The EMBO Journal vol.10 no.4 pp.981 - 985, 1991

A single-stranded DNA binding protein from *S.cerevisiae* specifically recognizes the T-rich strand of the core sequence of ARS elements and discriminates against mutant sequences

Almut M.A.Schmidt, Sabine U.Herterich and Gerhard Krauss

Laboratorium für Biochemie, Universität Bayreuth, Postfach 10 12 51, D-8580 Bayreuth, Germany.

Communicated by M.Sprinzl

A protein named ssARS-T binding protein has been purified from yeast that specifically binds to the T-rich strand of the consensus core sequence of yeast autonomously replicating sequence (ARS) elements. As assayed from gel mobility shift experiments the ssARS-T protein shows characteristics of a sequence specific single-stranded DNA binding protein. The complementary A-rich strand of the ARS core sequence is bound much more weakly and no binding can be detected for the double-stranded form of the core sequence. Three single base substitutions in the core sequence that are known to abolish ARS function *in vivo* also lead to weaker binding of the core sequence to the ssARS-T protein *in vitro*. The strong correlation between the binding of mutated sequences *in vitro* and the ARS properties of these sequences *in vivo* points to an essential function of the ssARS-T protein during replication initiation in yeast ARS elements.

Key words: ARS/sequence specific single-stranded DNA binding protein/*Saccharomyces cerevisiae*

Introduction

The replication of eukaryotic chromosomal DNA initiates at discrete sites called origins. The question of whether origins correspond to specific sequences has proven difficult to resolve. Sequences required for origin functions have been identified to date only for yeast whereas in higher eukaryotes, the search for specific initiation sequences has led to conflicting results. In yeast, sequence elements could be identified which, when cloned into plasmids, confer autonomous replication to these plasmids (Hsiao and Carbon, 1979; Stinchcomb *et al.*, 1979). The sequence elements were called autonomously replicating sequences or ARS elements and it could be shown that plasmid replication initiates close to or within the ARS elements (Brewer and Fangman, 1987; Huberman *et al.*, 1987). Initiation of chromosomal DNA replication in yeast also starts at positions within ARS elements (Huberman *et al.*, 1988; Linskens and Huberman, 1988). The relationship between ARS elements and origins is however a complex one since not all ARS elements in yeast chromosomal DNA are used as replication origins at any one time (Umek *et al.*, 1989).

The sequence requirements for ARS elements to function as origins in yeast plasmids or in yeast chromosomal DNA are not yet fully established. All ARS elements examined to date appear to be composed of three functionally distinct domains, named A-, B- and C-domains (Celniker *et al.*, 1984; Broach *et al.*, 1983). Domain A shows an 11 bp core consensus sequence 5'-(A/T)TTAT(A/G)TTT(A/T)-3' (Broach *et al.*, 1983). The significance of the core sequence for replication has been confirmed by construction and analysis of point mutations (Van Houten and Newlon, 1990; Kipling and Kearsey, 1990; Bouton *et al.*, 1987; Bouton and Smith, 1986; Srienc *et al.*, 1985; Celniker *et al.*, 1984; Kearsey, 1984). Due to the strict sequence requirements the core sequence has been proposed to represent a recognition element for the specific binding of an initiator protein.

The core sequence alone is unable to promote autonomous replication of yeast plasmids. 3' flanking sequences have been shown to be important as well (Palzkill and Newlon, 1988; Bouton and Smith, 1986; Srienc *et al.*, 1985; Celniker *et al.*, 1984). Domain B extends 50–100 bp 3' to the core consensus, is AT-rich and contains 1–5 near matches of the core sequence (Palzkill and Newlon, 1988). Specific protein binding to the B-domain has been reported (Della Seta *et al.*, 1990a and b; Halfter *et al.*, 1989a and b; Walker *et al.*, 1989; Francesconi and Eisenberg, 1989; Diffley and Stillman, 1988; Sweder *et al.*, 1988; Eisenberg *et al.*, 1988; Buchman *et al.*, 1988; Shore *et al.*, 1987) but none of the proteins identified bind to the consensus sequence or to its near matches. The role of the 3' flanking sequences has also been interpreted in terms of thermal stability (Umek and Kowalski, 1988). It has been shown that yeast replication origins are readily underwound in supercoiled plasmids and that nuclease sensitivity correlates with replication efficiency (Umek and Kowalski, 1988). These observations point to a preferential unwinding at ARS elements and to the production of single-stranded regions at ARS sites during initiation.

The search for initiator proteins in yeast has been unsuccessful to date. None of the proteins described as ARS binding proteins bind to the core consensus sequence and show sensitivity to point mutations in this region. In this article we describe the characterization and partial purification of a protein that binds specifically to one of the complementary strands of the ARS core sequence *in vitro*. Protein binding shows sensitivity to point mutations in the core region which correlates with origin properties of the mutated sequences *in vivo*.

Results

Purification of a single-strand specific ARS binding protein

Earlier attempts in our group to purify proteins from yeast that bind specifically to the double-stranded form of the ARS core sequence have been unsuccessful. However we noticed the presence of a protein that binds specifically to the T-rich strand of the ARS core sequence. During purification from baker's yeast the presence of this protein was assayed from gel mobility shift experiments. The 44mer

oligodeoxynucleotide 5′-TCG ATT TTT **ATT TAT GTT TTC** TTC TTC ACA CAT GGG TTA CTG CA-3′ (= ssARS-T) containing the ARS consensus sequence (bold letters) was used as a probe for specific DNA binding. Starting from a crude extract of commercial baker's yeast the ssARS-T binding protein was purified by chromatography on DEAE−cellulose, heparin−Sepharose, the cation exchange resin Fractogel EMD-SO_3^--650 and Blue Sepharose. It has not yet been possible to purify the ssARS-T binding protein to homogeneity. However during purification the enrichment of a protein of 65 kd can be seen (Figure 1A). A polypeptide of the same size is detected when the specific retarded band (see below) from a gel mobility shift experiment is excised, protein is eluted and analyzed on an SDS gel (Figure 1B). From gel filtration on Sephacryl S200 HR, an apparent molecular weight of 65 kd is determined (data not shown) which suggests that the protein exists as a monomer of ~65 kd.

DNA binding specificity

For the detection of specific protein−ssDNA complexes we used gel mobility shift experiments. The 44mer 5′-TCG ATT TTT **ATT TAT GTT TTC** TTC TTC ACA CAT GGG TTA CTG CA-3′ (= ssARS-T) was used as a probe and the specificity of the complexes observed was judged from competition experiments with various nonspecific single-stranded DNAs. The sequence used contains the core sequence ATTATGTTTT and corresponds except for two minor changes outside of the core to positions 166−200 of ARS307 of yeast chromosome III (Palzkill and Newlon, 1988). This ARS element was formerly named C2G1 ARS (Palzkill *et al.*, 1986). For the T-rich strand of ARS307 a retarded band is detectable up to a 12 000-fold excess of single-stranded M13 DNA (Figure 2). At a low excess of nonspecific competitor DNA a second slower migrating band is observed that disappears at a higher degree of competition (Figure 2). As judged from the competition behaviour the faster migrating band represents a distinct specific complex.

The complementary 36mer oligodeoxynucleotide ssARS-A 5′-GTA ACC CAT GTG TGA AGA AGA **AAA CAT AAA TAA** AAA-3′ containing the A-rich part of the ARS core forms a complex with the ssARS-T binding protein only at low concentrations of competitor DNA, indicating a much lower affinity of the protein for this sequence (Figure 3). Using the duplex between ssARS-T and ssARS-A as a probe, we could not detect complexes, even in the absence of competitor DNA; this demonstrates the absolute requirement for a single-stranded form of the binding substrate (Figure 4). This conclusion is also supported by hybridization competition experiments where a complex between the ssARS-T binding protein and the 44mer ssARS-T oligodeoxynucleotide was preformed and subjected to competition with the complementary ssARS-A oligodeoxynucleotide. Figure 5 shows that the 36mer ssARS-A is able to displace the binding protein from the preformed complex with ssARS-T at a ratio of ssARS-T to ssARS-A of about 1:1. The 12mer 5′-TAA ACA TAA AAT-3′ complementary to the core sequence however is unable to displace the ssARS-T binding protein. Presumably the core sequence is covered by the binding protein and is not accessible for hybridization by the 12mer. Alternatively the thermal stability of the hybrid between the 12mer and ssARS-T is not high enough for competition to occur. A displacement of the ssARS-T binding protein from the specific complex by the complementary 36mer ssARS-A oligodeoxynucleotide is also observed in the presence of 1 mM ATP (data not shown). One would not expect a dissociation of the specific protein−DNA complex if the ssARS-T binding protein contained a helicase activity.

To check whether specific complex formation is influenced by the presence of the flanking sequences of the core sequence we investigated the binding of the 24mer 5′-CCC CGA **TTT TAT GTT TAG** ACC CCC-3′, the 19mer 5′-ATG CGA **TTT TAT GTT TAG** A-3′ and their complementary counterparts. For both sets of oligodeoxy-

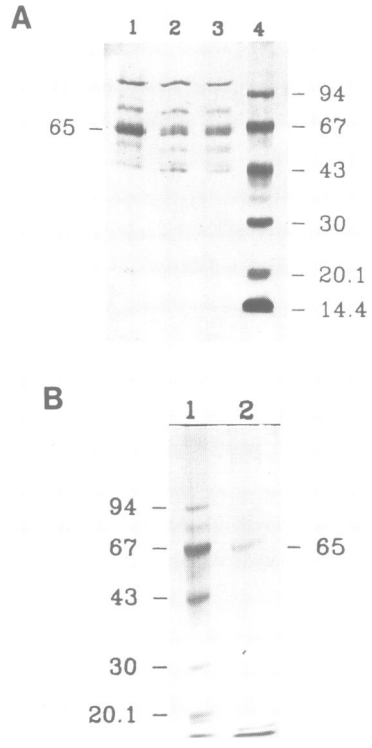

Fig. 1. SDS gel electrophoresis of partially purified ssARS-T binding protein: (**A**) Lanes 1−3: ssARS-T binding activity containing Blue Sepharose fractions from three different purifications. Lane 4: molecular weight standard. (**B**) Lane 1: molecular weight standard. Lane 2: excised retarded band from a gel mobility shift experiment.

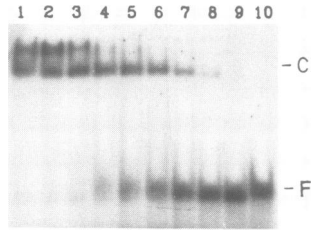

Fig. 2. Sequence specific binding of the 44mer ssARS-T oligodeoxynucleotide by the ssARS-T binding protein. Reactions contained 0.5 ng of ^{32}P-labelled ssARS-T oligodeoxynucleotide and 5 μl of binding protein fraction after Blue Sepharose. The effect of increasing concentrations of M13mp8 ssDNA as nonspecific competitor DNA is shown. In lanes 1−9 the ratios (w/w) of labelled ssARS-T probe to M13ssDNA were 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400 and 1:12800, respectively. Lane 10: as lane 9, without added ssARS-T binding protein. (F = free DNA, C = specific protein−DNA complex)

nucleotides the same preference for the T-rich strand was observed. The complexes are however more sensitive to competition with single-stranded M13 ssDNA and have already disappeared at a competition of 1:5000 (data not shown).

Further support for the high specificity of the complexes with the 44mer ARS-T oligodeoxynucleotide comes from binding experiments with oligodeoxynucleotides that are not related to the ARS core sequence. Both strands of the Nuclear Factor I consensus sequence (Guggenheimer *et al.*, 1984) present in the 27mers 5′-AAT TAT TTT GGA TTG AAG CCA ATA TGA-3′ (1) and 5′-AAT TTC ATA TTG GCT TCA ATC CAA AAT-3′ (2) are bound very weakly and in a nonspecific manner by the ssARS-T binding protein as judged from competition with M13mp8 ssDNA (data not shown).

We can rule out the possibility that the observed specific complexes are due to a hybridization of the 44mer ssARS-T oligodeoxynucleotide to contaminating nucleic acids. Treatment of the protein extract with phenol abolishes binding and digestion with RNase A does not destroy the retarded bands (data not shown).

Choice of competitor DNA

The choice of the competitor DNA could be critical for the evaluation of the specificity of the binding reaction. Therefore we studied a series of polyribo- and polydeoxynucleotides in mobility shift experiments as nonspecific competitors for the specific binding of the T-rich 44mer (Figure 6). A poor competition is observed for poly(C), poly(dA), poly(dC) and poly(dI). M13mp8 ssDNA is the best competitor. Poly(dT) and poly(U) are slightly less effective in competition, showing that T-stretches or U-stretches alone are not sufficient for specific complex formation. In contrast to the nonspecific sequences, the unlabelled specific sequence is efficient in competition (Figure 7, lanes 1–4). In the presence of poly(dA), poly(dC) and poly(C), the specific complex shows a different electrophoretic mobility as compared with the presence of the other competitor DNAs. This behaviour is difficult to explain unless more detailed information about the structure of the complex is available.

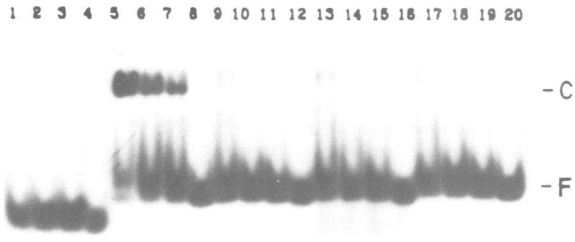

Fig. 3. Binding of the A-rich strand and of mutant ARS core sequences. 1 ng of labelled DNA probe and 5 μl of ssARS-T binding protein fraction after Blue Sepharose were assayed for binding in the presence of 100 ng, 500 ng and 1000 ng of competitor M13mp8 ssDNA. Lanes 1–3: ssARS-A. Lane 4: ssARS-A, without protein. Lanes 5–7: ssARS-T. Lane 8: ssARS-T without protein. Lanes 9–11: mutant ssARS-T-918. Lane 12: mutant ssARS-T-918 without protein. Lanes 13–15: mutant ssARS-T-1048. Lane 16: mutant ssARS-T-1048 without protein. Lanes 17–19: mutant ssARS-T-305. Lane 20: mutant ssARS-T-305 without protein.

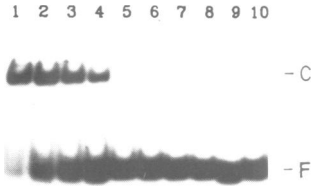

Fig. 4. Binding of single-stranded compared with double-stranded form of the recognition sequence. Binding reactions contained 5 μl of ssARS-T binding protein as in Figure 2, 0.5 ng of labelled DNA probe and 250 ng, 500 ng, 1000 ng and 2000 ng of competitor M13mp8 ssDNA, respectively. Lanes 1–4: ssARS-T. Lanes 5–8: hybrid between ssARS-T and ssARS-A. Lanes 9 and 10: sARS-T and hybrid without added protein.

Binding of mutant ARS core sequences

Support for a function of the observed complexes during replication *in vivo* has to come from binding experiments with mutated ARS core sequences that are defective in their ability to promote autonomous replication. Such mutant ARS sequences have been constructed and examined in detail (Kearsey, 1984; Van Houten and Newlon, 1990). We investigated three mutant sequences carrying single-base changes for binding to the ssARS-T binding protein. In the synthetic 44mer oligodeoxynucleotides used the flanking sequences of the core sequence were the same as in the wild-type ssARS-T sequence. The oligodeoxynucleotides ssARS-T-918 (5′-TCG ATT TTT AAT TAT GTT TTC TTC TTC ACA CAT GGG TTA CTG CA-3′) and ssARS-T-1048 (5′-TCG ATT TTT ATT TAT GTT CTC TTC TTC ACA CAT GGG TTA CTG CA-3′) are derived from earlier work of Kearsey (1984) and carry substitutions of A for T and C for T, respectively. Both mutants have been shown to confer an ARS$^-$ phenotype in *in vivo* experiments (Kearsey, 1984). The mutated core sequence ATT TTT GTT TT of the 44mer ssARS-T-305 has been examined recently (Van Houten and Newlon, 1990) and shown to be ARS$^-$ too. Figure 3 illustrates that all three mutant sequences bind

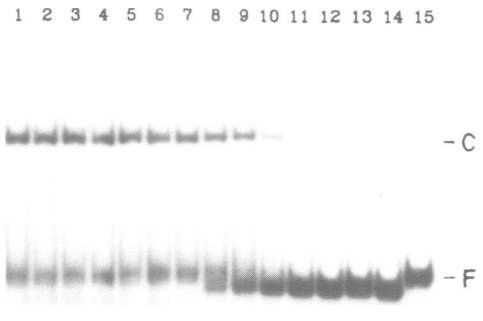

Fig. 5. Displacement of ssARS-T binding protein from the specific protein–DNA complex by complementary sequences. Binding reactions contained 1 ng of labelled ssARS-T, 3 μl of ssARS-T binding protein fraction as in Figure 2, 1 μg of M13mp8 ssDNA and complementary oligodeoxynucleotide as indicated below. Protein was preincubated with labelled ssARS-T before addition of the complementary A-rich strands. Lanes 1 and 7: no complementary oligodeoxynucleotide. Lanes 2–6: complementary 12mer 5′-TAA ACA TAA AAT-3′ in a molar ratio of 1:0.25, 1:0.5, 1:1, 1:2 and 1:4 repectively. Lanes 7–14: complementary 36mer ssARS-A in a molar ratio of 1:0.125, 1:0.25, 1:0.5, 1:1, 1:2, 1:4 and 1:8, respectively. Lane 15: as lane 1, without protein.

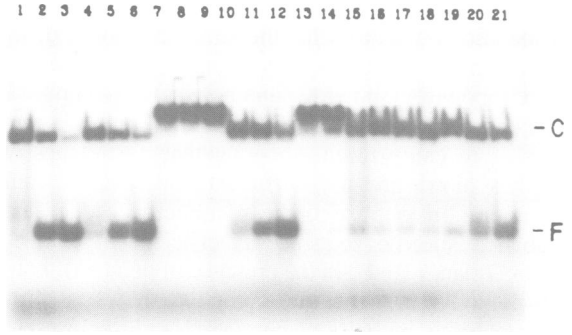

Fig. 6. Comparison of the efficiency of various nonspecific single-stranded competitor DNAs. Binding reactions contained 0.5 ng of the 44mer ssARS-T and 5 μl of protein as in Figure 2. Competition ratios of 1:500, 1:2000 and 1:8000 were assayed with M13mp8 ssDNA (lanes 1–3), poly(dT) (lanes 4–6), poly(dA) (lanes 7–9), poly(U) (lanes 10–12), poly(dC) (lanes 13–15), poly(C) (lanes 16–18) and poly(dI) (lanes 19–21).

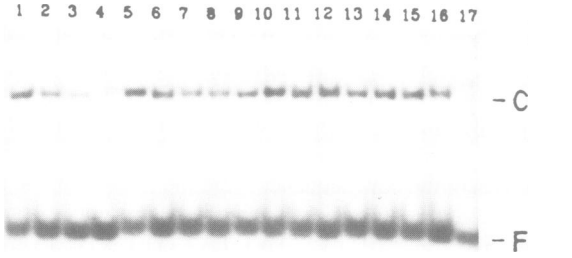

Fig. 7. Competition of the specific protein–DNA complex with the specific sequence and with mutant sequences. 1 ng of labelled ssARS-T, 5 μl of ssARS-T binding protein fraction as in Figure 2 and 2 μg of M13mp8 ssDNA were assayed for complex formation in the presence of ssARS-T and unlabelled mutant 44mers. Lanes 1–4: 0, 10, 20 and 50 ng of unlabelled ssARS-T. The same amounts of unlabelled mutant 44mer oligodeoxynucleotides were added in lanes 5–16. Lanes 5–8: ssARS-T-918, lanes 9–12: ssARS-T-1048, lanes 13–16: ssARS-T-305. Lane 17: no protein added.

with a much lower affinity to the ssARS-T binding protein as compared with the wild-type sequence. As judged from the competition experiments, the mutant sequences, however, bind more strongly to the ssARS-T binding protein than the A-rich strand of the ARS core sequence, indicating that some specificity of binding is preserved upon the single-base changes. A weaker binding of the mutant sequences is also visible in competition experiments between the wild-type and the mutant sequences (Figure 7). The experiments demonstrate, for the core sequences investigated, a correlation between binding to the ssARS-T binding protein *in vitro* and ARS function *in vivo*, pointing to an essential function of the ssARS-T binding protein during replication initiation in ARS sequences.

Discussion

The autonomously replicating sequences of yeast are (apart from the replication origin of SV40) the only sequence elements in eukaryotes for which an essential function during replication initiation has been established and whose sequence requirements for replication initiation have been defined. Information about proteins involved in replication initiation in yeast has been scarce however. The strict conservation of the ARS core sequence in yeast strongly argues in favour of the existence of initiation proteins that function via a specific recognition of the initiation sequence similarly to the large T antigen in the SV40 system. The protein we have purified fulfils the most critical criterion for the classification as a yeast ARS initiator protein, namely the differential recognition of wild-type sequences and mutant sequences, which when examined in *in vivo* experiments, show loss of ARS activity. The strong correlation between binding of the ssARS-T binding protein to wild-type and mutated sequences *in vitro* and function of these sequences *in vivo* makes the ssARS-T binding protein a good candidate for a specific initiator protein in yeast. The ssARS-T binding protein appears to be a single-stranded DNA binding protein with strict sequence recognition properties. Examples of such proteins are rare in the literature (Gaillard and Strauss, 1990; Wilkinson *et al.*, 1990; Pan *et al.*, 1990; Gaillard *et al.*, 1988). Recently a sequence specific single-stranded DNA binding protein has been described that is involved in transcription of the SV40 late genes (Gaillard and Strauss, 1990).

In the present work the sequence specificity of the ssARS-T binding protein is inferred from competition experiments in gel mobility shift experiments. The complexes between the ARS binding protein and the core sequence remain stable in the presence of a more than 8000-fold excess of M13 ssDNA and other single-stranded DNAs. It is interesting to note that of the single-stranded DNAs tested the M13mp8 ssDNA is the most effective in competition. During attempts to obtain revertants of ARS$^-$ clones it has been found that the majority of the revertants originated from the M13mp9 vector sequence indicating that M13 contains sequences that are closely related to the ARS core sequence (Kipling and Kearsey, 1990). Such related sequences could be responsible for the competition behaviour of the M13ssDNA. The sequence specificity of the ARS binding protein cannot be explained in terms of recognition of T-rich stretches of ssDNA only since the mutant sequence ssARS-T-305 has an increased T-content and yet shows diminished binding as compared with the wild-type sequence.

The ssARS-T binding protein binds specifically only one strand of the ARS core sequence and exhibits only very low affinity for the double-stranded form of the ARS core sequence. Specific complexes must therefore only be able to form after generation of single-stranded ARS regions. A localized unwinding of ARS elements has been suggested from a mutational analysis of the B-region (Umek and Kowalski, 1988). ARS containing supercoiled plasmids are readily unwound at the ARS elements and the efficiency of ARS elements *in vivo* correlates strongly with the energy required for unwinding. From the observation of specific binding to the T-rich strand of the ARS core sequence only, it can be speculated that during replication initiation in ARS elements the two strands of the core are made functionally unequivalent by differential binding of the ssARS-T binding protein. The meaning of the specific complex formation with the T-rich strand is however unclear, due to the complete lack of knowledge of enzymatic processes involved in replication initiation in yeast. The ssARS-T binding protein seems not to be functionally equivalent to the SV40 large T antigen. It shows completely different DNA binding characteristics and up to now we have not been able to detect

a helicase acivity in the ssARS-T binding protein preparations.

Materials and methods

Nucleic acids

Oligodeoxynucleotides were prepared by chemical DNA synthesis on an Applied Biosystems 381 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany).

Preparation of yeast whole extracts

Commercial baker's yeast was obtained from GIEGOLD (Schwarzenbach/Saale, Germany). Cells (1 kg) were incubated overnight at room temperature in 1.5 l of 200 mM Tris−HCl pH 7.5, 300 mM NH_4Cl, 20 mM $MgSO_4$, 1 mM EDTA and 150 mM glucose, were then harvested by centrifugation and resuspended in 1.5 l of 20 mM Tris−HCl pH 7.5, 10 mM $Mg(OAc)_2$, 10 mM NH_4Cl, 10 mM KCl, 1 mM 2-mercaptoethanol, 0.1 mM phenylmethanesulphonylfluoride, 0.1 mM EDTA and 10% (v/v) glycerol. A bead mill (Netsch, Selb, Germany) was used for disintegration. A S-100 supernatant was prepared by centrifugation at 100 000 *g* and loaded on a 2×13 cm column of DEAE−Sephadex A25 (Pharmacia, Freiburg, Germany) preequilibrated with disintegration buffer without NH_4Cl. The flow-through was precipitated by addition of $(NH_4)_2SO_4$ (70% saturation) and dialysed against 20 mM K-phosphate, pH 7.0, 2 mM 2-mercaptoethanol, 20 μM phenylmethanesulfonylfluoride and 50% (v/v) glycerol. The resulting protein extract was stored in aliquots at −60°C.

Purification of ssARS-T binding protein

A 5 ml heparin−Sepharose column was equilibrated with 20 mM K-phosphate, pH 7.0, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.2 mM EDTA and 50 mM KCl (buffer A). 5 ml of protein extract were mixed with 3 vol. of buffer A and loaded onto the column, which was then washed with 5 vol. of buffer A containing 100 mM KCl. Proteins were eluted with a stepwise gradient from 200 to 600 mM KCl in buffer A. The peak of ssARS-T binding activity eluting at 250 mM KCl was pooled, diluted 3-fold with buffer A and loaded onto a 2 ml Fractogel EMD SO_3^--650 (M) (Merck, Darmstadt, Germany) column preequilibrated with 20 mM K-phosphate, pH 7.0, 5 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 100 mM KCl (buffer B). The column was washed with 5 vol of buffer B containing 150 mM KCl and eluted with a gradient from 200 to 600 mM KCl in buffer A. ssARS-T binding activity eluted at 300 mM KCl. Active fractions were combined and loaded onto a Blue Sepharose (Biorad, München, Germany) column preequilibrated with buffer B. The column was washed with 200 mM KCl in buffer B and eluted with a stepwise gradient from 300 to 600 mM KCl. Binding activity eluted at 400 mM KCl. Active fractions were pooled and dialysed against buffer D containing 50 mM K-phosphate, pH 7.0, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 150 mM KCl and 50% (v/v) glycerol and stored at −20°C.

Gel retardation assay

For gel retardation experiments protein−nucleic acid complexes were allowed to form in 25 mM K-phosphate, pH 7.0, 1 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol, 150 mM KCl and 10% (v/v) glycerol in a volume of 10 μl. Protein extract (2−5 μl) and various amounts of competitor DNA were preincubated for 15 min at room temperature. Radioactive DNA probe (0.5−2 ng) was added and incubation was continued for 15 min at room temperature. Electrophoresis was performed on 8% polyacrylamide gels in Tris−acetate, pH 7.6 at 10 V/cm for 2 h at 8°C.

For preparative purposes retarded bands were excised, homogenized and extracted with electrophoresis buffer containing 2% SDS, 100 μg/ml myoglobin and 1 mM 2-mercaptoethanol. Samples were concentrated (Amicon Centricons 30000, Amicon, Witten, Germany), precipitated by TCA and analysed by SDS−PAGE.

SDS-polyacrylamide gel electrophoreses

Protein samples were TCA precipitated, pellets were collected by centrifugation, washed with ether−ethanol 1:1 (v/v) and resuspended in buffer containing 20% (v/v) glycerol, 0.01% bromphenol blue, 2% (w/v) SDS, 1.4 M 2-mercaptoethanol, 5 mM bicine pH 8.3 and 5 mM Tris−HCl pH 8.3. After incubation at 95°C for 3 min, samples were analysed using PhastGel Homogeneous 12,5 from Pharmacia (Freiburg, Germany). Gels were silver-stained using the Pharmacia Silver Stain Kit.

Acknowledgements

We thank Mrs C.Förster for providing the yeast crude extracts and Mr C.Reiser for reading the manuskript.

References

Bouton,A.H. and Smith,M.M. (1986) *Mol. Cell. Biol.*, **6**, 2354−2363.
Bouton,A.H., Stirling,V.B. and Smith,M.M. (1987) *Yeast*, **3**, 107−115.
Brewer,B.J. and Fangman,W.L. (1987) *Cell*, **51**, 463−471.
Broach,J.R., Li,Y.-Y., Feldman,J., Jayaram,M., Abraham,J., Nasmyth,K.A. and Hicks,J.B. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **47**, 1165−1173.
Buchman,A.R., Kimmerly,W.J., Rine,J. and Kornberg,R.D. (1988) *Mol. Cell. Biol.*, **8**, 210−225.
Celniker,S.E., Sweder,K.S., Srienc,F., Bailey,J.E. and Campbell,J.L. (1984) *Mol. Cell. Biol.*, **4**, 2455−2466.
Della Seta,F., Ciafre,S.A., Marck,C., Santoro,B., Prescutti,C., Sentenac,A. and Bozzoni,I. (1990a) *Mol. Cell. Biol.*, **10**, 2437−2441.
Della Seta,F., Treich,J., Buhler,J.-M. and Sentenac,A. (1990b) *J. Biol. Chem.*, **265**, 15168−15175.
Diffley,J.F.X. and Stillman,B. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 2120−2124.
Eisenberg,S., Civalier,C. and Tye,B.K. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 743−746.
Francesconi,S.C. and Eisenberg,S. (1989) *Mol. Cell. Biol.*, **9**, 2906−2913.
Gaillard,C. and Strauss,F. (1990) *J. Mol. Biol.*, **215**, 245−255.
Gaillard,C., Weber,M. and Strauss,F. (1988) *J. Virol.*, **62**, 2380−2385.
Guggenheimer,R.A., Stillman,B.W., Nagata,K., Tamanoi,F. and Hurwitz,J. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3069−3073.
Halfter,H., Müller,U., Kavety,B., Vanderkerckhove,J., Kiefer,F. and Gallwitz,D. (1989a) *EMBO J.*, **8**, 4265−4272.
Halfter,H., Winnacker,E.L. and Gallwitz,D. (1989b) *EMBO J.*, **8**, 3029−3037.
Hsiao,C.-L. and Carbon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3829−3833.
Huberman,J.A., Spotila,L.D., Nawotka,K.A., ElAssouli,S.M. and Davis,L.R. (1987) *Cell*, **51**, 473−481.
Huberman,J.A., Zhu,J., Davis,L.R. and Newlon,C.S. (1988) *Nucleic Acids Res.*, **16**, 6373−6384.
Kearsey,S.E. (1984) *Cell*, **37**, 299−307.
Kipling,D. and Kearsey,S.E. (1990) *Mol. Cell. Biol.*, **10**, 265−272.
Linskens,M.H.K. and Huberman,J.A. (1988) *Mol. Cell. Biol.*, **8**, 4927−4935.
Palzkill,T.G. and Newlon,C.S. (1988) *Cell*, **53**, 441−450.
Palzkill,T.G., Oliver,S.G. and Newlon,C.S. (1986) *Nucleic Acids Res.*, **14**, 6247−6264.
Pan,W.T., Lin,Q. and Bancroft,T. (1990) *J. Biol. Chem.*, **265**, 7022−7028.
Shore,D., Stillman,D.J., Brand,A.H. and Nasmyth,K.A. (1987) *EMBO J.*, **6**, 461−467.
Srienc,F., Bailey,J.E. and Campbell,J.L. (1985) *Mol. Cell. Biol.*, **5**, 1676−1684.
Stinchcomb,D.T., Struhl,K. and Davis,R.W. (1979) *Nature*, **282**, 39−43.
Sweder,K.S., Rhode,P.R. and Campbell,J.L. (1988) *J. Biol. Chem.*, **263**, 17270−17277.
Umek,R.M and Kowalski,D. (1988) *Cell*, **52**, 559−567.
Umek,R.M., Linskens,M.H.K., Kowalsky,D. and Huberman,J.A. (1989) *Biochim. Biophys. Acta*, **1007**, 1−14.
Van Houten,J.V. and Newlon,C.S. (1990) *Mol. Cell. Biol.*, **10**, 3917−3925.
Walker,S., Francesconi,S.C., Tye,K. and Eisenberg,S. (1989) *Mol. Cell. Biol.*, **9**, 2914−2921.
Wilkinson,W.O., Min,H.Y., Claffey,K.P., Satterberg,B.L. and Spiegelman,B.M. (1990) *J. Biol. Chem.*, **265**, 477−482.

Received on December 11, 1990; revised on January 16, 1991