An ARS/silencer binding factor also activates two ribosomal protein genes in yeast

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ABSTRACT

GFI is an abundant yeast DNA-binding protein, capable of binding to both ARS sequences and to the upstream regions of a number of nuclear genes coding for mitochondrial proteins (Dorsman et al., Nucl. Acids Res., 16 [1988] 7287–7301). GFI binding sites conform to the consensus RTCRYN$_5$ACG, an element also present in the binding sites of factors designated SUF and TAF. These factors act as trans-activators of the constitutive transcription of the genes for ribosomal proteins S33 and L3 respectively. We now present evidence that GFI, TAF and SUF are probably the same protein. We speculate that one of the functions of GFI is the adjustment of the expression of a number of gene families to cell growth rate.

INTRODUCTION

Transcription in eukaryotic cells is activated by a number of proteins acting in concert with RNA polymerase (see ref. 1 for review). In the yeast *Saccharomyces cerevisiae* a number of such transcriptional activators have been characterized both genetically and biochemically. Some of these, such as the GCN4 and GAL4 proteins (2,3), are specifically required for the expression of only a limited number of genes, while others, such as the PHO2 and TUF (alias RAP or GRF1) proteins (4–6) may act as global regulators of genes involved in many apparently unlinked metabolic activities.

We have previously described the properties of two abundant DNA-binding proteins, desigated as GFI and GFII (7). Both factors bind to the upstream regions of a number of nuclear genes coding for mitochondrial proteins, while GFI is in addition capable of binding to ARS sequences. Features of its recognition site suggest that GFI is identical to, or at least highly similar to two other DNA-binding proteins, namely SBF-B and ABFI, which were initially characterized in terms of their ability to bind to silencer and ARS elements respectively (8,9). More recently, we have noted that the consensus derived for the GFI binding site can also accommodate the recognition sequences for SUF and TAF, two other independently identified DNA-binding proteins that act as trans-activators of the constitutive transcription of the genes for ribosomal proteins S33 and L3 respectively (6,10). We now show by a combination of retardation-competition and binding site studies that GFI, TAF and SUF are likely to be the same protein. We suggest that GFI is a DNA binding protein with several different functions in cell division and transcriptional regulation. One of these may be the adjustment of the expression of a number of gene families with respect to cellular growth rate.

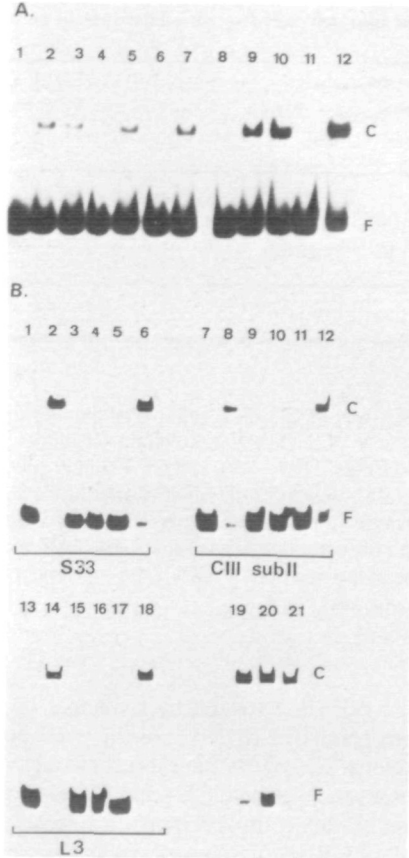

Figure 1: *Mobility -shift assays of complexes containing the factors GFI, TAF, or SUF*
A: A [^{32}P]-labelled DNA fragment encompassing the region between positions −408 and −102 relative to the initiator codon of the gene for subunit II of the yeast ubiquinol-cytochrome c reductase was used as a GFI binding site (7). The fragment was incubated with a partially-purified GFI preparation in the presence of the following competitor DNAs: pEMBL9 (lanes 2,3); unlabelled CYC1 gene sequences, either intact, or cleaved in the GFI binding site by Tth111 I (lanes 4,5); unlabelled L3 sequences, intact, or cleaved in the TAF binding site by RsaI (lanes 6,7); pEMBL9 (lanes 9−10); unlabelled S33 sequences, intact or cleaved in the SUF-binding site by XbaI (lanes 11,12). In each case, F and C indicate the position of the free DNA fragment and the DNA-factor complex respectively.
B: [^{32}P]-labelled oligonucleotides designed to specifically bind each of the three factors were incubated with the same protein fraction as in (A).
Lanes 1−6, 19: SUF-binding oligonucleotide.
Lanes 7−12, 20: GFI-binding oligonucleotide.
Lanes 13−18, 21: TAF-binding oligonucleotide.
Lanes 2,8,14,19,20 and 21 show the complexes formed in the absence of yeast competitor DNA. Unlabelled, competing oligonucleotides were present at a 50-molar excess as follows:
Lanes 3,9,15: SUF-binding oligonucleotide.
Lanes 4,10,16: GFI-binding oligonucleotide.
Lanes 5,11,17: TAF-binding oligonucleotide.
Lanes 6,12,18: mutant oligonucleotide (cf Fig. 2).

MATERIALS AND METHODS

Fragment mobility-shift assays

Procedures for retardation-competition experiments have been described previously (6,7). Competitor DNAs were derived from p9-CYC1-II (7), pTCM (3.2)(ref 11) and pUC S33-Taq-Taq. This last plasmid contains the same 5'-flanking region of the S33 gene as described in (6). A partially purified GFI fraction was prepared according to Diffley and Stillman (12).

Methylation-interference footprinting

Oligonucleotides bearing the SUF recognition sequence (see Fig. 2) were labelled at their 5'-termini using polynucleotide kinase and γ-[^{32}P]-ATP. After annealing with the complementary unlabelled oligonucleotide, labelled products were purified by gel electrophoresis and dissolved in 50 mM sodium cacodylate (pH 8.0), 10 mM MgCl2, 1 mM EDTA. Dimethylsulfate (0.005% final concentration) was then added and the mixture was incubated for 4 min at 25°C. After addition of sodium acetate and β-mercaptoethanol, repeated ethanol-precipitations were performed. Methylated DNA was used for the band-shift assay (6). Free DNA and complex band were eluted from the gel in 50 mM MOPS, 500 mM NaCl, followed by a purification step using a QIAGEN column (DIAGEN GmbH, Düsseldorf). After ethanol-precipitation, the DNA was treated with piperidine according to Maxam and Gilbert (13) and loaded on a 7% polyacrylamide gel.

DNA manipulation

Restriction and other enzymes used in DNA manipulation were obtained from Boehringer Mannheim asnd New England Biolabs and used as recommended by the manufacturers. All other chemicals used were of the highest purity available.

RESULTS AND DISCUSSION

1. Retardation-competition

First indications of the relationship between the DNA-binding factors GFI, SUF and TAF were obtained by examination of the ability of DNA fragments containing the various binding sites to compete for protein binding in gel mobility shift assays. Fig. 1A shows the results of one such experiment, in which a fragment containing a GFI binding site was used as labelled probe in binding to a partially purified preparation of factor GFI. Specific competition was given by a 20-molar excess of unlabelled fragments containing a GFI site (CYC1; Fig. 1A, lane 4), a TAF site (ribosomal protein L3; Fig. 1A, lane 6) and a SUF site (ribosomal protein S33; Fig. 1A, lane 11), whereas competition did not occur when each of the competitor DNAs was cleaved in the binding site for the respective factors (Fig. 1A, lanes 5,7,12).

This interpretation of the results was subsequently confirmed by use of short synthetic oligonucleotides, each of which had been designed to specifically bind one of the three factors (see Figs 1B and 2). As can be seen in Fig. 1B (lanes 19–21), the complexes formed with each of the oligonucleotides display the same degree of retardation. Since the oligonucleotides are of comparable lengths, this suggests that the molecular weight of the protein involved is the same in each case. Additional support for this view comes from the observation that the molecular weights established for ABFI, TAF and SUF are in the same range of 130–150 kDa (4,6; J.C. Dorsman, A. Gozdzicka-Jozefiak, unpubl. obs).

Factor		Sequence			Ref.
SUF	-143	ATGCGTG	GTCACTCTAGACG	GCCGCGTCTGTAC	-175	5
SUF*	-143	ATGCGTG	GTCACTCTAGACc	GCCGCGTCTGTAC	-175	
TAF	-236	TAGTA	ATCGTTTTGTACG	TTTTTCAAGAA	-208	4
GFI	-210	AAAATTTCTG	ATCATTCCCAACG	AACCAATAGA	-178	1
GFI consensus			RTCRYNNNNNACG			

Figure 2: *Sequence comparison of factor binding-motifs*
Footprint areas are underlined. Oligonucleotides used in competition studies contained the sequences shown above, extended with SalI (SUF and TAF), or XhoI (GFI) cohesive ends. SUF* is a mutant oligonucleotide that is unable to bind the SUF-factor as the result of a G to C mutation within the consensus region. The GFI site shown is that present in the upstream region of the nuclear gene for subunit II of mitochondrial ubiquinol-cytochrome c reductase. Note that the GFI consensus sequence differs at two positions from that previously proposed (7).

2. Methylation-interference footprinting

One of the most characteristic features of the GF1 consensus sequence is the presence of two short conserved elements separated by a region which is variable in sequence but constant in length (Fig. 2; ref 7). This spacing is readily accounted for by separation of the two elements by exactly one turn of B-helix DNA, suggesting that contacts of GFI with its recognition site involve only one face of the helix. Two observations point in a similar direction. First, as is shown in Fig. 3, results of methylation-interference footprinting show that modification of both elements drastically reduces complex formation. Second, as shown by Buchman et al. (9) and Hamil et al. (10) for ABFI and TAF respectively, point mutations in the T and C residues of the first element reduce binding, while alteration in the spacing of the elements by only one nucleotide abolishes binding (9). In addition substitution of the last G in the consensus sequence by a C prevents binding of the factor (see Figs. 1B and 2).

GFI is clearly a multi-functional DNA-binding protein. As SBF-B, it has been shown to be capable of mediating repression of transcription at the silent mating type locus HMR (14), while as ABFI it has been implicated in DNA replication, since the region B of ARS1, which contains the ABFI binding site, is necessary for optimal ARS function (15). Moreover, the binding site plays a role in the phenomenon of antagonism between HMR-E and CEN6-mediated plasmid segregation (14), which may depend on plasmid attachment to some higher order nuclear structure other than the spindle body.

The role of the factor in binding to the 5'-flanks of other genes has so far been less clear. For example, although the binding site for ABFI in the DED1 gene was capable of stimulating transcription, its effects were observed to be more limited than other UAS elements. A role as an accessory transcription factor, or as boundary factor between adjacent regions of the yeast genome was thus postulated (9). On the other hand, mutational studies have shown that one role of this factor is that of trans-activator of transcription. Binding of TAF or SUF to the upstream activation sites of the genes for ribosomal proteins L3 and S33 can be directly correlated with transcriptional activation (10; W.H.. Mager and R.J. Planta, unpubl. obs) and, as Fig. 4 shows, GFI binds at, or close to the UAS elements of several other genes. In each case, the ability of GFI to exert a particular effect appears to be dependent on interaction with additional proteins. SBF-B, for example, activates

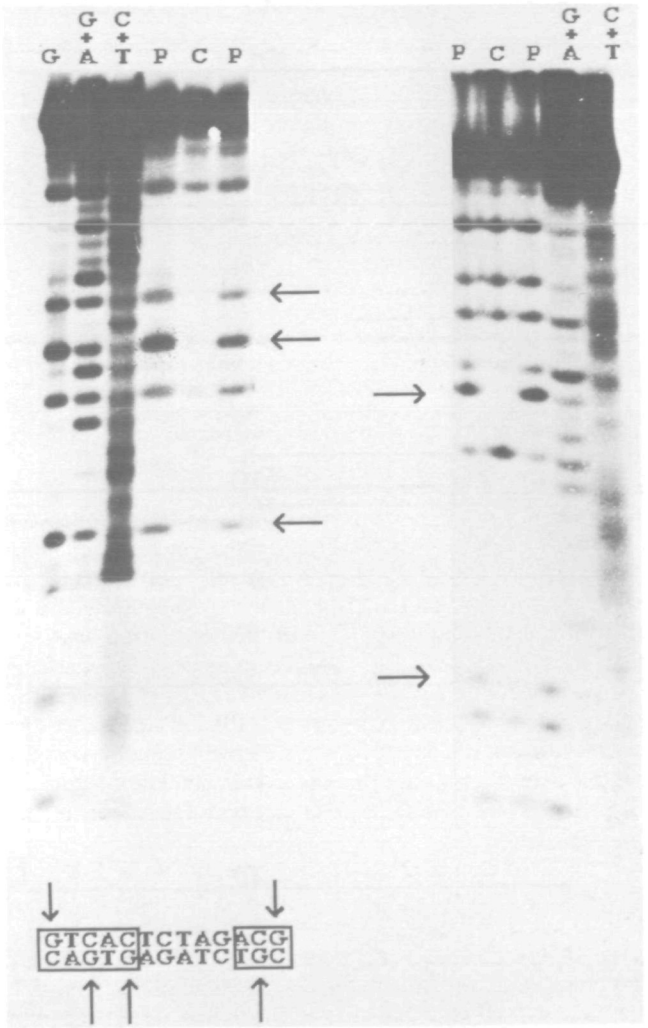

Figure 3: *Methylation interference study of the SUF-binding site.*
Arrows indicate the G- residues whose methylation interferes with complex formation. The upper strand is shown on the right, the lower strand on the left. See also the sequence in the lower part of the figure. Conserved regions of the binding-site are boxed. P= free probe, C= complex. G, G+A, C+T refer to the corresponding Maxam and Gilbert reactions.

transcription at HMR when it alone is bound to its target site (9). However, when bound to DNA in combination with GRF1 (alias RAP1, or TUF), which recognizes the adjacent element E, repression of transcription is observed (14,16).
The competition experiments described here provide direct evidence for the identity of at least three independently identified DNA-binding proteins, but more cases seem likely to exist. One possible additional candidate is an abundant factor binding to the modulator (M) domain of the promoter for the yeast phosphoglycerate kinase gene (17). The domain

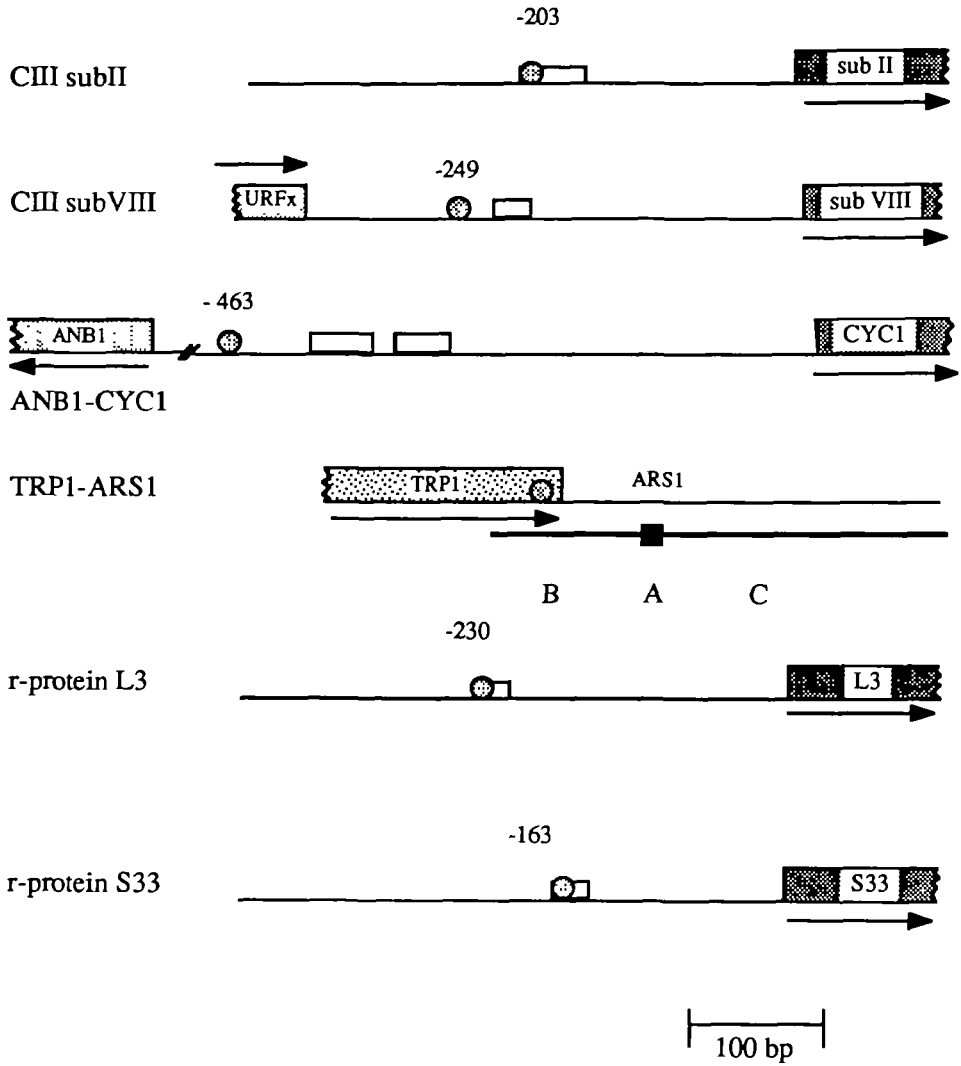

Figure 4: *Sequence organization of the DNA regions tested for GFI binding*
Shaded boxes represent protein-coding regions, with arrows indicating the direction of transcription. White bars denote UAS elements required for transcriptional activation. The sites of GFI binding are shown as shaded circles. For further information on the organization of the regions shown, see ref 19 (gene for subunit VIII ubiquinol-cytochrome c reductase); ref 20 (CYC1 gene); ref 21 (TRP1-ARS1 gene region); ref 10 (L3 gene).

contains a sequence motif that resembles the GFI consensus and interestingly, binding activity of this factor is dependent on the carbon source used for growth, with higher activity being present in glucose-grown cells. The constitutive transcription of genes for ribosomal proteins is also increased in cells grown on glucose, presumably to meet increased demand for protein synthetic capacity in response to increased rate of growth (18). A possible function of GFI and related factors may therefore be to adjust transcriptional levels of

a number of gene families, including those involved in mitochondrial biogenesis (7), to cell growth rate. Further characterization of this factor and its binding specificity will be of great interest.

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