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A Yeast Protein Possesses the DNA-Binding Properties of the Adenovirus Major Late Transcription Factor

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The adenovirus major late transcription factor (MLTF), or upstream stimulatory factor, is a human promoter-specific transcription factor which recognizes the near-palindromic sequence GGCCACGTGACC (R. W. Carthew, L. A. Chodosh, and P. A. Sharp, Cell 43:439–448, 1985; L. A. Chodosh, R. W. Carthew, and P. A. Sharp, Mol. Cell. Biol. 6:4723–4733, 1986; M. Sawadogo and R. G. Roeder, Cell 43:165–175, 1985). We describe here a protein found in the yeast *Saccharomyces cerevisiae* which possesses DNA-binding properties that are virtually identical to those of human MLTF. These two proteins recognize the same DNA-binding site, make the same purine nucleotide contacts, and are affected in the same manner by mutations in the MLTF-binding site.

Studies of yeast and mammalian transcription systems have provided increasing evidence for the evolutionary conservation of essential elements of the eucaryotic transcription apparatus. Beyond the similarities in structure of yeast and mammalian promoters, this conservation appears to extend to the proteins involved in transcription in these two groups of organisms (13), including the subunits of RNA polymerase II (1, 10), the heat shock transcription factor (18), the TATA-box-binding protein (3a), and the transcriptional activators AP-1 and GCN4 (2, 19, 20). Similarly, a yeast transcriptional activator composed of a complex of the HAP2 and HAP3 gene products has been shown to have DNA-binding properties that are virtually identical to those of the multisubunit human CCAAT-binding protein, CP1 (6, 9, 15). Furthermore, the subunits of the yeast and human CCAAT-binding proteins are functionally interchangeable (9). Thus, both the DNA and the intersubunit recognition properties of the yeast and human proteins have been evolutionarily conserved.

The adenovirus major late transcription factor (MLTF) is a 46-kilodalton promoter-specific transcription protein which stimulates transcription in vivo and in vitro from the adenovirus major late promoter, the rat γ-fibrinogen (γ-FBG) promoter, and the mouse metallothionein I promoter (4, 5, 7, 8, 16). Recently, a yeast protein and a human protein have been identified that recognize sequences with a 25-base-pair region present in the GAL2 promoter (3). This 25-base-pair region also contains a sequence related to the conserved yeast centromere DNA element, CDEI, an element which is itself related to the consensus binding site sequence of MLTF. Although the human and yeast proteins both bind within the same 25-base-pair region, the relationship between the recognition sequences of these two proteins has not previously been defined. We show here that a yeast protein possesses DNA-binding properties that are virtually indistinguishable from those of MLTF and thereby provide additional evidence for the evolutionary conservation of sequence-specific DNA-binding proteins in eucaryotic organisms.

The gel electrophoresis DNA-binding assay (4, 11, 12) was used to search for proteins in extracts from the yeast *Saccharomyces cerevisiae* which would specifically recognize an oligonucleotide DNA probe containing a high-affinity MLTF-binding site (Fig. 1a). An oligonucleotide DNA probe, MLP, containing the high-affinity MLTF-binding site present in the adenovirus major late promoter was incubated with the nonspecific competitor DNA poly(dI-dC) · poly(dI-dC) and either a partially purified chromatographic fraction containing human MLTF (Fig. 1a, lane 1) or yeast whole-cell extract (lane 7). Protein-DNA complexes were resolved by electrophoresis through low-ionic-strength native polyacrylamide gels. Addition of partially purified MLTF to the MLP probe generated a protein-DNA complex with mobility and appearance characteristic of purified MLTF (4, 8). Addition of yeast whole-cell extract to the MLP probe generated a single protein-DNA complex with mobility slightly lower than that of the MLTF-DNA complex. To determine whether the binding of the yeast protein to the MLTF probe was specific, binding reactions were performed in the presence of a 40-fold molar excess of an unlabeled competitor DNA fragment containing either the high-affinity MLTF-binding site, MLP, or the double-point mutation of this binding site, MLP-dm, which abolishes MLTF binding. Binding of the yeast protein probe was effectively competed for by the addition of the unlabeled competitor DNA fragment containing the wild-type MLTF-binding site, MLP (Fig. 1a, lane 8). Binding of the yeast protein was not competed for by the addition of the unlabeled competitor DNA fragment MLP-dm (Fig. 1a, lane 9). Control reactions demonstrated that binding of human MLTF was effectively competed for by the addition of the competitor DNA fragment MLP but not by MLP-dm (Fig. 1a, lanes 2 and 3). Thus, this yeast protein specifically recognizes a probe containing a high-affinity MLTF-binding site. The observation that the same double-point mutation which abolishes MLTF binding also abolishes the binding of this yeast protein to the MLP fragment suggests that these two proteins recognize similar features of this sequence. Therefore, we refer to this yeast binding activity as the yeast MLTF-related factor (MRF).

The binding specificities of yeast MRF and human MLTF were further compared by determining the efficiencies with which three mutant MLTF elements derived from the rat γ-FBG promoter were able to compete for the binding of

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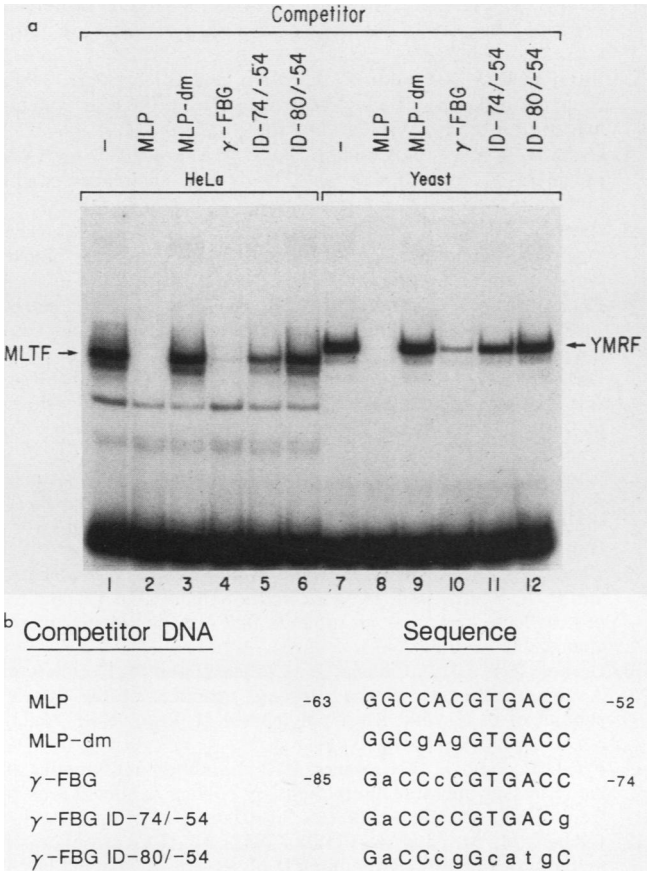

FIG. 1. MLTF and a yeast protein recognize similar features of the MLTF-binding site. (a) Gel electrophoresis DNA-binding assay of complexes formed with a probe containing a high-affinity MLTF-binding site and extract from either HeLa or yeast cells. Either 9 μg of MLTF-containing HeLa fraction AA (lanes 1 through 6) or 5 μg of yeast whole-cell extract (lanes 7 through 12) was added to a mixture of 20,000 cpm (0.2 ng) of the DNA probe, MLP, containing the adenovirus MLTF-binding site, 2 μg of poly(dI-dC) · poly(dI-dC), and either no additional DNA (lanes 1 and 7) or a 40-fold molar excess of an unlabeled competitor DNA fragment. Competitor DNA fragments were derived from either the adenovirus MLP, containing sequences from −47 to −67 (lanes 2 and 8); a mutant adenovirus MLP, containing sequences from −47 to −67 with a double-point mutation in the MLTF-binding site (lanes 3 and 9); the rat γ-FBG promoter, containing sequences from −39 to −95 (lanes 4 and 10); or internal deletion mutants of the γ-FBG promoter that lacked sequences from −54 to −74 (lanes 5 and 11) or from −54 to −80 (lanes 6 and 12). (b) MLTF-like sequences of the DNA competitor fragments used for panel a. Uppercase nucleotides are the same as in the high-affinity MLTF-binding site in the adenovirus MLP; lowercase nucleotides are different from those in the MLTF-binding site in the MLP. Numbers refer to the position of the MLTF-binding site in the MLP or γ-FBG promoter.

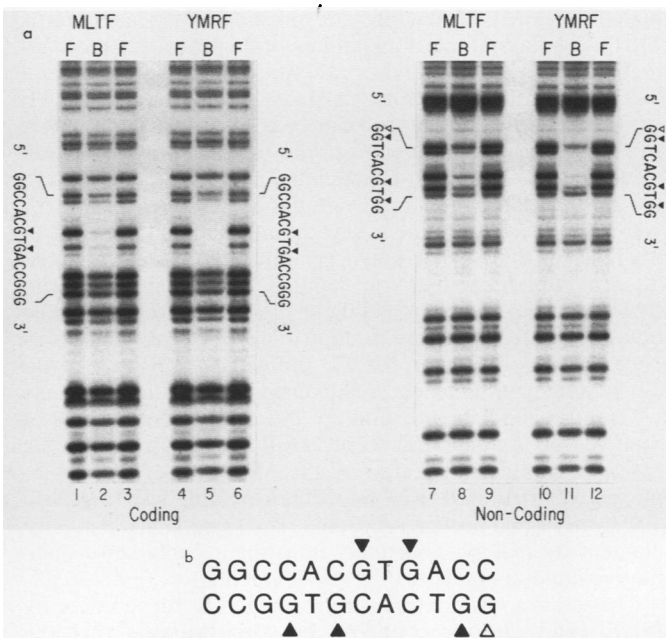

FIG. 2. MLTF and yeast MRF make the same set of DNA contacts with the MLTF-binding site. (a) Methylation interference analysis of the binding of human MLTF and yeast MRF to the adenovirus MLP. A partially methylated MLP probe (400,000 cpm) was incubated in a standard binding reaction with 10 μg of poly(dI-dC)·poly(dI-dC) and either 45 μg of HeLa fraction AA (lanes 1 through 3 and 7 through 9) or 10 μg of yeast whole-cell extract (lanes 4 through 6 and lanes 10 through 12). Bound (B) and free (F) probes were separated by native gel electrophoresis, visualized by autoradiography, eluted, purified, and cleaved with piperidine as previously described (7). An equal number of counts of free and bound DNA were electrophoresed on denaturing polyacrylamide gels. Coding and noncoding sequences are displayed along with a summary of the effects of partial methylation on the binding of MLTF and yeast MRF as below. (b) Summary of effects of methylation on the binding of human MLTF and yeast MRF to the adenovirus MLP. Symbols: ▲, ▼, △, and ▽, residues at which methylation interfered with the binding of MLTF or yeast MRF; closed symbols indicate a greater degree of interference than open symbols.

these proteins to the wild-type MLP probe. A 40-fold molar excess of an unlabeled competitor DNA fragment containing the wild-type γ-FBG MLTF element competed less efficiently than the adenovirus MLTF element for the binding of both human MLTF and yeast MRF to the MLTF probe in a binding reaction containing $MgCl_2$ (Fig. 1a, lanes 4 and 10). The γ-FBG MLTF element differs from the adenovirus MLTF element at two positions in the 5′ half of the binding site (Fig. 1b). This cellular element has been shown to bind MLTF in the presence of $MgCl_2$ with an affinity that is at least 10- to 20-fold lower than the affinity with which the MLP binds MLTF (7). A 40-fold molar excess of an unlabeled competitor DNA fragment containing the γ-FBG internal deletion mutant ID−74/−54 competed less efficiently than the wild-type γ-FBG fragment for the binding of both human MLTF and yeast MRF to the MLP probe (Fig. 1a, lanes 5 and 11). The γ-FBG mutant ID−74/−54 (14) contains an internal deletion which alters the 3′ ultimate nucleotide in the MLTF-binding site (Fig. 1b). This mutation has been shown to decrease MLTF binding and MLTF-mediated transcriptional stimulation from the γ-FBG promoter (7). Finally, addition of a 40-fold molar excess of the γ-FBG mutant ID−80/−54 failed to compete for the binding of either human MLTF or yeast MRF to the MLP probe (Fig. 1a, lanes 6 and 12). The γ-FBG mutant ID−80/−54 (14) contains an internal deletion which alters 5 base pairs in the 3′ half of the MLTF-binding site in the γ-FBG promoter (Fig. 1b). This mutation has been shown to abolish MLTF binding and MLTF-mediated transcription stimulation of the γ-FBG promoter (7). Thus, human MLTF and yeast MRF recognize similar features of the MLTF binding site.

Methylation interference (17) was used to compare the DNA contacts made by yeast MRF and human MLTF on the

high-affinity MLTF-binding site present in the adenovirus MLP (Fig. 2a). An MLP oligonucleotide probe was partially methylated with dimethylsulfate and used as substrate in a binding reaction with either partially purified human MLTF or MRF derived from a yeast whole-cell extract. After separation of free and bound probe fragments by native gel electrophoresis, protein-DNA complexes were excised from the gel. DNA fragments present in these excised complexes were purified, piperidine cleaved, and resolved by electrophoresis in denaturing polyacrylamide gels. Guanine residues at which methylation reduces or abolishes protein-DNA complex formation will be underrepresented in the bound relative to the free fraction. The methylation interference patterns of human MLTF and yeast MRF were virtually identical with respect to the positions of critical contacts within the binding site and to the quantitative effect of methylation at each of these positions. Each of the six essential contacts detected in the MLTF and MRF complexes was centered over the MLTF-binding site (Fig. 2b). Thus, these two proteins recognize the same DNA sequence element by making a virtually indistinguishable set of guanine residue contacts within that element.

The striking similarities between DNA recognition by MLTF and DNA recognition by MRF suggest that the binding domains of these two proteins have been conserved during evolution. Interestingly, a protein which may be similar or identical to MRF has previously been shown to bind to a highly conserved yeast centromere DNA element which is important for centromere function in *S. cerevisiae* (3). Thus, these results raise the possibility that MLTF plays a role in the function of mammalian centromeres in addition to its role in transcription. Nevertheless, partially purified yeast MRF could not be shown to stimulate transcription from the adenovirus MLP in an in vitro transcription system derived from HeLa cells (data not shown). As such, the possibilities remain that yeast MRF is not a transcriptional activator and that human MLTF and yeast MRF have made use of the same DNA recognition domain to perform different cellular functions.

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