A yeast homolog of the human UEF stimulates transcription from the adenovirus 2 major late promoter in yeast and in mammalian cell-free systems

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ABSTRACT

We report the identification and purification of a yeast factor functionally homologous to the human upstream element factor (UEFh). Although the yeast protein (UEFy) has a higher molecular weight than the HeLa UEF (60 kD versus 45 kD) both have identical DNA-binding properties: the purified UEFy recognizes the Adenovirus 2 (Ad2) major late promoter upstream element (MLP-UE ; from nucleotide −49 to −67) as well as the IVa2 upstream element (IVa2-UE; from nucleotide −98 to −122) with a higher affinity for the MLP-UE than for the IVa2-UE. Based on its DNA binding specificity, size and thermostability, the UEFy protein appears also similar or equivalent to the centromere binding protein CP1. In a competition assay with oligonucleotides containing the MLP-UE binding site, a drastic reduction of Ad2 MLP transcription was observed both in a HeLa and in a yeast cell free system, which was restored by addition of either the purified UEFh or UEFy proteins. We conclude that both UEFh and UEFy activate transcription from the Ad2 MLP upon binding to the upstream element, whatever is the *in vitro* cell-free system (yeast or HeLa). This indicate that some regulatory function represented by the upstream element and its cognate factor, is well conserved between human and yeast.

INTRODUCTION

Eukaryotic promoters result from the assembly of various motifs, each composed of a short DNA sequence. The TATA-box, the most conserved of these motifs, is located 25−30 bases pairs (bp) and 40−120 bp upstream of the transcription start site in higher and lower eukaryotes respectively. A transcription factor that specifically binds to this sequence (BTF1), has been identified in HeLa cells (1), Drosophila (2) and more recently in yeast (3, 4). In addition to the transcription factors involved in the basal transcription machinery (5), a number of proteins which bind further upstream from various promoters have been characterized in higher eukaryotes. Examples of such proteins are Sp1, HSTF, CBP and UEF (for a review see 6, 7). On the other hand, the basic molecular mechanisms that control initiation of transcription are known to be conserved from yeast to human: i) the yeast transcriptional transactivator GAL 4 can activate a minimal TATA-box containing promoter in HeLa cells (8) and a human inducible enhancer factor, the oestrogen receptor, can activate a minimal promoter in yeast (9), ii) the subunits of a yeast and a mammalian CAAT-box factor are functionally interchangeable in a DNA binding assay (10), iii) a yeast TATA-box recognizing protein is able to substitute for the mammalian BTF1 (TFIID) in an *in vitro* transcription system (3, 4). These results prompted us to look for the presence of yeast factors functionally homologous to other (general or specific) transcription factors.

The Adenovirus-2 major late promoter (Ad-2 MLP) has an upstream element (MLP-UE) (11) which is also found in several mammalian genes (12, 13) and which is the target of the Upstream Element Factor (UEF) (14, 15) also called MLTF (16) or USF (17). The binding of this protein to the MLP-UE produces a 3 to 5-fold stimulation of MLP in a HeLa *in vitro* transcription system. This factor which has been purified from HeLa cells (5, 18, 19, 20) has also been detected in lymphocytes (our unpublished results). A yeast centromere binding protein was also found to recognize sequences homologous to the MLP-UE (21, 22). In this report, we describe the purification of UEFy, a yeast factor that is functionally equivalent to the HeLa UEF (UEFh) based on its specific DNA binding properties and its activity in a yeast and in a HeLa in vitro transcription system.

MATERIAL AND METHODS

Purification of the yeast UEF

An S-100 extract (5 ml; 37 mg/ml) of the yeast *S. cerevisiae* (strain C13 AB Y) prepared as previously described (23) was

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dialyzed 12 h against buffer A (50 mM Tris-HCl pH 7.9, 50 mM KCl, 17.4% glycerol, 2 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM PMSF) and incubated 15 min at 4°C with 100 μg poly(dI-dC)(dI-dC)/ml (Pharmacia) and 0.1% NP-40 and then 15 min at 4°C with 1 ml of the sequence-specific DNA affinity resin [prepared with the oligomer of the Ad2 MLP-UE (from nucleotide −41 to −71 relative to the MLP initiation site) as described in 24]. The resin was then packed in a Pasteur pipette and washed with 10 column volumes of buffer B (50 mM Tris-HCl pH 7.9, 50 mM KCl, 17.4% glycerol, 2 mM $MgCl_2$, 1 mM DTT, 0.1% NP-40), 5 column volumes of buffer B containing 0.2 M KCl and 5 column volumes of buffer B containing 1 M KCl. After dialysis against buffer B, the 1 M KCl fraction was incubated 15 min at 4°C with 50 μg poly(dI-dC)(dI-dC)/ml and reapplied on the DNA affinity column. The 1M KCl eluate from this second affinity column (~2 μg/ml) was dialysed against buffer C (50 mM Tris-HCl pH 7.9, 50 mM KCl, 25% glycerol, 0.5 mM DTT, 0.1 mM EDTA) and stored at −80°C. Protein were analysed by electrophoresis on 9% SDS-polyacrylamide gels. The UEFh was purified by the DNA affinity column using the same procedure as above but starting with the SP0.35 fraction prepared as described previously (5, 20). The purified UEFh fraction (5 μg/ml) contains two polypeptides of 43 and 45 kD (20).

DNAse I footprinting and gel retention assays

For labelling the non-coding strand, pM677 (15) was digested by SacII at −245, dephosphorylated with calf intestinal phosphatase (Boehringer), 5′ end-labelled with [gamma ^{32}P] ATP and T4 polynucleotide kinase and digested with BamHI at +33. The resulting SacII-BamHI (−245/+33) DNA fragment was purified on a 6% acrylamide gel. For labelling the coding strand, pM677 was linearized at the BamHI site at +33 , ^{32}P phosphorylated, digested by SacII at −245, and the fragment was purified as described above.

DNAse I footprinting reaction (15) consisted of a 10 min preincubation at 24°C in a 18 μl reaction volume containing variable amounts of the protein fraction, ~1 ng (10000 cpm) of the 5′ end labelled DNA fragment, 50 ng of poly(dI-dC)(dI-dC), 4 mM $MgCl_2$ in buffer D (50 mM Tris-HCl pH 7.9, 50 mM KCl, 8.7% glycerol, 0.1 mM EDTA, 0.5 mM DTT). After the preincubation, 2 μl of DNAse I (10 μg/ml) (Worthington) was added for 2 min and DNA digestion products were analyzed on 8% acrylamide 8.3 M urea gels, followed by autoradiography. Sequencing reaction were performed as described in the Maxam and Gilbert DNA sequencing protocol (25).

The gel retention assay (26) consisted of a 15 min incubation step at 24°C identical to that of the footprinting assay in a 10 μl reaction volume containing ~0.1 ng of the end-labelled DNA fragment (pM677 SacII-BamHI) and 100−250 ng of poly(dI-dC)(dI-dC) (Pharmacia). 1 μl of 87% glycerol was added and the mixture electrophoresed immediately on a 4.5% polyacrylamide gel (ratio polyacrylamide-bisacrylamide: 80−1). The electrophoresis buffer was 6.7 mM Tris-HCl pH 7.9, 3.3 mM sodium acetate, 1 mM EDTA. The gel was dried and autoradiographed.

In vitro transcription

Yeast nuclei were prepared from the yeast strain S. cerevisiae C13 AB Y, essentially as described in 27. Details and characterization of the nuclear extracts will be published elsewhere (B.B. Amati, S.M. Gasser, A. Sentenac, R. Stalder, J.M. Verdier, manuscript in preparation). Briefly, the nuclei (from 30 g of cells) were resuspended in 15 ml of 10 mM Tris-acetate pH 7.9, 50 mM KOAc, 10 mM $MgSO_4$, 20% glycerol, 3 mM DTT, 2 mM EDTA, 1 mM PMSF and proteases inhibitors (0.04 mM benzamidine, 0.04 μM pepstatine A, 1.2 nM leupeptine, 0.15 nM chymostatine) and extracted with 0.5 M NH_4SO_4 final by addition of a 3 M neutralized stock solution. After 30 min. under stirring, the solution was centrifuged 30 min. at 35400 rpm in a sw41 rotor at 2°C. The supernatant was precipitated by addition of 0.35 g NH_4SO_4/ml of solution, centrifuged 20 min. at 25000 rpm in a sw41 rotor at 2°C, and the pellet was resuspended in 2 ml of 20 mM Hepes KOH, 10 mM $MgSO_4$, 10 mM EGTA, 20% glycerol, 5 mM DTT, 1 mM PMSF and proteases inhibitors as above, and dialysed against the same buffer.

In vitro transcription in HeLa WCE was as described in 5. For yeast *in vitro* transcription system, the reaction mixture contained 60 μg of nuclear extract, 40 mM Hepes-KOH pH 7.6, 5 mM $MgSO_4$, 5 mM $Mg(OAc)_2$, 70 mM KOAc, 5 mM EGTA pH 8, 2.5 mM DTT, 4 mM phosphoenolpyruvate pH 7, 0.4 mM each of ATP, UTP, GTP and CTP, 10% glycerol and 100 ng of template.

The template used was p17-UE (generous gift from J. White and C. Brou) and will be described elsewhere ; briefly the plasmid p17-UE (5.2 Kb) has a 17 mer GAL 4 binding site and a UEF binding site (separated by 32 bp) upstream of the MLP TATA-box. These promoter sequences are fused to the rabbit β-globin gene. For quantitative S1 nuclease analysis, a ^{32}P 5′ end-labelled single stranded probe was created by extension of a primer (complementary to +39 to +60 of rabbit β-glogin gene) along a single stranded M13mp19 template containing the 1250 bp EcoRI fragment from pAL10 (M. Ponglikitmongkol, J. White and P. Chambon, manuscript in press, EMBO.J.). Yeast or HeLa extracts were preincubated 15 min. at 22°C with the competitor (200 ng ; 100 fold molar excess) and the purified UEFh or UEFy, and then incubated 45 min. at 22°C with 350 ng of template. Hybridization and S1 nuclease treatment were as described in 28. Initiation at the +1 MLP initiation site should yield a 115 nucleotides protected fragment.

Competition assays were done with the following synthetic oligonucleotides (only the non-coding strand is shown) : MLP-UE wt: 5′ TTTATAGGTGTAGGCCACGTGACCGGGTGT 3′; MLP-UE mut: 5′ TTTATAGGTGTAGACTACGTGACCGG-GTGT 3′ (point mutations at −60 and −62 underlined ; see 15).

RESULTS

Purification of a yeast homolog of the HeLa UEF

The binding activity of a yeast UEF homolog (UEFy) was monitored by both gel retention and DNAse I footprinting assays on the Ad2 MLP-UE (using a DNA fragment encompassing the sequence −245 to +33 relative to the MLP initiation site). In order to isolate this UEFy, a yeast S-100 extract was applied twice to a sequence-specific DNA affinity column (containing a multimer of the Ad2 MLP-UE from nucleotide −41 to −71; see material and methods). A protein present in the 1 M KCl fraction of the second DNA-affinity column was able to form a nucleoprotein-complex with the labelled DNA (Figure 1B, lane 3). This complex migrated slightly faster than the yeast S-100 complex (Figure 1B, lane 2) suggesting the elimination of other(s) DNA-binding protein(s). As shown by SDS-PAGE, this fraction contained a major polypeptide of molecular weight 60 kD (Figure

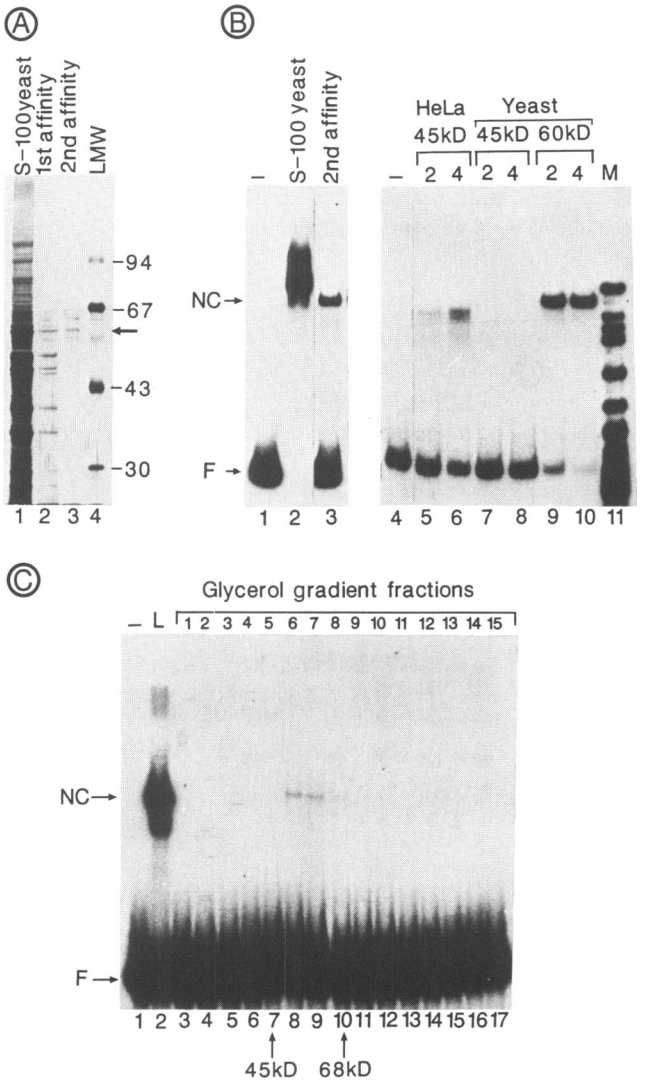

Figure 1: A. SDS polyacrylamide gel analysis of each stage of purification. Lane 1 : 0.5 μl of yeast S-100 extract. Lane 2 and 3 : 100 μl of the 1 M KCl eluate of the first and second DNA affinity column respectively. Lane 4 : molecular weight markers. The arrow indicates the 60 kD polypeptide (UEFy). **B.** Nucleoprotein complexes formation between the yeast or HeLa UEF and the ^{32}P labelled SacII-BamHI fragment of pM 677. Binding reaction (10 μl) included 250 ng (lanes 1−3) or 100 ng (lanes 4−10) of poly(dI-dC)(dI-dC), 0.1 ng of the labelled template and either 0.1 μl of yeast S-100 extract (lane 2), 0.05 μl of the purified UEFy (lane 3), 2 and 4 μl of the renatured 45 kD HeLa UEF (lanes 5−6), 2 and 4 μl of the renatured yeast 45 kD region (lanes 7−8), 2 and 4 μl of the renatured 60 kD yeast polypeptide (lanes 9−10) or no protein (lanes 1 and 4). Lane 11 : size markers : ^{32}P end labelled MspI fragments of pBR322. NC and F indicate the nucleoprotein-complexes and the free DNA respectively. **C.** Analysis by glycerol gradient centrifugation of the UEFy. 150 μl of the purified UEFy were loaded on a 5−25% glycerol gradient and centrifuged at 58 kD for 10 h at 4°C in a sw.60 rotor. 15 fractions were collected from the top of the tube and tested in the gel retention assay. Lane 1 : free DNA. Lane 2 : load (L): 0.1 μl of the purified UEFy. Lanes 3−17 : 5 μl of each of the 15 glycerol gradient fractions. The positions of BSA (68 kD) and ovalbumin (43 kD) in parrallel marker gradients are noted.

1A, lane 3). [The two additional bands visible in the 60 kD region are the silver staining artefacts previously described in the litterarure (29) and are also visible in the markers lane]. To confirm that this purified protein possesses DNA-binding activity, the 60 kD polypeptide was renaturated following elution from the SDS polyacrylamide gel (30). In a gel retention assay, this renaturated 60 kD polypeptide formed a complex with the labelled DNA (Figure 1B, lanes 9−10) which migrated with the same electrophoretic mobility as the complex formed with the native purified protein. No DNA-binding activity was recovered from other areas of the SDS polyacrylamide gel, including the 45 kD region (lanes 7−8) which corresponds to the molecular weight of HeLa UEF (UEFh) (compare with the complex formed with the renaturated 45 kD UEFh in lanes 5−6). By glycerol gradient sedimentation of the purified UEFy, the Ad2MLP-UE-binding activity was detected in fractions corresponding to a molecular weight of 58 ± 8 kD (Figure 1C, lanes 8−10) in complete agreement with the molecular weight observed on SDS polyacrylamide gel. This suggests that, in solution, UEFy is a monomer, as is UEFh (31). Starting with a yeast S-100 extract, UEFy was purified 12,000 fold by DNA affinity chromatography, with an estimated yield of 50% (see Table 1). The high recovery is probably due to the fact that UEFy was purified in a very short time, from a protease-deficient yeast strain, with a simplified purification procedure (compare with the purification process of UEFh ; see 5, 18, 19, 20).

The yeast and human UEF interact with identical DNA sequences

In order to precisely delineate the DNA region that interacts with the yeast protein, DNAse I footprint experiment was performed. On the non-coding strand, the purified UEFy protected the DNA from nucleotide −50 to −66 (MLP-UE) and from nucleotide −100 to −122 (IVa2-UE) (Figure 2A, lane 4−6). On the coding strand, the MLP-UE was protected from nucleotide −49 to −67 (with a strong DNAse I hypersensitive site at −69) and the IVa2-UE from nucleotide −98 to−120 (Figure 2A, lanes 10−12). In addition, in a DMS protection experiment performed on the coding strand with UEFy or UEFh, the same G residues are either protected (at positions −53, −58 and −60) or more accessible (at positions −52 and −61) to DMS, as was previously observed (14, 22). In spite of the difference in size of the yeast and human protein, the DNAse I footprinting pattern as well as DMS protection pattern (not shown) were identical to those previously described for UEFh (compare lanes 2 and 6, Figure 2C; see also 15, 20 for an extensive description of the UEFh footprint; for a summary of the footprinting data see Figure 2B).

The binding specificity of UEFy was determined by a competition assay. A 50-fold molar excess of the unlabelled wild-type oligonucleotide (MLP-UE wt ; see material and methods) competed for the binding of both UEFh (Figure 2C, lane 3) and UEFy (lane 7) whereas a similar excess of the mutated fragment [the MLP-UE mut. has a double point mutation at −60/−62 which was previously shown to prevent the binding of UEF to the MLP-UE (15)] had no effect on the footprinting pattern of either UEFh (lane 4) or UEFy (lane 8).

The affinity of UEFy for the MLP-UE and IVa2-UE was studied in parallel by gel retention and DNAse I footprint assays using a labelled DNA fragment which contains the two binding sites. When increasing amounts of the purified UEFy were added to the labelled DNA, we first observed the formation of nucleoprotein-complex I (Figure 2D, lane 2) which disappeared progressively, with a concomittant appearance of nucleoprotein-complex II (lanes 3−7). At a low protein concentration (1 ng), when only complex I was apparent (Figure 2D, lane 2), only the MLP-UE was protected (Figure 2A, lanes 4 and 10) ; at a higher concentration of UEFy (4 ng), complex II was detected (Figure 2D, lane 5) and both MLP-UE and IVa2-UE were

Table 1. Summary of UEFy purification

	volume (ml)	protein (mg/ml)	total protein (mg)	units (a)	specif. act. (units/mg)	purification (fold)	yield (%)
Yeast S-100	5	37	185	50000	270	–	–
DNA-affinity 1	4	0.02	0.1	33000	3.3×10^5	1200	66
DNA-affinity 2	4	0.002	0.008	26600	3.3×10^6	12000	53

a) One unit is defined as the amount of protein that produces retention of 0.05 ng of the pM677 SacII-BamHI fragment in the gel retention assay.

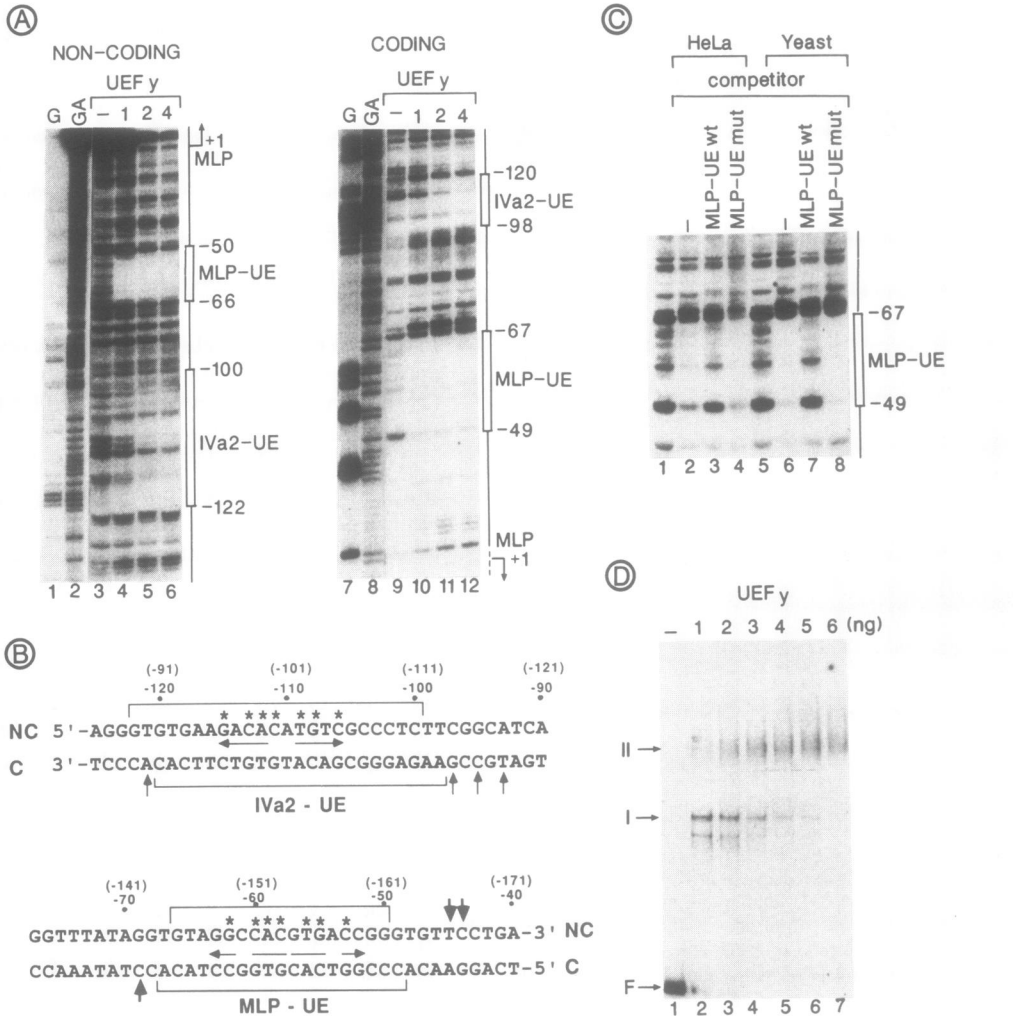

Figure 2: A. DNAseI footprint on both strands of the MLP-IVa2 intergenic region. Increasing amounts of UEFy, as indicated (in ng), were incubated with ~1 ng of the DNA fragment ^{32}P-labelled either on the non-coding strand (lanes 1–6) or on the coding strand (lanes 7–12). Lanes 1–2 and 7–8 show the sequence ladder of the G and GA Maxam and Gilbert reaction. Lanes 3 and 9 : naked DNA. The positions of the MLP-UE, IVa2-UE and MLP initiation sites are indicated. **B.** Summary of DNA sequences in the MLP-IVa2 intergenic region protected by the UEFy in DNAse I footprinting. The nucleotide sequences of the RNA non-coding (NC) and coding (C) strands of the MLP upstream regions are shown. The region protected from DNAse I digestion by factor binding are shown by brackets and DNAse I hypersensitives sites are indicated by arrows. The bases pointed out by stars denote the homology between the two protected regions. The regions exhibiting a dyad symetry are indicated by arrows pointing in opposite orientations. The numbers above the sequence show the positions with respect to the MLP initiation site. The position of the same sequences with respect to the IVa2 initiation site are given by the numbers in parentheses. **C.** Sequence specificity of the UEFy. 1 ng of either the purified HeLa UEF (lanes 2–4) or the purified yeast UEF (lanes 6–8) were incubated with ~1 ng (10000 cpm) of the pM677 SacII-BamHI DNA fragment (^{32}P labelled on the coding strand) and a 50 fold molar excess of either the wild type MLP-UE from nucleotides −41 to -71 (MLP-UE wt ; lanes 3 and 7) or the fragment mutated at positions −60 and −62 (MLP-UE mut ; lanes 4 and 8). Lanes 1 and 5 : naked DNA. DNAse I footprint was performed as described in Material and Methods. The position of the MLP-UE is indicated. **D.** Formation of nucleoprotein complexes between the yeast UEF and the DNA fragment encompassing both upstream elements (MLP-UE and IVa2-UE). Increasing amount of purified UEFy, as indicated (in ng), were incubated with the ^{32}P-labelled SacII-BamHI fragment of pM677. Free (F) and protein-bound DNA (complexes I and II) were separated on a non denaturing polyacrylamide gel. The nucleoprotein-complex which migrates faster than complex I probably results from partial proteolysis of UEFy (see figure 3B, lane 3).

protected (Figure 2A, lanes 6 and 12). This result was exactly identical to what was observed with the UEFh (20). Both complexes can be competed out by the MLP-UE wild-type oligonucleotide, but neither by the MLP-UE mutant nor by poly(dIdC) (data not shown). Thus, UEFy behave like UEFh toward the two binding sites; it binds with a higher affinity to the MLP-UE (K_D: $\sim 10^{-11}$ M) than to the IVa2-UE (K_D: $\sim 10^{-10}$ M) (31 and data not shown). Moreover, no cooperativity was apparent upon simultaneous binding of UEFy to these two sites as was observed with UEFh (20).

DNA-binding properties of the UEFy and UEFh

Many sequence specific DNA-binding proteins involved in transcriptional control, have structurally distinct DNA binding and activation domains. To determine whether UEFy (60 kD) and UEFh (45 kD) have a protease resistant DNA-binding domain of identical size, the purified proteins were complexed with the labelled DNA (at a low protein concentration, to restrict binding to the MLP-UE; see Figure 2D lane 2) and digested with increasing concentrations of proteinase K. The resulting digested complexes were analyzed using the gel retention assay. In both cases (Figure 3, panel A for UEFh and panel B for UEFy), three distinct complexes appeared sequentially (designated A, B, C for UEFh and A′, B′, C′′ for UEFy). Complexes A and A′ (lane 2 in panel A and B, respectively) correspond to the initial complexes in the absence of proteinase K treatment. As the concentration of proteinase K increased, complexes A and A′ were first transformed into transient complexes B and B′, respectively, (panel A, lanes 3−4 for UEFh; panel B, lane 4 for UEFy), which were themselves later transformed into complexes C and C′′, respectively, (panel A, lanes 4−7 for UEFh; panel B, lanes 5−7 for UEFy). Higher concentrations of proteinase K led to the disappearance of all nucleoprotein complexes (data not shown). A DNAse I footprint performed in parallel on the three proteinase K-treated complexes showed that, in all cases, the protection was identical to that obtained with the intact protein (see Figure 2A and data not shown). This indicated that the faster-migrating complexes were indeed derived from the slower-migrating ones and that they contained the UEF DNA binding domain. Thus, in both cases, a limited domain of the protein interacts with the DNA. However, as judged from the slower migration of each yeast complex compared to its HeLa counterpart (compare the migration of C with that of C′′ relative to the size marker in panels A and B, respectively) the DNA-binding domains of the yeast and HeLa proteins somewhat differed either by their size and/or by their charge . An identical pattern of nucleoprotein complexes was obtained when the proteins were treated by proteinase K before being complexed with the DNA (data not shown) which suggests that the DNA did not detectably protect the protein.

To further compare the DNA-binding properties of UEFh and UEFy, the stability of the complexes was analyzed under various experimental conditions. A number of transcription factors are metallo-proteins or require metal ions for their activity. The effect of Mg^{++} concentration on the UEF-DNA interaction was studied in gel retention assays. The Mg^{++} concentration required for optimum binding was between 3 and 4 mM for UEFh and between 1 and 2 mM for UEFy (Figure 3, panel C). More strikingly, in the absence of Mg^{++}, the binding of UEFh to the MLP probe was weak (10% of the optimal complex formation),

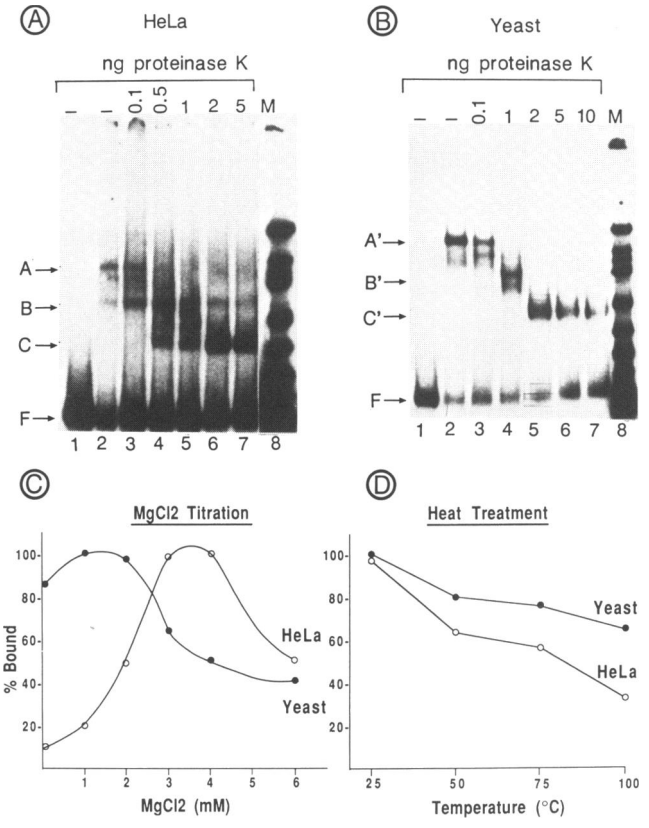

Figure 3: DNA binding properties of UEFh and UEFy. **A** and **B:** Proteinase K sensitivity of the HeLa or yeast nucleoprotein complexes. Nucleoprotein complexes were formed on the MLP-UE with 1 ng of either the HeLa (panel A) or yeast (panel B) UEF and incubated for 5 min. at 24°C with increasing amount of proteinase K (as indicated in ng). The resulting digested complexes were immediately separated by the gel retention method. The three complexes have been called A, B, C for HeLa and A′, B′, C′′ for yeast (the yeast nucleoprotein-complex A corresponds to complex I in fig 2D). F: free DNA. Lanes 8: size markers as in figure 1B. **C** and **D:** Physical parameters of UEFh or UEFy binding to the MLP-UE determined by the gel retention assay. **Panel C:** the nucleoprotein-complexes were formed in the presence of various concentrations of Mg^{++} for 15 min at 24°C with either UEFh (HeLa) or UEFy (Yeast). **Panel D:** either UEFh (HeLa) or UEFy (Yeast) were heated for 10 min. at the indicated temperature before complexed with the DNA. In all cases, reaction parameters and gel electrophoresis were as described in materials and methods. Quantitation of binding products were done by scanning of the autoradiograms.

whereas there was only a slight effect on the binding of UEFy (86%).

Dissociation rate constants were measured in the presence of a 50-fold molar excess of the unlabelled MLP-UE binding site in the presence or in the absence of Mg^{++}. The half-life of the UEFh-DNA complex was 15 min in the absence of Mg^{++} and was reduced to 5 min in the presence of the optimal Mg^{++} concentration. This difference was also observed with UEFy: half-lives of 6 min and less than 1 min were estimated in the absence or in the presence of Mg^{++} respectively (data not shown). Heat treatment of the two factors showed that both proteins are quite stable; even after 10 min at 100°C, the HeLa and yeast UEF retained 30% and 65% of their DNA-binding activity, respectively (Figure 3, panel D).

UEFy activates transcription in vitro

It was previously demonstrated that addition of UEFh to a HeLa

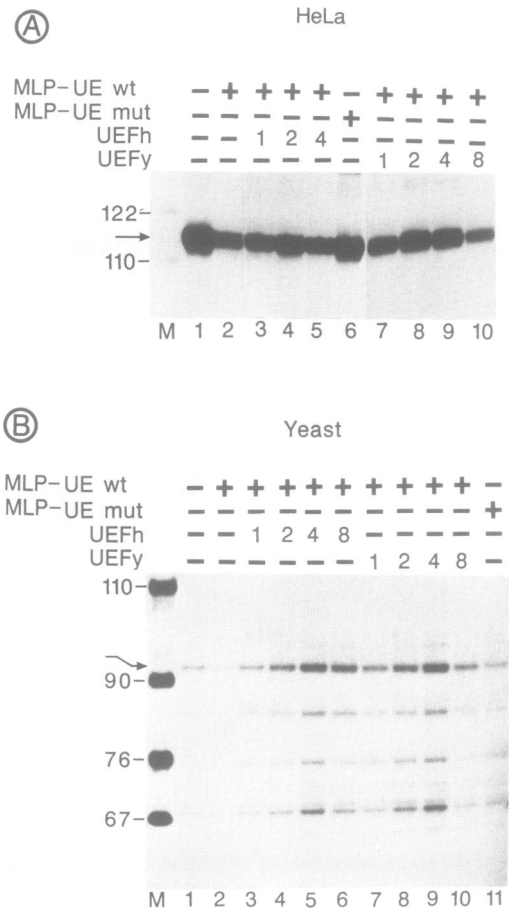

Figure 4. Stimulation of Ad2 MLP *in vitro* transcription by the UEFy or UEFh in a HeLa WCE or a yeast nuclear extract. Transcription from the Ad2 MLP in the HeLa WCE (panel A) or in the yeast nuclear extract (panel B) was detected by S1 nuclease analysis. Reactions were carried out as described under Materials and Methods. The oligonucleotides MLP-UE wt (200 ng) or MLP-UE mut (200 ng) (100 fold molar excess), the UEFy and the UEFh (indicated in μl) have been included in the reaction as indicated at the top of the panel. The arrows indicate the specific protected RNA in HeLa (115 nucleotides ; panel A) and the longer RNA protected in yeast (92 nucleotides ; panel B). Size markers (M) are MspI fragments of pBR322 and their length in nucleotides is noted on the left.

in vitro transcription system stimulates Ad2 MLP transcription (5, 18, 19, 20). The similarity of DNA-binding activities between UEFy and UEFh suggests that the yeast protein could have a stimulatory function in transcription. Yeast nuclear extracts which are able to initiate accurately from yeast promoters and to respond to upstream activators have been described recently (32−34). We observed that, while in the HeLa *in vitro* system, initiation take place 30 bp downstream of the TATA box, in our yeast nuclear extract, initiation from the MLP occurs approximately 55 bp downstream of the TATA element. In fact, three others initiation sites (at approximately 62, 71 and 76 bp downstream of the MLP TATA element) were detected by S1 mapping (Fig 4B, lane 1). Production of all of these transcripts was sensitive to alpha-amanitin at 10 μg/ml, a concentration known to inhibit RNA polymerase B *in vitro* (data not shown). To investigate the role of UEFy in the Ad2 MLP transcription, we have used a transcription competition assay since no reconstituted system was yet available from yeast. The same experiment has been done in parallel with HeLa and yeast extracts. Preincubation of the MLP-UE wt oligonucleotide with the yeast (fig 4B, lane 2) or the HeLa (Fig 4A, lane 2) extract, led to a marked reduction of the MLP transcription, while addition of the MLP-UE mut oligonucleotide has almost no effect on the extent of transcription (Fig 4A, lane 6 and fig 4B, lane 11 for HeLa and yeast, respectively). When transcription reaction containing inhibitory amounts of the MLP-UE wt competitor were supplemented with either UEFh or UEFy, transcription from the MLP was stimulated both in HeLa (Fig 4A) and in yeast (Fig 4B) extracts. Thus the stimulation of MLP transcription was independant of the source of purified factors or extracts. For both UEFh and UEFy, the stimulatory effect appeared much more efficient in the yeast extract since addition of either UEFh or UEFy not only restored the basal transcription of the MLP, but even improved its transcription efficiency (fig 4B, compare lanes 1, 5 and 9) while in the HeLa system this level was not fully restored (fig 4A, compare lanes 1 and 2 with lanes 4 and 8, respectively) ; this suggest that in our yeast extract, the UEF is in limiting amount. However, we cannot exclude that such a difference in the response to UEF is due to a difference in the mechanism of stimulation. Addition of higher amounts of either UEFh or UEFy in either HeLa or yeast, produces a decrease in transcription (Fig 4A for HeLa, lane 5 for UEFh and lane 10 for UEFy ; and Fig 4B for yeast, lane 6 for UEFh and lane 10 for UEFy). This effect can be due to a squelching mechanism as recently described (35, 36).

Taken together, these results demonstrated that UEFh and UEFy are functionnaly interchangeable in the yeast and HeLa *in vitro* transcription systems, which strongly suggest that a similar mechanism of stimulation is used in HeLa and yeast.

DISCUSSION

We and others have previously reported the purification from HeLa and the stimulatory transcription activity of the UEF (UEFh) toward the Ad2 MLP. We report here the purification from yeast of a factor (UEFy) functionally similar to the UEFh. Starting from a yeast S-100 extract containing 185 mg of protein, the purification was performed in two steps using a sequence specific DNA affinity column and gave 8 μg of UEFy (purity $>$ 95%) with an estimated yield of 50%. The UEFy protein, which exists as a monomer in solution, displays an apparent molecular weight of 60 kD as determined by SDS-PAGE and glycerol gradient centrifugation. We have compared the DNA-binding properties of the yeast factor with those of the human factor. As previously observed with the HeLa UEF, the yeast factor UEFy recognizes two binding sites of the MLP-IVa2 intergenic region with an identical pattern: the MLP-UE (from nucleotide −49 to −67) and the IVa2-UE (from −98 to −122 relative to the MLP initiation site) (see Figure 1 and ref 20). Both factors have a stronger affinity for the MLP-UE (K_D: $\sim 10^{-11}$ M) than for the IVa2-UE (K_D: $\sim 10^{-10}$ M) (18, 31 and unpublished data). Althought the IVa2-UE presents a sequence homology with the MLP-UE, various nucleotide changes at critical positions could be responsible for this difference in affinity. We also showed by S-1 mapping analysis that UEFy can stimulate *in vitro* transcription from the Ad2 MLP in a yeast nuclear extract, as well as in a HeLa WCE. Likewise, UEFh can stimulate *in vitro* transcription from the Ad2 MLP in the yeast and in the HeLa extracts (Figure 4). Since our S-1 mapping template also contained a GAL4 binding site which may inluence the transcription, we have also reproduce the stimulation of *in vitro* transcription in a yeast nuclear extract by the UEFy, using

the G-free cassette assay with the plasmid pML$(C_2AT)_{19}$ (37 and data not shown). In both S-1 mapping analysis and G-free cassette assay, the decrease of transcription was observed by competition with the wild-type MLP-UE oligonucleotide, but not with the mutated MLP-UE oligonucleotide (Figure 4 and data not shown). In addition, while increasing amounts of purified UEF restore the transcription level in the presence of the wild-type competitor, there was no effect in the presence of the mutated oligonucleotide (Figure 4 and data not shown). From the results presented here we can postulate that some features of the UEF factor must be conserved between yeast and man to allow it to interact with both basic transcription machinery. However, using the reconstituted mammalian basic system (BTF2, BTF3, STF, RNA polymerase B) (5) with either the mammalian or yeast BTF1, we have not been able, under our experimental conditions, to detect a stimulation of transcription by the UEFy. This may be due to the absence or low abondance in the reconstituted system of some additional transcription factor (probably lost or diluted during the purification process) required to mediate an optimal stimulation by the UEF. Indeed, in support of this hypothesis, it has to be noted that the stimulation by the purified UEFh in a HeLa reconstituted system was always weaker ($\sim$ 2-fold) than with crude fractions of the UEFh ($\sim$ 10 fold) as described by us (5, 20) as well as others (18, 19). This additional protein may be required to allow the interaction of the UEF with the basic transcriptionnal machinery.

Interestingly, the consensus sequence of the Ad2 MLP UEF ($CAC^A/_GTG$) is very similar to the consensus binding site of the yeast centromere binding protein CP1 (21). The CP1 protein has been recently purified as a 58 kD (38) or 64 kD (39) polypeptide and shown to be required for the optimal function of the yeast centromeres (38). Furthermore, the CP1 consensus sequence was noted upstream of several yeast genes (21) which suggest a possible role for CP1 as transcription factor, a hypothesis which is supported by the present work, if it is established that CP1 and UEFy factors correspond to the same protein. However, others yeasts proteins have been found to recognize a similar DNA sequence (40–41) but display a different molecular weight.

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