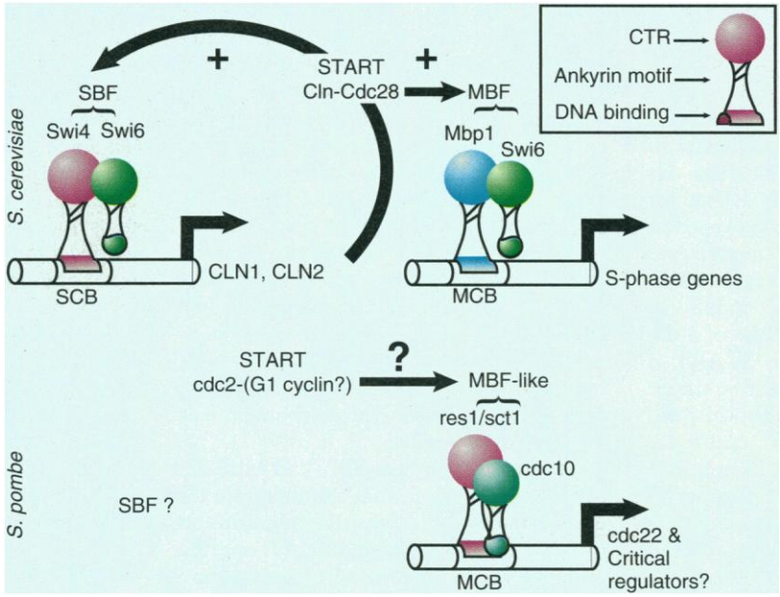
Gene Expression and the Cell Cycle: A Family Affair

Brenda J. Andrews and Stephen W. Mason

The periodic activation of transcription during the cell cycle is not just an interesting example of gene regulation. It is itself a key feature of the transition that marks irreversible entry into a new cell division cycle. In particular, the Swi4 and Swi6 proteins of the budding yeast *Saccharomyces cerevisiae*, which are components of a cell cycle–sensitive transcription factor, are at the heart of models explaining the molecular basis of cell cycle commitment. In this issue of *Science*, Koch and coworkers (*1*) describe the isolation and characterization of *MBP1*, a gene that encodes a new member of the Swi4-Swi6 family of transcription factors. The shared structural and functional features of the Swi4-Swi6-Mbp1 family members from budding yeast and other fungi reveal a highly conserved means of coordinating the events at the beginning of the cell cycle.

In *S. cerevisiae*, as in other eukaryotic cells, commitment to cell division occurs at a point known as START in late G1 phase, the period preceeding a new round of DNA replication (S phase). Passage through START (and through other regulatory transitions of the cell cycle) requires the product of the *CDC28* gene, a protein kinase that is functionally conserved from yeast cells to humans (*2*). The activity of Cdc28 depends on its association with different members of a large family of regulatory subunits called cyclins. Activation of Cdc28 at START requires association with at least one of three G1-specific cyclins encoded by the *CLN1*, *CLN2*, and *CLN3* genes (*3*). It is presumed, but as yet unproven, that the Cln-Cdc28 kinase complex is uniquely capable of phosphorylating substrates that are integral to the START transition. Potential substrates include transcription factors that control G1 phase–specific gene expression. A paradigm for coupling transcription to the cell cycle has emerged from studies of a large collection of genes from budding yeast that are maximally expressed in the late G1 and S phases of the cell cycle in a Cdc28-dependent manner. These genes can be grouped into two sets according to the upstream regulatory sequence that controls their cell cycle–regulated expression. The first group includes *CLN1*, *CLN2*, another cyclin-like gene called *HCS26*, and the *HO* gene (which encodes an endonuclease that initiates mating-type switching); expression of these genes is governed by the SCB regulatory element (Swi4-Swi6 cell cycle box, consensus CACGAAA) (*4*, *5*). G1-specific expression of the second set of genes requires a distinct upstream regulatory sequence, the MCB (MluI cell cycle box, consensus ACGCGTNA). The roster of MCB-driven genes includes enzymes required for DNA synthesis as well as a pair of cyclin genes, *CLB5* and *CLB6*, that regulate entry into S phase (*6*, *7*).



The Swi4-Swi6-Mbp1 family of transcription factors from yeast.

Biochemical and genetic analysis has revealed that SCB binding factor (SBF), the transcription factor responsible for the activity of the SCB sequences, contains two proteins, Swi4 and Swi6. Deletion of either the *SWI4* or *SWI6* gene prevents SBF complexes from forming in vitro and reduces or eliminates expression of SCB-dependent genes in vivo. Under normal growth conditions, neither Swi4 nor Swi6 is essential for cell viability. However, in the absence of both proteins, cells succumb to inadequate expression of *CLN1* and *CLN2* and a resulting inability to negotiate START (*5*). A picture of the individual roles of the Swi4 and Swi6 subunits within the SBF complex has begun to emerge. Swi4 has the functional hallmarks of a respectable transcription factor: It is responsible for specific recognition of the SCB sequences through a unique DNA binding domain in its amino terminus and, when overproduced, can activate transcription through the SCB sequences in the absence of Swi6 (*8*, *9*). By contrast, Swi6 has only nonspecific DNA binding activity and is bound to Swi4 through an interaction between the carboxyl-terminal regions (CTRs) of both proteins (*8*, *10*) (see figure). Although the exact role of Swi6 within the SBF complex remains a mystery, the view that Swi6 functions as a regulatory subunit was greatly strengthened by the remarkable discovery that Swi6, but not Swi4, is a component of MCB binding factor [MBF, also known as DSC1 (DNA synthesis control)], the factor that binds the MCB motif (*11*, *12*). Consistent with a regulatory role for Swi6 in MBF, transcription of MCB-driven genes persists in the absence of Swi6, but is insensitive to cell cycle position.

The isolation by Koch and co-workers (*1*) of the MCB binding subunit of MBF beautifully completes the analogy between SBF and MBF. The same group previously found a protein, dubbed p120, which could be crosslinked to an MCB-containing probe (*12*). The new report now describes a gene called *MBP1*, which they identified by screening a yeast library with a probe corresponding to the DNA binding domain of Swi4. Its product copurifies with Swi6 during the isolation of MBF and probably corresponds to the previously identified p120. Mbp1 is functionally similar to the Swi4 protein, since it binds the MCB through an amino-terminal DNA binding domain that is 50% identical to the same region of Swi4. The functional similarity is likely to extend to an identical means of interacting with Swi6, since the carboxyl terminus of Mbp1 resembles the CTR of Swi4, which is known to mediate interac-

The authors are in the Department of Molecular and Medical Genetics, University of Toronto, Toronto M5S 1A8 Canada.

tion with Swi6. Mbp1 shares a third region of similarity with both Swi4 and Swi6; the central portion of all three regulators contains two copies of the 33–amino acid cdc10-Swi6 [or ankyrin (ANK)] repeat that is known to be important for protein-protein interactions in other systems (*13*). The similar organization of the ANK-motif region in all three proteins (and, as described below, in other members of the family) presumably indicates an important regulatory role that remains to be determined. A very satisfying model emerges from the common architecture of the constituents of MBF and SBF: Specific regulation may be imposed by dedicated DNA binding subunits (Swi4 and Mbp1), while common regulatory needs may be met through interaction with a shared regulatory component (Swi6).

Koch and co-workers (*1*) assessed the importance of Mbp1 in MCB-driven gene expression and cell division with deletion mutants of *MBP1*. Cells grow normally in the absence of Mbp1—this may be explained by the fact that in cells deleted for *MBP1*, MCB-driven genes are expressed at normal levels but, as in a *swi6* mutant, are transcribed throughout the cell cycle. However, deletion of both *MBP1* and *SWI4* causes cells to die, largely because of a defect at START. The arrest at START is not caused by a failure to transcribe the S-phase genes but rather by drastically reduced levels of SBF-driven G1 cyclin expression. Since deletion of *SWI6* in a *swi4* mutant cell causes a similar phenotype, there may be cross talk between the SBF and MBF pathways. That is, the residual G1 cyclin expression in a *swi4* mutant may be due to MBF. The idea of cross talk is supported by the fact that MCB and SCB sequences cross-compete in DNA binding assays (*8*).

Other observations suggest an additional layer of complexity in the regulation of the SBF-MBF pathways. Several observations point to the use of alternative, but as yet unidentified, regulators to activate transcription of MCB target genes in the absence of MBF. First, the residual expression of at least one MCB-driven gene (*TMP1*) in the absence of MBF is still dependent on the MCB sequences. Second, the expression of two MCB-containing genes remains high in cells lacking both Swi4 and Mbp1, suggesting an alternative means of activating these genes. Finally, in the absence of Swi6, the common and essential component of both SBF and MBF, there is still sufficient expression of SCB- and MCB-containing genes for cell viability. Perhaps, other regulators work with Mbp1 and Swi4 in this instance. Thus, the yeast cell may have redundant fail safe mechanisms to ensure expression of the essential genes that are controlled by MBF and SBF: (i) the use of alternative regulators and (ii) cross talk between the pathways.

What is the biological importance of G1-specific gene expression through the SBF and MBF pathways? For SBF, the answer is relatively clear. Several lines of evidence place SBF within a positive feedback loop that generates a burst of Cdc28 kinase activity at START (*5*, *14*) (see figure). In this model, SBF is activated by the Cln-Cdc28 kinase; this increases the expression of *CLN1* and *CLN2* resulting in increased Cln-Cdc28 kinase activity. Restriction of *CLN1* and *CLN2* transcription to G1 by the SBF-dependent mechanism is critical for proper cell cycle progression, because constitutive overproduction of *CLN2* messenger RNA from an SBF-independent promoter interferes with subsequent phases of the cell cycle (*15*). The importance of MBF activity at START is less clear. Many of the known targets of MBF encode stable enzymes whose abberent expression throughout the cell cycle has no detectable effect on cell division. Of the known targets of MBF, the S-phase cyclin genes *CLB5* and *CLB6* may encode products whose de novo synthesis in each cell cycle is important for cell cycle progression (*7*). In the case of MBF, it is possible that periodic expression is largely a means of ensuring that enzymes essential to a critical cell cycle function (DNA synthesis) are present precisely when needed.

Are there analogous SBF- and MBF-dependent mechanisms that are important for regulating cell cycle–sensitive transcription in other organisms? Certainly, G1-specific gene expression appears to be a general and important feature of the cell cycle in yeast and mammalian cells. The identification of Swi4-Swi6-Mbp1 family members in other yeast species suggests that the SBF-MBF paradigm for linking transcription and the cell cycle might be broadly followed. An MBF-like activity (DSC1) described in *Schizosaccharomyces pombe* (*16*) contains two factors (*16*, *17*), cdc10 and res1-sct1, which are most similar to Swi6 and Swi4-Mbp1, respectively. Mutation of either cdc10 or res1-sct1 causes cells to arrest at START, indicating a complete intolerance for the absence of MBF-like activity in *S. pombe*. Genes that closely resemble *MBP1* and *SWI6* have also been identified in the yeast *Kluyveromyces lactis* (*1*). Some general structural themes for the Swi4-Swi6-Mbp1 family emerge from a consideration of this collection of factors. First, it is apparent that all family members contain a central ANK-motif domain. Second, the Swi6-like branch of the family contains a CTR that mediates dimerization, while members of the Swi4-Mbp1 branch boast both a distinct CTR as well as an amino-terminal DNA binding domain. Heteromeric transcription factors, such as SBF and MBF, are formed by association between a representative of each branch of the family. The *S. pombe* cdc10 protein represents an interesting variation on the theme that invites speculation regarding the evolution of the Swi4-Swi6-Mbp1 family—cdc10 most resembles Swi6 yet contains what may be a vestigial Swi4-like DNA binding domain in its amino terminus (*8*). Perhaps proteins with unique CTRs evolved from a common ancestor in order to promote heterodimerization and allow more specific responses to a wider range of regulators.

The high degree of conservation of the Swi4-Swi6-Mbp1 regulators in three yeast species raises a new question: Are there Swi4-Swi6-Mbp1 homologs in higher eukaryotes? As of yet, none have been described. Nonetheless, the human transcription factor E2F/DRTF1 defines an analogous pathway with some tantalizing parallels to the SBF-MBF systems. E2F binds a site that is remarkably similar to the SCB-MCB sites, associates with regulatory proteins and cyclin-dependent kinases, and is involved in the G1-specific regulation of at least one DNA synthesis gene (*6*, *18*). However, two proteins that specifically bind the E2F binding site (E2F-1 and DP-1) display little apparent similarity to the Swi4-Swi6-Mpb1 family (*1*, *19*). It is still possible that SBF-MBF–like factors function with E2F to regulate target genes. Alternatively, a distinct regulatory pathway in vertebrates that is truly homologous to the SBF-MBF pathway may await discovery.

References

1. C. Koch *et al.*, *Science* **261**, 1551 (1993).
2. K. Nasmyth, *Curr. Opinion Cell Biol.* **5**, 166 (1993).
3. H. E. Richardson *et al.*, *Cell* **59**, 1127 (1989); M. Tyers *et al.*, *EMBO J.* **11**, 1773 (1992).
4. L. Breeden and K. Nasmyth, *Cell* **48**, 389 (1987).
5. J. Ogas *et al.*, *ibid.* **66**, 1015 (1991); K. Nasmyth and L. Dirick, *ibid.*, p. 995.
6. G. F. Merrill *et al.*, *BioEssays* **14**, 823 (1992).
7. C. Epstein and F. Cross, *Genes Dev.* **6**, 1695 (1992); E. Schwob and K. Nasmyth, *ibid.* **7**, 1160 (1993).
8. M. Primig *et al.*, *Nature* **358**, 593 (1992).
9. L. Breeden and G. Mikesell, *Genes Dev.* **5**, 1183 (1991).
10. B. Andrews and L. Moore, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11852 (1992); J. Sidorova and L. Breeden, *Mol. Cell Biol.* **13**, 1069 (1993).
11. N. F. Lowndes *et al.*, *Nature* **357**, 505 (1992).
12. L. Dirick *et al.*, *ibid.*, p. 508.
13. P. Michaely and V. Bennett, *Trends Cell Biol.* **2**, 127 (1992)
14. F. Cross and A. Tinkelenberg, *Cell* **65**, 875 (1991); L. Dirick and K. Nasmyth, *Nature* **351**, 754 (1991).
15. K. Nasmyth, personal communication.
16. N. F. Lowndes *et al.*, *Nature* **355**, 449 (1992).
17. K. Tanaka *et al.*, *EMBO J.* **11**, 4923 (1992); M. Caligiuri and D. Beach, *Cell* **72**, 607 (1993).
18. J. Nevins, *Nature* **358**, 375 (1992).
19. N. B. La Thangue and W. R. Taylor, *Trends Cell Biol.* **3**, 75 (1993).