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A cell cycle-responsive transcriptional control element and a negative control element in the gene encoding DNA polymerase α in *Saccharomyces cerevisiae*

(DNA replication/yeast/G_1 phase)

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ABSTRACT **Transcription of the *POL1* gene of *Saccharomyces cerevisiae*, which encodes DNA polymerase α, the DNA polymerase required for the initiation of DNA replication, has previously been shown to be cell cycle regulated. To understand how the *POL1* gene senses cell cycle position, we have investigated the cis-acting elements that respond to the factors that govern cell cycle progression. In this report we demonstrate that a region of 54 nucleotides containing the repeated element ACGCGT, which conforms to an *Mlu* I restriction endonuclease recognition site, contains all information necessary for transcriptional activation and cell cycle responsiveness. Although oligonucleotides lacking either one or both of the repeated *Mlu* I sites can function as an upstream activating sequence, the presence of at least one *Mlu* I site stimulates expression and, moreover, is absolutely essential for cell cycle regulation. A synthetic oligonucleotide corresponding to a 19-base-pair sequence in the *POL1* promoter containing one *Mlu* I site can function as an autonomous cell cycle-responsive upstream element (upstream activation sequence) with temporal regulation indistinguishable from that previously described for the *POL1* gene. Thus, the *Mlu* I site is an essential part of a cis-acting element responsible for the observed periodic activation. This sequence differs from previously defined cell cycle-responsive transcriptional control elements in the yeast HO endonuclease and histone genes. We also present evidence for a negative regulatory element in the 5′ flanking region of the *Mlu* I upstream activation sequence.**

Cell cycle-regulated genes in the budding yeast *Saccharomyces cerevisiae* fall into several classes based on the time of expression in the cell cycle and the function of the gene products. The DNA replication genes, for example, undergo a burst of transcription at a point late in the G_1 phase of the cell cycle just before the periodically regulated histone genes are activated (for review, see ref. 1). The fact that such genes are expressed at a discrete time during the cell cycle implies that they are somehow responsive to the molecules that regulate the cell cycle. The study of such cell cycle-regulated genes should, therefore, provide insights into the basic mechanisms involved in controlling orderly cell cycle progression.

We have been interested in the regulation of *POL1* (2, 3), which encodes DNA polymerase α, the polymerase thought to be involved in the initiation of DNA replication. The periodic regulation of *POL1* is well documented (4, 5). Furthermore, in cells arrested by α-factor or a *cdc28* temperature-sensitive mutation, transcription was suppressed (4). *CDC28* encodes the 34-kDa subunit of a protein kinase thought to be critical in initiating a cascade of events culminating in the initiation of DNA synthesis (6). By understanding how *POL1* transcription responds to *CDC28*, we may be able to define events set in motion by *CDC28*. As a prerequisite to such studies, we have carried out experiments designed to define the upstream elements that regulate the *POL1* promoter.

Many other genes involved in DNA metabolism have been shown to have similar temporal regulation: *POL3* (7), *POL30* (8), *CDC8* (9), *CDC9* (10), *CDC21* (11), *PRI1* (12), *PRI2* (13), and *TS26* (14). Comparison of the promoters of these genes revealed the presence of one or two copies of the 6-base-pair (bp) consensus sequence ACGCGT, at about the same position in each promoter. This sequence conforms to the recognition site for the restriction enzyme *Mlu* I. It has been proposed that the *Mlu* I site is part of a cis-acting site responsible for the cell cycle regulation of these promoters (15, 16).

Here we identify cis-acting sequences in the *POL1* promoter that are at least partly responsible for the complex regulation of the *POL1* gene. We show that a 19-bp oligonucleotide containing one *Mlu* I site is sufficient to act as an upstream activation sequence (UAS) with the same pattern of cell cycle regulation as the chromosomal *POL1* gene. McIntosh *et al.* (17) have come to similar conclusions about the regulation of *CDC21*.

MATERIALS AND METHODS

Strains. All plasmid propagation and subcloning were carried out in *Escherichia coli* HB101 (18). *S. cerevisiae bar1-1* (*Mat***a**, *bar1-1, rme, leu1, met, trp5, ura3, can1, cir+*) was used exclusively for all yeast manipulations (obtained from Ambrose Jong, University of Southern California Medical School, Los Angeles). The *bar1-1* mutation reduces degradation of α-factor (19). Cells were synchronized according to Mendenhall *et al.* (20). The budding index after α-factor release was determined on plasmid-containing and plasmid-free cells under the same growth conditions, and only very slight differences were observed.

Plasmids. pLacZSal is a derivative of pSEYC102 (21) constructed by inserting a *Sal* I linker into the single *Sma* I site (Carl Parker, personal communication). Plasmid pLGΔBS (22) contains a *CYC1–lacZ* fusion gene lacking a functional yeast UAS.

Construction of the *POL1–lacZ* Fusion Gene. All sites in the *POL1* gene are referred to by the numbering system used in Pizzagalli *et al.* (16). A *POL1–lacZ* gene fusion was constructed using the *POL1* fragment from the *Eco*RI site at position −2333 to position +343 bp in the *POL1* gene inserted

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Abbreviations: UAS, upstream activation sequence; MCB, *Mlu* I-containing cell cycle box.

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into the *Eco*RI–*Bam*HI sites of the vector pLacZSal to give pBS(PD1) (see Fig. 1).

Promoter Deletions. The *Sal* I–*Bam*HI fragment from pBS(PD1) was subcloned into placZSal to give pPD1-1 (Fig. 1). Plasmid pPD1-2 was constructed by digesting pPD1-1 with *Hin*dIII and religating, creating an internal deletion from −435 to −376 bp in *POL1*. Plasmid pPD1-3 was made by cutting pPD1-1 with *Eco*RI and *Hin*dIII, filling-in the ends with the Klenow fragment of DNA polymerase I, and recircularizing the plasmid. Thus, pPD1-3 lacks all the *POL1* promoter sequences 5′ to −375 bp. pPD1-4 was constructed in three parts. (*i*) The *Sal* I–*Bam*HI fragment from pBS(PD1) was subcloned into the Stratagene Bluescript vector to give pBS(PD1-1). (*ii*) pBS(PD1-1) was digested with *Mlu* I and religated generating an internal deletion from −207 to −173 bp but regenerating a single *Mlu* I site. (*iii*) The *Sal* I–*Bam*HI fragment was inserted into the placZSal. The plasmid pPD1-5 is similar to pPD1-4 except that the *Mlu* I sticky ends were removed with S1 nuclease before religating and subcloning into placZSal. Therefore, the internal promoter deletion in this plasmid is from −207 to −169 bp.

Promoter Fusions. Plasmid pPU1 contained the 60-bp oligonucleotide PU1 cloned into the plasmid pLGΔBS. pPU1 contains 54 bp of *POL1* sequence from −217 to −164 bp plus 6 bp to reconstitute the *Sal* I and *Bgl* II sites after insertion into pLGΔBS. The *POL1* sequence in PU1 is TCTATTCAAACGCGTTAAAATTTTTTGTTAGCCTATAAGTAAAACGCGTCGCG.

Similarly, the plasmid pPU2 contains a similar 54-bp oligonucleotide to PU1 except that the *Mlu* I site from −173 to −168 bp has been deleted. pPU3 contains a 54-bp oligonucleotide missing the *Mlu* I site from −208 to −203 bp. Finally, pPU4 contains a 48-bp oligonucleotide missing both *Mlu* I sites. pPU5 contains a 25-bp oligonucleotide missing the sequence between the two *Mlu* I sequences in PU1 but retaining a single reconstituted *Mlu* I site. Therefore, it contains 19 bp present in the *POL1* promoter. To construct plasmid pPU7, the *Sal* I–*Mlu* I *POL1* fragment (bp −683 to −204) was purified and the 11-bp oligonucleotide PU7 was synthesized. PU7 had *Mlu* I and *Bgl* II cohesive ends enabling it to ligate to the *Sal* I–*Mlu* I fragment to give a *Sal* I–*Bgl* II fragment that contained all the PU5 sequence plus all the *POL1* promoter 5′ up to the *Sal* I site at −683 bp. The *Sal* I–*Mlu* I fragment, the PU7 oligonucleotide, and pLGΔBS were ligated together and the plasmid pPU7, containing the desired construct, was verified (see Fig. 2). Plasmid pPU8 was prepared by purifying the *Mlu* I–*Bgl* II fragment from pPU5. Dimerization of this fragment through the *Mlu* I site and insertion into the *Bgl* II site of pLGΔBS gives rise to a construct containing the 3′ *Mlu* I site and the 4-bp sequence CGCG 3′ to that region from *POL1* but no other sequences.

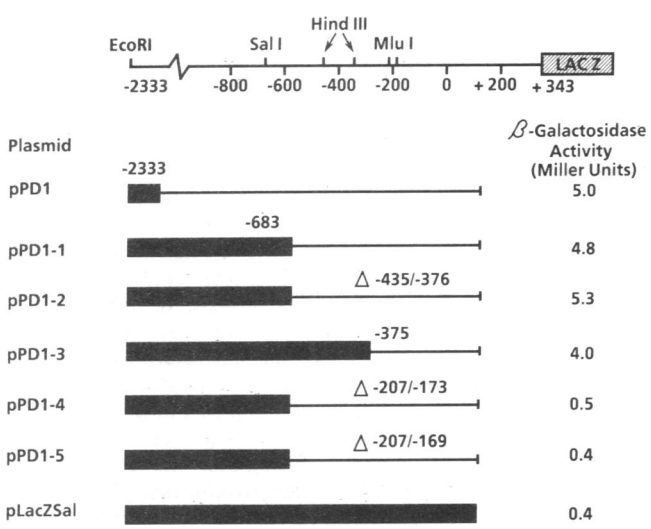

FIG. 1. Deletion analysis of the *POL1* promoter. The diagram shows the *POL1* promoter and the location of the two *Mlu* I sites as hatch marks under the *Mlu* I label. A *POL1–lacZ* in-frame gene fusion was constructed in the vector placZSal. The effects of various promoter deletions on transcription were then estimated by the amount of β-galactosidase activity obtained (23). β-Galactosidase activity was given in Miller units (24) and represents the average of two determinations or two individual transformants. Coordinates are relative to the ATG translation start site of the *POL1* gene.

RESULTS

Promoter Deletions. To identify cis-acting sites involved in the regulation of expression, a *POL1–lacZ* fusion was constructed in the vector placZSal. As shown in Fig. 1, yeast cells carrying pPD1 (−2333 bp to +343 bp in *POL1*) had 12 times more β-galactosidase activity than cells with placZSal.

To define the extent of the *POL1* promoter, a series of deletions were introduced into the pPD1 vector (see Fig. 1). To estimate the effect of each promoter deletion on *POL1* gene expression, the level of β-galactosidase activity in cultures of cells carrying each plasmid was determined, as shown in Fig. 1.

Two main conclusions can be drawn. (*i*) Since no dramatic drop in activity occurred when 1958 bp from −2333 bp to −375 bp was removed, a 375-bp DNA fragment is sufficient for activating transcription. (*ii*) The 29 bp between the two *Mlu* I sites are important for activating transcription, since the removal of this region caused the level of activity to drop to that of the plasmid placZSal without any insert. It is interesting to note that this reduction in the level of the β-galactosidase activity was observed even though the plasmid carrying this deletion (pPD1-4) retains one intact *Mlu* I site. Thus, the *Mlu* I site alone is not sufficient to promote transcription in the context of the *POL1* promoter.

Activity of *POL1* Promoter Sequences in a UAS Tester Plasmid. Our results suggest that the *POL1* sequence between the two *Mlu* I sites acts as a positive effector of transcription. If this conclusion were correct, we would expect that an oligonucleotide corresponding to this sequence should act as a UAS and activate transcription from a heterologous promoter. To assay for autonomous UAS activity, various sequences from the *POL1* promoter were tested for their ability to promote transcription in plasmid pLGΔBS (22), which contains a *CYC1–lacZ* fusion gene lacking a functional UAS. The UAS activity of a DNA sequence can be estimated by measuring the β-galactosidase activity obtained when the tester sequence is present in pLGΔBS. Consistent with promoter-deletion studies, when the *Sal* I–*Mlu* I (bp −683 to −204) fragment was cloned into pLGΔSB, no β-galactosidase was expressed (Fig. 2). Next, a double-stranded oligonucleotide, PU1, containing 54 bp of the *POL1* promoter from −217 bp to −164 bp spanning both *Mlu* I sites and including 9 bp upstream and 4 bp downstream of these sites was synthesized and cloned into pLGΔBS. As shown in Fig. 2, yeast cells containing the PU1 oligonucleotide pPU1 had almost 100 times more β-galactosidase activity than cells carrying pLGΔBS with no insert. This observation clearly demonstrates that a single copy of PU1 can function as a UAS in yeast.

To investigate if PU1 functioned as a cell cycle-regulated UAS, a synchronous culture of a strain carrying pPU1 was prepared by α-factor block/release. Samples were taken at 10-min intervals after removal of α-factor, and primer-extension analysis was used to determine the amount of *CYC1–lacZ* transcript present. The results obtained are shown in Fig. 3*a*. When using the lacI primer, which is specific for the *lacZ* portion of the CYC1–lacZ fusion mRNA,

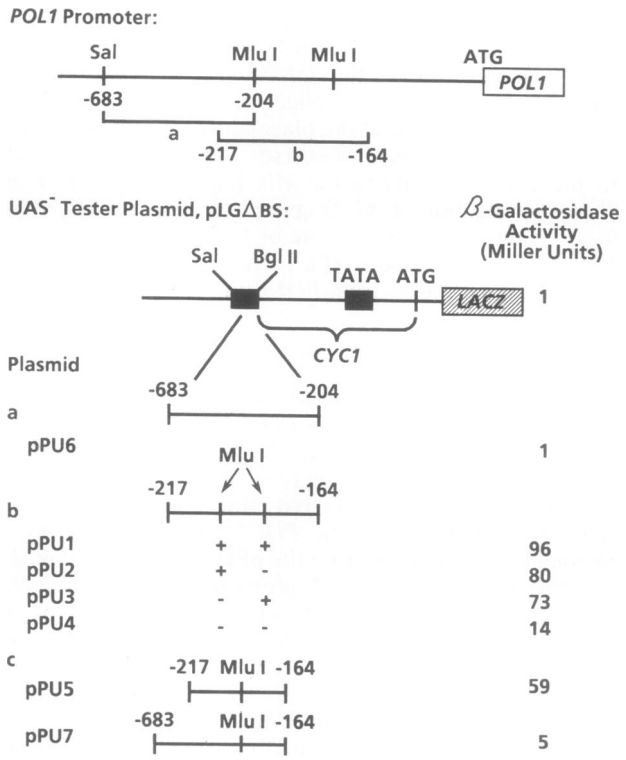

FIG. 2. Activity of various *POL1* promoter sequences in the UAS tester plasmid pLGΔBS. The figure shows diagrammatically the essential features of plasmid pLGΔBS. The plasmid contains a *CYC1–lacZ* fusion gene lacking a functional UAS. UAS activity was estimated by introducing the different plasmid constructs into yeast and assaying the amount of β-galactosidase obtained. The *POL1* promoter sequences tested are shown. Sequence a is the fragment from −204 to −683 bp. Sequence b is the 54-bp oligonucleotide *POL1* sequence present in the PU1 and its derivatives PU2, PU3, and PU4. The presence or absence of the *Mlu* I sites in each oligonucleotide is shown by + or −, respectively. Sequence c is oligonucleotide PU5 that is missing the 35-bp sequence interval between the two *Mlu* I sites but with a single *Mlu* I site reconstituted.

four main extension products were obtained that correspond to the sites expected if the previously determined *CYC1* transcription start signals were being used (ref. 25; data not shown). Fig. 3*a* clearly demonstrates that the level of these products varies dramatically during the cell cycle. First, cells blocked by α-factor in early G_1 phase do not transcribe the test gene in pPU1 (first two samples after α-factor release, Fig. 3*a*). Thus, pPU1 responds to the same suppression of transcription in early G_1 phase as described for the *POL1* gene (5). Second, the PU1-driven CYC1–lacZ transcripts appear periodically in the synchronous cycle after removal of α-factor. Furthermore, timing of the peaks in CYC1–lacZ transcript level is the same as observed for the POL1 mRNA. This was determined by correlating the variation in transcript level with the budding index and comparing this periodicity with the reported fluctuation of the POL1 transcript (refs. 4 and 5 and unpublished results). As an aperiodic control, the same RNA samples were subjected to primer-extension analysis using ACT1, a primer specific for actin mRNA. The level of ACT1 primer-extension products remained constant during the experiment (Fig. 3*a*). These results clearly show that the 54-bp segment of *POL1* present in PU1 contains all the information required to act as a cell cycle-regulated UAS with temporal regulation identical to the *POL1* gene.

An *Mlu* I Site Is Required for Cell Cycle Regulation. To investigate the role of the *Mlu* I sites, oligonucleotides PU2, PU3, and PU4 were synthesized. PU2 contains the same DNA sequence as PU1 but lacks the 3′ *Mlu* I site. PU3 is

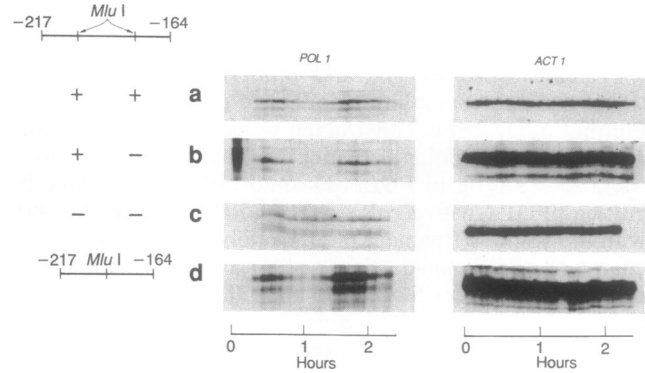

FIG. 3. Level of CYC1–lacZ and actin mRNA in synchronous cultures of cells carrying various synthetic UASs. Synchronous cultures of cells containing various synthetic UASs were prepared by α-factor block/release. The synchronous cultures were sampled every 10 min and total RNA was prepared. The level of CYC1–lacZ and actin transcript was then investigated by primer-extension analysis (25) using primers specific for *CYC1–lacZ*, CGCGCGGGAGAGGCGGTTTGCG, or ACT1, GGGAAGACAGCACGAGGAGGGTC, in strain *bar1.1* + pPU1 (*a*), strain *bar1.1* + pPU2 (*b*), strain *bar1.1* + pPU4 (*c*), and strain *bar1.1* + pPU5 (*d*). For reference, the presence or absence of *Mlu* I sites is shown. In *b*, the first primer-extension reaction using the lacI primer, specific for CYC1–lacZ mRNA, gave an aberrant result. Other primer-extension reactions using lacI with the same RNA sample repeatedly gave low levels of CYC1–lacZ mRNA (data not shown). All experiments were repeated at least twice.

missing the 5′ *Mlu* I site; PU4 lacks both *Mlu* I sites (see Fig. 2). The data shown in Fig. 2 demonstrate that the β-galactosidase activity observed in asynchronous cultures of cells carrying pPU2 or pPU3, in which only one of the two *Mlu* I sites found in PU1 was present, was only slightly lower than that measured for cells carrying pPU1 (76 and 83%, respectively). However, for cells containing pPU4, in which both *Mlu* I sites were deleted, the β-galactosidase activity was ≈5 times lower than the value obtained for cells with pPU1. The levels of β-galactosidase in the absence of *Mlu* I sites is still 14-fold higher than for cells carrying the vector pLGΔBS without any insert, however. These results support two conclusions. (*i*) One *Mlu* I site is sufficient for UAS function, but either *Mlu* I site alone (that in PU2 or PU3) is almost as efficient as the two together. (*ii*) Neither *Mlu* I site alone constitutes the sole UAS in the *POL1* promoter, since the region between the two *Mlu* I sites by itself has substantial transcriptional activation activity and since deletion of these sequences in the context of the *POL1* promoter leads to loss of transcriptional activation (see Fig. 1).

To investigate the role of the *Mlu* I sites in cell cycle regulation, the level of CYC1–lacZ transcript was determined in synchronous cultures of strains containing either pPU2 (one *Mlu* I site) or pPU4 (no *Mlu* I sites). As with strains carrying pPU1, the level of CYC1–lacZ transcript was found to vary periodically during the cell cycle in strains carrying pPU2. The timing of the peaks, at samples 4 and 12, was the same as that obtained with pPU1. The actin levels, as expected, remained constant throughout the experiment. With pPU4, lacking both *Mlu* I sites, by contrast, the level of the CYC1–lacZ transcript stayed relatively constant during the cell cycle. Only in the first two samples after α-factor release, as with the strains containing all the plasmids tested, was the amount of CYC1–lacZ transcript low. Once again the levels of the actin transcript used as an aperiodic control remained constant.

Two main conclusions can be inferred from the results obtained with synchronous cultures. (*i*) At least one *Mlu* I site is essential for proper timing of *POL1* expression in the cell cycle, since removal of both *Mlu* I sites from the PU1 UAS

eliminates the periodic regulation of the CYC1–lacZ transcript. (*ii*) The presence of a single *Mlu* I site is sufficient to entrain proper timing of the *POL1* expression. These results strongly suggest that the *Mlu* I site is part of a cis-acting DNA sequence responsible for cell cycle regulation of the *POL1* gene. (*iii*) A third result worth noting is that, in the synchronous culture containing pPU4 (Fig. 3*c*), although the CYC1–lacZ transcript level was found to be relatively constant during most of the cycle, it was still almost undetectable in the first two samples taken after α-factor release. The results in Fig. 3 *a* and *c* raise the intriguing possibility that transcription driven by the UAS sequences lying between the *Mlu* I sites still responds to the early G_1 phase restriction imposed by α-factor but does not respond to any early G_1 phase restriction in the cell cycles after α-factor release.

Minimal Sequence Requirement for the *Mlu* I-Containing UAS. An oligonucleotide missing the 35 bp between the two *Mlu* I sites but retaining a hybrid *Mlu* I site was synthesized (PU5). As no β-galactosidase activity could be detected in cells carrying pPD1-6, which contains the same deletion (Fig. 1), little β-galactosidase activity was expected from the PU5-driven *CYC1–lacZ* gene. Surprisingly, however, the level of β-galactosidase observed with pPU5 was only slightly lower than that obtained with pPU2 or pPU3 and four times higher than that found with pPU4 (Fig. 2). In addition, the transcription was cell cycle regulated (Fig. 3*d*) with the same timing as the chromosomal *POL1* gene. Thus, the entire region between the *Mlu* I sites is dispensable for cell cycle regulation. Since the 5′ *Mlu* I site plus 4 bp 3′ to it are functional, the minimal essential site is the 15 bp.

The fact that PU5 acts as a functionally regulated UAS was surprising because the PU5 sequence is present in pPD1-4, one of the promoter deletions that conferred little transcriptional activity on the *POL1–lacZ* fusion (see Fig. 1). One explanation is that *POL1* promoter sequences upstream of the PU5 sequence in pPD1-4 somehow repress the PU5 UAS activity. To test this possibility, the region from −683 to −217 bp in the *POL1* promoter was cloned upstream of PU5 in pPU5 to give pPU7. The result shown in Fig. 2 demonstrates that the amount of β-galactosidase activity obtained with cells carrying pPU7 was less than 10% of that found with pPU5. These results are entirely consistent with the results observed with pPD1-4. Therefore, it appears that a sequence between −683 and −217 bp in the *POL1* promoter represses the "potential" high UAS activity of the sequence encoded by the PU5 oligonucleotide.

Finally, a 10-bp UAS was constructed containing only the 3′ *Mlu* I site and 4 bases 3′ to it—ACGCGTCGCG. This sequence functioned efficiently (59 units of β-galactosidase activity) leaving a minimal size of 10 bp for the UAS.

DISCUSSION

This report describes the identification of a cell cycle-regulated transcriptional control element in *S. cerevisiae*. We show that the 6-bp *Mlu* I sites found twice in the *POL1* promoter are part of cis-acting sites responsible for the cell cycle regulation of the yeast *POL1* gene. The critical experiments involved testing various regions of the *POL1* promoter for UAS activity by inserting the appropriate synthetic DNA oligonucleotides into a plasmid that contains a *CYC1–lacZ* fusion gene lacking a functional *CYC1* UAS. We propose that this cis-acting sequence be called the *Mlu* I-containing cell cycle box or MCB element. The fact that the MCB site can confer periodic regulation on a heterologous *CYC1–lacZ* fusion gene, which contains no *POL1* sequences, indicates that the cell cycle regulation must be, at least partly, at the level of transcription. These experiments provide no evidence for or against a second level of control on the periodic stability of the POL1 transcript. Similar conclusions have been reached about the role of the *Mlu* I site in cell cycle regulation of the *CDC21* gene (17).

Two other classes of genes also show bursts of RNA synthesis in G_1 phase: the *HO* gene, involved in mating-type switching, and the histone genes. Previous studies have identified the sequence $CACGA_4$ as being necessary and sufficient for the periodic regulation of the *HO* gene and the 16-bp sequence $GCGA_5NTRGAAC$ (where N is any nucleotide and R is a purine) as being required for the cell cycle regulation of the histone genes (26, 27). The 54-bp sequence present in PU1, identified here as a cell cycle-regulated UAS, bears no obvious similarity to the consensus sequences responsible for cell cycle regulation of the *HO* or histone genes. Thus, the MCB element represents a distinct class of cell cycle-regulatory sequence and indicates that at least three pathways exist in yeast for the control of periodic gene expression. Although we have shown that the *Mlu* I site is necessary for cell cycle regulation, our studies do not allow us to conclude if the 6-bp site is sufficient. However, the observation that oligonucleotide PU5, in which the 35 bp between the two *Mlu* I sites in the PU1 oligonucleotide is missing, still works as a cell cycle-regulated UAS allows us to limit the size of a functional MCB element to 19 bp. To determine what sequences, if any, within this 19 bp are required in addition to the 6-bp *Mlu* I site for periodic regulation will require further experiments. That an exact match to the 6-bp *Mlu* I site may not be absolutely required is indicated by the fact that two other cell cycle-regulated DNA synthesis genes, *TS26* and *PRI1*, do not have any *Mlu* I sites in their promoters. However, *PRI1* has two 5/6 matches and *TS26* has one 5/6 match. All of these putative MCB sites appear at approximately the same position, between −109 and −207 bp upstream of the ATG translation start site of the respective genes. As we have shown that the MCB site is essential for cell cycle regulation of the *POL1* gene, we expect that the *Mlu* I and *Mlu* I-related sites are part of sites responsible for cell cycle regulation of all the above DNA metabolism genes. We do not know if there is a difference between the regulation of promoters with one or two *Mlu* I sites, but in the *POL1* promoter the two *Mlu* I sites do not appear to act synergistically, as oligonucleotides PU2 and PU3, containing only one or the other *Mlu* I site, appear to function as UASs with activities similar to the activity of PU1 oligonucleotide carrying both *Mlu* I sites (Fig. 2).

Sequences other than the MCB sequences (oligonucleotide PU4) can confer transcriptional activation (Fig. 2). These sequences do not function as a cell cycle-regulated UAS but still appear to be repressed by the presence of α-factor (Fig. 3*c*, first two samples). How this repression occurs is not known, but one possibility is that it is responding to the presence of α-factor by way of the pheromone signal transduction pathway. Irrespective of the mechanism, the data shown in Fig. 3 suggest that the α-factor repression is independent of the cell cycle regulation in the *POL1* promoter.

A 37-bp deletion between the two *Mlu* I sites of the *CDC21* promoter resulted in an 8 times reduction in transcriptional activity (15). It is striking that a deletion between the two *Mlu* I sites in the *POL1* promoter of a *POL1–lacZ* fusion gene also resulted in a 12 times reduction in transcriptional activity to essentially background levels. It is surprising though that these sequences in the two promoters contain no obvious similarities, except a high A+T content. To investigate the possible role of the 29-bp sequence between the two *Mlu* I sites in *POL1* further, an additional oligonucleotide (PU5) with an internal deletion between the two *Mlu* I sites but with a single reconstituted *Mlu* I site was constructed (see Figs. 2 and 3). PU5 was indeed found to behave as a cell cycle-regulated UAS, but the UAS activity was about 61% of that obtained with PU1.

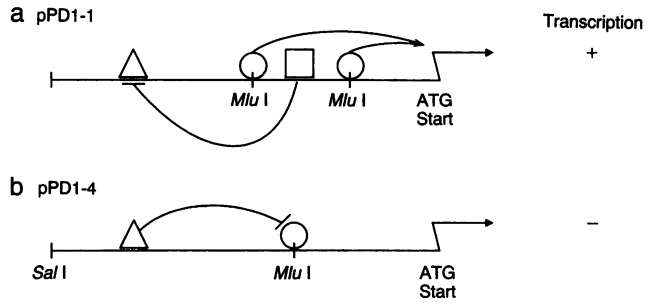

FIG. 4. Data implying that at least two cis-acting sequences act to regulate the cell cycle-regulated MCB box. One element (△) acts negatively on the MCB UAS activity, whereas the other (□) "suppresses" the repression. In the wild-type promoter in pPD1-1 (*a*), □ and △ are present allowing the cell cycle MCB box to operate. However, in pPD1-4 (*b*), the □ sequence is absent allowing △ to repress the MCB box and allowing little transcription to occur.

That PU5 acts as a cell cycle-regulated UAS seemed, initially, to contradict the fact that the promoter deletion pPD1-4 confers little transcriptional activity on the *POL1–lacZ* gene fusion even though it contains the complete PU5 sequence. An explanation for this apparent contradiction is offered by the fact that the presence of additional *POL1* promoter sequences 5′ to PU5 caused a 10 times reduction in the amount of UAS activity obtained (see Fig. 2). This strongly suggests that a cis-acting inhibitor of the MCB box in PU5 is found somewhere in this 406-bp sequence. Furthermore, an additional cis-acting site must be postulated that is somehow involved in the removal of the potential MCB repression to give the transcriptional activity found with the intact *POL1* promoter. A model summarizing the cis-acting elements involved in *POL1* transcription is shown in Fig. 4.

The latter form of regulation is not without precedent. Negative control of the *S. cerevisiae HO* gene must be overridden for the cell cycle box to become active (ref. 28; see also ref. 29; discussed in ref. 1). The negative element has not been defined in either the *HO* or the *POL1* promoter, but comparison of the relevant *HO* and *POL1* promoter sequences displayed no obvious similarities. In addition, negative control seems to account in part for the regulation of the *HTA1–HTA2* histone genes. Deletion of a 21-bp negative regulatory element (called the CCR) results in elevated expression and at least partial loss of cell cycle control (27). The CCR sequence is not found in the *POL1* promoter anywhere between position −480 and the ATG. Although we have not specifically defined the *POL1* negative regulatory sequence, three copies of the sequence TTAAAGAA and two copies of the sequence TCTATC occur in the region upstream of the *Mlu* I sites and might constitute additional regulatory sequences (16).

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