenzyme complexes interact with MCM proteins via the CTD of pol II (Yankulov *et al.* 1999). We proposed that in higher eukaryotes MCM proteins are involved in pol II transcription. In this article we demonstrate that a deletion of pol II CTD partially reverses the *ts* phenotype in *S. cerevisiae*, which is caused by a mutation in *MCM5*. We also demonstrate that RNA polymerase II is recruited to ARS1.

MATERIALS AND METHODS

Plasmids: pARS1/wtA is an ARS1/CEN4/*URA3*-based vector described in Marahrens and Stillman (1992). The derivative pARS1/-B23/G24 (Li *et al.* 1998) contains ARS1 in which the B2 and B3 elements are mutated and a GAL4-binding site is inserted next to the B3 element. pARS1wtA/2.2-kb/URA3 and pARS1/-B23/G24/2.2 kb/URA3 contain a 2.2-kb cDNA fragment derived from human *MCM2*, which is inserted in the *Sph*I site between the ARS1 and the *URA3* elements, respectively. This fragment contains no promoter elements. YCp50CDC46 is an ARS1/CEN4/*URA3* plasmid containing a 15.6-kb *MCM5* genomic fragment on the YCp50 vector (Chen *et al.* 1992). YIp122CDC46 is a *LEU2* integrating vector containing the 6.5-kb *Afl*II *MCM5* genomic fragment cloned into the *Sma*I site of YIp122. pFL35CDC46 is a *TRP1* integrating vector containing the *Sal*I-*Afl*II genomic *MCM5* fragment cloned in *Sal*I/*Xho*I sites of pFL35. pFL26RPB1Δ104 is a *LEU2* integrating vector encoding *rpb1Δ104* (Nonet *et al.* 1987). pFL38RPB1 (McNeil *et al.* 1998) is an ARS1/CEN4/*URA3* plasmid containing the 6042-bp *Eco*RI-*Pst*I fragment of *RPB1*. pGBKT7 (CLONTECH, Palo Alto, CA) is a 2μ/*TRP1* vector encoding the DNA-binding domain (amino acids 1–147) of GAL4 under the control of the *ADH1* promoter. pGBKT7-CTDwt encodes the mouse CTD (52 heptad repeats) fused to the DNA-binding domain of GAL4. pGBKT7-CTDmut encodes 15 mutant (S5 → A) heptad repeats fused to the DNA-binding domain of GAL4. pGBKT7-dacB expresses the *Escherichia coli* DAC-B protein fused to the DNA-binding domain of GAL4.

Yeast strains and growth conditions: The names and genotypes of the yeast strains used in this study are listed in Table 1. *rpb1Δ104mcm5* was produced by transforming the *mcm5* strain with pFL26*RPB1*Δ104 linearized by *Bsi*WI and selecting on SC-Leu plates. *MCM5* was produced by transforming the *mcm5* strain with YIp122CDC46 linearized by *Bsp*HI and selecting on SC-Leu plates and then on YPD plates at 37°. *rpb1Δ104 MCM5* was produced by transforming the *rpb1Δ104mcm5* strain with pFL35CDC46 linearized by *Bsp*HI and selecting on SC-trp plates.

Minichromosomes were introduced by electroporation. Yeast cultures were grown in SC (synthetic complete) medium plus 2% glucose or 2% galactose. Uracil, tryptophan, or leucine were omitted as indicated. Cells containing pARS1/-B23/G24 were grown on SC-Ura/Galactose medium.

Minichromosome stability assay: The rate of plasmid loss per generation was estimated as described (Dani and Zakian 1983) with modifications that improve precision of the measurement and accuracy of calculation (Kramer *et al.* 2002). Briefly, cells grown in selective medium were diluted to <5 cells/ml in SC medium and 150 μl were dispensed in 96-well tissue culture plates. The plates were incubated at specified temperatures until single colonies were visible in the wells. Colonies were resuspended by pipetting and cells were counted in a hemacytometer chamber. Wells with the equal lowest number of total cells (A) corresponding to the same number of generations [N calculated as $N = \mathrm{Log}(A,2)$] were selected as mini-cultures originating from a single cell and were further analyzed. Aliquots were briefly sonicated and cells were plated on selective and nonselective plates. Percentage of plasmid-containing cells (F) was calculated as F = number of colonies on selective plates/number of colonies on nonselective plates. We presumed that cultures that produced colonies on selective plates had originated from a single plasmid-containing cell. Plasmid loss was calculated as $1 - F^{1/N}$. A loss of 100% indicated a mini-culture that had originated from a single cell without a plasmid.

Measurement of total *de novo* RNA synthesis: Cells were grown overnight under specified conditions to an early exponential phase and diluted with prewarmed medium to OD_{600} = 0.2. [5,6-^{3}H]Uridine was added to 15 μCi/ml final concentration. Aliquots of 0.2 ml were removed after 20, 40, and 60 min and immediately added to 1 ml ice-cold stop solution

TABLE 1

Strains

Strain	Genotype/Phenotype	Reference
mcm5[a]	*mcm5-3 ura3-52 leu2-3,112 his3-11,15 MAT*α; *ts* at 37°C	Maine *et al.* (1984)
rpb1Δ104mcm5[a]	*mcm5-3 ura3-52 leu2-3,112 his3-11,15 rpb1Δ104::LEU2 trp1::HIS3 MAT*α; *ts* at 37°C	This study
MCM5[a]	*mcm5-3 ura3-52 leu2-3,112 his3-11,15 MCM5::LEU2 MAT*α	This study
rpb1Δ104MCM5[a]	*mcm5-3 ura3-52 leu2-3,112 his3-11,15 rpb1Δ104::LEU2 trp1::HIS3 MCM5::TRP1 MAT*α; *ts* at 37°C	This study
Z26	*ura3-52 leu2-3,112 his3-200 rpb1Δ187::HIS3 MAT***a**	Nonet *et al.* (1987)
Z551[b]	*ura3-52 leu2-3,112 his3-200 rpb1Δ187::HIS3, MAT***a**, [pRY2128 (*LEU2,CEN, RPB1*)]	Nonet *et al.* (1987)
pY1WT(10)[b]	*ura3-52 leu2-3,112 his3-200 rpb1Δ187::HIS3 MAT***a**, [pY1wt10 (*LEU2,CEN, rpb1(CTD)10)*]	West and Corden (1995)
pY1WT(12)[b]	*ura3-52 leu2-3,112 his3-200 rpb1Δ187::HIS3 MAT***a**, [pY1wt12 (*LEU2,CEN, rpb1(CTD)12)*]	West and Corden (1995)
DF5	*ura3-52 trp1-901 leu2-3,112 his3-200 gal4Δ MAT***a**	Clontech

[a] These strains are derivatives of 8534-8C (Maine *et al.* 1984).
[b] These strains are derivatives of Z26 (Nonet *et al.* 1987).

(15% trichloroacetic acid, 50 mm pyrophosphate) containing 0.2 ml unlabeled stationary-phase yeast culture. Cells were washed (five times for 10 min) in stop solution and once in EtOH. Radioactivity was determined by scintillation counting in a Beckman (Fullerton, CA) LS6500 counter.

Measurment of *de novo* poly(A)$^+$ RNA synthesis: Cells were grown and labeled for 1 hr as described for total RNA synthesis and then harvested in ice-cold water plus 0.5 ml of unlabeled stationary-phase yeast culture and washed four times with ice-cold water. RNA was isolated by a SV total RNA isolation system (Promega, Madison, WI) according to the instructions of the manufacturer. RNA yields were estimated by OD_{260}. Poly(A)$^+$ containing RNA was isolated by a poly(A) tract mRNA isolation kit (Promega). Radioactivity in the RNA samples was determined by scintillation counting in a Beckman LS6500 counter. *De novo* synthesis of RNA and poly(A)$^+$ RNA was expressed as counts per minute per microgram RNA.

Microarray analysis of gene expression: Cells were grown under specified conditions to OD_{600} = 0.2–0.5 and harvested on crushed ice. Total RNA was isolated by the lithium chloride method and cDNA was synthesized from 10 μg of total RNA by reverse transcribing with SuperScript II (GIBCO BRL, Gaithersburg, MD) in the presence of amino-allyl dUTP. *N*-hydroxy-succinimide Cy5 and Cy3 dyes (Amersham-Pharmacia) were coupled to the amine-modified cDNA according to the instructions of the manufacturer. Microarrays containing all 6200 open reading frames from the *S. cerevisiae* genome were purchased from the Microarray Centre at the Ontario Cancer Institute, Toronto. Hybridization was for 18 hr at 37°. The microarrays were scanned with the Axon GenePix 4000a microarray scanner and analyzed with the GeneSpring v4.0.1 software package (Silicon Genetics). Three different replica samples were analyzed. Differentially expressed genes were identified as twofold up- or downregulated.

Chromatin immunoprecipitation: This was performed according to the procedure described in Strahl-Bolsinger *et al.* (1997) with some modifications. Cells containing pARS1/2.2 kb/URA3 or pARS1/-B23/G24/2.2 kb/URA3 were grown in SC-Ura medium to O_{D600} = 1.5, crosslinked with 1% formaldehyde, and sonicated to an average DNA size of 100–1000 bp. Sheared chromatin was immunoprecipitated with monoclonal antibodies against pol II CTD (8WG16; Thompson *et al.* 1989) or with the corresponding amount of fetal bovine serum IgG as a control. The immunoprecipitated chromatin was eluted with 1% SDS, DNA was uncrosslinked and precipitated, and aliquots were subjected to 20–23 cycles of PCR in the presence of 2 μCi [γ-^{32}P]dCTP with the primers described below. Amplification of CEN4, 2.2 kb, and ARS1 DNA was performed in multiplex PCR reactions with three pairs of primers. The *URA3* fragment was amplified separately. PCR products were resolved on native polyacrylamide gels and exposed to X-ray films.

PCR primers: These were designed to specifically amplify the plasmid-borne, but not the endogenous ARS1, CEN4, and *URA3* elements. One of the primers annealed to ARS1, CEN4, and *URA3*, respectively, while the corresponding reverse primers annealed to the pUC119 backbone (see Figure 8A). Another pair of primers was designed to amplify a 400-bp fragment from the 2.2-kb insert positioned between the ARS1 and *URA3* elements. The amplified fragment is ∼1 kb away from both ARS1 and *URA3*. The CEN4 amplified fragment is 1.2 kb away from ARS1. The sequences of the used primers and the PCR conditions are available upon request.

RESULTS

Truncation of RNApol II CTD partially reverses *mcm5* phenotype: The biochemical interaction between the human pol II holoenzyme and MCM proteins raises the issue of a similar interaction in *S. cerevisiae*. We explored this possibility by disrupting the *RPB1* gene with an *rpb1Δ104* encoding 11 out of 26 CTD repeats (Nonet *et al.* 1987) in strains that harbored *mcm* mutations. The parental *mcm* strains, *mcm2-1*, *mcm3-3*, *mcm5-1*, and *mcm5-3*, were produced by ethyl methanesulfonate mutagenesis and have conditional *ts* phenotypes (Maine *et al.* 1984; Hennessy *et al.* 1991; Tye 1999b). Strains that encode *rpb1* with 10–12 CTD repeats can also display conditional *cs* and *ts* phenotypes (Nonet *et al.* 1987; West and Corden 1995). Initially we compared the *cs* and *ts* phenotypes of single *mcm* mutants *vs.* double *mcmrpb1Δ104* mutants. We did not see any obvious alteration of the phenotypes of *rpb1Δ104mcm2-1*, *rpb1Δ104mcm3-3*, and *rpb1Δ104mcm5-1* relative to the corresponding parental *mcm* strains. The *rpb1Δ104mcm5-3* mutant (from now on referred to as *rpb1Δ104mcm5*), grew significantly better at 37° than did the parental *mcm5* strain (Figure 1). We focused our studies on this strain.

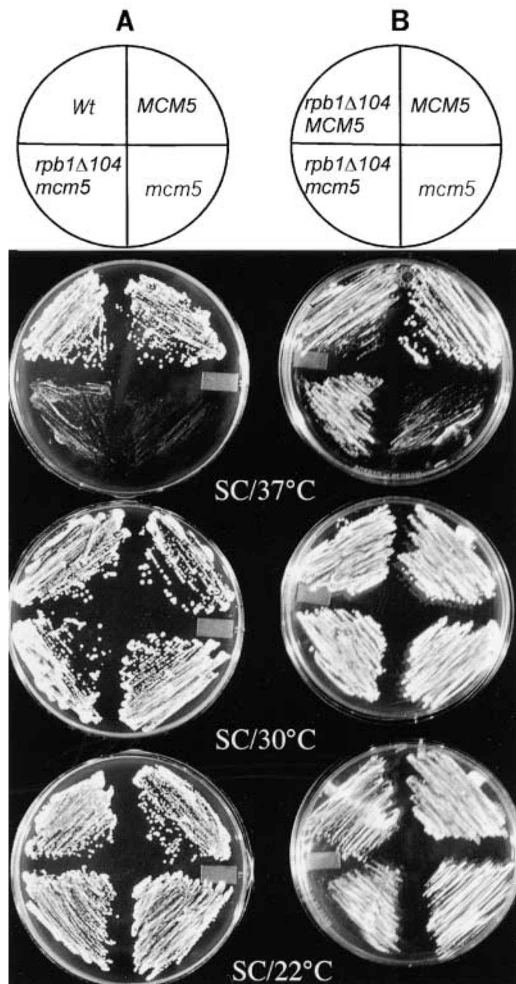


Figure 1.—Truncation of pol II CTD partially suppresses the *ts* phenotype of *mcm5*. *rpb1Δ104mcm5*, *mcm5*, *MCM5*, and *rpb1Δ104MCM5* and an unrelated wild-type strain were streaked on SC or SC-Leu and grown at the temperatures indicated below each plate. The positions of the strains are diagrammed above each column of plates. A and B represent two separate experiments.

The phenotype of *rpb1Δ104mcm5* could be specific to *mcm5* or, alternatively, could be a consequence of mutations introduced during the mutagenesis of the parental strain. We addressed this issue by complementation with *MCM5*. To avoid any effects from poor maintenance of plasmids, we inserted the *MCM5* gene in the genomes of *mcm5* and *rpb1Δ104mcm5* strains, respectively. The resulting isogenic strains were designated *MCM5* and *rpb1Δ104MCM5*. The growth of all strains was comparable at room temperature and at 30° (Figure 1). As expected, insertion of *MCM5* in the *mcm5* strain completely reversed its *ts* phenotype (Figure 1). Introduction of *MCM5* in the *rpb1Δ104mcm5* strain resulted in some growth advantage (Figure 1), yet *rpb1Δ104MCM5* did not grow as fast as *MCM5* at 37° (Figure 1B and data not shown). The temperature sensitivity of *rpb1Δ104MCM5* could be attributed to the *rpb1Δ104* mutation, which had shown a similar phenotype in an unrelated strain (Nonet *et al.* 1987). Neither of the mutant strains grew at 38.5° (data not shown). These initial results indicated that the observed phenotypes of *mcm5* and *rpb1Δ104mcm5* strains were specific to the *mcm5* and *rpb1Δ104* mutations.

Minichromosome stability is enhanced in *rpb1Δ104 mcm5*: In a separate set of experiments we attempted to complement each of the mutations in the double *rpb1Δ104/mcm5* strain by expressing wild-type (wt) *RPB1* or *MCM5* from CEN4/ARS1/*URA3* minichromosomes. As expected, expression of *MCM5* significantly increased the growth rate of both *mcm5* (not shown) and *rpb1Δ104mcm5* (Figure 2B) strains in SC-Ura medium at 30°. Surprisingly, *rpb1Δ104mcm5* cells expressing RPB1 from a plasmid-borne gene (pFL38RPB1) grew slightly slower relative to cells with a control plasmid (Figure 2B). One possibility for the observed kinetics could be that expression of *RPB1* may interfere with the maintenance of pFL38RPB1 in *rpb1Δ104mcm5*, resulting in slower growth. We tested this possibility by analyzing cell growth of the *rpb1Δ104mcm5* and *mcm5* strains containing the same pARS1/wtA (CEN4/ARS1/URA3) plasmid. Figure 2C shows that the *mcm5* strain grew significantly slower than *rpb1Δ104mcm5* in selective SC-Ura medium. These results are consistent with the idea that truncation of the CTD in *RPB1* partially reverses the effect of *mcm5-3* on minichromosome stability (Figure 2C). Hence, we specifically analyzed the loss rate of pARS1/wtA in *mcm5*, *rpb1Δ104mcm5*, *MCM5*, and *rpb1Δ104MCM5* strains grown in nonselective medium at both normal and restrictive temperatures.

Minichromosome stability is estimated by measuring the percentage of minichromosome-containing cells after a period of growth in nonselective medium (Tye 1999b). The major advantage of this assay is that it is direct and no assumption is made about other phenotypes that may or may not be associated with some deficiency in DNA replication (Tye 1999b). At 30° the *mcm5* strain showed a loss rate of 22.3 ± 2.2%/generation, whereas the *MCM5* strain had a loss rate of 5.2 ± 0.8% (Figure 3). These data are in good agreement with previously reported studies on pARS1/wtA maintenance in other strains (Marahrens and Stillman 1992; Li *et al.* 1998; Hu *et al.* 1999; Stagljar *et al.* 1999) or maintenance of similar minichromosomes in the *mcm5* strain (Maine *et al.* 1984; Chen *et al.* 1992). The loss rate in *rpb1Δ104mcm5* was 16.1 ± 2.6% (Figure 3), which was higher than that in *MCM5*, but lower than that in the single *mcm5* mutant. The loss rate in *rpb1Δ104MCM5* was 9.67 ± 1.5% (Figure 3). Similar relative levels of minichromosome loss per generation were observed at 37°. The *MCM5* strain continued to lose plasmids at ∼5%/generation (Figure 3). The loss rate in the *rpb1Δ104 mcm5*, *mcm5*, and *rpb1Δ104MCM5* mutants increased to 25.3 ± 3.4%, 36.7 ± 0.4%, and 16.2 ± 1.38%, respectively (Figure 3).

We considered the possibility of recombination between the direct repeats, which were produced from

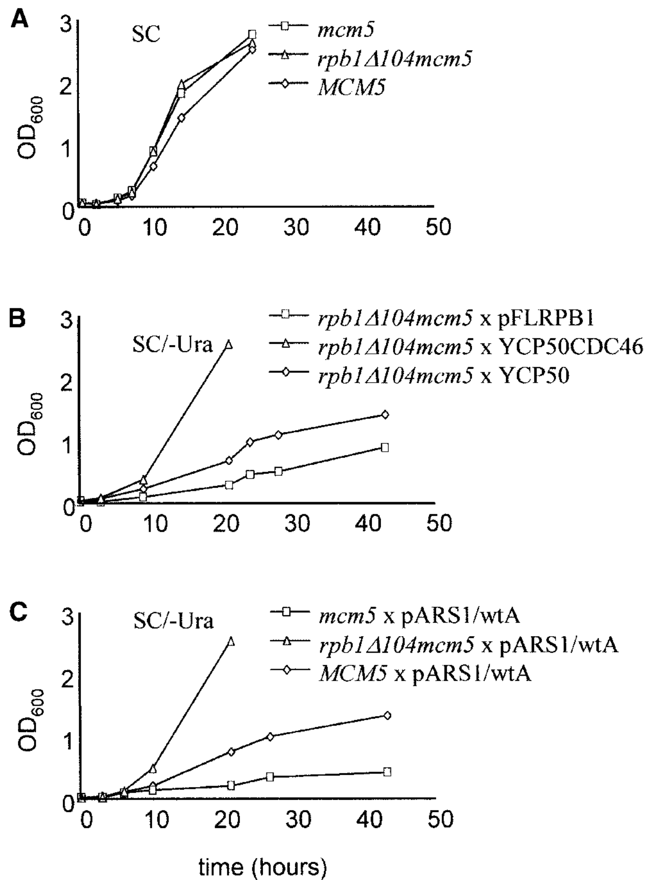


Figure 2.—Analysis of cell growth of *mcm5*, *rpb1Δ104mcm5*, and *MCM5*. Cells were grown at 30° in SC or SC-Ura liquid cultures as indicated. Time course measurements of OD_{600} are plotted. (A) Growth in SC medium. (B) Complementation of *rpb1Δ104mcm5* by expressing *RPB1* from an ARS1/CEN4 minichromosome retards its growth in selective medium. *rpb1Δ104mcm5* cells were transformed with YCP50 (ARS1/CEN4/*URA3*), YCP50CDC46, and pFL38RPB1 (ARS1/CEN4/*URA3*) and grown in SC-Ura medium. (C) Growth rate of *mcm5*, *rpb1Δ104mcm5*, and *MCM5* cells transformed with pARS1/wtA in SC-Ura medium.

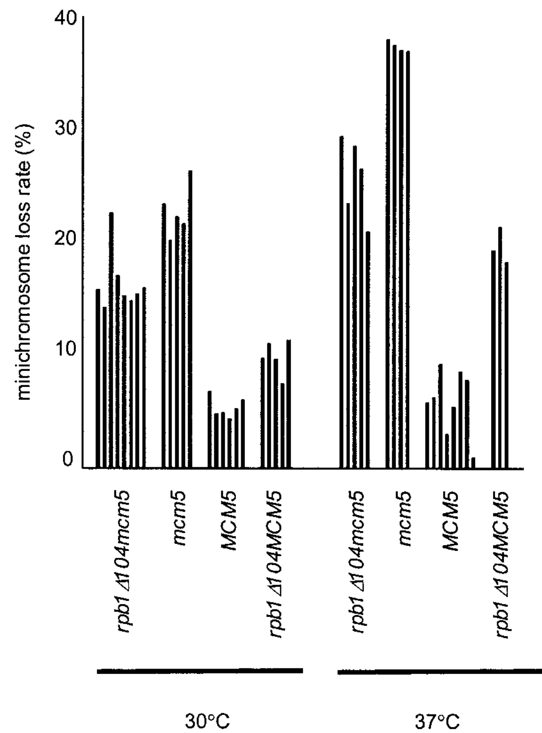


Figure 3.—Minichromosome stability in *rpb1Δ104mcm5*, *mcm5*, *MCM5*, and *rpb1Δ104MCM5*. Cells were transformed with pARS1/wtA. Single colonies were grown in SC-Ura medium as stock cultures, diluted in SC medium at 5 cells/ml, dispensed in 96-well tissue culture plates, and grown in SC at 30° or 37°. Minichromosome loss per generation (%) was calculated as $X = 1 - F^{1/N}$ (*F*, percentage of minichromosome-containing cells; *N*, number of generations) as described in materials and methods. Each bar represents the calculation of minichromosome stability from an individual mini-culture in the strain indicated below the graph.

the integration of *rpb1Δ104* or *MCM5* in the genome of the recipient strains. If this was the case, the LEU2 and TRP1 marker genes would be lost from the *rpb1Δ104 mcm5* and *MCM5* or *rpb1Δ104MCM5* strains, respectively. We controlled against such recombination events by selecting for the Leu+ and Trp+ phenotypes before each experiment and confirming it after growth in nonselective SC medium. In five independent experiments we consistently observed lower levels of minichromosome loss in the *rpb1Δ104mcm5* strain relative to the single *mcm5* mutant (data not shown). We also consistently observed increased minichromosome loss in the *rpb1Δ104MCM5* relative to *MCM5* (data not shown).

Analysis of transcription: The suppression of the *ts* phenotype and of minichromosome loss in *rpb1Δ104 mcm5* could be a consequence of aberrant transcription resulting from the truncation of pol II CTD. Initially we tested this possibility by analyzing the rate of *de novo* total RNA and mRNA synthesis and steady-state mRNA levels at different temperatures. The rate of total RNA synthesis was assessed by incorporation of [5,6-^{3}H]uridine in exponentially growing cells at 30° and 37°. Cells were harvested at the 20th, 40th, and 60th minute after addition of the label and incorporation was measured by scintillation counting. At 30° the *rpb1Δ104mcm5* and *mcm5* incorporated [5,6-^{3}H]uridine at comparable rates (Figure 4A). *rpb1Δ104MCM5* incorporated the label at higher levels relative to *rpb1Δ104mcm5* and *mcm5*, but did so more slowly than *MCM5* (Figure 4A). A similar rate of total RNA synthesis was observed at 37° with the exception of the significant difference between *MCM5* and the mutant strains (Figure 4A, bottom). Again, *rpb1Δ104mcm5* and *mcm5* incorporated the label at similar levels, while the *rpb1Δ104MCM5* strain incorporated at a higher rate. Rates of mRNA synthesis were assessed by exposing cells to [5,6-^{3}H]uridine for 1 hr and isolating mRNA on oligo(dT) magnetic beads. *De novo* total RNA and mRNA synthesis were expressed as counts per minute per microgram RNA. As shown in Figure 4, B and C, no sub-

stantial difference in the levels of total RNA and mRNA synthesis between *rpb1Δ104mcm5* and *mcm5* was observed at both temperatures. *rpb1Δ104MCM5* synthesized RNA at slightly higher levels than did *rpb1Δ104mcm5* and *mcm5* probably because of its slightly higher growth rate (not shown). In summary, our results did not point out any significant variation in the ratio of mRNA/total RNA in the mutant strains. Importantly, they did not reveal any considerable differences in total or mRNA transcription between *rpb1Δ104mcm5* and *mcm5* that might explain the difference in growth rate and plasmid maintenance.

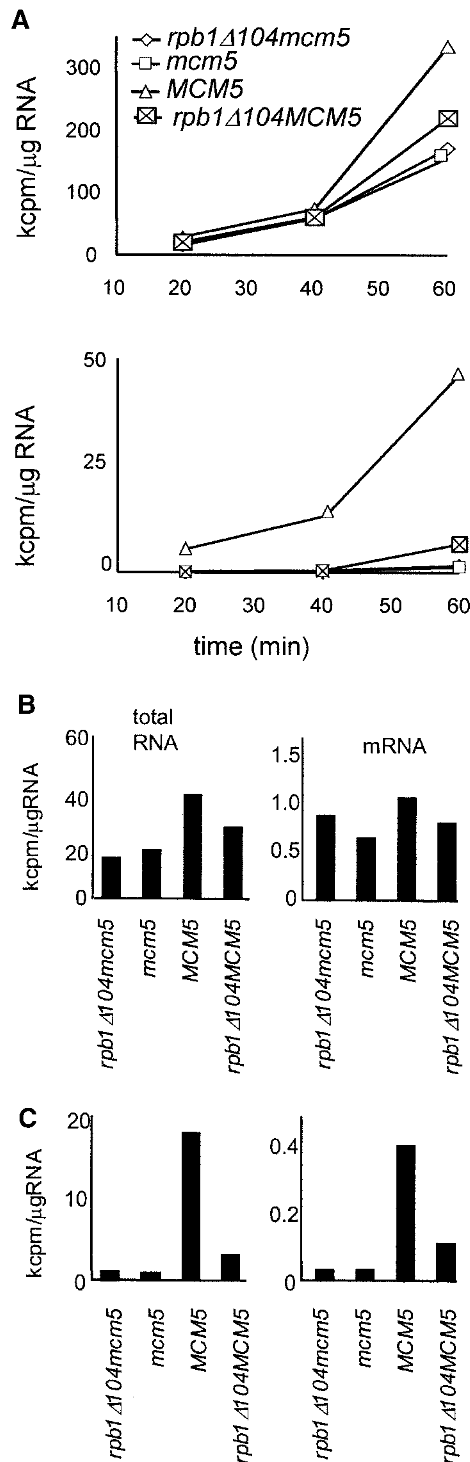


Figure 4.—Rate of *de novo* total RNA and mRNA synthesis at 30° and 37°. (A) Rate of *de novo* RNA synthesis. Cultures were grown in SC medium at 30° (top) or 37° (bottom) to $OD_{600} = 0.2$. [5,6-^{3}H]Uridine was added at 15 μCi/ml. Aliquots were collected at the 20th, 40th, and 60th minute. Incorporation of the label was measured as described in materials and methods. (B) Rate of *de novo* mRNA synthesis at 30°. (C) Rate of *de novo* mRNA synthesis at 37°. Cultures were grown in SC medium at 30° (B) or 37° (C) to $OD_{600} = 0.2$. [5,6-^{3}H]Uridine was added at 15 μCi/ml and cells were grown for 1 hr. Isolation of total RNA and mRNA is described in materials and methods. Incorporation of the label is plotted as counts per minute per micrograms RNA.

Analysis of specific gene expression in *rpb1Δ104mcm5* and *mcm5*: The differences in cell growth and minichromosome maintenance between *rpb1Δ104mcm5* and *mcm5* could result from changes in the expression of specific genes, which cannot be detected by global analysis of mRNA. We therefore performed analysis of gene expression using microarrays. Expression profiles of *rpb1Δ104 mcm5* and *mcm5* were compared at both 30° and 37°. Control experiments with *mcm5* at 30° and 37° and *rpb1Δ 104mcm5* at 30° and 37° were also performed. We analyzed three independent replicas for each couple of samples. The number of differentially expressed genes in *mcm5 vs. rpb1Δ104mcm5* was 89 at 30° and 173 at 37° (see supplementary data at http://www.uoguelph.ca/mbgwww/faculty/yankulov/appendix_ky082001/appendix_ky082001.html). Most of these genes encode ribosomal proteins and proteins involved in the regulation of metabolic processes, RNA metabolism, and translation and are referred to as environmental stress response (ESR) genes (Gasch *et al.* 2000). Three of the genes that were not found in the ESR cluster (Gasch *et al.* 2000) and were upregulated in *rpb1Δ 104mcm5* only at 37° (see supplementary data at http://www.uoguelph.ca/mbgwww/faculty/yankulov/appendix_ky082001/appendix_ky082001.html) had been previously implicated in regulation of DNA replication and cell growth. *POL32* is a subunit of DNA polymerase δ. *PSP1* and *YAC1* are high-copy-number suppressors of cell growth (Garrett *et al.* 1991; Akada *et al.* 1997; Formosa and Nittis 1998). Another group of genes (*ZDS1*, *CYC8*, *TUP1*, *POP2*, *SPT5*, *SPT8*, *SNF5*, and *GAL11*), which positively or negatively regulate pol II transcription, were upregulated in *rpb1Δ104mcm5* at both temperatures. Analysis of gene expression in *rpb1Δ104mcm5* at 30° *vs.* 37° and in *mcm5* at 30° *vs.* 37° showed a significantly broader range of differentially expressed genes in both strains (not shown), which probably reflects the combination of the effects of temperature change, slower growth, and mutations in *mcm5* and *rpb1*.

In conclusion, the comparison of gene expression profiles of *rpb1Δ104mcm5* relative to *mcm5* did not show

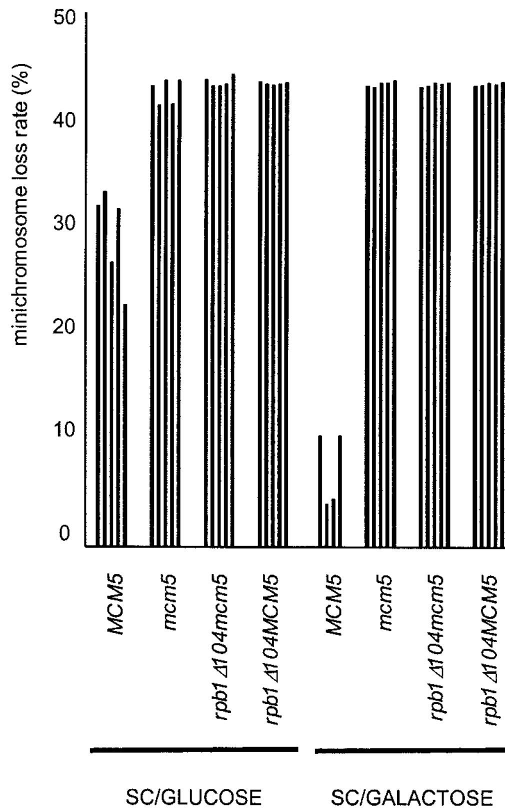


Figure 5.—Stability of pARS1/-B23/G24 in *rpb1Δ104mcm5*, *mcm5*, *MCM5*, and *rpb1Δ104MCM5*. Cells were transformed with pARS1/-B23/G24 and single colonies were grown in SC/Galactose. Stock cultures were diluted in SC/Glucose or SC/Galactose medium at 5 cells/ml and dispensed in 96-well tissue culture plates. Minichromosome loss per generation (%) was calculated as $X = 1 - F^{1/N}$ (*F*, percentage of minichromosome containing cells; *N*, number of generations) as described in materials and methods. Each bar represents the calculation of minichromosome stability from an individual mini-culture in the strain indicated below the graph.

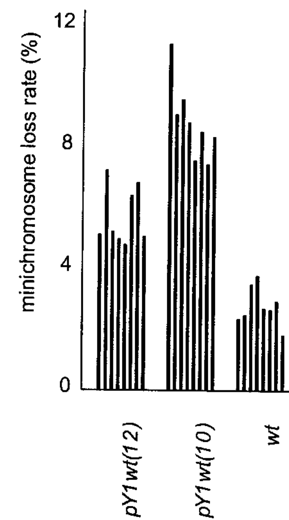


Figure 6.—Stability of pARS/wtA in strains containing *RPB1* with 10 and 12 CTD repeats. *pYwt(10)*, *pYwt(12)*, and *Z551(wt)* strains were transformed with pARS1/wtA and selected on SC-Ura-Leu. Single colonies were grown in SC-Ura-Leu and diluted in SC-Leu. Minichromosome loss per generation (%) was calculated as $X = 1 - F^{1/N}$ (*F*, percentage of minichromosome containing cells; *N*, number of generations) as described in materials and methods. Each bar represents the calculation of minichromosome stability from an individual mini-culture in the strain indicated below the graph.

a gene or a group of genes whose expression pattern could explain the increased stability of minichromosomes in *rpb1Δ104mcm5*.

Activation of DNA replication by GAL4 is abolished in *mcm5*, *rpb1Δ104mcm5*, and *rpb1Δ104MCM5*: We performed three additional experiments, which addressed the effects of pol II CTD on DNA replication. Earlier reports demonstrated a direct role of an array of transcriptional activators in stimulating origins of DNA replication (Marahrens and Stillman 1992; Li *et al.* 1998; Hu *et al.* 1999; Li 1999). In most cases the effects on replication were measured by the stability of pARS1/-B23/G24 in which the *ABF*1-binding site in ARS1 is transformed to a GAL4-binding site (Li *et al.* 1998). pARS1/-B23/G24 is very poorly maintained; however, its loss is dramatically decreased if cells are grown in galactose, presumably because GAL4 replaces the function of *ABF*1 (Li *et al.* 1998; Stagljar *et al.* 1999). First we tested whether this effect of GAL4 is influenced by the truncation of pol II CTD by measuring the loss rate of pARS1/-B23/G24 in *rpb1Δ104mcm5*, *mcm5*, *rpb1Δ104 MCM5*, and *MCM5* strains in the presence or absence of galactose. Because the mutant strains did not grow in galactose at 37° the experiment was performed at 30° only. In SC/GLU the mutant strains were losing the minichromosome at a very high rate of ∼40–42% (Figure 5). *MCM5* was losing the pARS1/-B23/G24 at 29.2 ± 4.4% (Figure 5). When the strains were grown in SC/GAL, pARS1/-B23/G24 gained significant stability only in *MCM5* (Figure 5). We observed similar results in three independent experiments. We concluded that truncation of pol II CTD or a mutation in *MCM5* completely abolished the positive effect of GAL4 on the activity of a GAL4-responsive synthetic origin of DNA replication.

Truncation of pol II CTD impairs minichromosome stability: In Figure 3 we show that the loss rate of pARS1/wtA was higher in *rpb1Δ104MCM5* than in the corresponding *MCM5* strain (Figure 3). We furthered these observations by testing whether truncation of the CTD would have similar effects in the unrelated Z26 strain (Nonet *et al.* 1987), which carries a disrupted genomic *RPB1*. The Z26 strain is complemented by *RPB1*, which contains 10 [strain pY1WT(10)], 12 [strain pY1WT(12)], or 26 (wt, Z551) CTD repeats, respectively (Nonet *et al.* 1987; West and Corden 1995). The strains containing truncated CTD did not exhibit any *ts* or *cs* phenotype (West and Corden 1995). *pY1WT(10)*, *pY1WT(12)*, and Z551 were transformed with pARS1/wtA and minichromosome stability was assessed as described earlier. Z551

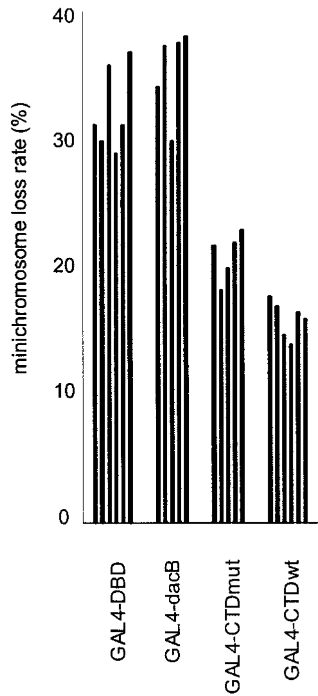


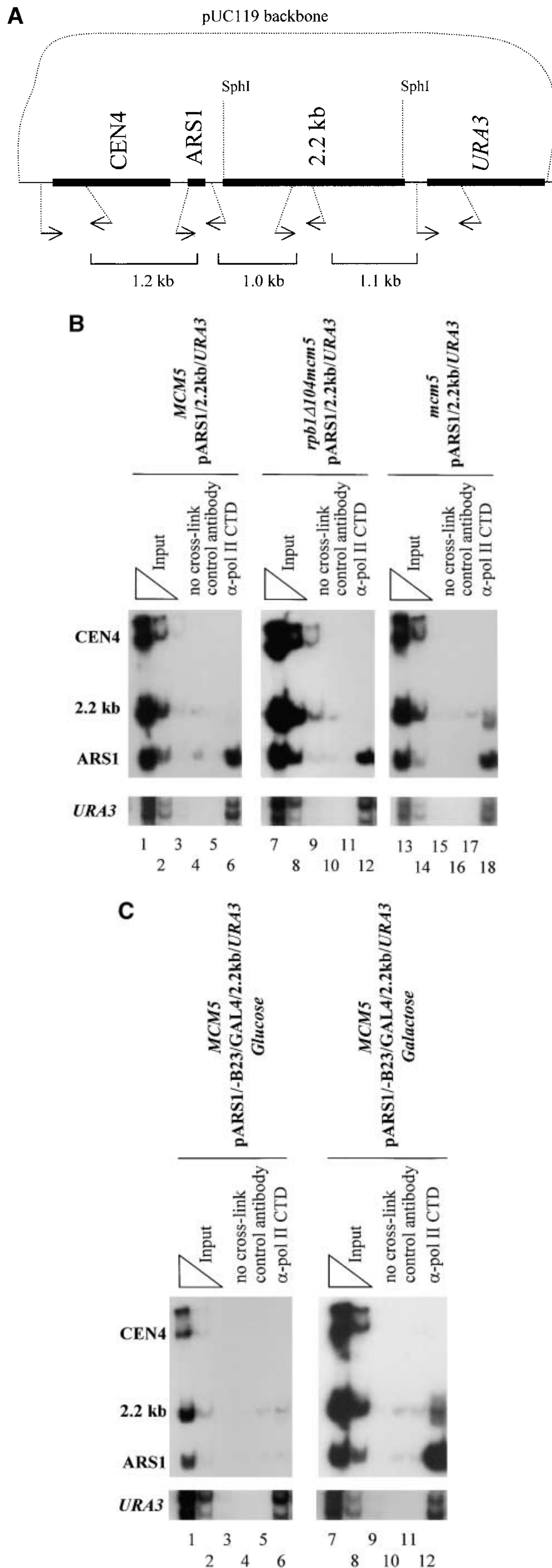
Figure 7.—Recombinant CTD stimulates stability of pARS1/-B23/G24. *DF5* cells were transformed with pGBKT7 plasmids encoding different GAL4-fusion proteins and pARS1/-B23/G24 and selected on SC-Ura-Trp. Single colonies were grown in SC-Ura-Trp and diluted in SC-Trp. Minichromosome loss per generation (%) was calculated as $X = 1 - F^{1/N}$ (*F*, percentage of minichromosome containing cells; *N*, number of generations) as described in materials and methods. Each bar represents the calculation of minichromosome stability from an individual mini-culture in the strain indicated below the graph. GAL4-DBD, GAL4 DNA-binding domain; GAL4-dacB, GAL4 DNA-binding domain fused to the *E. coli* DAC-B protein; GAL4-CTDwt, GAL4 DNA-binding domain fused to *wt* pol II CTD; GAL4-CTDmut, GAL4 DNA-binding domain fused to 15 mutant (S5 → A) heptad repeats.

lost pARS1/wtA at 2.75 ± 0.59%/generation (Figure 6). Truncation of pol II CTD to 12 or 10 repeats [*pY1WT(12)* and *pY1WT(10)*] increased the loss rate to 5.67 ± 0.90% and 8.82 ± 1.18%, respectively (Figure 6). Thus, truncation of pol II CTD resulted in a small but consistent decrease in minichromosome stability in two unrelated strains (Figure 3 and Figure 6). Furthermore, stability of the test minichromosme was lower in the strain with the shorter CTD (Figure 6).

The observed deficiency in minichromosome stability in *pY1WT(12)* and *pY1WT(10)* could be a consequence of the aberrant transcription of genes, which are involved in the regulation of DNA replication. We tested this possibility by comparing the gene expression profiles of *pY1WT(10)* and Z551. There were significant differences in the expression of numerous genes from the ESR cluster (Gasch *et al.* 2000), but no alteration in the expression of genes involved in DNA replication was observed (see supplementary data at http://www.uoguelph.ca/mbgwww/faculty/yankulov/appendix_ky082001/appendix_ky082001.html). The possibility of minor changes in the expression of such genes still exists; however, we obtained no evidence in support of this idea.

Artificial recruitment of CTD stimulates origins of replication: If the CTD can influence DNA replication independently of its role in pol II transcription, then recruitment of CTD to origins of replication may have an effect on plasmid stability. We tested this possibility by measuring the loss rate of the pARS1/-B23/G24 in a *gal4Δ* strain (DF5), in which we expressed the DNA-binding domain of GAL4 (GAL1-147) fused to the wild-type mouse CTD (GAL4-CTDwt), to 15 synthetic mutant CTD repeats (GAL4-CTDmut), or to the *E. coli dacB* gene product (GAL4-dacB). These recombinant proteins were expressed from pGBKT7 (TRP/2μ). DF5 cells were cotransformed with the test minichromosome and the expression plasmid and stability of pARS1/-B23/G24 were measured. Loss rate of the test minichromosome in the presence of GAL4(1-147) and GAL4-dacB was 32–35% (Figure 7). Upon expression of GAL4-CTDwt and GAL4-CTDmut the loss rate decreased by ~12 and 16% relative to the controls. It is noteworthy that both the mutant and wild-type CTD exerted a positive effect on the activity of the synthetic origin of pARS1/-B23/G24, whereas only GAL4-CTDwt was reported to stimulate transcription from the GAL4-responsive promoter *in vivo* (Yuryev *et al.* 1996).

RNA polymerase II is recruited to ARS1: A key question in our study was whether pol II itself is recruited to origins of DNA replication. Previous studies indicated that artificial tethering of pol II or pol III complexes can substitute for transcriptional activators (Stagljar *et al.* 1999; Bodmer-Glavas *et al.* 2001), but direct association of pol II to unmodified origins has not been shown. We addressed this question by performing chromatin immunoprecipitation experiments on minichromosomes with antibodies against the CTD of pol II. Cells containing minichromosomes were grown in selective medium, crosslinked with formaldehyde, and sonicated to average size of DNA of ~100–1000 bp as in Strahl-Bolsinger *et al.* (1997). Sheared chromatin was immunoprecipitated with anti-pol II CTD antibodies and DNA was un-crosslinked and analyzed by 20–23 cycles of PCR. Under these conditions the PCR of the immunoprecipitated DNA was within the linear range as judged by the at least 200 times higher signal obtained in parallel PCR with 10 ng of pARS1wtA/2.2 kb/URA3 (not shown). An important part of our experimental design was the elimination of noise signals coming from the pol II transcribed *URA3* gene. We cloned a 2.2-kb DNA fragment in the *Sph*I site between ARS1 and *URA3* on the pARS1/wt and pARS1/-B23/GAL4, respectively. The subsequent PCR analysis was performed with primers that specifically amplified minichromosome-borne DNA elements, which were ~1000 bp away from each other (Figure 8A). In these experiments signals from the amplification of the URA3 and ARS1 elements and the absence (or significant decrease) of signals from the amplification of the



CEN4 and the 2.2-kb insert elements means that pol II is independently crosslinked to URA3 and ARS1.

Initially we performed experiments with pARS1wtA/2.2 kb/URA3 in *MCM5*, *rpb1Δ104mcm5*, and *mcm5* cells (Figure 8B). In all immunoprecipitates we observed very low or no signals from the amplification of the CEN4 and the 2.2-kb fragment as compared to the strong input signals (Figure 8B, lanes 6, 12, and 18). Similar low signals from the amplification of the URA3 and ARS1 elements were detected in the control immunoprecipitates without crosslinking (Figure 8B, lanes 4, 10, and 16) and with control antibody (Figure 8B, lanes 5, 11, and 17). In the anti-pol II CTD precipitates there was a clear increase in the signals resulting from amplification of the *URA3* and ARS1 fragments (Figure 8B, lanes 6, 12, and 18). These results indicate that pol II was independently crosslinked to ARS1 and *URA3*. We did not attempt to measure the amounts of immunoprecipitated DNA relative to the input signal between the three strains because the truncation of the CTD in *rpb1Δ 104mcm5* could contribute to altered efficiency of immu-

Figure 8.—RNA polymerase II is crosslinked to ARS1 in *MCM5*, *rpb1Δ104mcm5*, and *mcm5*. (A) A diagram of pARS1-wtA/2.2 kb/URA3. The locations of the CEN4, ARS1, the 2.2-kb insert, and the *URA3* gene are shown in scale. The positions of the pairs of PCR primers and the distance between the amplified fragments are shown below the diagram. In pARS1/-B23/G24/2.2 kb/URA3 the ARS1 element is replaced by ARS1/-B23/G24. (B) PCR amplification of minichromosome-borne CEN4, ARS1, 2.2-kb, and *URA3* fragments immunoprecipitated with anti-pol II CTD. Cells were transformed with pARS1wtA/2.2 kb/URA3 and grown in SC-Ura. The strain used for preparation of crosslinked chromatin is shown above each panel of lanes. In lanes 4, 10, and 16 the samples were immunoprecipitated with anti-pol II CTD antibody without prior crosslinking with formaldehyde. In lanes 5, 11, and 17 the samples were crosslinked and immunoprecipitated with control antibody. In lanes 6, 12, and 18 samples were crosslinked and immunoprecipitated with anti-pol II CTD. The following amounts of DNA were amplified and resolved on polyacrylamide gels: 1% of the input in lanes 1, 7, and 13; 0.02% of the input in lanes 2, 8, and 14; 0.02% of the input in lanes 3, 9, and 15; 10% of the immunoprecipitate in lanes 4, 5, 6, 10, 11, 12, 16, 17, and 18. Amplification of CEN4, 2.2-kb, and ARS1 DNA was performed in multiplex PCR reactions with three pairs of primers. The *URA3* fragment was amplified separately. The position of each amplified fragment is indicated on the left. (C) PCR amplification of minichromosome-borne CEN4, ARS1/-B23/GAL4, 2.2-kb, and *URA3* fragments immunoprecipitated with anti-pol II CTD. *MCM5* cells transformed with pARS1/-B23/G24/2.2 kb/URA3 and grown in SC-Ura/glucose (lanes 1–6) or SC-Ura/galactose (lanes 7–12). In lanes 4 and 10 the samples were immunoprecipitated with anti-pol II CTD antibody without prior crosslinking. In lanes 5 and 11 the samples were crosslinked and immunoprecipitated with control antibody. In lanes 6 and 12 the samples were crosslinked and immunoprecipitated with anti-pol II CTD. The following amounts of DNA were amplified and resolved on polyacrylamide gels: 1% of the input in lanes 1 and 7; 0.02% of the input in lanes 2 and 8; 0.02% of the input in lanes 3 and 9; 10% of the immunoprecipitate in lanes 4, 5, 6, 10, 11, and 12. Amplification of fragments and description are as in Figure 8B.

noprecipitation with the anti-CTD antibody. In addition, the proportion of minichromosomes relative to genomic DNA between the three strains is different (see Figure 3), which could further complicate the interpretation of data.

We performed similar experiments with pARS1/-B23/G24/2.2 kb/URA3 in *MCM5* cells grown on glucose and galactose, respectively (Figure 8C). It was previously shown that the GAL4-binding site, which replaces the B3 element in wild-type ARS1, can be activated when cells are grown on galactose (Marahrens and Stillman 1992; Li *et al.* 1998). In Figure 8C we show virtually no crosslinking of pol II to pARS1/-B23/G24 when cells are grown in glucose (Figure 8C, lane 6). In cells grown in galactose we observed signals from crosslinking of pol II to pARS1/-B23/G24, which were significantly higher than the signals from the 2.2-kb linker fragment (Figure 8C, lane 12). This result indicates that the crosslinking of pol II to ARS1 is mediated by association of a *trans*-activator to the B1 element.

DISCUSSION

RNA polymerase II is involved in regulation of origins of DNA replication: Previous studies have indicated that in *S. cerevisiae* transcriptional activators regulate origins of DNA replication (Marahrens and Stillman 1992; Li *et al.* 1998; Hu *et al.* 1999). Furthermore, artificial recruitment of pol II holoenzyme/pol II transcription factors and also pol III transcription factors can substitute for the function of transcriptional activators (Stagljar *et al.* 1999; Bodmer-Glavas *et al.* 2001). Whereas the recruitment of pol II complexes can be linked to their ability to bind *trans*-activators, the way by which pol III complexes stimulate replication is not clear. In all these cases the likely cause of stimulation is chromatin remodeling (Hu *et al.* 1999; Bodmer-Glavas *et al.* 2001); however, the mechanisms by which remodeling factors are recruited under normal conditions are not known.

The present study provides a significant advancement toward understanding these mechanisms. First, we show that RNA polymerase II is recruited to ARS1 (Figure 8). Second, we show a genetic interaction between a component of the prereplicative complex, *MCM5*, and the CTD of pol II (Figures 1–3). Third, we correlate the phenotypes of *rpb*Δ*104mcm5* and *mcm5* to the stability of an ARS1/CEN4 minichromosome and to the response of an artificial origin of replication (ARS1/-B23/G24) to *trans*-activators (Figure 5). Taken together, these experiments indicate that RNA polymerase II could be directly involved in regulating origins of DNA replication. Truncation of the pol II CTD improves chromosome stability in the *mcm5* strain (Figures 2 and 3), but decreases chromosome stability if the CTD is truncated in strains with no mutations in *MCM5* [*rpb1*Δ*104MCM5*, *pYwt(10)*, *pYwt(12)*; Figures 3 and 6]. Thus, truncation of the pol II CTD can have a positive or a negative effect on minichromosome stability depending on the genetic context of the strain. While the actual mechanism by which pol II exerts these effects on replication origins is still enigmatic, one possibility is that the correct recruitment and arrangement of the chromatin remodeling factors is mediated at least in part by pol II.

Previous reports have indicated that some CTD deletions, which leave 8–14 heptapeptad repeats (Nonet *et al.* 1987; West and Corden 1995) in *rpb1*, produce conditional *ts*, *cs*, or other growth phenotypes presumably because pol II is not transcribing correctly *in vivo*. Our results suggest that some of these phenotypes could be caused in part by concomitant effects on DNA replication. The subtle decrease in minichromosome stability in strains with 10–12 CTD heptad repeats [*rpb1*Δ *104MCM5*, *pYwt(10)* and *pYwt(12)*; Figures 3 and 6] could explain why these effects had not been noticed in screens for *mcm* mutants (Tye 1999b) and in analyses of strains with CTD truncation where minichromosome stability has not been exclusively tested.

Does the CTD truncation directly affect DNA replication? A central issue in this study is whether the partial deletions of CTD directly affected DNA replication or if the observed effects were a consequence of aberrant pol II transcription. It is important that the minichromosome assay directly measures efficiency of DNA replication and is independent of other phenotypes that may be associated with deficiencies in DNA replication or in other processes (Tye 1999b). Therefore, if truncation of the CTD affects minichromosome stability indirectly, it should be through altered expression of genes, which are directly involved in DNA replication. We therefore conducted microarray analyses to compare the expression profiles of *mcm5* and *rpbD104mcm5* and of *Z551* and *pY1WT(10)*, respectively. We did not see any altered gene expression that could explain the differences in minichromosome stability between *Z551* and *pY1WT(10)* (see supplementary data at http://www.uoguelph.ca/mbgwww/faculty/yankulov/appendix_ky082001/appendix_ky082001.html). A more complex situation was observed between *mcm5* and *rpb*Δ*104mcm5* (see supplementary data at http://www.uoguelph.ca/mbgwww/faculty/yankulov/appendix_ky082001/appendix_ky082001.html). Several genes (*ZDS1*, *CYC8*, *TUP1*, *POP2*, *SPT5*, *SPT8*, *SNF5*, and *GAL11*), which function in repression/activation of transcription and in chromatin remodeling (http://www.proteome.com/databases/YPD/YPDsearch-quick.html and the references therein), are upregulated in *rpb1*Δ*104mcm5 vs. mcm5* at both 30° and 37°. To our knowledge these genes have never been implicated in direct regulation of DNA replication. *SNF5* encodes a component of the SWI/SNF global transcription activator complex. Inactivation of SWI/SNF specifically cripples the maintenance of minichromosomes containing ARS121, but not the maintenance of ARS1, ARS309, or ARS307 minichromosomes (Flanagan and

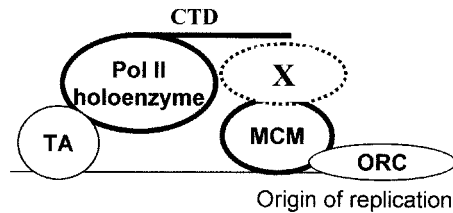


Figure 9.—A model for the possible interaction between *MCM5* and pol II CTD. TA, *trans*-activator; ORC, origin recognition complex; CTD, carboxyterminal domain of pol II.

Peterson 1999). The overexpression of another gene, *ZDS1*, can enhance the stability of linear, but not circular minichromosomes (Roy and Runge 1999). Because we use circular ARS1 containing plasmids (pARS1/wtA and pARS1/-B23/G24) upregulation of *SNF5* and *ZDS1* is unlikely to directly influence their maintenance. Three other genes that function in cell growth and DNA replication were expressed above the twofold-increase threshold in *rpb1Δ104mcm5 vs. mcm5* at 37° (see supplementary data at http://www.uoguelph.ca/mbgwww/faculty/yankulov/appendix_ky082001/appendix_ky082001.html). *POL32* encodes a subunit of DNA polymerase δ. It is present at higher concentrations than the catalytic subunit POL3 and its overproduction *in vivo* does not result in an increase of DNA polymerase δ activity (Burgers and Gerik 1998). *PSP1* is a suppressor of a *cdc17* mutation (Formosa and Nittis 1998) and its overproduction leads to growth inhibition (Akada *et al.* 1997). *YAK1* is a protein kinase that might work in controlling exit from G_1 to G_0 (Garrett *et al.* 1991). Its overexpression also inhibits cell growth (Garrett *et al.* 1991). Our current understanding of the function of these genes argues that their increased production cannot enhance cell growth and DNA replication in *rpb1Δ104mcm5*. While the possibility that changes in gene expression contribute to the observed characteristics of *rpb1Δ104mcm5* is still applicable, we have not obtained any evidence pointing in this direction. In addition, subtle limitations in the production of some replication factor(s) that might be caused by truncation of the CTD cannot explain why we see positive or negative effects in different genetic contexts (Figures 3 and 6). This reasoning leads us to the hypothesis that the truncation of pol II CTD could affect DNA replication independently of transcription.

Mechanism of CTD effects on DNA replication: A simple and straightforward explanation of our results is that pol II is recruited to origins of replication where its CTD participates in the formation of prereplicative complexes (Figure 9). This idea is in tune with observations in several previous studies. For example, artificial tethering of pol II holoenzyme (in which the CTD plays a central role; Kim *et al.* 1994; Hengartner *et al.* 1995; Myers *et al.* 1997) to ARS1 significantly stimulates origin function (Stagljar *et al.* 1999; Bodmer-Glavas *et al.* 2001). In addition, a tight correlation between the potency of *trans*-activators to enhance pol II transcription and DNA replication has been well documented (Marahrens and Stillman 1992; Li *et al.* 1998; Hu *et al.* 1999; Li 1999). Most of the *trans*-activators used in these studies directly interact with pol II holoenzyme in the absence of promoter DNA (Gold *et al.* 1996; Anderson *et al.* 1998; Neish *et al.* 1998; Yankulov *et al.* 1999) and stimulate transcription presumably by recruiting pol II holoenzyme to promoters. It is conceivable that the *trans*-activators can establish the same contacts at origins of DNA replication. If this is the case, truncation of CTD, which destabilizes pol II holoenzyme, will prevent the effect of *trans*-activators on DNA replication, as is the case in the experiment shown in Figure 5, and will impair plasmid maintenance mediated by Abf1, as shown in Figures 3 and 6. In addition, recruitment of pol II holoenzyme components that bind the CTD would have a stimulatory effect on origin function as shown in Figure 7.

Our hypothesis presumes a contact between pol II holoenzyme and the MCM protein complex, which could explain the genetic interaction between *mcm5* and the pol II CTD (Figures 1–3). Such a contact has been described in metazoan cells (Yankulov *et al.* 1999); however, it was not revealed in yeast extracts by the methods we used in higher eukaryotes (X. Song and K. Yankulov, unpublished data). Alternatively, pol II holoenzyme and MCM proteins may independently bind a factor(s), which is important for origin function. In this case improper contacts with either *MCM5* or CTD would have a negative effect on minichromosome stability, as is the case in the *mcm5*, *rpb1Δ104MCM5*, *pYwt(10)*, and *pYwt(12)* strains, while mutations in both *MCM5* or CTD could reverse this effect, as is the case in *rpb1Δ104mcm5*. Both possibilities suggest the intriguing idea that in *S. cerevisiae* there is a mechanism of coordinating pol II transcription and DNA replication, which is mediated by the CTD of pol II. More *in vivo* studies are needed to address this question in detail.

We thank B. Tye, R. Scalfani, J. Corden, R. Young, D. Mangroo, and R. Lu for yeast strains; R. Li for the pARS1/wtA and pARS1/B23/G24 plasmids; and J. Bag, A. Wildeman, D. Evans, L. Holland, and J. Philips for valuable suggestions and discussion. This study was supported by a grant from the Canadian Institutes of Health Research (MOP-36371) to K. Yankulov.

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Communicating editor: B. J. Andrews