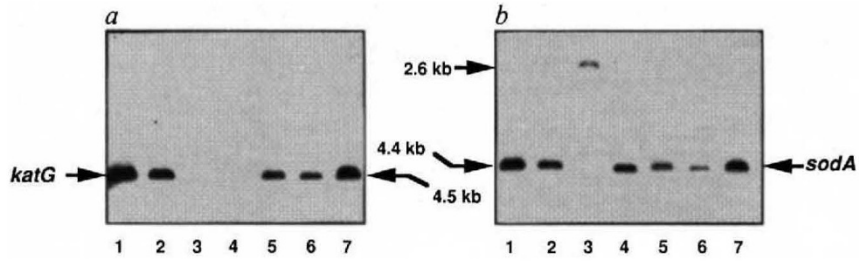

FIG. 4 Southern blots prepared using genomic DNA from different *M. tuberculosis* strains, digested with *KpnI*, were probed with *a*, *katG* (the 4.5-kb *KpnI* fragment), and *b*, the *sodA* gene (1.1-kb *EcoRI–KpnI* fragment; ref. 18). Labelling of probes and processing of blots was as described[15]. Lane 1, H37Rv; lane 2, strain 12, IC_{min} 1.6 μg ml^{-1} INH; lane 3, B1453, $IC_{min} > 50$ μg ml^{-1} INH[20]; lane 4, strain 24, $IC_{min} > 50$ μg ml^{-1} INH; lane 5, 79112, INH-sensitive[21]; lane 6, I2646, INH-sensitive[21]; lane 7, 79665, INH-sensitive[21]. INH susceptibilities were confirmed by inoculation of Lowenstein-Jensen slopes containing differing concentrations of INH.

multiple-drug-resistant strains in which there is a correlation between INH resistance and decreased catalase activity are particularly important because, owing to the contagiousness of tuberculosis, these strains pose a public health threat to both HIV-infected and healthy individuals[3]. An improved understanding of the mechanisms of drug resistance will enable rapid tests for drug-resistance isolates to be developed and should facilitate the design of antituberculosis drugs. □

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Anatomy of a transcription factor important for the Start of the cell cycle in *Saccharomyces cerevisiae*

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ENTRY of yeast cells into the mitotic cell cycle (Start) involves a form of the CDC28 kinase that associates with G1-specific cyclins encoded by *CLN1* and *CLN2* (ref. 1). The onset of Start may be triggered by the activation of *CLN1* and *CLN2* transcription in late G1 (ref. 2). SWI4 and SWI6 are components of a factor (SBF) that binds the CACGAAAA (SCB) promoter elements[3–5] responsible for activation in late G1 of the *HO* endonuclease, *CLN1* and *CLN2* genes[6,7]. A related factor (MBF) containing SWI6 and a 120K protein[8] binds to the ACGCGTNA (MCB) promoter elements responsible for late G1-specific transcription of DNA replication genes[9–12]. Nothing is known about how these heteromeric proteins bind DNA. We show here that SWI4 contains a novel DNA-binding domain at its N terminus that alone binds specifically to SCBs and a C-terminal domain that binds to SWI6. SWI4's DNA-binding domain is similar to an N-terminal domain of the *cdc10* protein that is a component of an MBF-like factor from *Schizosaccharomyces pombe*[13] and is required for Start[14,15]. An involvement of this kind of DNA-binding domain in transcriptional controls at Start may therefore be a conserved feature of eukaryotic cells.

To determine whether SWI4 or SWI6 alone can bind SCBs, we translated both proteins in reticulocyte lysates. Full-length SWI6 (846 residues[16]) is made efficiently but much of SWI4 (1,094 residues[4,17]) is either degraded or prematurely terminated (Fig. 1*a*). We tested the ability of the proteins to bind an oligonucleotide from the *CLN2* promoter (pCL2) that contains three potential SCBs and forms a complex with SBF isolated from yeast[6]. Using a gel retardation assay, we observe a heterogenous set of SWI4:pCL2 complexes (Fig. 1*b*), all of which are recognized by a SWI4-specific antibody but not by preimmune serum (data not shown and Fig. 1*c*). The heterogeneity may be due to the variable size of the SWI4 protein. No complexes were observed using the SWI6 protein (Fig. 1*b*). That SWI4 but not SWI6 can bind SCB DNA is consistent with experiments showing that SWI4 overproduction allows *HO* to be transcribed without SWI6[17,18] (but not vice versa) and that *CLN2* can be partially activated by SWI4 in *swi6* mutants[6,19].

Cotranslated SWI4 and SWI6 proteins form a new complex (with pCL2) containing both proteins that migrates with a mobility similar to that of the complexes formed by partially purified SBF from yeast (Fig. 1*b*, *c*). The complex formed with *in vitro* translated proteins seems to migrate slightly faster than that formed by yeast proteins and could conceivably lack a third component or modification. A truncated version of SWI4 lacking 144 amino acids from its C-terminal end (SWI4E) cannot form complexes with SWI6 although it still binds pCL2 (Fig. 1*d*). *HO* expression due to modest overproduction of such a protein in yeast is largely SWI6-independent[17]. Likewise, a version of SWI6 lacking its most C-terminal 89 amino acids (SWI6C) cannot form complexes on pCL2 with SWI4 (Fig. 1*e*). SWI4 and SWI6 might therefore interact by their C termini. This part of SWI6 is highly conserved in *Kluyveromyces lactis*

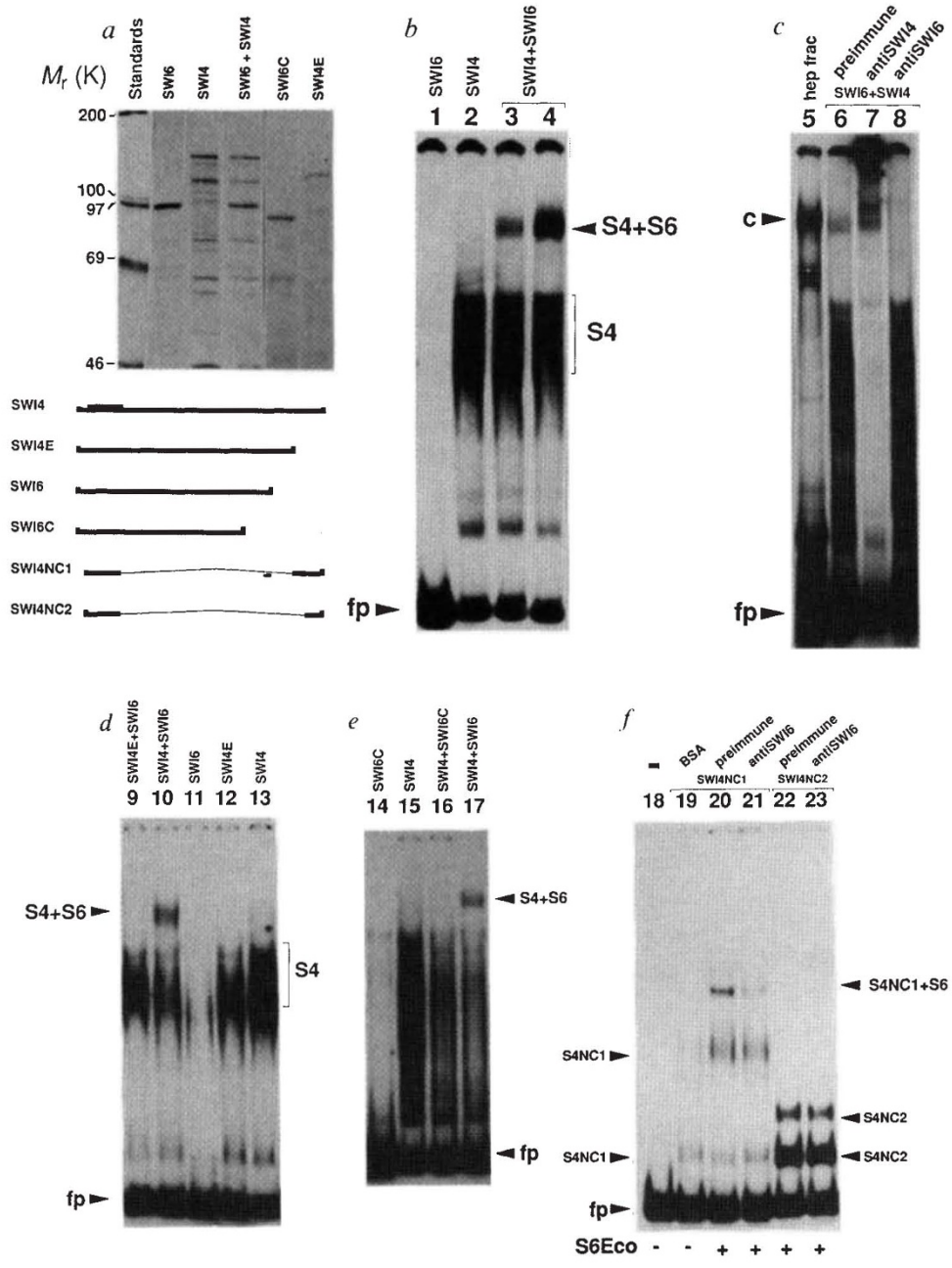


FIG. 1 Binary and ternary complexes formed by SWI4 and SWI6 on SCB DNA. fp, Free probe. *a*, Translation products labelled with ^{35}S-Methionine and examined on SDS polyacrylamide gels[24]. SWI4E refers to a truncated form of SWI4 lacking its C-terminal 144 amino acids due to digestion of the DNA template with *Eco*RI. SWI6C refers to a version of SWI6 that lacks its C-terminal 89 amino acids due to digestion of the DNA template with *Cla*I. SWI4's predicted M_r is 123K but it runs as a 150K protein. *b*, SWI4 binds DNA and recruits SWI6. Labelled DNA (0.5 ng) was incubated with 10 μl reticulocyte lysates containing SWI6 (lane 1), SWI4 (lane 2) and both proteins (lanes 3 and 4). Four times less SWI4 RNA was added to the translation mix in lane 4 compared to lane 3. Note that the ternary complexes containing SWI4 and SWI6 are much more uniform than the binary complexes containing SWI4 alone. We presume that this is due to the involvement of SWI4's C-terminus in its interaction with SWI6 (see *d*); that is very few of the incomplete SWI4 molecules will be able to form ternary complexes. *c*, *In vitro* SWI4–SWI6 complexes are recognized by SWI4- and SWI6-specific antibodies and have a similar electrophoretic mobility to *in vivo* complexes (marked c). Yeast SBF (1 μl) purified by heparin agarose chromatography (hep frac) was incubated with 0.5 ng labelled probe (lane 5). A reticulocyte lysate containing both SWI4 and SWI6 (lanes 6 to 8) was mixed with 1 μl preimmune serum (lane 6), anti-SWI4 serum[8] (lane 7) and anti-SWI6 serum[5] (lane 8). *d*, The C-terminus of SWI4 is required for interaction with SWI6. Labelled probe (0.5 ng) was incubated with 10 μl lysates containing SWI4 (lane 13), SWI4E (lane 12), SWI6 (lane 11) as well as SWI4 and SWI4E cotranslated with SWI6 (lanes 10 and 9). Lysate alone (not shown) gave the same pattern as SWI6 protein. *e*, The C terminus of SWI6 is required for interaction with SWI4. Labelled probe (0.5 ng) was incubated with 10 μl lysates containing SWI6C (lane 14), SWI4 (lane 15), SWI6C plus SWI4 (lane 16), and SWI6 plus SWI4 (lane 17). *f*, The C terminus of SWI4 is sufficient for interaction with SWI6. Labelled probe (0.5 ng) was incubated with a control lysate (lane 18), with SWI4NC1 (lanes 19–21) and SWI4NC2 (lanes 22 and 23). SWI6 (0.2 μg) purified from *E. coli*[5] (S6Eco) was added in lanes 20–23 as indicated at the bottom of the panel. Preimmune serum (1 μl) diluted 1:10 was added in lanes 20 and 22. SWI6 antiserum diluted 1:10 was added in lanes 21 and 23. The SWI4NC-dependent complexes are marked S4NC1 and S4NC2. The SWI4NC1:SWI6 complexes are only partially abolished by SWI6 anti-serum because of the large amount of SWI6 protein present.

METHODS. RNA synthesized by T7 RNA polymerase[25] was added to a rabbit reticulocyte lysate system (Promega L4210) at concentrations of 10–50 μg ml^{-1}. SWI4 RNA was synthesized at a 30-fold molar excess of ^{m7}GpppG CAP analogue (Pharmacia) versus rGTP. Labelled oligonucleotide (0.5 ng) and 3–15 μl reticulocyte lysate was incubated in 20 mM Tris pH 7.5, 3 mM $MgCl_2$, 1 mM DTT, 50 mM NaCl, 0.1 mM EDTA, 5 mM spermidine, 50 μg ml^{-1} BSA, 0.5 μg ml^{-1} salmon sperm DNA and 5% glycerol for 15–30 min in a final volume of 20 μl. For each μl of lysate, 0.5 μg Poly d(I).d(C) from Pharmacia was added. Complexes were analysed on 4% 20:1 acrylamide:bisacrylamide gels in 0.25 × TBE buffer (1 × TBE is 89 mM Tris, 89 mM borate and 2.4 mM EDTA) that were prerun at 10 V cm^{-1} for 1 h. In competition experiments, unlabelled oligonucleotides were added at a 500-fold molar excess before addition of the lysate samples. Antibodies were added last (10 min before electrophoresis). Gels were run at 10 V cm^{-1} dried and exposed overnight at −80 °C with an intensifying screen. The following oligonucleotides were used to construct the *in vitro* expression vectors of SWI4, SWI6, SWI4NC1 and SWI4NC2: SWI6-1, taattcgaattc-**ccATGG**CGTTGGAAGAAG; SWI6-2, aaaatttcgaaggaggaaa**tggCCA**GTCTC; SWI4-1, gatccg**ggCC**ATTTGATGTTTTGATATCAA; SWI4-2, tcgatc**agatct**-TTATGCGTTTGCCCTCAA; SWI4-A, ttggcg**ctgcaG**TGTAGCTCGATGGAGAAG; SWI4-B, gatcga**ctgcaG**AAAAGCAAAATTTTATTC; SWI4-C, gatcga**ctgcaG**-AGACGTTGAGACTAGCAA; To construct the SWI6 expression plasmid, pT7$_{755-1024}$ was linearized with *Nco*I and *Bam*HI and triple ligated to a SWI6 PCR fragment cut with *Nco*I and *MSc*I plus a *Msc*I–SWI6–*Bgl*II restriction fragment from pBd159 (a SWI6 *Hin*dIII fragment cloned into the *Hin*dIII site of pIC19H). To obtain the SWI4 vector, pT7$_{755-1024}$ was digested with *Nco*I, end repaired with Klenow and redigested with *Bam*HI. The linearized plasmid was ligated to a SWI4 PCR fragment that was digested with *Hae*III and *Bgl*II. The SWI4NC1 fusion was a triple ligation of 2 PCR fragments obtained with the oligonucleotides SW14Δ8 and SWI4-A as well as SWI4-B and SWI4-2; the former was cut with *Nco*I and *Pst*I, the latter with *Pst*I and *Bgl*II and ligated to pT7$_{755-1024}$ treated as described for SWI6. SWI4NC2 was produced the same way except that the oligonucleotide SWI4-C was used instead of SWI4-B.

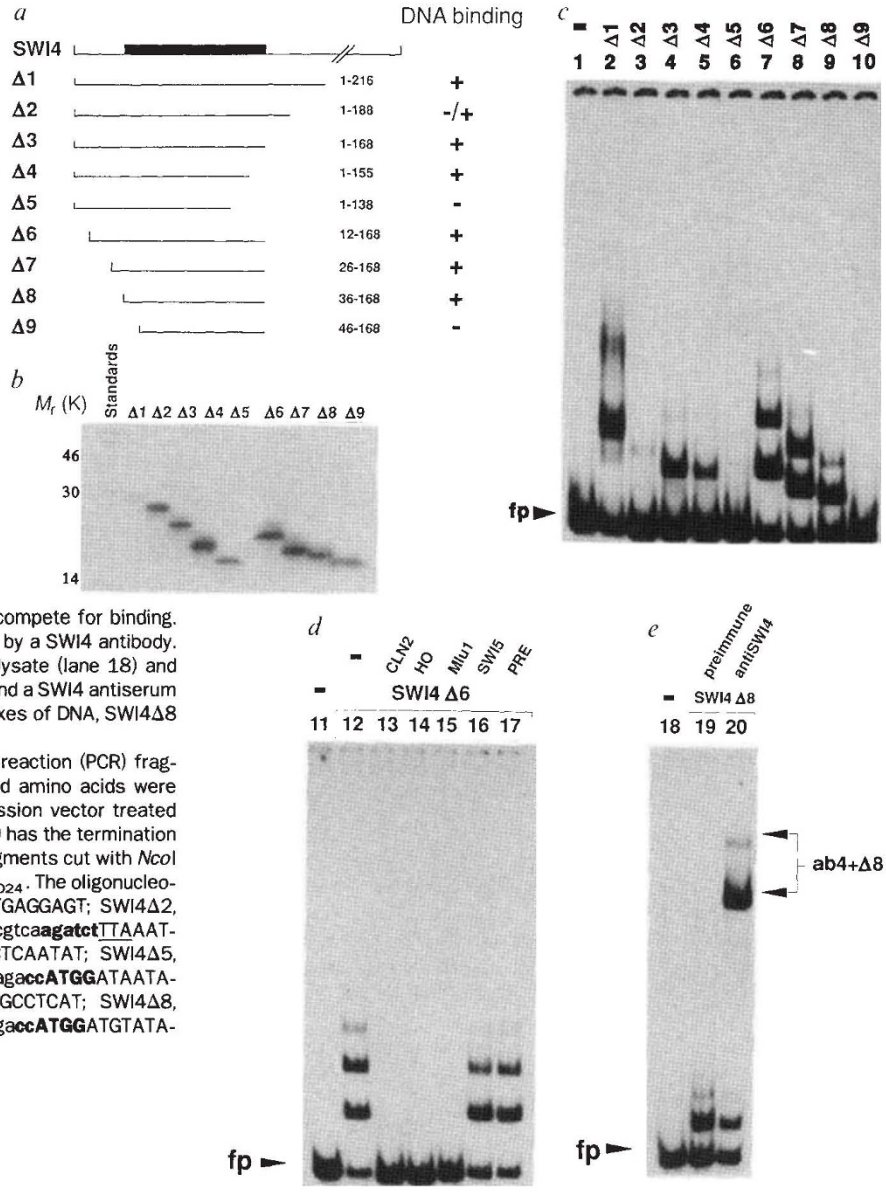


FIG. 2 Defining SWI4's DNA-binding domain. *a*, Deleted versions of SWI4 and a summary of their DNA binding. The black bar indicates the DNA-binding domain. Amino-acid end points are indicated by numbers on the right. *b*, Translation products of the above deletions. Size markers (K) on the left. The low abundance of Δ1 might be a loading artefact. *c*, DNA-binding assays. Labelled probe (0.5 ng) was incubated with 4 μl reticulocyte lysates containing the translation products of RNAs encoding viral coat proteins (lane 1) and SWI4 mutants (Δ1–Δ9, lanes 2–10). fp, Free probe. *d*, SWI4 DNA binding is competed by oligonucleotides containing SCBs. Labelled probe (0.5 ng; pCL2) was mixed with a control lysate (lane 11) and SWI4Δ6 (lanes 12–17). Unlabelled competitors were added at a 500-fold molar excess in lanes 13–17. The oligonucleotide competitors are: unlabelled probe, pCL2[6] (lane 13); an *HO* promoter fragment containing three SCBs, sRS2[5] (lane 14); a *TMP1* promoter fragment containing two MCBs, MCB-TMP1[8] (lane 15); an *HO* promoter fragment containing a SWI5-binding site[26] (lane 16); and a fragment containing PREs bound by STE12 (lane 17) (M.P. and G. Ammerer, manuscript in preparation). Note that MCBs as well as SCBs can compete for binding. *e*, SWI4Δ8:pCL2 gel retardation complexes are shifted by a SWI4 antibody. Labelled probe (0.5 ng) was incubated with a control lysate (lane 18) and SWI4Δ8 (lanes 19 and 20). A preimmune serum (1 μl) and a SWI4 antiserum (1 μl)[8] were added in lanes 19 and 20. Ternary complexes of DNA, SWI4Δ8 protein and SWI4 antibody are marked (ab4 + Δ8).

METHODS. As described for Fig. 1. Polymerase chain reaction (PCR) fragments with either ATG of TAA codons at the indicated amino acids were digested with *Hae*III and *Bgl*II and ligated to an expression vector treated as described for SWI4 to yield SWI4Δ1-Δ5; SWI4Δ6-Δ9 has the termination codon from SWI4Δ3 and were made by cloning PCR fragments cut with *Nco*I and *Bgl*II between the *Nco*I and *Bam*HI sites of $PT7_{755-1024}$. The oligonucleotides used were: SWI4Δ1, tcgatcagatctTTAGAACTGTTGAGGAGT; SWI4Δ2, tcgatc**agatct**TTAATTCGCAGCTGTAGT; SWI4Δ3, tcgtca**agatct**TTAAATGTAGCTCGATGG; SWI4Δ4, tcgtca**agatct**TTAAGTCTTCCTCAATAT; SWI4Δ5, tcgatc**agatct**TTAATCGAATTGAAAAGT; SWI4Δ6, actaga**ccATGG**ATAATACCAATCACCAG; SWI4Δ7, actaga**ccATGG**TTCTTTTGGCGCCTCAT; SWI4Δ8, actaga**ccATGG**TGATTGAAATAGCTACG; SWI4Δ9, actaga**ccATGG**ATGTATACGAATGCTATA.

(M. Neuberg, personal communication) and is similar to an equivalent domain of the *cdc10* protein (Cdc10)[16].

To define SWI4's DNA-binding domain more precisely, we compared the binding activity of various truncated forms (Fig. 2*a*). SWI4 polypeptides lacking all amino acids C-terminal to position 155 can still form binary complexes, as can polypeptides lacking the N-terminal 36 amino acids (Fig. 2*a*, *b*, *c*). But deleting C-terminal to residue 36 or N-terminal to residue 155 abolishes binding. A SWI4 polypeptide containing only residues 36 to 168 (Δ8) forms complexes almost as efficiently as larger forms. The only anomaly of this deletion series is Δ2 which binds poorly. The complexes formed by Δ8 contain SWI4 protein (Fig. 2*e*) and those formed by Δ6 are competed by oligonucleotides containing SCBs from the *CLN2* and *HO* promoters but not by unrelated oligonucleotides (Fig. 2*d*). An oligonucleotide containing MCBs from the *TMP1* promoter also competes. The amino acids 36–155 that form SWI4's DNA-binding domain are, in contrast to much of SWI4's primary sequence, highly conserved in a related protein for *K. lactis* (M. Neuberg, personal communication) and are sufficient for binding to the *HO* promoter *in vivo* (N. Jones, personal communication). But this domain alone cannot activate *HO* transcription[17]. Most of the SWI4 polypeptides capable of binding to pCL2 give rise to two and often three complexes. The number of complexes is related to the number of SCBs in the DNA (Fig. 3*a*, *c*). An oligonucleotide containing only a single SCB (pCL2Δ2) forms only one type of complex with either SWI4Δ7 or SWI4Δ8, whereas an oligonucleotide containing two SCBs forms two types of complex (Fig. 3*a*). Cotranslation of SWI4Δ1 and SWI4Δ8 produced complexes with the expected mobilities but did not produce any complexes with an intermediate mobility indicative of hybrids (data not shown). These data imply that SWI4 binds as a monomer to single SCBs and that it can bind simultaneously to all three SCBs in pCL2. To determine the binding site of SWI4 on pCL2Δ2 more precisely, we have determined at which adenine or guanine residues carbethoxylation interferes with binding. Interference is restricted to bases in the original SCB consensus sequence[20] (Fig. 3*b*). The pattern suggests that SWI4 makes contacts along an extensive stretch of the major groove.

To test whether SWI4's C terminus is sufficient for forming ternary complexes with SWI6 on DNA, we analysed complex formation with deleted versions of SWI4 lacking the central region due to fusion of SWI4's DNA-binding domain directly to C-terminal sequences. Neither the DNA-binding domain alone (data not shown) nor a fusion containing the C-terminal 65 residues (SWI4NC2) can recruit SWI6 but a larger fusion

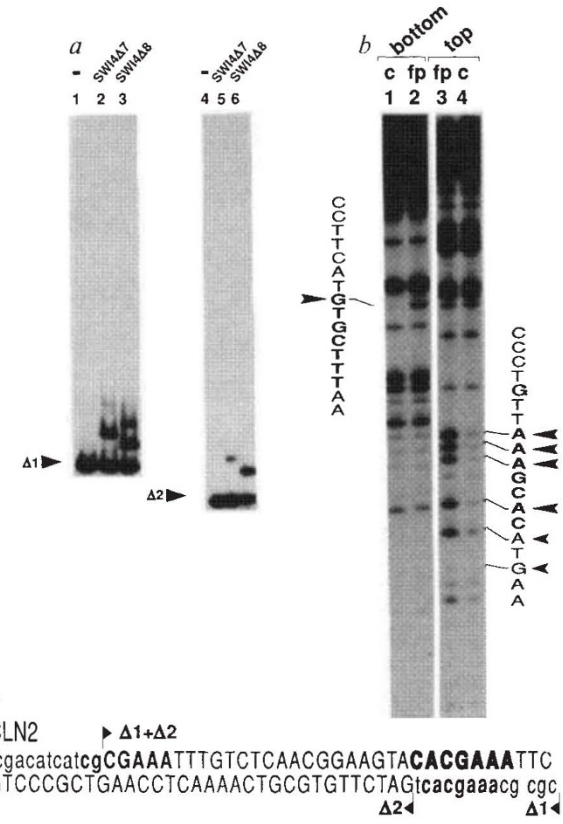
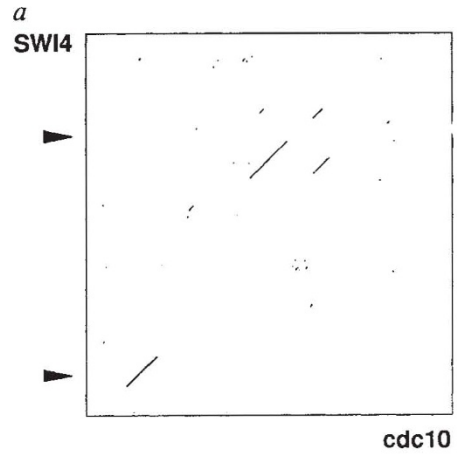


FIG. 3 The SWI4's DNA-binding domain recognizes a single SCB element. *a*, End-labelled pCL2Δ1 (0.5 ng) (lanes 1–3) and pCL2Δ2 (lanes 4–6) probes were incubated with control lysates (lanes 1 and 4) as well as SWI4Δ7 (lanes 2 and 5) and SWI4Δ8 (lanes 3 and 6). *b*, Carbethoxylation interference[28]. An end-labelled DEPC-treated[8] oligonucleotide (pCL2Δ2) containing a single SCB from the *CLN2* promoter was incubated with SWI4Δ8. Unbound (fp, lanes 2 and 3) and complexed DNAs (c, lanes 1 and 4) were separated by gel electrophoresis as in *a*, and the hydrolysed products analysed on a 10% sequencing gel[8]. Interfering bases are indicated by arrowheads. *c*, The sequences of pCL2, pCL2Δ1 and Δ2. The pCL2 sequence is shown on two lines and the end points of Δ1 and Δ2 are flagged. Large bases are those in pCL2Δ2 whose SCB is in bold letters. Small bases are deleted at the 5′-end of pCL2Δ1 or both at the 5′- and 3′-end of pCL2Δ2.

containing 146 C-terminal residues (SWI4NC1) allows SWI6 to form complexes on SCB DNA (Fig. 1*f*). Our data imply that SWI4's terminal 149 amino acids are both necessary and sufficient (with the DNA-binding domain) for recruiting SWI6 into ternary complexes *in vitro* but do not exclude other sequences also contributing.

We have found only a single protein that exhibits significant similarity to SWI4's DNA-binding domain. This is the Cdc10 protein from *Schizosaccharomyces pombe*[21]. A DIAGON comparison of SWI4 and Cdc10 (Fig. 4*a*) reveals two regions of similarity, one in the N-terminus that has not previously been recognized and a second in the middle of both proteins that corresponds to the two 33 amino acid 'Notch' repeats also found in SWI6[16] and many other proteins[22,23]. The N-terminal region of similarity extends from residues 42 to 143 in SWI4, which corresponds almost exactly to its DNA-binding domain. Recently, Cdc10 has been shown to be part of a transcription factor[13] that binds to the Start-dependent MCB element found in the promoters of many DNA replication genes in *S. cerevisiae*[9–12]. We suggest that this region of Cdc10 may be involved in the recognition of MCBs. It seems likely that the *S. cerevisiae* p120 protein that forms complexes with SWI6 on

FIG. 4 The region of SWI4 covering its DNA-binding domain is conserved in Cdc10 from *S. pombe*. The results of a DIAGON (*a*) and a peptide-sequence alignment (*b*) obtained with the GCG package[27]. The sequences of SWI4 and Cdc10 were aligned with the BESTFIT program. The DOTPLOT parameters of the COMPARE program were window 35 with a stringency of 18.0. Identical residues and conservative changes are indicated by thick and thin lines, respectively. A SWI4 gene cloned and sequenced by us[17] had an identical sequence to that published by Andrews and Herskowitz[4]. One problem with the proposal that the N-terminal domain of Cdc10 may bind DNA is that a fragment of Cdc10 lacking this domain is capable at high copy number of suppressing the lethality of a temperature-sensitive Cdc10 mutant[21]. It is possible that the mutation whose phenotype is suppressed may lie in the C-terminal half of Cdc10 and the suppressing fragment may only enable the mutant protein with an intact DNA-binding domain to function.



b

SWI4

42 IEIATYSETDVYECYIRGFETKIVMRRTKDDWINITQVFKIAQFSKTKRTKILEKESNDMQHEKVQGGYGRFQGTWIPLDSAKFLVNKYEIIDPVVNSILTF 143

67 LYAVECSGMKYME.LSCG..DNVALRRCPDSYFNISQILRLAGTSSSENAKELDDIIESGDYENVDSKHPQIDGVWVPYDRAISIAKRYSFYEI.LQPLISF 164

cdc10

MCB elements[8] will also turn out to have a similar DNA-binding domain. SCBs and MCBs are related in sequence and when present at high concentration can compete with each other for the binding of SWI4/SWI6 (SBF) and p120/SWI6 (MBF) complexes (ref. 8, Fig. 2*d*, and data not shown). Nevertheless, SBF and MBF bind preferentially to SCB and MCB elements, respectively[6,8].

Apart from sharing two Notch repeats, the C-terminal half of Cdc10 is unlike that of SWI4. It instead has extensive similarity to the C-terminal half of SWI6[16]. The Cdc10 protein therefore has the appearance of a hybrid between SWI4 and SWI6. In contrast to Cdc10, the N terminus of SWI6 has very little resemblance to SWI4. It may therefore not possess a functional DNA-binding domain, which is consistent with it not binding DNA in *in vitro* assays (Fig. 1*b*). One explanation for the hybrid character of Cdc10 is that all of these proteins are descended from a Cdc10-like ancestor, one of whose descendants (SWI4) has, apart from the Notch repeats, diverged extensively in its C terminus and another (SWI6) has diverged extensively in its N terminus. The conservation of SWI4's DNA-binding domain suggests that it may be found in Start-dependent transcription factors in many eukaryotic organisms. □

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Candidate proto-oncogene *bcl-3* encodes a subunit-specific inhibitor of transcription factor NF-κB

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THE NF-κB subunits p50 and p65 and the product of the *rel* proto-oncogene are members of a growing class of transcription factors with a unique DNA-binding and dimerization domain[1–13]. Nuclear transfer of each of these factors is controlled by cytoplasmic inhibitors, and regulated by specific stimuli. The inhibitors IκB-α and -β and pp40 recognize either p65 or the c-*rel* protein[14–16]. We show here that the proto-oncogene *bcl-3*, believed to be involved in certain human B-cell leukaemias[17], encodes a protein that functions as an IκB-like molecule for native NF-κB but is specific for the p50 subunit. The ankyrin repeat domain of the *bcl-3* product is shown to mediate complex formation with NF-κB dimers by contacting the conserved dimerization domain of NF-κB.

On the basis of structural similarities in a repeat domain of the *bcl-3* protein (Bcl-3) and a region in the C-terminal domain of p105 (refs 3–6) and MAD-3/IκB-α (ref. 18), we predicted as a common function the interaction with Rel-like transcription factors and reported that DNA binding of p50 is inhibited by the repeat domain of p105 and by Bcl-3 (ref. 19). We now report that native NF-κB is a target for Bcl-3.

In a band-shift assay with nuclear extracts from HeLa cells, Bcl-3 specifically inhibited nuclear NF-κB induced with the phorbol ester phorbol myristate acetate (PMA) (Fig. 1*a*, lanes 3 and 4), but did not affect endogenous octamer-binding transcription factor 1 (OTF-1) binding (lanes 5, 6). The inhibition was achieved using an amount of Bcl-3 comparable to the amount of MAD-3/IκB (ref. 18) necessary for inhibition of native NF-κB (not shown), demonstrating that Bcl-3 is an alternative form of IκB. We next analysed the subunit specificity of Bcl-3 for various Rel-like factors. DNA binding of p50, or of truncated p50 containing only the conserved Rel domain (p50Δ), was effectively inhibited by Bcl-3 (Fig. 1*b*, lanes 1 to 4). Both the c-*rel* protein and truncated p65 were recognized (lanes 5 to 8), but less efficiently. In contrast, the *Drosophila* morphogen *dorsal* was not a target (lanes 9, 10). The affinities of Bcl-3 and MAD-3/IκB were compared for both subunits of NF-κB (Fig. 1*c*). MAD-3 has a strong preference for p65, but can at high concentrations also inhibit p50 (lanes 5–8, 13–16). Bcl-3 clearly has an inverse specificity, with a higher affinity for p50 than for p65 (lanes 1–4, 9–12). These results establish that Bcl-3 is a previously undetected IκB species with a novel specificity, and suggest that IκB molecules recognize the Rel domain in a conserved manner.

A deletional analysis of the Bcl-3 molecule (Fig. 2) revealed that the ankyrin repeat domain is necessary and sufficient for inhibition (Fig. 2*b*, lanes 1 to 4), and that removal of either part of the first repeat (lanes 5 and 6), or of the seventh and part of the sixth (lanes 7 and 8), results in inactivation. This is in contrast to erythrocyte ankyrin and GA binding protein β in which a subset of the ankyrin repeats can mediate protein–protein interaction[20,21], but is similar to the ankyrin repeat domains of p105 and MAD-3 (ref. 19; and E. Hatada and C.S., unpublished observation).

To determine which domain of p50 is contacted by Bcl-3, deletion mutants of p50 were assayed for interaction with immobilized Bcl-3 (Fig. 3). At the C terminus, amino acids up to and including the conserved nuclear transfer signal could be deleted (p50Δ and C1) without affecting binding to the Bcl-3 matrix, but further deletion of 18 (C2) or 38 (C3) amino acids abrogated interaction (Fig. 3*b*). For unknown reasons, N-terminal deletions led to low levels of nonspecific binding to the control matrix (N2 and N3). Nevertheless, deletion of either the N-terminal 75 (N1) or 201 (N2) amino acids did not reduce binding to Bcl-3 compared to full-length p50; and the level of specific binding was significantly higher than to the immobilized

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