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TUF, the yeast DNA-binding factor specific for UAS_{rpg} upstream activating sequences: Identification of the protein and its DNA-binding domain

(pore-gradient electrophoresis/nuclease-protection assay/transcription regulation/limited proteolysis/*Saccharomyces cerevisiae*)

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ABSTRACT **The factor TUF interacts specifically with RPG or HOMOL1 sequences, which are present upstream of many genes coding for the yeast translational apparatus. Here we present evidence that the RPG and HOMOL1 motifs are variants of a consensus UAS_{rpg} (upstream activating sequence) recognized by the same factor. Factor TUF was identified by using two highly selective methods. (*i*) The DNA–protein complex was isolated by pore-limit electrophoresis in polyacrylamide gradient gels and found to contain a single polypeptide of 150 kDa. (*ii*) In a two-step protein-blotting/nuclease-protection ("footprinting") procedure, the same 150-kDa polypeptide blotted on nitrocellulose exhibited the same specific DNA-binding properties as TUF factor. A 50-kDa DNA-binding domain of TUF was isolated by selective proteolysis. This suggests a bipolarization of the TUF protein, with distinct functional domains.**

The mechanisms by which eukaryotic genes are regulated at a distance by DNA-binding factors are not well understood. In yeast, upstream activating sequences (UASs) are analogous in many respects to enhancer elements found in animal cells (1, 2). The function of UASs has been investigated in a number of genetically characterized systems such as *CYC1*, *GAL4*, and the general amino acid control (3). They are the target of specific cellular proteins such as RC2 (4), GAL4 (5), or GCN4 (6), function bidirectionally, and act at long and variable distances from the initiation site. How these bound proteins in turn activate transcription is not known.

Recently, we and our coworkers (7) have discovered in yeast a DNA-binding factor, termed TUF, that binds selectively to conserved sequence elements present upstream of *TEF1* and *TEF2* genes, coding for the elongation factor EF1α, and of the gene encoding ribosomal protein RP51A. These conserved motifs, RPG (ACCCATACAT$^{TT}_{CA}$) and HOMOL1 (AACATC$^{CG}_{TA}$T$^{A}_{G}$CA), first were detected by computer analyses in the majority of the 20 ribosomal protein genes examined (8, 9) and later were found to be essential *in vivo* for transcription of L25 (10) and RP39A (11) genes. The case of factor TUF is particularly interesting, as there is the possibility that this component regulates the expression of a family of genes encoding components of the yeast translational apparatus (7). This system is attractive in view of the large number of "housekeeping" genes potentially involved. To obtain some insight into the mode of action of TUF, our primary goal was to identify the polypeptide(s) recognizing the RPG and HOMOL1 sequences. In this paper we present evidence showing that a unique component recognizes these two conserved sequences. TUF was identified by two independent approaches: by directly isolating the protein–DNA complex and by a two-step protein-blotting/nuclease-protection ("footprinting") procedure. The protein is a 150-kDa polypeptide, and a 50-kDa DNA-binding domain was isolated by selective proteolysis.

MATERIALS AND METHODS

TUF Factor and DNA Probes. TUF factor used in this work was isolated on heparin-Sepharose as described (7) and further purified by DEAE-Sephadex chromatography. The DEAE step brought about a further 6- to 10-fold purification, based on the gel electrophoretic retardation assay, and did not change the binding specificity of the factor. This partially purified preparation will be referred to as TUF factor. Three DNA probes were used. Probe A is a 662-base-pair (bp) *Eco*RI–*Hin*fI DNA fragment, from pLB25-1, carrying the *Nar* I–*Sca* I upstream region of the *TEF2* gene (7). Probe B is a 172-bp *Eco*RI–*Hph* I DNA fragment from pLB25-1; TUF binding sites are located 70–100 bp away from the 3′ end-labeled *Eco*RI site. Probe C is a synthetic, double-stranded 32-mer (GGAATTCTAACATCCGTACATCTTTGAATTCC) containing the consensus HOMOL1 sequence (8) flanked with *Eco*RI sites and ^{32}P-labeled by kinase at both ends. The plasmids pLB25-1 and p51A-P, harboring the promoters of *TEF2* and *RP51A* genes, have been described (7).

Analysis of TUF–DNA Complexes. Protein–DNA complexes were formed and analyzed by gel electrophoretic retardation assay (7) or by pore-gradient electrophoresis in 4–30% or 2–16% polyacrylamide gradient gels (Pharmacia) for 18–24 hr at 4°C, at a constant voltage of 125 V (12). The complexes and free DNA were located by autoradiography. For identification of TUF, the proteins in complex C_1 or P_1 were electroeluted into a membrane trap with a Tris/glycine/NaDodSO$_4$ buffer, precipitated with acetone, and analyzed by NaDodSO$_4$/PAGE (13).

"Blot and Footprint" Procedure. TUF factor (7 μg of protein) was fractionated by NaDodSO$_4$/10% PAGE and the proteins were transferred to nitrocellulose electrophoretically. The membrane strips were treated with nonfat milk (14) and then incubated for 1 hr at 4°C in 1 ml of binding buffer (10 mM Tris·HCl, pH 8/50 mM NaCl/0.1 mM EDTA/1 mM dithiothreitol/0.25% nonfat dry milk) containing 10^5 cpm of ^{32}P-labeled *TEF2* probe B. The filters were washed in three changes of binding buffer over 30 min and autoradiographed wet for 4 hr in a sealed plastic bag. Pieces of membrane were pooled and incubated (≈3000 cpm Cerenkov; 160 μl/cm^2) for 15 min at 25°C in footprint buffer (20 mM Tris·HCl, pH 8/70 mM NaCl/15 mM $MgCl_2$/0.5 mM $CaCl_2$/0.5 mM dithiothreitol/0.1 mM EDTA) and then for 1 min with DNase I (50 ng/ml). The reaction was stopped and the solution was

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Abbreviation: UAS, upstream activating sequence.

withdrawn for recovery and analysis of the DNA fragments in a sequencing gel as described (7). The same procedure was followed for exonuclease treatment, except that the NaCl was removed and digestion was allowed for 5 min at 25°C with bacteriophage λ exonuclease (25 units/ml; New England Biolabs). Details on this method are available upon request.

RESULTS

The Same Factor Binds to RPG and HOMOL1 Sequences. TUF factor was originally defined by its ability to bind to HOMOL1- and RPG-like sequences on *TEF2*, *TEF1*, and *RP51A* genes. Several observations suggested that a single component interacted with these two conserved sequence elements, which are largely overlapping (7). To strengthen this conclusion, we have made a synthetic oligonucleotide harboring the consensus HOMOL1 motif for DNA binding and competition experiments using the gel retardation assay. Fig. 1 shows complex formation between the factor and a *TEF2* ^{32}P-labeled promoter fragment in the presence of various competitor DNAs. As previously shown (7), this *TEF2* probe harbors one strongly binding RPG box (ACCCACACATTT) and a contiguous, RPG-related site of lesser affinity, giving rise to two retarded bands of complexes C_1 and C_2 (R_f = 0.38 and 0.2) by gel electrophoresis. In complex C_1, the factor protects 22 bp over the RPG box, and an extended nuclease-insensitive region ("footprint") was found in complex C_2 (7). With the present factor preparation, an additional complex, C_3 (R_f = 0.1), was detected, upon binding of a third molecule of factor to an adjacent, HOMOL1-related sequence (Fig. 1, lanes 1 and 2; see Fig. 7 for sequence and binding data). A 20-fold molar excess of the *RP51A* promoter (with two HOMOL1 boxes) or of a synthetic HOMOL1 oligonucleotide prevented the formation of both complex C_2 and complex C_3, while complex C_1 accumulated (lanes 6–9). The HOMOL1 oligonucleotide also inhibited formation of complex C_1 on the RPG box when added at a ≥60-fold molar excess (lanes 10 and 11). *TEF2* promoter (with three binding sites) was a 10-fold better competitor than the oligonucleotide (compare lanes 5 and 10). TUF factor interacted with the ^{32}P-labeled oligonucleotide to give only one complex by gel retardation, even when the protein was present at a concentration 7 times that required to bind all the probe (see Fig. 2 *Right*). Formation of this complex was inhibited by *RP51A* or *TEF2* promoters, and again, *TEF2* was a better competitor than *RP51A* or the HOMOL1 oligonucleotide itself (results not shown).

We compared the protease sensitivity of the component binding to *TEF2* and to the HOMOL1 oligonucleotide (Fig. 2). Interestingly, limited proteinase K treatment brought about a drastic increase in the electrophoretic mobility of complexes C_1 and C_2 formed with the *TEF2* probe A, to give complexes P_1 and P_2 migrating very close to the free DNA probe (Fig. 2 *Left*). Similar results were obtained with papain or Pronase (results not shown). This suggested that the proteases had clipped off a large portion of the factor molecule without affecting its DNA binding affinity, since the yield of complexed DNA was not reduced. Under the same conditions, only one complex (C_1; R_f = 0.26) was formed with the ^{32}P-labeled oligonucleotide, which also gave rise to a protease-resistant, fast-migrating complex (P_1; R_f = 0.63) upon treatment with proteinase K, again with no change in DNA affinity (Fig. 2 *Right*). The protease concentration producing the fast-migrating complexes was the same with the *TEF2* and HOMOL1 probes (Fig. 2, lanes 5 and 6). These observations and the competition experiments supported the previous contention that the same component interacted with RPG and HOMOL1 sequences, which are probably variants of a consensus UAS_{rpg} sequence.

To characterize factor TUF further, we subjected the factor–DNA complex to gel electrophoresis in a 4–30% gradient of polyacrylamide for 18–24 hr. In gels of graded porosity, the electrophoretic migration rate of proteins tends asymptotically to zero as they reach their pore-exclusion limit, and the distance migrated can be experimentally correlated with molecular size (12, 15). To be adapted to the analysis of protein–DNA complexes, the method required that the complex did not dissociate during such prolonged running times. Remarkably, although the measured half-life of the factor–*TEF2* complexes in low-ionic-strength buffers and with EDTA was much shorter than the duration of electrophoresis, two complexes were clearly obtained on the gel, which presumably corresponded to C_1 and C_2 complexes (Fig. 3). The position of these complexes depended on the length of the DNA fragment, which was 662 bp (Fig. 3, lane 2) or 172 bp (lane 5). The distance migrated by these complexes corresponded to that of the largest protein marker, thyroglobulin (669 kDa). A better resolution of these

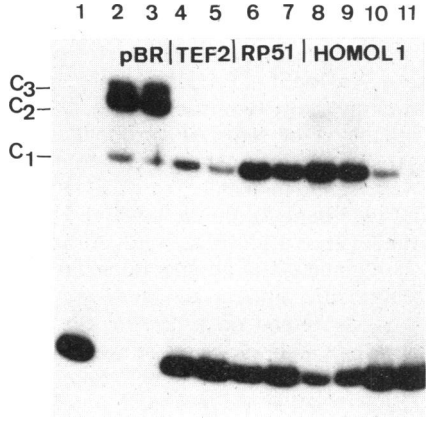

FIG. 1. Competition experiment using the gel electrophoretic retardation assay. TUF factor (170 ng) was incubated as described (7), in a volume of 20 μl, with *TEF2* probe A (8 fmol; 6000 cpm) in the presence of various competitor DNAs. The amount of vector pBR322 DNA was kept constant at 800 ng. After a 10-min incubation at 25°C, protein–DNA complexes were subjected to gel electrophoresis and revealed by autoradiography. Lanes: 1, control (DNA probe); 2 and 3, pBR322 (800 ng); 4 and 5, pLB25-1 DNA (*TEF2*; 500 and 800 ng); 6 and 7, p51A-P DNA (*RP51A*; 500 and 800 ng); 8–11, HOMOL1 32-mer (2.5, 5, 10, and 50 ng, respectively).

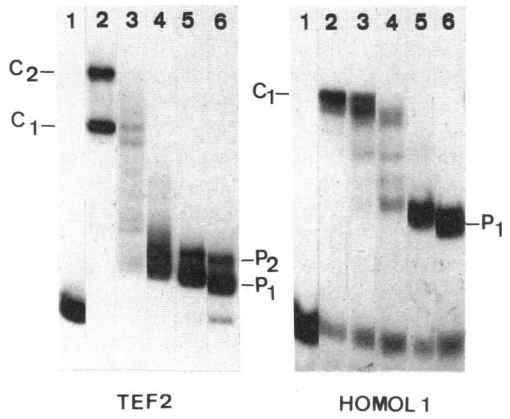

FIG. 2. Limited proteolysis of TUF–DNA complexes. TUF factor (88 ng) was incubated in 20 μl with *TEF2* probe A or the HOMOL1 32-mer probe C (each 8 fmol; 4000–6000 cpm) in the presence of pBR322 DNA (120 ng) and various amounts of proteinase K. After 10 min at 25°C, protein–DNA complexes were subjected to gel electrophoresis for 3.5 hr (*TEF2*) or 1.5 hr (HOMOL1). Lanes: 1, control (DNA probe); 2, no protease; 3–6, proteinase K (0.025, 0.1, 1.25, and 10 ng, respectively).

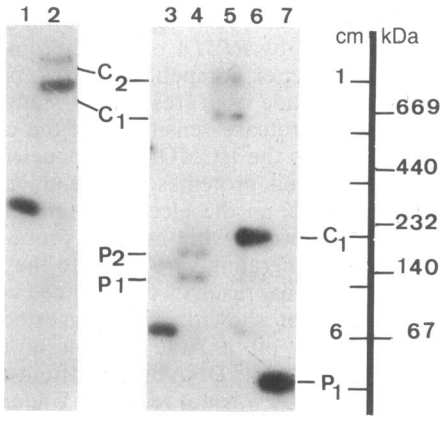

FIG. 3. Analysis of TUF–DNA complexes by pore-gradient electrophoresis. TUF factor (88 ng) was incubated with probe A (lanes 1 and 2), B (lanes 3–5), or C (lanes 6 and 7) and the complexes were separated by electrophoresis for 15 hr at 4°C in a 4–30% gradient gel. Lanes 4 and 7: proteinase K (10 ng) was present during incubation. Lanes 1 and 3: control (DNA probes). The distance migrated (in cm) and the apparent molecular mass of protein markers are indicated on the right.

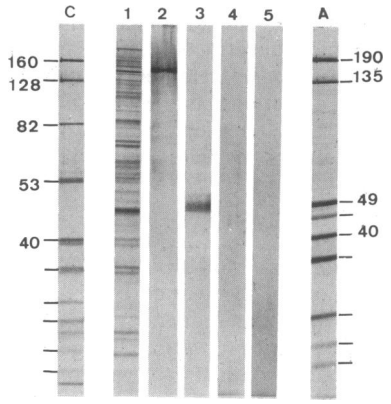

FIG. 4. Identification of TUF protein in native and proteolyzed complexes. The binding reaction mixture (400 μl) contained TUF (21 μg), probe B (118 fmol; 300,000 cpm), pLB25-1 DNA (6 μg) cut with *Eco*RI and *Hph* I (to generate probe B), carrier pBR322 DNA (14 μg), and, where indicated, proteinase K (0.25 μg). After 10 min at 25°C, TUF–probe B complex was isolated by electrophoresis in a 2–16% gradient gel (other conditions of electrophoresis were as in Fig. 3). Complexes C_1 and P_1 were electroeluted and subjected to $NaDodSO_4$/10% PAGE together with protein markers and factor TUF. Lanes: 1, TUF (1.75 μg); 2, proteins in complex C_1; 3, proteins in complex P_1 (proteolyzed); 4, control sample incubated without probe B DNA and treated similarly; 5, as in lane 4, but with proteinase K. Lanes C and A: subunits of yeast RNA polymerases C and A. Proteins were stained with silver.

complexes was obtained in a 2–16% gradient gel (results not shown). The contribution of the DNA moiety to the gel retardation effect being unknown, no information on the size of the complexed protein can be drawn directly from the migration of a given complex. However, we assumed that the difference in migration between C_1 and C_2 complexes should reflect the binding of a second molecule of factor TUF to complex C_1, to give complex C_2. This corresponded to an apparent mass increase of about 200 kDa (± 50 kDa) estimated on 2–16% gels. This value was confirmed by the analysis of complex C_1 formed with the HOMOL1 32-mer. In that case, only complex C_1 was formed (one unique protein binding site), and the contribution of DNA was minimized. During electrophoresis the complex migrated as a protein of 200 kDa (Fig. 3, lane 6), suggesting that TUF factor was a large component in that apparent molecular mass range, probably smaller than 200 kDa, in view of the presence of the oligonucleotide. When the proteolyzed factor–oligonucleotide complex was subjected to the same electrophoretic analysis, the protease-resistant complex P_1 migrated like an ≈43-kDa protein (Fig. 3, lane 7). This value corresponded well to the apparent mass increase of ≈45 kDa deduced from the migration of proteolyzed complexes P_1 and P_2 obtained with the 172-bp *TEF2* probe B (Fig. 3, lane 4).

Identification of TUF Factor. To identify the protein bound to the UAS_{rpg} sequence, we followed two independent approaches. In the first one, the protein–DNA complexes were isolated on a preparative scale by electrophoresis in polyacrylamide gel of graded porosity, as described above. The mixture of partially purified factor, *TEF2* DNA fragment (172 bp), and carrier pBR322 DNA was scaled up 40-fold and separated in a 2–16% gradient gel, which provides the best resolution for large components. As the factor–DNA complex migrated with an apparent molecular mass of 660 kDa (see Fig. 3, lane 5), we thought that few if any contaminants would migrate at the same place in the gel. In addition, after extensive electrophoresis (≥18 hr) the protein–DNA complex is conveniently concentrated as its migration rate tends to zero. The complex (C_1) was therefore easily located by autoradiography, excised, and electroeluted for analysis of its polypeptide content by $NaDodSO_4$/PAGE (Fig. 4). The partially purified factor preparation contained many polypeptides that stained with silver (lane 1). In contrast, a single polypeptide was recovered from the gel slice containing the protein–DNA complex. By comparison with the large subunits of yeast RNA polymerases A and C, its apparent molecular mass was 150 kDa. In a control experiment where the protein sample was subjected in parallel to the same fractionation procedure in the absence of *TEF2* DNA fragment, no protein band was recovered from a similar gel slice cut at the same level (lane 4). The proteolyzed complex P_1 formed with the same DNA fragment was electrophoresed, eluted, and analyzed in the same gel. It contained a major protein band at about 50 kDa with two minor bands corresponding to slightly larger size (Fig. 4, lane 3). Again, no band was recovered from a protein sample proteolyzed and electrophoresed in the absence of *TEF2* DNA (lane 5). The protein bound to the HOMOL1 oligonucleotide was isolated in the same way and also found to migrate as a single polypeptide of 150 kDa (results not shown).

In the second approach, the specific binding protein was detected by protein blotting (16). The factor preparation was fractionated by $NaDodSO_4$/PAGE, the proteins were transferred electrophoretically to nitrocellulose, and the filter was incubated with the ^{32}P-labeled *TEF2* probe B. The buffer used in binding and subsequent washing steps contained no Mg^{2+} ions, which were found to destabilize TUF–DNA complexes. As shown in Fig. 5 (lane 4), the radioactive DNA probe was retained at the level of a 150-kDa band. Although the factor preparation contained many polypeptides (lane 2), the background of nonspecific binding was very low. Similarly, no radioactivity was retained on a control filter with blotted RNA polymerase A subunits (not shown). Binding of the *TEF2* probe on the 150-kDa band was inhibited selectively by unlabeled *TEF2* or *RP51A* DNA and much less so with pBR322 DNA (results not shown). These results are in good agreement with the previous finding of a 150-kDa polypeptide in the complex isolated from the polyacrylamide gel. Attempts to retain the labeled HOMOL1 oligonucleotide on the filter were not successful. We suspect that the complex was too unstable (half-life <30 sec with Mg^{2+} ions) to withstand the washing procedure (results not shown).

To firmly establish that the 150-kDa polypeptide was factor TUF, we next showed that the DNA retained by the filter-bound protein was specifically bound at the level of the RPG

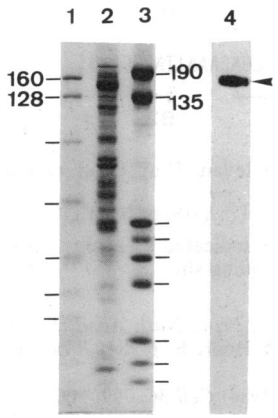

FIG. 5. Identification of TUF by protein blotting. TUF factor (7 μg) was fractionated by NaDodSO$_4$/PAGE, proteins were stained or transferred to nitrocellulose, and the filter was incubated with ^{32}P-labeled probe B as described in *Materials and Methods*. Lanes 1 and 3: subunits of RNA polymerases C and A. Lane 2: TUF factor (7 μg) stained with Coomassie blue. Lane 4: autoradiograph of the membrane strip. Arrowhead shows the 150-kDa band.

box. We reasoned that unbound regions of DNA should be normally accessible to nuclease attack, since double-stranded DNA does not bind to nitrocellulose. Bacteriophage λ exonuclease was first used to map the 5′ boundary of the complex. This nuclease degrades DNA processively, starting from the 5′ ends of the molecule (17). DNA labeled at the 3′ end was incubated with the factor in solution, or the strip of filter with the blotted proteins. Then the complex was digested with λ exonuclease. In both cases, the same pattern of nuclease-resistant DNA bands was formed (Fig. 6, lanes 5 and 9). The control of DNA digestion in the absence of factor showed no accumulation of fragments of that size (lane 4). The length of the protected fragments on the sequencing gel indicated that the nuclease had met the border of the complex 1, downstream from the RPG box, at positions −412 and −416 (B1 and B1′), and the border of complex 2 (B2) at −400 (see Fig. 7). The finding of two main borders reflected the binding of either one or two molecules of factor. The −400 border (B2) of the complex as defined by the exonuclease experiment corresponded well to the outermost boundary of the footprint of the same protein–DNA complex in solution (lane 2). Remarkably, the DNA retained by the filter-bound protein was protected from DNase I digestion exactly as in solution (lane 8). We noted in these footprinting experiments an extension of the protected region (from position −441 to −460) when compared to the footprint observed previously with a less purified factor preparation (7). The sequence protected showed a strong homology (10/12 nucleotides) with the HOMOL1 consensus. This extended partial footprint probably corresponded to complex C_3 seen in Fig. 1. These results indicate that the 150-kDa polypeptide interacted with the three RPG- or HOMOL1-related binding sites on the *TEF2* promoter. A summary of these binding data is given in Fig. 7. In the same experiment we also found that the proteolyzed factor in solution protected the same region of DNA over and around the RPG box (Fig. 6, lane 3).

DISCUSSION

Since the discovery of factor TUF, we have been interested in studying the mechanisms by which this DNA-binding component regulates a large family of housekeeping genes. The main conclusions reached in the present work concern the existence of a unique component interacting with the RPG and HOMOL1 conserved sequences, the identification

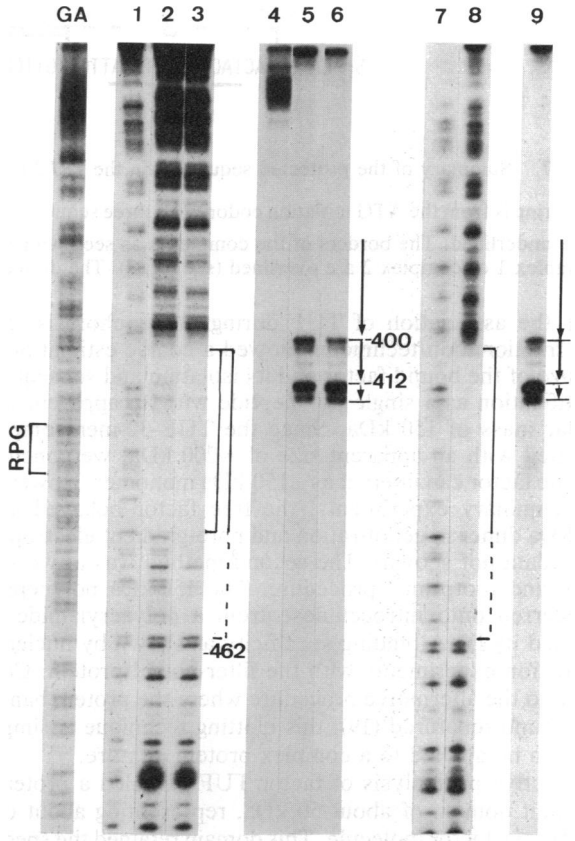

FIG. 6. "Blot and footprint" experiment. Factor TUF (70 μg) was electrophoresed, transferred to nitrocellulose, and incubated with DNA (see Fig. 5). The filter-bound protein–DNA complex was then digested with DNase I (lane 8) or λ exonuclease (lane 9) as described in *Materials and Methods*. TUF–DNA complexes were also footprinted in solution with DNase I (lanes 2 and 3) or λ exonuclease (lanes 5 and 6). Lanes 1, 4, and 7: controls of DNA degradation without factor. Lane GA: degradation products of the G+A sequencing reaction, with location of the RPG box. Lanes 3 and 6: complexes were treated with proteinase K (0.5 μg/ml). Distances to the ATG codon (−400, −412, and −460 bp) are indicated. See Fig. 7 for a summary of the binding data.

of this protein, and the isolation of its DNA-binding domain by selective proteolysis.

We have carried out binding and competition experiments showing that the same 150-kDa component recognizes three related binding sites (including the RPG box) present in *TEF2* and a consensus HOMOL1 oligonucleotide. Furthermore, the factor binding to both DNA probes displayed the same protease sensitivity and gave rise, upon limited protease treatment, to a similar fast-migrating, protease-resistant domain with unaltered DNA-binding properties. These results convincingly showed that RPG and HOMOL1 sequences must be variants of a consensus UAS_{rpg} recognized by factor TUF. This general UAS_{rpg} remains to be defined precisely by mutagenesis. A comparison of the affinity of TUF for a number of binding sequences already indicated that the optimal UAS_{rpg} is similar to the RPG box (M.-L. Vignais and L. P. Woudt, personal communication).

TUF factor was identified in a partially purified preparation by two highly selective methods. These are worth emphasizing as they might be of general use for the characterization of nucleic acid-binding proteins. In the first approach, the protein was isolated as a specific complex with DNA, by pore-limit electrophoresis in a steep polyacrylamide gradient. It was remarkable that the complexes remained stable during prolonged running times far in excess of their measured half-life in solution. A "cage effect" of the gel network might

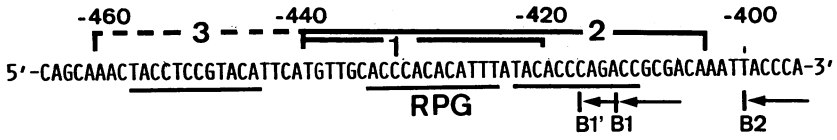

FIG. 7. Summary of the protected sequences in the *TEF2* promoter. The sequence of the relevant 5' upstream region of *TEF2* is shown. Numbering is from the ATG initiation codon. The three sequences related to RPG (ACCCATACAT$^{TT}_{CA}$) (9) or HOMOL1 (AACATC$^{CG}_{TA}$T$^{G}_{A}$CA) (8) are underlined. The borders of the complexes as seen with λ exonuclease (B1, B1', B2) are indicated by arrows. DNA regions protected in complex 1 or complex 2 are overlined (see ref. 7). The dotted line shows the third, weak binding site.

favor the association of TUF during electrophoresis (18). This fractionation technique allowed a coarse estimation of the size of the bound factor and its isolation and subsequent identification as a single polypeptide with an apparent molecular mass of 150 kDa. Since the TUF–32-mer complex migrated with an apparent size of ≈200 kDa, we conclude that the factor can interact as a 150-kDa monomer. However, in preliminary experiments, the free factor behaved as a 300-kDa dimer in gel filtration and pore-gradient electrophoresis (data not shown). The second method was a two-step "blot and footprint" procedure. The 150-kDa polypeptide transferred onto nitrocellulose from a polyacrylamide gel retained its DNA binding specificity as shown by nuclease-protection experiments with the filter-bound protein. Compared to the alternative procedure where the protein band is eluted and renatured (19), this blotting technique is simpler and can be applied to a complex protein mixture.

Selective proteolysis of factor TUF revealed a protease-resistant domain of about 50 kDa, representing about one-third of the factor molecule. This domain retained the specific DNA-binding activity of native TUF, as shown by nuclease protection experiments (Fig. 6). This observation might be important for understanding the mechanism of gene activation, as it suggests a bipolarization of the protein, with a DNA-binding domain and a larger domain endowed with regulatory functions. Functional domains of other DNA-binding factors have been separated by proteolytic cleavage (20, 21). Construction of hybrid GAL4 proteins also showed that the DNA-binding and gene-activation functions lie on distinct regions of the protein (22).

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1. Struhl, K. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7865–7869.
2. Guarente, L. & Hoar, E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7860–7864.
3. Guarente, L. (1984) *Cell* **36,** 799–800.
4. Arcangioli, B. & Lescure, B. (1985) *EMBO J.* **4,** 2627–2633.
5. Giniger, E., Varnum, S. M. & Ptashne, M. (1985) *Cell* **40,** 767–774.
6. Hope, I. A. & Struhl, K. (1985) *Cell* **43,** 177–188.
7. Huet, J., Cottrelle, P., Cool, M., Vignais, M.-L., Thiele, D., Marck, C., Buhler, J.-M., Sentenac, A. & Fromageot, P. (1985) *EMBO J.* **4,** 3539–3547.
8. Teem, J. L., Abovich, N., Kaufer, N. F., Schwindinger, W. F., Warner, J. R., Levy, A., Woolford, J., Leer, R. J., van Raamsdonsk-Duin, M. M. C., Mager, W. H., Planta, R. J., Schultz, L., Friesen, J. D. Fried, H. & Rosbash, M. (1984) *Nucleic Acids Res.* **12,** 8295–8312.
9. Leer, R. J., van Raamsdonsk-Duin, M. M. C., Mager, W. H. & Planta, R. (1985) *Curr. Genet.* **9,** 273–277.
10. Wouldt, L. P., Smit, A. B., Mager, W. H. & Planta, R. (1986) *EMBO J.* **5,** 1037–1040.
11. Rotenberg, M. O. & Woolford, J. L. (1986) *Mol. Cell. Biol.* **6,** 674–687.
12. Anderson, L.-O., Borg, H. & Mikaelsson, M. (1972) *FEBS Lett.* **20,** 199–202.
13. Jacobs, E. & Clad, A. (1986) *Anal. Biochem.* **154,** 583–589.
14. Miskimins, W. K., Roberts, M. P., McClelland, A. & Ruddle, F. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6741–6744.
15. Felgenhauser, K. (1974) *Hoppe-Seyler's Z. Physiol. Chem.* **355,** 1281–1290.
16. Bowen, B., Steinberg, J., Laemmli, U. K. & Weintraub, H. (1980) *Nucleic Acids Res.* **3,** 1–20.
17. Little, J. W. (1967) *J. Biol. Chem.* **242,** 679–686.
18. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
19. Briggs, M. R., Kadonaga, J. T., Bell, S. P. & Tjian, R. (1986) *Science* **234,** 47–52.
20. Marzouki, N., Camier, S., Ruet, A., Moenne, A. & Sentenac, A. (1986) *Nature (London)* **323,** 176–178.
21. Miller, J., McLachlan, A. D. & Klug, A. (1985) *EMBO J.* **4,** 1609–1614.
22. Brent, R. & Ptashne, M. (1985) *Cell* **43,** 729–736.