Volume 321, number 2,3, 153–158 FEBS 12368 April 1993

Identification of a protein from *Saccharomyces cerevisiae* with E2F-like DNA-binding and transactivating properties

Bernard Mai and Martin Lipp

Institut für Biochemie der Ludwig-Maximilians-Universität München, Am Klopferspitz 18a, D-8033 Martinsried, Germany

Received 9 February 1993; revised version received 4 March 1993

The promoter of the human proto-oncogene MYC has been the first cellular target shown to be subject to regulation by the E2F transcription factor. E2F also has binding sites in other promoters regulated by cell proliferation and during the cell cycle. We have analyzed *Saccharomyces cerevisiae* for the presence of an E2F-analogous protein. GAL1-based promoter constructs carrying the E2F binding site of the MYC or the adenovirus E2 promoter showed transcriptional activity in yeast cells. A DNA-binding factor, designated YE2F, binds specifically to the E2F consensus sequence and was partially purified from yeast extracts. YE2F showed identical contact points within the MYC binding site as authentic E2F protein from mammalian cells. The results suggest the existence of an E2F-like protein in the yeast *S. cerevisae*.

E2F; MYC; Transcription activation; Transactivating protein; *Saccharomyces cerevisiae*

1. INTRODUCTION

Transcription factors are of critical importance for the tissue- and differentiation-specific transcription of eukaryotic genes. These factors determine the frequency of transcription initiation by interacting specifically with regulatory DNA elements [1,2]. Recently it became clear that, apart from sequence-specific DNA interactions, transcription factors may also interact with other proteins. Such proteins include other transcription factors, factors of the common transcription machinery, or proteins which are able to cooperate with or dissociate such transcription initiation complexes, but themselves do not bind to DNA.

E2F was originally identified as a cellular transcription factor involved in the regulation of adenovirus early promoters and enhancers by interacting with the virus-encoded early E1A protein [3]. Besides the c-*myc* gene [4] there are now several more cellular E2F-binding sites known, for example in the promoters of the DNA polymerase α [5], a histone H2A-H2B gene pair [6] and the hamster DHFR gene [7]. It has been shown recently that E2F forms complexes not only with the adenovirus E4-gene product [8,9], but also with important cellular proteins like cyclin A [10], cdk2 kinase [11], the tumor suppressor gene product RB [12–14] and another protein, p107, closely resembling RB [15,16]. Viral antigens such as adenovirus E1A, papillomavirus E7 and SV40 large T-antigen are capable of dissociating these complexes and therefore change the activity of E2F [17]. Alterations in the composition of E2F complexes may therefore play a role in E1A-mediated oncogenesis [18].

In this context much attention has been given to the regulation of the cellular proto-oncogene MYC. The cellular transcription factor E2F was found to bind efficiently to an element within the P_2 promoter [4]. Binding of E2F is essential for basic expression and for transactivation of the P_2 promoter by adenovirus E1A protein [19,20].

We have analyzed the yeast *Saccharomyces cerevisae* for the presence of E2F-analogous proteins by using GAL 1-based promoter constructs with E2F binding sites of the MYC P_2 promoter or the adenovirus E2 promoter. We report here the existence of a factor specifically binding to the E2F consensus sequence. The partially purified protein, designated YE2F, behaves like authentic E2F isolated from mammalian cells as shown by its effects on transcription, protection and contact point analyses.

2. MATERIALS AND METHODS

2.1. *Yeast strains and media*

For all experiments the *S. cerevisiae* strain MGDL4a (leu2, ura3, his3, trp1, lys2, cyh^r, MATa) was used. Yeasts were grown at 30 °C in YPD, containing 2% glucose, or in selection medium lacking uracil.

2.2. *Plasmids*

For the transcription activation tests oligonucleotides with wild-type and mutated E2F-binding sites were cloned in the *Xho*I site of pLR1Δ1 [21] (see Fig. 1). For the footprint experiments the oligonucleotide MYC-wt$^+$ was cloned into pBluescript to yield pBSM910. The bacterial strain used routinely for cloning was *E. coli* DH5α [22].

2.3. *Preparation and purification of yeast extracts*

Yeasts were grown in YPD medium to OD_{600nm} = 1, harvested by

Correspondence address: M. Lipp, Institut für Biochemie der Ludwig-Maximilians-Universität München, Am Klopferspitz 18a, D-8033 Martinsried, Germany. Fax: (49) (89) 8578 2470.

centrifugation and suspended in 1/100 vol. of a buffer containing 25 mM HEPES pH 7.5, 1 mM DTT and 15% glycerol. Protein extracts were prepared by incubation with Zymolyase-100T (Miles Scientific) (1 mg/g cell pellet) for 30–60 min at 37°C. After two centrifugations at 20,000 rpm the supernatant was stored as crude extract at −80°C or loaded onto a DEAE-Sepharose CL6B column (Pharmacia) equilibrated with buffer FA (40 mM HEPES pH 7.5, 1 mM EDTA, 1 mM DTT, 15% glycerol) containing 100 mM NaCl. After washing with 3 vols. buffer FA containing 100 mM NaCl proteins were eluted with a linear gradient from 0.1 to 1 M NaCl in buffer FA. Active fractions (0.4–0.7 M NaCl) were pooled, diluted to 200 mM NaCl and loaded onto a Heparin-Sepharose or a FPLC-MonoQ column (Pharmacia) equilibrated with buffer FA containing 100 mM NaCl. After washing with 2 vols. buffer FA containing 100 mM NaCl proteins were eluted with a linear gradient from 0.1 to 1 M NaCl in buffer FA. Active fractions (0.5–0.6 M NaCl in the case of MonoQ; 0.7–0.8 M NaCl in the case of Heparin-Sepharose) were stored at −80 °C.

2.4. *Yeast transformation and β-galactosidase assay*

Yeast cells were transformed with 3–5 μg DNA of each construction by the LiAc technique [23] and transformants were selected on selection medium lacking uracil. Fresh transformants were used to inoculate liquid selection medium and protein extracts were prepared at 4°C with a bead mill (Retsch), centrifuged at 15,000 rpm and stored at −80°C. β-Galactosidase assays were performed from at least three independent transformants and repeated at least four to six times as previously described [24]. β-Galactosidase units presented are averages from multiple assays.

2.5. *Gel retardation assay*

Gel retardation assays were performed as described by Schneider [25]. A reaction volume of 30 μl usually contained 50 fmol of double-stranded oligonucleotide end-labeled by Klenow polymerase, 1 μg poly[d(A-T)], 200 ng sonicated salmon sperm DNA, 40 mM HEPES pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl and up to 20 μg of protein. The binding reaction was carried out for 10 min at room temperature. DNA–protein complexes were separated from free DNA on 8% polyacrylamide gels running in 0.5 × TBE.

2.6. *Methylation interference footprint analysis*

The 72-bp *Bam*HI/*Pst*I fragment from pBSM910 was isolated, end-labeled with reverse transcriptase and methylated partially with dimethylsulfoxide (DMSO) [26]. Gel retardation experiments, scaled up 20- to 30-fold, were performed with the DMSO-treated probes. DNA–

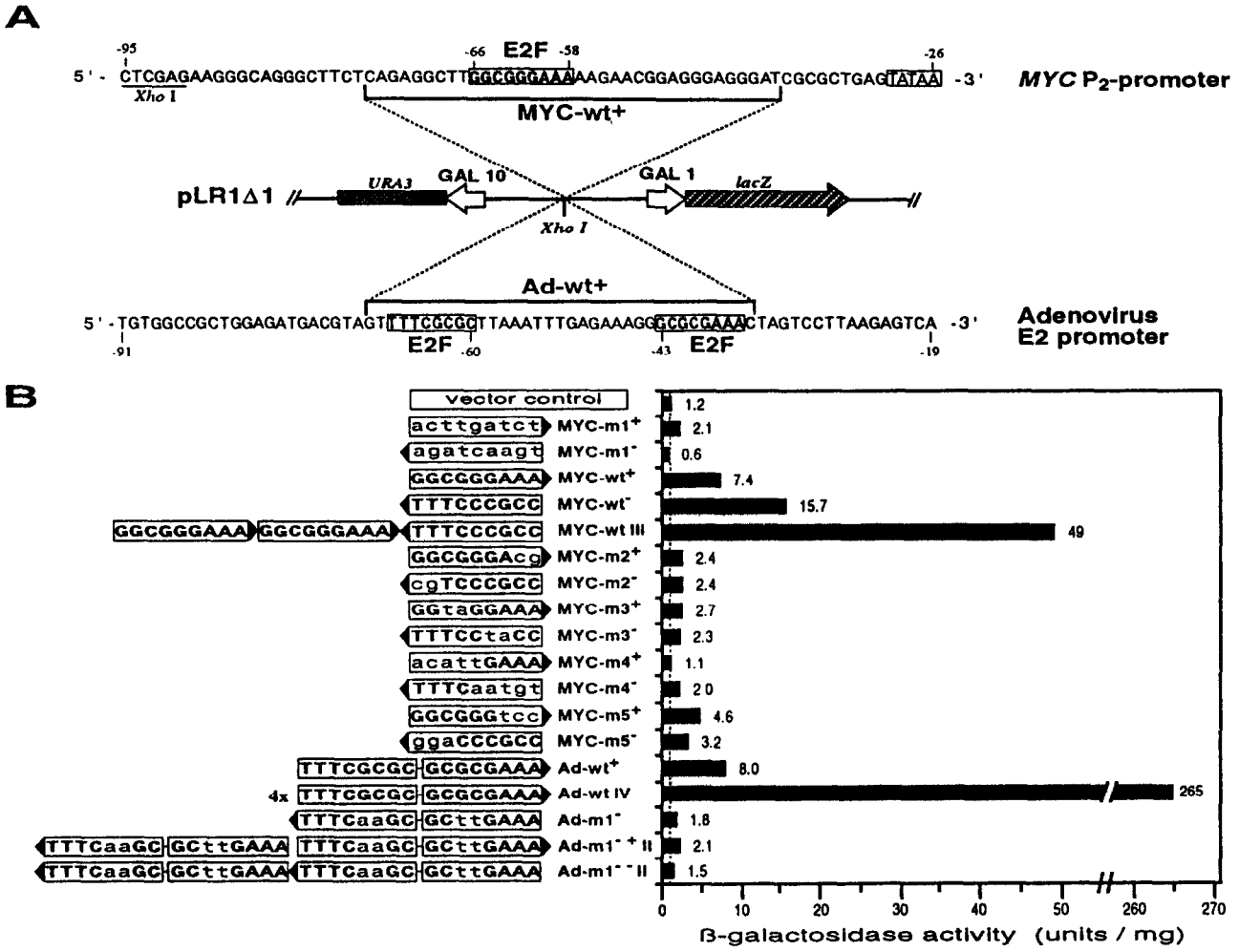

Fig. 1. Transactivation of GAL1 based promoter constructions mediated by E2F binding sites from the human MYC P_2 promoter and the adenovirus E2 promoter. (A) Sequence of the human MYC P_2 promoter and the adenovirus E2 promoter with the derived wild-type-oligonucleotides which were cloned into the *Xho* I site of the reporter plasmid pLR1Δ1 [19]. (B) Average β-galactosidase units/mg protein for the constructions with the wild-type and mutated E2F binding sites. Boxes show the upper strand of the binding site with small letters for the exchanges in the E2F binding site. Black triangles depict the orientation of the binding sites with respect to the wild-type situation. The broken line marks the vector background.

protein complexes were excised, the DNA isolated with electro-isotachophoresis [27] and purified by ethanol precipitation. After treatment with NH_4Cl and piperidine [26] the DNA was analyzed on a 8% urea sequencing gel. As reference we used a Maxam–Gilbert sequence of the same probe.

2.7. DNase I protection analysis

The fragment was labeled as described above. A gel retardation reaction mix was scaled up 20-fold. After incubation for 10 min on ice DNase I was added to a final concentration of 100 to 300 ng/ml. After 1 min incubation at 37°C DNA–protein complexes and free DNA were separated on a 8% polyacrylamide gel. Shifted DNA was excised and eluted as described above. Positions of DNase I cleavage sites were determined by coeletrophoresis of Maxam–Gilbert sequencing reaction of the same probe on a 8% urea sequencing gel.

2.8. Renaturation of proteins

Partially purified extracts were run on a 16% Tricine-gel [28], the gel was cut in small (1–2mm) horizontal sections, and the proteins of these strips were eluted in an elution buffer (50 mM Tris pH 7.9, 0.1% SDS, 0.1 mg/ml BSA, 1 mM DTT, 0.1 mM PMSF, 2.5% glycerol) overnight at 4°C. The supernatant was precipitated with 5 vols. methanol/acetone at −80°C for at least 2 h. The dry pellet was dissolved in renaturation buffer (20 mM Tris pH 7.6, 150 mM urea, 10 mM KCl, 2 mM DTT, 10 μM PMSF). After at least 12 h at 4°C the renatured proteins were tested for DNA binding in the gel retardation assay or loaded on a 16% Tricine-gel to determine their size.

3. RESULTS AND DISCUSSION

In view of the interactions of E2F with proteins involved in growth regulation and the cell cycle (Rb, p107, Cyclin A, cdk2) and the fact that many important proteins are conserved between yeasts and higher eukaryotes the presence of an E2F-like activity in the yeast *Saccharomyces cerevisiae* would not be surprising. This assumption is also supported by the observation that a number of *S.cervisiae* genes contain potential E2F-binding sites (see Table I).

3.1. Yeast E2F activates transcription in vivo

To investigate whether the E2F-specific binding sites are functional in yeast cells, we cloned oligonucleotides carrying the wildtype E2F-binding site from the MYC-P_2 promoter, the adenovirus E2-promoter or different mutations of the binding sites in various copy numbers and orientations into the reporter plasmid pLR1Δ1 [21]. This plasmid carries a GAL1/lacZ-fusion-gene under the control of the GAL10/GAL1-intergenic region, from which the UAS-element has been removed (Fig. 1). Most oligonucleotides were inserted in both orientations. These constructions were used for transformation of yeast cells. As shown in Fig. 1 only the E2F wildtype-binding sites yielded significant expression of the reporter-gene. On comparison with the negative control – vector without binding site oligonucleotide – the transcription activity was more than six times higher for one E2F-binding site (MYC-P_2 or Ad E2 promoter), more than twelve times higher for the inverted orientation (MYC-P_2 promoter) and nearly forty times higher for the triple-binding site construction. The repetition of four double-E2F-binding sites from the adenovirus E2 promoter yielded the strongest transcriptional activation. The sequence around the E2F-binding site or a mutated binding site only resulted in a weak transcriptional activation (at most 4-fold for construction MYC-m5$^+$). Both E2F-binding sites, from the MYC-P_2 and the adenovirus E2 promoter showed comparable stimulatory activities when they were inserted as single copies (Fig. 1). The higher transcriptional stimulation for the MYC-wt$^-$ construction with an inverted E2F-binding might be explained by a more favourable conformation of the bound protein and the RNA polymerase or accessory proteins. The presence of multiple binding sites led to a dramatic increase in β-galactosi-

Table I

Potential E2F-binding site in *S. cerevisiae* promoter sequences

Gene	ID	Sequence and location		
Phosphogycerate kinase UAS	SCPGKUAS	−358	atGGCGGGAAAggg	−345[a]
Growth regulation protein (WHI2)	SCWH12	−17	ctGGCGGGAAAtta	−30[a]
Ribosomal protein 29	SCRP29	−232	taGGCGGGAAAtaa	−245[a]
Mitochondrial ribosomal protein L8	SCMRPL8	−123	gaGGCGGGAAAagt	−110[b]
Alcohol dehydrogenase (ADR2)	SCADR2	−348	caGGCGGGAAAcca	−335[b]
Sporulation and meiosis segregation gene	SCRED1	−436	gtcGCGGGAAActt	−429[a]
Putative G1 cyclin (HCS26)	SCG1CYC	−306	ccgGCGCGAAAttt	−293[b]
Histon H4	SCH3H402	−266	cggGCGCGAAAtgc	−253[b]
Ribosomal protein L44	SCRGAP44	−203	gacGCGCGAAAcca	−190[a]
Recombination gene (RAD54A)	SCRAD54A	−67	tttGCGCGAAActt	−80[a]
Casein kinase I	SCYCK1	−32	aatGCGCGAAAttt	−45[b]
cAMP-dependent kinase	SCTPKA	−338	ataGCGCGAAAaaa	−351[b]

[a] position relative to the transcription start
[b] position relative to the ATG

The EMBL Nucleotide Sequence Database, Release 31 (June 1992) was searched for potential E2F-binding sites (GGCGGGAAA and GCGCGAAA). The listed sequences are a selection of E2F-binding sites which lay upstream of the transcription start or the start codon of *Saccharomyces cerevisiae* genes.

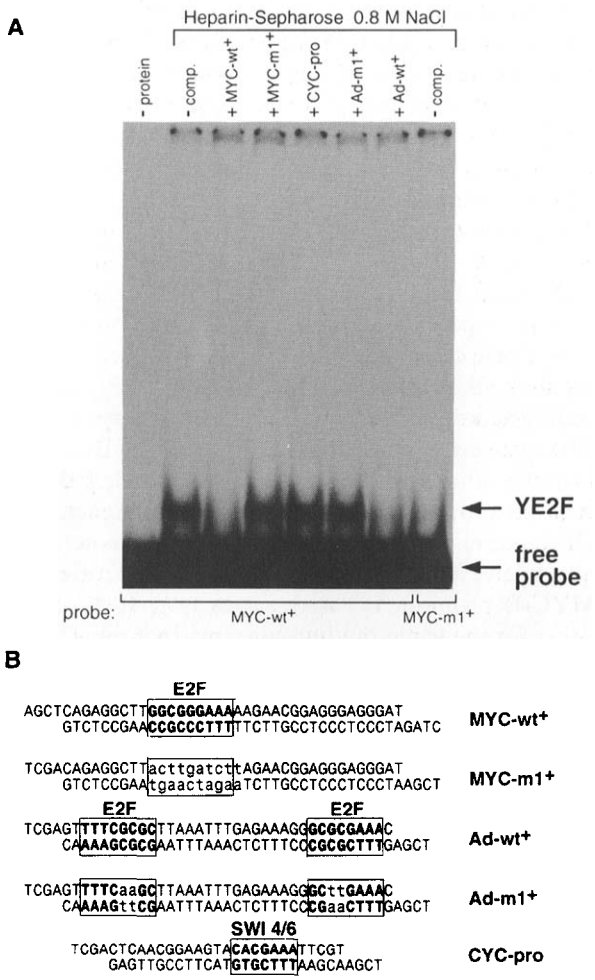

Fig. 2. Gel retardation, using a partially purified yeast extract, shows a YE2F-specific complex. (A) A Heparin-Sepharose gradient fractions was tested for the presence of a YE2F-specific complex. The sequence of the probe and the competitor oligonucleotides are shown in (B), with the E2F- or SWI 4/6-binding site in boxes and the mutations in small letters. MYC-wild-type and a MYC mutant oligonucleotide were used as probes for the binding reaction. All oligonucleotide-competitors were present in 60-fold molar excess. The first lane shows a reference binding reaction with no protein.

dase activity. This behaviour suggests a cooperative binding of the yeast E2F-like activity, and this is in good agreement with the observations made with the mammalian E2F [31].

3.2. *Specific binding pattern of an E2F-analogous protein in yeast cell extracts*

In order to further investigate the putative E2F-analogous protein we have used the technique of gel retardation. The end-labeled double-stranded oligonucleotides MYC-wt$^+$ and MYC-m1$^+$ (Fig. 2) were routinely employed as probes for analyzing DNA–protein complexes. With this test we partially purified a yeast crude extract, by using a combination of DEAE-Sepharose, MonoQ and Heparin-Sepharose columns. As shown in Fig. 2, a gradient fraction of a Heparin-Sepharose run gave rise to only one specific DNA-protein complex (indicated with YE2F). The specificity of the complexes was demonstrated by competition analysis with a 60-fold excess of unlabeled oligonucleotides. The only competing oligonucleotides were those carrying a wildtype E2F-binding site from the MYC-P_2 promoter or the adenovirus E2 promoter. Neither oligonucleotides with mutated binding sites nor an oligonucleotide with a SWI 4/6 binding site from the CLN2 promoter [29] were able to prevent the formation of YE2F-DNA complexes. These demonstrates that there is no relationship between YE2F and the SWI 4/6 complex, although the E2F-binding site possesses some homologies to the SWI 4/6 binding sequence [29]. Probe MYC-m1$^+$, which does not contain an E2F-binding site, did not result in DNA-protein complex formation with the same extract (Fig. 2). This shows that the sequences surrounding the E2F-binding site are not responsible for the formation of the YE2F-DNA complex. The same results were obtained with yeast crude extracts (data not shown). In contrast to the gel retardation pattern of mammalian E2F, which gives rise to several specific complexes [4,32], yeast E2F only forms one specific complex, in crude extracts as well as in partially purified extracts. Since the different mammalian E2F complexes are due to interactions of E2F with other proteins [8–16], our results suggest that in the yeast system no similar interactions take place.

3.3. *Yeast E2F interacts with the human MYC-P_2 promoter*

To confirm the data obtained by gel retardation and competition experiments we performed methylation interference- and DNaseI-footprint analyses. In the sequence pattern of the complexed DNA, which has shown a YE2F-specific competition pattern, five G and two A residues interfered with protein binding after methylation (Fig. 3B). The DNase I-protected region clearly included the complete E2F-binding site (Fig. 3A). This indicated that the sequence GGCGGGAA is essential to form the YE2F-specific complex. These results prove conclusively that an E2F-analogous protein or a transcription factor with a similar recognition sequence exists in the budding yeast.

3.4. *The YE2F-specific binding activity has a size of ≈ 12 kDa and does not appear to exist as a hetero-complex*

Since human E2F forms complexes with several other proteins we also investigated whether the YE2F-specific DNA–protein complex consisted of hetero-complexes. We separated a partially purified yeast extract through a denaturing Tricine-SDS gel and cut the gel into horizontal stripes to obtain after elution and renaturation fractions of proteins with different sizes, the molecular weights of which were determined on denaturing

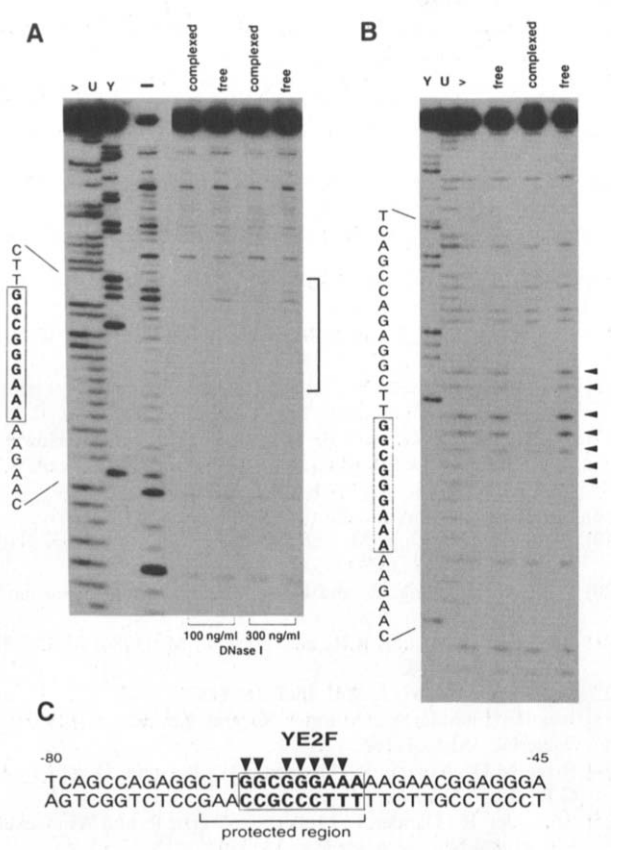

Fig. 3. DNaseI protection and methylation interference assay with partially purified yeast extract. (A) The oligonucleotide MYCwt$^+$ was cloned into pBluescript. After an upscaled gel retardation assay the DNA of the YE2F-specific band and the free probe were isolated and run on a sequencing gel. As reference the Maxam–Gilbert sequence of the same fragment is shown (> = G > A reaction; U = purines; Y = pyrimidines). The - lane contains the DNaseI digest of a reaction with no protein. The boxed sequence marks the E2F-binding site of the MYC-P_2 promoter. The brackets show the DNase I-protected region when YE2F is bound to the DNA. (B) The same labeled fragment was partially methylated with dimethylsulfate (DMS) in a G > A reaction and incubated with partially purified yeast extract. Beside the Maxam-Gilbert sequence (Y = pyrimidines; U = purines > = G > A reaction) the interference pattern of the free and bound DNA is shown. G and A nucleotides that interfere with YE2F-binding are indicated with black triangles. (C) MYC-P_2 promoter sequence from −80 to −45 with the E2F-binding site boxed. The black triangles indicate the G and A nucleotides which interfere with the binding of YE2F and the brackets show the DNase I-protected region.

Tricine-SDS gels (Fig. 4A). To determine which size fractions contained the YE2F-specific binding activity we assayed the renatured proteins with the standard gel retardation assay (Fig. 4B). Only one size fraction, from 11.1 to 12.0 kDa, yielded material that formed a YE2F-specific complex. In renaturation experiments with crude yeast extracts we obtained exactly the same size. DNA–protein complexes observed in higher size fractions were shown to be results of unspecific DNA–protein interactions as determined by competition experiments (data not shown). So the data from the renaturation experiments corroborate the results of gel retardation experiments: firstly the very fast migrating DNA/protein complex makes it very likely that a small protein is responsible for complex formation; secondly the complex consists of a single protein or an homodimer and not an heterodimer of YE2F and another protein. The size of YE2F (≈ 12 kDa) is smaller than that of other known yeast DNA-binding proteins such as MCBF (*Mlu*I-cell cycle box-binding factor; [33]), MBF (*Mlu*I-cell cycle box-factor; [34]) and DSCI (DNA synthesis control; [35]). These three proteins were shown to bind a similar sequence (5′-ACGCGCT-3′) and mediate cell cycle-regulated expression of genes. With South-Western analysis it was shown that MCBF has an apparent molecular weight of 17 kDa and MBF of approximately 120 kDa, whereas YE2F has a size of ≈ 12 kDa. Perhaps this 12 kDa protein is only a degradation product but the migration behaviour in the gel retardation assay did not change during the purification procedure and the size determinations from crude extracts and partially purified extracts revealed exactly the same size. Alternatively the nature of the interaction of YE2F with other putative partners could be extremely weak and there-

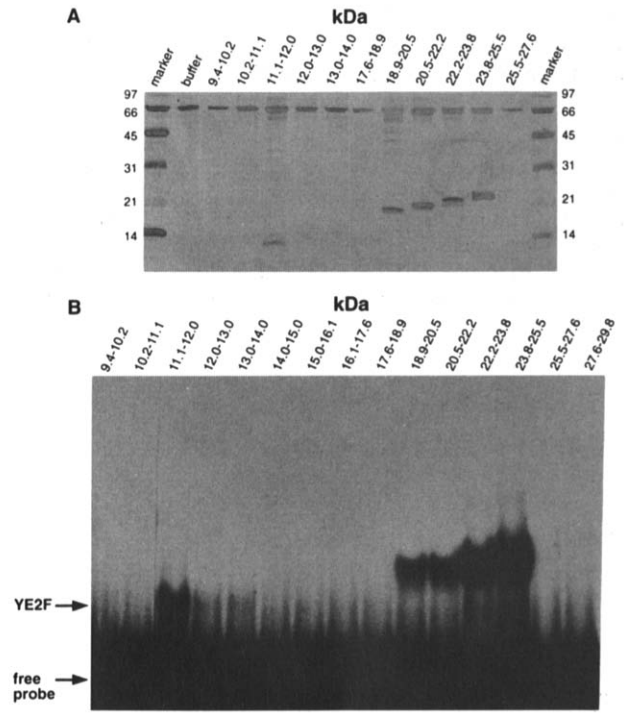

Fig. 4. Determination of the size of the YE2F activity with a protein renaturation experiment. (A) The size fractions of the renatured proteins were separated again on a denaturing 16% Tricine gel and silver stained. The strong band at approximately 66 kDa and the sometimes visible weak bands down to this occuring in each lane correspond to the BSA of the elution buffer. The boundaries of the size areas were determined with a standard curve using the shown marker proteins as fixed size-points. (B) The same size fractions were assayed for the existence of DNA-binding proteins. Binding reactions were done as described above and separated on a 8% native polyacrylamide gel. The size area of the proteins inserted in each binding reaction is indicated above each lane. The position of the free probe and the YE2F-specific complex is marked with arrows. The other complexes are unspecific.

fore not detectable by gel retardation assay. Nevertheless this small protein is responsible for a highly specific recognition of an E2F binding site.

3.5. *The YE2F-specific binding activity does not change during the cell cycle*

Since it is known from the mammalian E2F, that its DNA-binding and transactivation potential is regulated through the cell cycle [11,16] we investigated if there are any changes of the YE2F binding activity through the cell cycle. Therefore we synchronized an appropriate bar1⁻ yeast strain with a-factor treatment as previously described by Rhode [36]. The synchronization was controlled by FACS-analysis and protein extracts from the different cell cycle stages were tested with the gel retardation assay. But we did not find any significant changes through the cell cycle on protein level. So far there is only the DSCI-factor known to be regulated through the cell cycle detectable on protein level [36]. However as long as the YE2F-gene isn't cloned we could not analyse its product at the RNA-level. Since in 1992 a mammalian E2F-like gene was cloned [37,38] it should be possible to find the homologous gene in yeast. Cloning of the gene coding for YE2F will allow to analyse its regulation and function in *Saccharomyces cerevisiae* in more detail, as well as to evaluate which functional domains of YE2F may be evolutionarily conserved.

Acknowledgements: We thank Georg Arnold for the synthesis of oligonucleotides and Horst Ibelgaufts and Ingrid Wolf for critically reading the manuscript. B.M. was supported by a doctoral fellowship from the 'Boehringer Ingelheim Fonds'. The work was supported by the Deutsche Forschungs Gemeinschaft Forschergruppe 'Virus-Zell-Wechselwirkung' (FA 138/3-7).

REFERENCES

[1] Johnson, P.F. and McKnight, S.L. (1989) Annu. Rev. Biochem. 58, 799–839.
[2] Latchman, D.S. (1990) Biochem. J. 270, 281–289.
[3] Kovesdi, I., Reichel, R. and Nevins, J.R. (1986) Cell 45, 219–228.
[4] Thalmeier, K., Synovzik, H., Merz, R., Winnacker, E. and Lipp, M. (1989) Genes Dev. 3, 527–536.
[5] Pearson, B.E., Nasheuer, H. and Wang, T.S. (1991) Mol. Cell. Biol. 11, 2081–2095.
[6] Dobner, T., Wolf, I., Mai, B. and Lipp, M. (1991) DNA Sequence 1, 409–413
[7] Blake, M.C. and Azizkhan, J.C. (1989) Mol. Cell. Biol. 9, 4994–5002.
[8] Reichel, R., Neill, S.D., Kovesdi, I., Simon, M.C., Raychaudhuri, P. and Nevins, J.R. (1989) J. Virol. 63, 3643–3650.
[9] Raychaudhuri, P., Bagchi, S., Neill, S.D. and Nevins, J. R. (1990) J. Virol. 64, 2702–2710.
[10] Mudryj, M., Devoto, S.H., Hiebert, S.W., Hunter, T., Pines, J. and Nevins, J.R. (1991) Cell 65, 1243–1253.
[11] Pagano, M., Draetta, G. and Jansen-Dürr, P. (1992) Science 255, 1144–1147.
[12] Chellapan, S., Hiebert, S., Mudryj, M., Horowitz, J.M. and Nevins, J.R. (1991) Cell 65, 1053–1061.
[13] Bagchi, S., Weinmann, R. and Raychaudhuri, P. (1991) Cell 65, 1063–1072.
[14] Chittenden, T., Livingston, D.M. and Kaelin, W.G.J. (1991) Cell 65, 1073–1082.
[15] Cao, L., Faha, B., Dembski, M., Tsai, L., Harlow, E. and Dyson, N. (1992) Nature 355, 176–179.
[16] Shirodkar, S., Ewen, M., DaCaprio, J.A., Morgan, J., Livingston, D.M. and Chittenden, T. (1992) Cell 68, 157–166.
[17] Chellappan, S., Kraus, V.B., Kroger, B., Munger, K., Howley, P.M., Phelps, W.C., Nevins, J.R. (1992) Proc. Natl. Acad. Sci. USA 89, 4549–53.
[18] Bagchi, S. and Raychaudhuri, P. (1990) Cell 62, 659–669.
[19] Hiebert, S.W., Lipp, M. and Nevins, J.R. (1989) Proc. Natl. Acad. Sci. USA 86, 3594–3598.
[20] Lipp, M., Schilling, R. and Bernhardt, G. (1989) Oncogene 4, 535–541.
[21] West, R.W., Yocum, R.R. and Ptashne, M. (1984) Mol. Cell. Biol. 4, 2467–2478.
[22] Wood, W.B. (1966) J. Mol. Biol. 16, 118–133.
[23] Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol. 153, 163–168.
[24] Rose, M.D., Novick, P., Thomas, J.H., Botstein, D. and Fink, G.R. (1987) Gene 60, 237–243.
[25] Schneider, R., Gander, I., Müller, U., Mertz, R. and Winnacker, E.L. (1986) Nucleic Acids Res. 15, 1303–1317.
[26] Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[27] Ofverstedt, L.G., Hammerström, K., Balgobin, N., Hjeten, S., Pettersson, U. and Chattopadhaya, J. (1984) Biochim. Biophys. Acta 782, 120–126.
[28] Schägger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
[29] Ogas, J., Andrews, B.J. and Herskowitz, I. (1991) Cell 66, 1015–1026.
[31] Pidder, J.D., Boeuf, H. and Kedinger, C. (1989) EMBO J. 8, 3365–3370.
[32] Hiebert, S.W., Chellappan, S.P., Horowitz, J.M. and Nevins, J.R. (1992) Genes Dev. 6, 177–185.
[33] Verma, R., Patapoutian, A., Gorgon, C.B. and Campbell, J.L. (1991) Proc. Natl. Acad. Sci. USA 88, 7155–7159.
[34] Dirick, L., Moll, T., Auer, H. and Nasmyth, K. (1992) Nature 357, 508–513.
[35] Lowndes, N.F., Johnson, A.L. and Johnson, L.H. (1991) Nature 350, 247–250.
[36] Rhode, P.R., Elsaesser, S. and Campbell, J.L. (1992) Mol. Cell. Biol. 12, 1064–1077.
[37] Helin, K., Lees, J.A., Vidal, M., Dyson, N., Harlow, E. and Fattaey, A. (1992) Cell 70, 337–350.
[38] Kaelin, W.G., Krek, J.W., Sellers, W.R., DeCaprio, J.A., Ajchenbaum, F., Fuchs, C.S., Chittenden, T., Blanar, M.A., Livingston, D.M. and Flemington, E.K. (1992) Cell 70, 351–364.