A Role for the Transcription Factors Mbp1 and Swi4 in Progression from G1 to S Phase

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In budding yeast genes that encode G1 cyclins and proteins involved in DNA synthesis are transcriptionally activated in late G1. A transcription factor, called SBF, is composed of Swi4 and Swi6 proteins and activates transcription of G1 cyclin genes. A different, but related, complex called MBF binds to MCB elements (Mlu I cell cycle box) found in the promoter of most DNA synthesis genes. MBF contains Swi6 and a 120-kilodalton protein (p120). MBF was purified and the gene encoding p120 (termed *MBP1*) was cloned. A deletion of *MBP1* was not lethal but led to deregulated expression of DNA synthesis genes, indicating a direct regulatory role for MBF in MCB-driven transcription. Mbp1 is related to Swi4. Strains deleted for both *MBP1* and *SWI4* were inviable, demonstrating that transcriptional activation by MBF and SBF has an important role in the transition from G1 to S phase.

The G1-S transition is a central regulatory step of the eukaryotic cell cycle. In most cells, mitogenic signals and cell size are sensed at this stage. In *Saccharomyces cerevisiae*, a G1 cell can either enter a quiescent state, differentiate into a gamete, or start a new cell cycle. Cells only become committed to cell division at a specific point in late G1 called START, which may correspond to the restriction point of certain mammalian cell lines. It is becoming increasingly clear that gene activation plays an important role at this stage of the cell cycle.

START requires activation of the Cdc28 protein kinase by G1 cyclins encoded by *CLN1*, *CLN2*, and *CLN3* (*1*). *CLN1* and *CLN2* RNAs are absent in early G1 but appear suddenly at the G1-S boundary of the cell cycle (*2*). However, *CLN3* RNA is present throughout the cell cycle. Transcriptional activation of *CLN1* and *CLN2* requires a functional Cdc28 kinase, and therefore is thought to occur via a positive feedback loop (*3*, *4*). Both *CLN1* and *CLN2* are coordinately expressed with the *HO* endonuclease gene (*5*, *6*) and *HCS26* (encoding a cyclin-like protein) (*7*). The promoters of these genes contain copies of a cis-acting regulatory sequence called the SCB element (Swi4-Swi6 cell cycle box, CACGAAA), which confers *CDC28*-dependent transcription (*5*, *8*).

Most genes involved in DNA synthesis including *POL1* (DNA polymerase α), *TMP1* (thymidylate synthase, *CDC21*) (*9*), and the B-type cyclin genes *CLB5* and *CLB6* (*10*, *11*) are also transcriptionally activated at or soon after cells undergo START. Their transcription in late G1 depends on copies of a short sequence called the MCB element (Mlu I cell cycle box; ACGCGTNA) within their promoters (*9*). Many of these genes encode stable proteins whose synthesis is not rate-limiting for DNA replication, but others, like *CLB5*, *CLB6* (*10*, *11*), and possibly also *CDC6* (*12*) encode unstable proteins, whose de novo synthesis in late G1 participates in the initiation of S phase.

Two different, but related transcription factors have been implicated in driving expression from SCB and MCB elements respectively. SBF (SCB binding factor), a complex containing Swi4 and Swi6 binds to SCB elements (*8*, *13*) via a DNA binding domain near the NH_2-terminus of Swi4 (*14*). Swi6 does not seem to contact DNA directly and may have a regulatory function. Activation of SBF requires G1 cyclins, whereas repression of SBF-driven transcription in G2 is dependent on mitotic cyclins, suggesting that SBF activity may be directly modulated by different forms of the Cdc28 kinase (*15*).

A different DNA binding activity, called DSC1 or MBF (MCB binding factor) binds to MCB elements (*16*, *17*). MBF also contains Swi6, but ultraviolet crosslinking experiments suggested that MBF binds DNA via a 120-kD protein (p120) which is distinct from Swi4 (*17*). DNA synthesis genes are still expressed but are deregulated in *swi6* mutants (*16*, *17*). It is unclear whether this phenotype is due to a loss of MBF activity or to residual p120 function in the absence of Swi6.

We purified MBF and cloned the gene encoding its p120 subunit, which we call *MBP1*. Cells lacking *MBP1* are viable, but express certain DNA synthesis genes constitutively. *MBP1* is related to *SWI4*. We show that *mbp1 swi4* double mutants are inviable largely because of their failure to express G1 cyclins, demonstrating that the transcriptional program initiated at START participates in regulating entry into the cell cycle. The Swi4-Mbp1 class of transcription factors is highly conserved among ascomycete yeasts and may prove to be a universal feature of the eukaryotic cell cycle.

The components of MBF. MBF from crude yeast extracts was quantitatively bound to a heparin agarose column and eluted with an ammonium sulfate gradient (*18*) (Fig. 1, lane 3). SBF elutes just before MBF and the two peaks coincide with the elution profile of Swi6, as assayed by immunoblotting. Fractions containing the peak of MBF were further purified by DNA affinity chromatography with MCB elements from the *TMP1* promoter (*18*) (Fig. 1, lane 4). Whereas more than 50 percent of Swi6 was recovered after affinity chromatography, we did not detect MBF activity in the eluted material. The MBF activity could be recovered, however, when binding assays were performed in the presence of boiled reticulocyte lysates or *Escherichia coli* extracts. Thus, the loss of activity was probably due to the conditions of gel retardation which may not allow detection of MBF in very dilute protein solutions, rather than to the loss of an essential yeast component of MBF during chromatography.

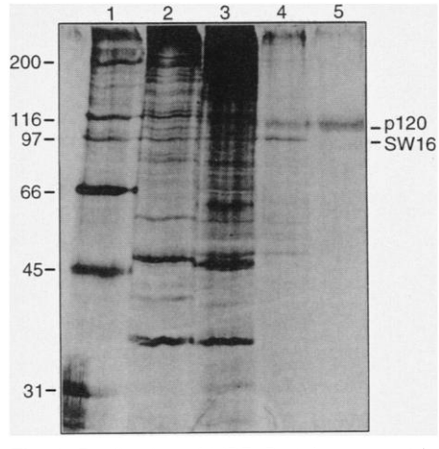

Fig. 1. Purification of MBF. Proteins present in whole cell extracts (lane 2), after heparin agarose chromatography (lane 3), DNA affinity chromatography (lane 4), and gel purification (lane 5) were visualized by silver staining; p120 and Swi6 are outlined to the right. Molecular markers (in kilodaltons) are shown to the left (lane 1).

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The DNA column eluates contained two major proteins (Fig. 1, lane 4): Swi6 (95 kD), and a 115-kD protein, which most likely corresponds to the 120-kD protein (p120) previously detected by ultraviolet crosslinking (*17*), since both can be coimmunoprecipitated with specific antibodies to Swi6 (*19*). The 115-kD protein was isolated by preparative SDS–polyacrylamide gel electrophoresis (Fig. 1, lane 5) and proteolytically cleaved with Lys C. Partial protein sequence was obtained from one Lys C peptide of this material (*18*).

The similar chromatographic properties of MBF and SBF and the finding that MCB and SCB elements cross-compete in gel retardation assays (*17*) suggested that p120 might be encoded by a gene related to *SWI4*. An oligonucleotide probe (*20*) derived from Swi4's DNA binding domain hybridized to two restriction fragments in yeast genomic DNA, one of which matched the size predicted for the *SWI4* gene (*8*). The other hybridizing fragment was isolated from an ordered genomic *Saccharomyces cerevisiae* λ phage library (*20*). DNA sequencing identified a gene encoding an 833–amino acid protein with a calculated size of 94 kD (Fig. 2A). Residues 372 to 387 of the deduced amino acid sequence matched the partial peptide sequence obtained for the Lys C peptide of p120 (*18*), suggesting that the gene encodes p120. We call this gene *MBP1* (MCB binding protein) (Fig. 2A). The sequence of Mbp1 is related to Swi4 (Fig. 2) (30 percent identical residues). Strong similarities were confined to three regions: The NH_2-terminus of Mbp1 (residues 1 to 112) was 50 percent identical to the NH_2-terminal DNA binding domain of Swi4 (Fig. 3A). The second region of similarity contained two copies of the 33–amino acid ankyrin repeat (*21*) (Fig. 3B). This sequence motif, also called the cdc10-Swi6 motif, has been found in many different regulatory proteins and has been implicated in protein-protein interactions (*21*). Residues 735 to 813 of Mbp1 were 44 percent identical to the COOH-terminus of Swi4 (Fig. 3C), which is important for the interaction between Swi4 and Swi6 (*22*).

Reticulocyte lysates programmed with *MBP1* mRNA produce a 100-kD translation product, which is not identical to the apparent size (115 kD) of p120 from yeast. The discrepancy may reflect differences in post-translational modification. In vitro translated Mbp1 forms gel retardation complexes with MCBs from the *TMP1* promoter in the absence of Swi6 (Fig. 4B, lane 5). No DNA binding was detected with in vitro translated Swi6 alone. Cotranslation of Mbp1 and Swi6 not only decreased the amount of binary Mbp1-DNA complexes but also generated new protein-DNA complexes which comigrate with MBF from yeast (Fig. 4B, lane 6). These data indicate that Mbp1, like Swi4 (*14*), can bind DNA independently of Swi6 and that Mbp1 and Swi6 may be the sole constituents of MBF.

The NH_2-terminal 124 residues of *MBP1* were translated in vitro and tested for binding to the *TMP1* promoter. The NH_2-terminal fragment forms two retarded protein-DNA complexes on the *TMP1* promoter, which may correspond to the occupation of either one or both MCB elements (Fig. 4B). Thus, Mbp1 and Swi4 have a common architecture; they have an NH_2-terminal DNA recognition motif and bind DNA as heteromeric complexes with Swi6.

Cell cycle regulation of DNA synthesis genes by *MBP1*. To assess the role of *MBP1* in controlling the expression of DNA synthesis genes, we generated an *mbp1* deletion mutant (*23*). The mutant has no obvious growth defect in haploids or homozygous diploids. MBF activity is absent from crude extracts of *mbp1Δ* mutants (Fig. 4A) providing independent proof that *MBP1* encodes p120. As previously shown (*16*, *17*), MBF activity is also absent in *swi6Δ* mutants, but detectable in *swi4Δ* mutants.

We found no major changes in the amounts of *TMP1*, *POL1*, or *CLB5* RNAs in asynchronous cultures of *mbp1Δ* mutants. This result explains why a deletion of *MBP1* does not affect viability, but it raises questions concerning the role of MBF in the transcription of MCB-driven genes. We therefore tested whether a deletion of *MBP1* affects the cell cycle regulation of DNA synthesis genes. A synchronous population of small G1 daughter cells was prepared from congenic wild-type and *mbp1* deletion strains by centrifugal elutriation and transferred to fresh medium at 30°C (Fig. 5). The relative timing of DNA synthesis (assayed by flow cytometric DNA analysis), bud emergence, and *HCS26* transcription was very similar in the wild-type and the *mbp1Δ* strain (Fig. 5A). Thus, deletion of *MBP1* has no gross effect on synchronous entry into the cell cycle or on the progression through S phase. In contrast, we observed a dramatic effect on the periodicity of MCB regulated gene expression. *TMP1*, *POL1*, and *CLB5* RNAs showed little or no cell cycle–dependent variation in the *mbp1Δ* strain (Fig. 5B).

The deregulation of *TMP1* and *POL1* expression in *mbp1Δ* mutants was confirmed with cultures synchronized by manipulating G1 cyclin expression (Fig. 6). A strain lacking the *CLN1*, *CLN2*, and *CLN3* genes, which was kept alive because of ectopic expression of *CLN2* from the repressible *MET3* promoter, becomes arrested in G1 when grown in the presence of methionine. Congenic wild-type and *mbp1Δ* strains were arrested in G1 and

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  1  MSNQIYSARY SGVDVYEFIH STGSIMKRKK DDWVNATHIL KAANFAKAKR TRILEKEVLK ETHEKVQGGF GKYQGTWVPL
 81  NIAKQLAEKF SVYDQLKPLF DFTQTDGSAS PPPAPKHHHA SKVDRKKAIR SASTSAIMET KRNNKKAEEN QFQSSKILGN
161  PTAAPRKRGR PVGSTRGSRR KLGVNLQRSQ SDMGFPRPAI PNSSISTTQL PSIRSTMGPQ SPTLGILEEE RHDSRQQQPQ
241  QNNSAQFKEI DLEDGLSSDV EPSQQLQQVF NQNTGFVPQQ QSSLIQTQQT ESMATSVSSS PSLPTSPGDF ADSNPFEERF
321  PGGGTSPIIS MIPRYPVTSR PQTSDINDKV NKYLSKLVDY FISNEMKSNK SLPQVLLHPP PHSAPYIDAP IDPELHTAFH
401  WACSMGNLPI AEALYEAGTS IRSTNSQGQT PLMRSSLFHN SYTRRTFPRI FQLLHETVFD IDSQSQTVIH HIVKRKSTTP
481  SAVYYLDVVL SKIKDFSPQY RIELLLNTQD KNGDTALHIA SKNGDVVFFN TLVKMGALTT ISNKEGLTAN EIMNQQYEQM
561  MIQNGTNQHV NSSNTDLNIH VNTNNIETKN DVNSMVIMSP VSPSDYITYP SQIATNISRN IPNVVNSMKQ MASIYNDLHE
641  QHDNEIKSLQ KTLKSISKTK IQVSLKTLEV LKESSKDENG EAQTNDDFEI LSRLQEQNTK KLRKRLIRYK RLIKQKLEYR
721  QTVLLNKLIE DETQATTNNT VEKDNNTLER LELAQELTML QLQRKNKLSS LVKKFEDNAK IHKYRRIIRE GTEMNIEEVD
801  SSLDVILQTL IANNNKNKGA EQIITISNAN SHA
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B

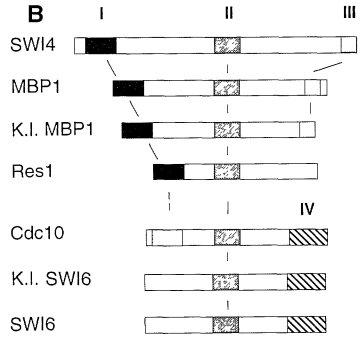

Fig. 2. The Swi4-Swi6 family of transcription factors. (**A**) Amino acid sequence of Mbp1 (EMBL database accession number X74158). (**B**) Schematic representation of the Swi4-Swi6 family of transcription factors. Regions of similarity are shaded. (I) DNA binding domain, (II) central region encompassing two copies of the ankyrin repeats, (III) COOH-terminal similarity of Swi4 and Mbp1, and (IV) COOH-terminal similarity of Swi6 and Cdc10. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

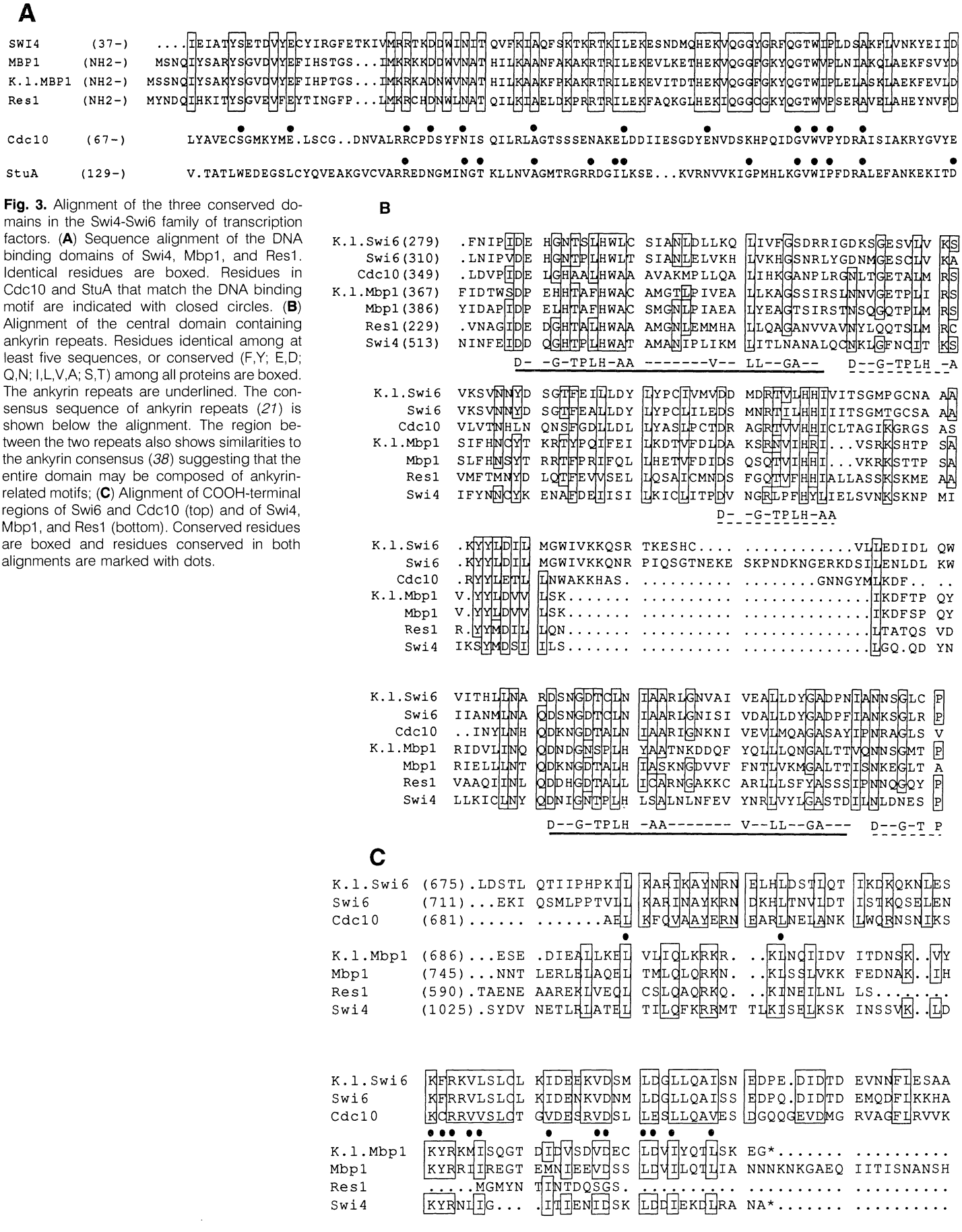

Fig. 3. Alignment of the three conserved domains in the Swi4-Swi6 family of transcription factors. (**A**) Sequence alignment of the DNA binding domains of Swi4, Mbp1, and Res1. Identical residues are boxed. Residues in Cdc10 and StuA that match the DNA binding motif are indicated with closed circles. (**B**) Alignment of the central domain containing ankyrin repeats. Residues identical among at least five sequences, or conserved (F,Y; E,D; Q,N; I,L,V,A; S,T) among all proteins are boxed. The ankyrin repeats are underlined. The consensus sequence of ankyrin repeats (*21*) is shown below the alignment. The region between the two repeats also shows similarities to the ankyrin consensus (*38*) suggesting that the entire domain may be composed of ankyrin-related motifs; (**C**) Alignment of COOH-terminal regions of Swi6 and Cdc10 (top) and of Swi4, Mbp1, and Res1 (bottom). Conserved residues are boxed and residues conserved in both alignments are marked with dots.

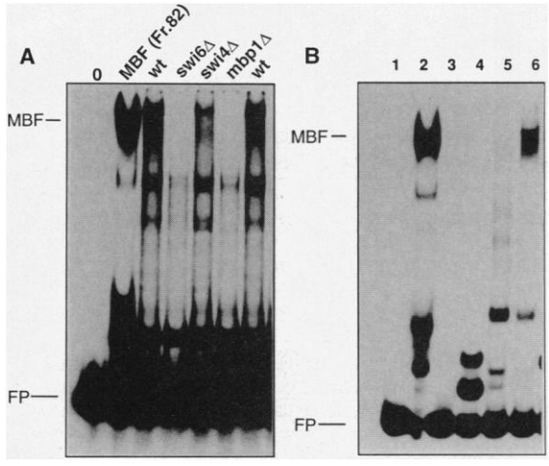

Fig. 4. Separation of the components of MBF. (**A**) Whole cell extracts (*6*) (20 μg of protein) were assayed for MBF activity by gel retardation (*36*). The strains used were as indicated: wild-type (wt) (K1107); *swi6Δ* (K2004); *swi4Δ* (K1940); *mbp1*Δ (K3294) (*23*); 0, no extract; MBF (Fr.82), 1 μl of heparin agarose fraction. The free DNA probe (FP) and the MBF complex (MBF) are markers. The reduction of MBF activity observed in the *swi4* mutant extract is not typical and presumably due to difficulties in obtaining high-quality extracts from this mutant. (**B**) In vitro translated Mbp1 bound MCB elements and formed complexes with Swi6. A sample of ^{32}P-labeled DNA (MCB-TMP1) (lane 1) was incubated with purified MBF (2 μl of the heparin fraction) (lane 2), unprogrammed reticulocyte lysate (lane 3), lysate containing the NH_2-terminus of Mbp1 (lane 4), full-length Mbp1 (lane 5), and with full-length Mbp1 cotranslated with Swi6 (lane 6). Templates for in vitro translation were generated by the polymerase chain reaction (PCR) and cloned (Nco I–Bam HI) in the T7-expression vector pT7(755–1024) as described (*14*). pCK 45 (residues 1 to 124 of Mbp1) was generated with oligonucleotides C47 (5′-ACT AGA CCA TGG CTA ACC AAA TAT ACT CAG CGA GAT ATT CG) and C51 (5′-GGC GGA TCC TCT AAT CCA CCT TCG AGG CAT GGT GAT GTT TTG G). The Swi6 template has been described (*14*). pCK51 (full-length Mbp1) was generated by cloning a Xba I–Bgl II fragment of pCK13 (*20*) in pCK45 (Xba I–Bam HI). In vitro translation with a coupled transcription-translation system (Promega TNT) was done according to the manufacturers instructions. For each assay (20 μl), portions (7.5 μl) of the in vitro translation reactions were used (*36*).

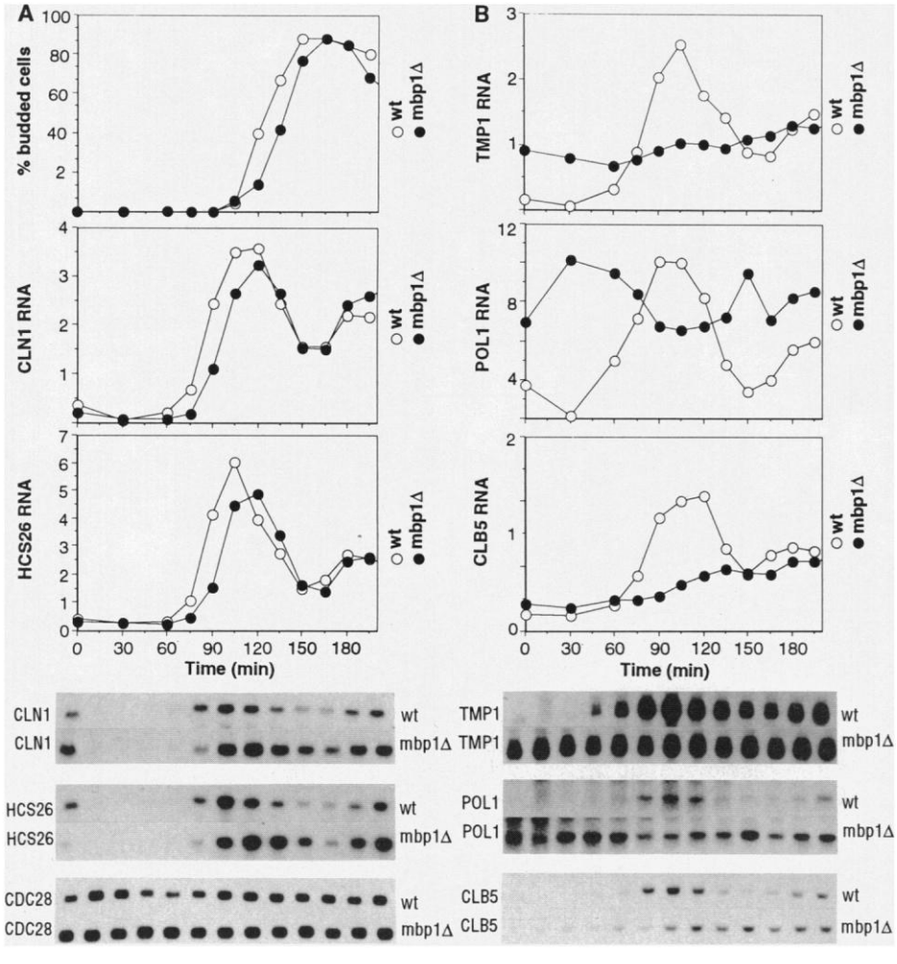

Fig. 5. Deregulated expression of S phase genes in *mbp1* mutants. Small G1 daughter cells from congenic wild-type (wt) (K1107) and *mbp1*Δ strains (K3294) (*23*) were isolated by centrifugal elutriation (>95 percent unbudded cells) (*11*) and resuspended in YEPD medium (*11*) at 30°C. Samples were taken at the indicated time points and processed for isolation of RNA (*37*) and for flow cytometry (*10*). The culture of mutant cells entered S phase about 10 to 15 minutes later than the wild-type population (determined by flow cytometry). This may be due to differences in the size distribution of the cells in the two starting populations or may indicate that the *mbp1* mutants are in G1 for a longer time because they undergo START at a slightly larger size. This was not investigated further. (**A**) Analysis of budding index (determined by light microscopy) and Northern analysis of SBF-driven transcripts in wild-type (open circles) and *mbp1* mutant (closed circles) cells (*37*). For each time point the levels of *CLN1* and *HCS26* were determined by Northern analysis and are graphically displayed relative to the level of *CDC28* RNA as internal control. In the first lane of each autoradiograph cycling cells were analyzed; (**B**) Northern analysis of MCB-driven genes. The amounts of *TMP1, CLB5*, and *POL1* RNAs were determined and quantified relative to *CDC28* RNA as above.

subsequently released into the cell cycle by removal of methionine. The *HCS26* RNAs oscillated in a similar manner in wild-type and the *mbp1*Δ mutant, but *TMP1* and *POL1* RNAs were periodic only in the wild-type strain. Under these circumstances, *CLB5* transcription remained periodic in the *mbp1* mutant, indicating that other factors can substitute for its regulation, at least during induction synchrony which causes cells to become very large. The different behavior of *CLB5* and *TMP1* shows that not all promoters of DNA synthesis genes are equivalent. Although most DNA synthesis genes carry MCB-like sequences in their promoters, their ability to bind MBF and their importance for late G1-specific transcription has been well characterized only in a few cases, such as *TMP1* (*17, 24*).

Using three different synchronization regimes (including release from alpha factor arrest), *TMP1* RNA levels in the *mbp1* mutant were intermediate between the peaks and troughs observed in wild-type cells, suggesting that MBF has a dual role in that it behaves as an activator of transcription at the G1-S boundary and as a repressor during other stages of the cell cycle. Transcriptional regulation of *MBP1* cannot be the cause of MBF's cell cycle–dependent activity because there is little or no variation in the amount of *MBP1* RNA or MBF activity (*17*) during the cell cycle.

We analyzed as to whether the deregulated transcription of *TMP1* in *mbp1*Δ mutants is dependent on MCB elements. The

TMP1 promoter contains two sequences with a perfect match to the MCB consensus (ACGCGTNA). Mutating the distal MCB (*24*) decreases *TMP1* transcription more than fivefold in both wild-type and in the *mbp1Δ* strain (*25*), suggesting that *TMP1* transcription remains largely MCB dependent in the absence of Mbp1. This indicates that MCB elements are recognized by factors other than the Mbp1-Swi6 complex in *mbp1* mutants.

An overlapping essential function for *MBP1* and *SWI4*. To test whether Swi4 substitutes for Mbp1, we analyzed the consequences of inactivating *SWI4* in a *mbp1Δ* mutant. No viable *swi4Δ mbp1Δ* double mutants were obtained among meiotic segregants of a heterozygous diploid, suggesting that *SWI4* and *MBP1* have an overlapping, essential function. In contrast, *mbp1 swi6* double mutants are viable but have the same morphological defect as *swi6* single mutants (6). This suggests that Mbp1 is inactive in *swi6* mutants.

The *mbp1* mutants carrying the temperature-sensitive *swi4-29* allele (*23*) are temperature-sensitive for growth. About 120 minutes after shifting from 25° to 37°C, *swi4-29 mbp1Δ* cells (but not wild-type or either single mutant) accumulated as unbudded cells with a 1 N DNA content (Fig. 7A). Some of the double mutant cells subsequently replicated their DNA and formed abnormal buds. Hence, entry into the cell cycle at START is one of the major defects of the double mutant. Similar results were obtained with *swi4-29 mbp1Δ* diploid cells.

We measured the RNA levels of S phase and G1 cyclin genes upon shifting cultures to the restrictive temperature (37°C). The amount of *TMP1* and *CLB5* RNAs remained high in all strains including the double mutant (Fig. 7B), implying that factors other than Mbp1 and Swi4 can activate these genes. In contrast, the RNAs of *CLN1* and *CLN2* drop rapidly in the *swi4-29* single mutant and the *swi4-29 mbp1* double mutant (Fig. 7B). The reduction was more severe in the double mutant, where *CLN1* and *CLN2* RNAs were reduced more than tenfold. Thus, most of the residual *CLN1* and *CLN2* transcription observed in *swi4* mutants is dependent on *MBP1* (Fig. 7B) and *SWI6* (6) and presumably due to MBF activity. MBF could either act through MCB-like elements in the upstream sequences of *CLN1* and *CLN2*, or alternatively, MBF could bind to SCB elements. The consensus sequences of SCBs (CACGAAA) and MCBs (ACGCGTNA) are similar and the elements cross-compete in gel retardation assays (*17*). Indeed, we have detected direct binding of the Swi4 DNA binding domain to MCB elements as well as the binding of the Mbp1 DNA binding domain to SCBs. An overlap in the function of Swi4 and Mbp1 could also explain the continued cell cycle regulation of *CLB5* RNA in *mbp1* mutants (Fig. 6) or *swi4* mutants (*10*) following induction synchrony; *CLB5* is fully deregulated in *swi6* mutants (*11*) which are defective for both MBF and SBF. Alternatively, there may exist additional Swi6-interacting factors that we have not detected in our analysis.

Our RNA analysis suggests that, like in *swi4 swi6* double mutants (6), the lethality of *swi4 mbp1* double mutants stems from a failure to express G1 cyclins. Consistent with this, we found that *swi4 mbp1* double mutants can be partially rescued by expression of *CLN2* from the *Schizosaccharomyces pombe ADH* promoter. However, the rescued cells grow more slowly than *swi4* or *mbp1* mutants at 37°C and have an abnormal morphology, suggesting that the deletion of *SWI4* and *MBP1* affects additional genes.

Genes regulated by MBF, like *POL1*, *TMP1* and others, are needed for DNA replication. Some, like *CLB5* and *CLB6*, encode proteins that must be newly synthesized for entry into S phase. MBF represses transcription of DNA synthesis genes in early G1. Its transition in late G1 from repressor to transcriptional activator may therefore be a necessary event leading to DNA replication in wild-type cells. The viability of *mbp1* mutants may be puzzling in this regard. We think that the viability of *mbp1* mutants may arise because of the binding to MCBs of factors whose access is normally blocked by MBF.

A family of transcription factors concerned with *CDC28*-dependent gene expression. Members of the Swi4-Swi6 class of transcription factors have recently been described in the fission yeast *S. pombe*. The Cdc10 protein (*26*) is similar to Swi6 (*21*), and the Res1 or Sct1 protein (*27*) is similar to Swi4. Both are components of a factor termed DSC1 (*28*) that binds to MCB elements within the promoter of the *cdc22* ribonucleotide reductase gene from *S. pombe*; hence, *res1* (*sct1*) may be the *S. pombe* homologue of *MBP1*. The activation in late G1 of MBF in *S. cerevisiae* and DSC1 in *S. pombe* may therefore be a conserved feature of S phase control. In contrast to *cdc10* and *res1* (*sct1*), *SWI6* and *MBP1* are not essential for entry into the cell cycle.

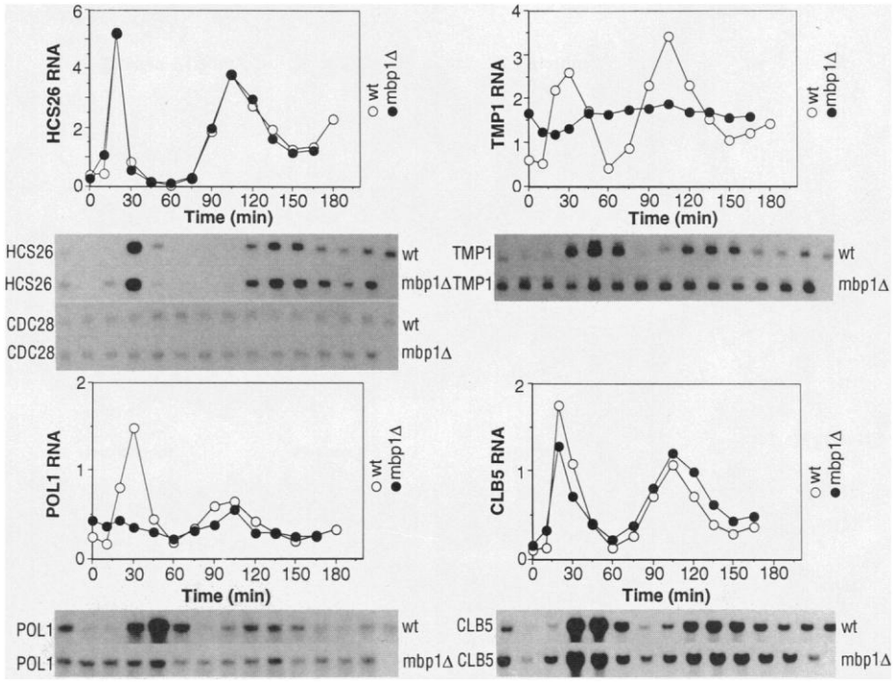

Fig. 6. Expression of late G1-specific transcripts in cells synchronized by manipulation of G1 cyclin activity. A strain deleted for G1 cyclins *CLN1, CLN2*, and *CLN3* and kept alive by expression of *CLN2* from the *MET3* promoter (K3413, *MATa cln1::HisG cln2Δ cln3::LEU2 MET3-CLN2::TRP1*) and a congenic *mbp1Δ* strain (K3488, *mbp1::URA3*) were grown to log phase in medium lacking methionine. Cells were then arrested in G1 by the addition of methionine (0.5 mM) for 3 hours. The cells were then filtered, washed, and resuspended in fresh medium lacking methionine. Samples were taken at the indicated time points, and RNA was isolated (*37*). [K3413; wild type (wt), open circles]; (K3488; *mbp1Δ*, closed circles). The levels of *HCS26, TMP1, CLB5* and *POL1* RNAs were determined by Northern analysis and graphically displayed relative to the amounts of *CDC28* RNA (*37*). In the first lane of each panel a sample was taken (as a control) prior to the G1 arrest and analyzed. K3488, (*mbp1::URA3*) was derived from K3413 by gene transplacement (*23*).

As discussed above, this may be due to extra transcription factors that bind MCB elements in *S. cerevisiae* rather than to major differences in the physiological functions of MBF and DSC1. The finding that a loss of MBF has little or no detrimental effect on viability, at least under laboratory conditions, suggests that the cell has means to ensure the successful coordination of cell cycle events even when DNA synthesis genes are expressed throughout the cell cycle.

We have started to address the evolutionary conservation of SBF and MBF transcription factors by isolating related genes from the yeast *Kluyveromyces lactis* (*29*) by low stringency hybridization. We isolated a gene that encodes a protein with 51 percent identity to Mbp1 (Fig. 2A) across the entire protein sequence (Figs. 2B and 3). This protein is much more similar to Mbp1 than to Swi4 and is presumably the *K. lactis MBP1* homologue. The degree of conservation between *MBP1* genes is much higher than that reported for other *K. lactis* homologues of *S. cerevisiae* DNA binding proteins like heat shock factor (*30*). Heat shock factor has been shown to be functionally conserved from yeast to humans (*30*), and yet it is much less conserved between *S. cerevisiae* and *K. lactis* (35 percent identity) than *MBP1*. It is therefore quite conceivable that *MBP1*-like genes also exist in animals and plants.

Although the *S. cerevisiae* and *K. lactis* Mbp1 proteins can be aligned throughout their whole sequences, the similarity is most pronounced in the regions similar to Swi4 (Fig. 2B), particularly the NH_2-terminal DNA binding domain, the central region containing two ankyrin repeats, and the most COOH-terminal region, which is required for the interaction of Swi4 with Swi6 (*22*). The DNA binding domains are 81 percent identical. This region is also most similar to *S. pombe* Res1 (Sct1). The DNA binding domain of this family of transcription factors is highly charged and rich in aromatic residues and it appears not related to any previously described DNA binding motif (Fig. 3A). It is similar, however, to a region of the *Aspergillus nidulans* StuA protein (*31*), which is involved in regulating cell pattern formation (*32*).

SBF and MBF are heteromeric complexes composed of a DNA binding protein (Swi4 or Mbp1) and a common regulatory subunit (Swi6) that does not contact DNA directly (Fig. 2B). DSC1 from *S. pombe* has a similar architecture and is thought to be composed of Res1 (Sct1) and Cdc10 (*27, 28*). All these proteins share a central domain flanked by two ankyrin repeats. Not only are the repeats conserved but also the sequences between them (Fig. 3B). We therefore propose that this class of proteins is derived from a common ancestor which combined the functions of both subunits. Swi4, Mbp1, and Res1 have retained the DNA binding domain while Swi6 and Cdc10 may have retained (or acquired) specific sequences at their COOH-termini including a leucine zipper (*22*). The COOH-termini of Swi4 and Mbp1 which are very similar to each other contain a small region which appears to be similar to the very COOH-termini of Swi6 and Cdc10 (Fig. 3C), lending further support for our hypothesis. There are few if any vestiges of an ancestral DNA binding domain in Swi6, but this domain has clearly persisted in Cdc10 (Fig. 3A). The conclusion that Swi6 has lost its DNA binding domain is supported by a comparison of Swi6 sequences from *S. cerevisiae* and *K. lactis*, which have virtually no similarity at their NH_2-termini, although the rest of their sequences are 55 percent identical.

Transcriptional activation at the G1-S boundary may be a conserved feature of the eukaryotic cell cycle. In vertebrates, transcripts from genes involved in S phase like those coding for dihydrofolate reductase (DHFR) and cyclin A appear at the G1-S boundary, whereas transcripts from cyclin E appear in late G1 (*33*). The E2F transcription factor has been implicated in the regulation of DHFR (*34*), but nothing is known about cyclin gene regulation. The conservation of Swi4-Swi6 type of transcription factors among fungi suggests that such factors may also have a function in the G1-S transition in animals and plants.

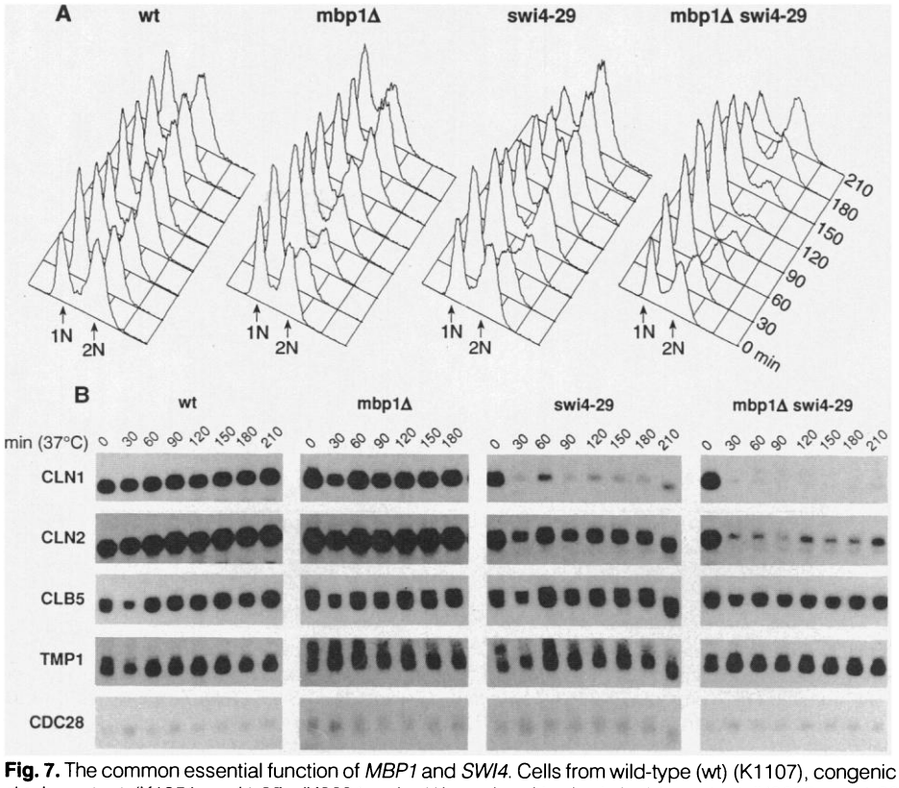

Fig. 7. The common essential function of *MBP1* and *SWI4*. Cells from wild-type (wt) (K1107), congenic single mutant (K1951, *swi4-29*), (K3294, *mbp1Δ*), and *swi4 mbp1* double mutant (K3295, *swi4-29 mbp1Δ*) strains were grown to early log phase at 25°C and then shifted to 37°C. (**A**) Flow cytometric DNA analysis of cells stained with propidium iodide (*10*) at various times after the temperature shift. The fluorescence signal of cells with a 1 N and 2 N DNA content are indicated by arrows. (**B**) Analysis of RNA (*37*) from S phase genes and G1 cyclin genes at the indicated time points.

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18. The protein p120 was purified from a 1000-liter culture of the protease-deficient strain BJ2168 essentially as described (*35*). Extracts (400 to 450 ml; protein 20 to 25 mg/ml) were prepared in batches of 500 to 600 g of cells and purified on a 400-ml heparin-agarose column (*19, 35*). Column fractions were assayed for MBF activity by gel retardation (*36*). Peak fractions of MBF activity were then dialyzed against $A_{50}N_{50}$ (*35*) and placed on an 8-ml specific DNA-sepharose column in the presence of sonicated salmon sperm DNA (0.1 mg/ml) at 0.3 ml/min. The column was washed with $A_{50}N_{50}$ containing 150 mM NaCl and eluted with 16 ml of buffer containing 500 mM NaCl (*35*). Fractions (1 ml) were assayed by silver staining and immunoblotting. The DNA-sepharose column was prepared as described (*35*) with concatemerized oligonucleotides from the *TMP1* promoter (5′-AGC TTG GAA ACG CGT CAA TTA AGG TCT TTT TCA TTT TTT CTA TTT AAC GCG TCA-3′; 5′-AGC TTG ACG CGT TAA ATA GAA AAA ATG AAA AAG ACC TTA ATT GAC GCG TTT CA-3′). Peak fractions eluted from the column were pooled, precipitated with trichloroacetic acid and separated by SDS-PAGE. The p120 band was excised from the gel, eluted in 50 mM tris-HCl (pH 7.5), 150 mM NaCl, 0.05 percent SDS, and 0.1 mM EDTA, and subsequently digested with Lys C (Boehringer) for 18 hours at 37°C. Peptides were isolated by reversed-phase chromatography and sequenced by automated Edman degradation (Applied Biosystem 477A). The sequence obtained for Lys C peptide 28 was as follows: X-(Leu)-Pro-Gln-Val-Leu-Leu-X-Pro-Pro-(Pro)-X-(Ser)-Ala-(Pro)-Tyr-(Ile); X denotes any amino acid. For residues in parenthesis more than one signal was detected, and the amino acid given corresponds to the strongest signal.
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20. Oligonucleotide CSW4, corresponding to residues 98 to 115 of Swi4 (5′-CAT GAA AAA GTT CAA GGT GGG TAT GGT AGG TTT CAA GGT ACT TGG ATC CCA CTG) was ^{32}P-labeled at the 5′ end. Hybridizations (50 μg of oligonucleotide per milliliter) were performed at 55°C in 6 × SCP (0.6 M NaCl, 0.2 M Na_2HPO_4, 0.01 M EDTA, pH 6.2), 10.8 percent (w/v) dextran sulfate, 2 percent (w/v) *N*-laurylsarcosine, and denatured salmon sperm DNA at 50 μg/ml. Filters were washed twice for 20 minutes in 4 × SCP, 1 percent SDS at 55°C and twice in 2 × SCP, 1 percent SDS at 55°C for 20 minutes. The ordered genomic *S. cerevisiae* λ phage library filters were hybridized under the same conditions. Two positive clones (6052 and 3955) were from chromosome V and code for SWI4. The other two positive phage (3769 and 3888) carried overlapping fragments of chromosome IV. The 7.5-kb Hind III fragment from phage 3769 encoding *MBP1* was subcloned to generate plasmid pCK13. The DNA sequence of *MBP1* was determined from both strands and was deposited in the EMBL database (accession number X74158).
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23. All genetic manipulations and yeast transformations were performed as described (*6*). *S. cerevisiae* strains used were congenic derivatives of the wild-type strain K1107 (*6*): K2471 (*MATa/MATα*), K2004 (*MATα swi6::TRP1*), K1951 (*MATα swi4-29*), K1940 (*MATα swi4::LEU2*) (*8*). The *mbp1::URA3* deletion was generated in K2471 by replacing the *MBP1* coding region (residues 18 to 625) by *URA3*. Spores from the resulting heterozygous diploid were dissected to isolate K3294 (*MATa mbp1::URA3*). Strains K3295 (*MATa mbp1::URA3 swi4-29*) and K3356 (*MATα mbp1::URA3 swi4-29*) were from a genetic cross (K3294 × K1951).
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29. The *Kluyveromyces lactis* homologue of *MBP1*, called *K.l. MBP1*, was cloned as a 4.5-kb Pst I–Asp 718 DNA fragment from genomic *K. lactis* DNA (K1748) with the use of the 781-bp Eco RV–Asp HI fragment from *SWI4* (residues 8 to 267) as a probe (*20*). A full-length clone was subsequently isolated from a YCp50 *K. lactis* library (provided by M. Stark) screened for plasmids that suppress the lethality of a *swi6Δ swi4-29 S. cerevisiae* strain (K2003) at 36°C. The *K. lactis SWI6* homologue (*K.l. SWI6*) was cloned as a 5.5-kb Eco RI–Bsp H1 718 fragment by low stringency hybridization with the 1.5-kb Sac I–Sph I fragment of *SWI6* (residues 284 to 801). DNA sequences were determined from both strands and deposited in the EMBL data base (accession numbers X74159, X74292).
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36. Gel retardation assays were done as described (*17*) with oligonucleotide probes (0.5 ng) from the *TMP1* promoter (MCB-TMP1) (*17*), carrying two MCB elements.
37. RNA was prepared (*4*) and analyzed by Northern analysis (*2*) with probes generated by random priming. Signals were quantified with a Phosphoimager (Molecular Dynamics); the amount of *CDC28* RNA, which does not vary during the cell cycle, was used as control.
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18 May 1993; accepted 18 August 1993