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A Single Domain of Yeast Poly(A)-Binding Protein Is Necessary and Sufficient for RNA Binding and Cell Viability

ALAN B. SACHS,[1] RONALD W. DAVIS,[2] AND ROGER D. KORNBERG[1]*

Departments of Cell Biology[1] and Biochemistry,[2] Stanford University School of Medicine, Stanford, California 94305

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The poly(A)-binding protein (PAB) gene of *Saccharomyces cerevisiae* is essential for cell growth. A 66-amino acid polypeptide containing half of a repeated N-terminal domain can replace the entire protein in vivo. Neither an octapeptide sequence conserved among eucaryotic RNA-binding proteins nor the C-terminal domain of PAB is required for function in vivo. A single N-terminal domain is nearly identical to the entire protein in the number of high-affinity sites for poly(A) binding in vitro (one site with an association constant of approximately 2×10^7 M^{-1}) and in the size of the binding site (12 A residues). Multiple N-terminal domains afford a mechanism of PAB transfer between poly(A) strands.

Saccharomyces cerevisiae poly(A)-binding protein (PAB) is representative of a family of eucaryotic RNA-binding proteins, which includes the A1, A2, and C polypeptides of mammalian heterogeneous nuclear ribonucleoprotein, the HD40 protein of *Artemia salina*, and the nucleolar RNA-binding protein nucleolin (5a; B. M. Merrill, K. L. Stone, A. Kumar, W. Szer, and K. R. Williams, submitted for publication). Members of this family have the following in common: (i) an N-terminal domain of 80 to 90 residues, which may be tandemly repeated; (ii) a degree of sequence conservation of the N-terminal domain, especially of the octapeptide sequence Arg/Lys-Gly-Phe/Tyr-Gly/Ala-Phe/Tyr-Val-X-Phe/Tyr; and (iii) a C-terminal domain, which can be removed, in some cases, by proteolysis. The N-terminal domains have been implicated in RNA binding, while the C-terminal domain may mediate protein-protein interaction, for example, through a keratinlike sequence in the A1 heterogeneous nuclear ribonucleoprotein. We report here on the physiologic importance of the various domains of the yeast PAB and on the roles of the domains in RNA binding in vitro.

PABs occur as nuclear and cytoplasmic proteins, the former differing from the latter by cleavage but not loss of a C-terminal domain (2, 15, 16). A single gene has been cloned that encodes both forms (1, 15). The gene sequence revealed four tandemly repeated N-terminal domains connected by a methionine- and proline-rich linker to the C-terminal domain. The sequence also disclosed an A-rich region of the mRNA upstream of the initiator codon that could be bound by PAB, raising the possibility of autoregulation of PAB synthesis. In the present study, we constructed a yeast strain dependent on a plasmid-borne copy of the PAB gene for growth. Mutations in the gene were assessed for effects on cell viability, and mutant proteins were expressed in *Escherichia coli* and analyzed in regard to poly(A) binding in vitro.

MATERIALS AND METHODS

Plasmids. Yeast plasmids containing *CEN4*, *ARS1*, and either *URA3* (pSE360) or *TRP1* (pSE358) markers subcloned between nucleotides 183 and 2674 of pUC18 were kindly provided by S. Elledge. A 2.9-kilobase (kb) *Sal*I-*Kpn*I fragment containing the yeast PAB gene was inserted into the polylinker of these plasmids. Mutant forms of the PAB gene (Table 1) were constructed by the deletion of restriction fragments, by *Bal* 31 deletion (10), or by site-specific mutagenesis (S. Inouye and M. Inouye, *in* S. Narang, ed., *DNA and RNA Synthesis*, in press) with the oligonucleotide 5′-CGGTAAGTCGAAGGGTTTGGGTTT-3′. This oligonucleotide corresponds in sequence to nucleotides 1935 to 1958 of the PAB gene, except that a T at position 1953 is replaced with a G (changing Phe-364 to Leu) and a T at position 1944 is replaced with a G (creating a *Taq*I site at position 1943).

A plasmid for expression of wild-type PAB (p68) in *Escherichia coli* was constructed by attachment of *Bam*HI linkers to nucleotides 885 to 3435 of the PAB gene and insertion at the *Bam*HI site of pAS1 (14) (unrelated to yeast strain AS1 described below). Plasmids for expression of p66, p55, and p35 (and F364) were identical except for deletion of nucleotides 1028 to 1131, 2274 to 2678, and 885 to 1702, respectively.

Yeast strains. A *HIS3* gene was inserted into a 3.4-kb *Sal*I-*Eco*RI fragment containing the PAB gene, replacing nucleotides 885 to 2438 of the PAB gene. Transplacement (11) of a diploid yeast strain (α/**a** *ura3-52/ura3-52 trpΔ1/trpΔ1 his3Δ/his3Δ leu2-3-12/+ +/ade2-101 can*^{r}*/can*^{s}) with this fragment gave strain AS1. Further transformation with pSE360 containing the entire PAB gene (nucleotides 1 to 2935), followed by sporulation on 1% potassium acetate medium for 4 days and dissection of tetrads, gave strain AS2 (α *his*$^+$ *ura*$^+$ *trp leu ade*).

Cell viability and growth rates. Yeast strain AS2 was transformed with pSE358 containing a mutant PAB gene and grown for 4 to 6 days on minimal medium (17) supplemented with uracil, leucine, and adenine. During this time, some *TRP*$^+$ cells lost pSE360 owing to random segregation. Transformants were plated on minimal medium supplemented with uracil, leucine, 0.2 strength adenine, and 1 mg of 5-fluoroorotic acid (SCM Speciality Chemicals, Gainesville, Fla.) per ml. Growth on this medium was revealed by an increase in colony size and by the appearance of a red color, which is indicative of actively metabolizing, *ade2* cells (13). Plasmid DNA in viable cells was examined by restriction analysis to ensure the loss of the wild-type PAB gene and the correct structure of the mutant gene. Generation times were obtained from the optical density at 600 nm of

* Corresponding author.

TABLE 1. Construction of PAB mutants

Construction[a]	Modification[b]	Nucleotides of PAB gene remaining[c]
1	None	1–2935
2	Δ*hpa*II-*spe*I	1–2152, 2678–2935
3	Δ*hin*dIII-*Spe*I	1–1692, 2678–2935
4	Δ*Hin*fI-*Spe*I	1–1483, 2678–2935
5	Δ*Pvu*II-*Hin*fI	1–884, 1703–2935
6	Δ*Pvu*II-*Pvu*II	1–884, 2438–2935
7	Δ*Eco*RV-*Nae*I	1–1027, 1132–2935
8	Δ*Taq*I-*Hin*fI	1–1378, 1483–2935
9	Δ*Hin*fI-*Hin*fI	1–1483, 1703–2935
10	Δ*Pvu*II-*Hin*fI, *Nde*I-*Spe*I	1–884, 1703–1893, 2678–2935
11	None	542–2935
12	*Bal* 31 deletion	542–764, 842–2935
13	Phe-364 → Leu	1–884, 1703–2935
14	Δ*Sph*I-*Spe*I	542–2274, 2678–2935
15	*Bal* 31 deletion; Δ*Sph*I-*Spe*I	542–764, 842–2274, 2678–2935

[a] Numbers correspond to those of proteins in Fig. 3.
[b] Deletion (Δ) of restriction fragment, *Bal* 31 deletion, or site-specific mutation.
[c] Numbering as in Sachs et al. (15). The A-rich upstream region extends from nucleotides 807 to 862, and the coding region extends from nucleotides 862 to 2592.

cells growing in YPD medium (17) at the indicated temperatures.

Protein and mRNA levels. Yeast strain AS2 was transformed with pSE358 containing PAB constructions (Table 1). Cells containing both the wild-type PAB gene and the mutant gene were grown to the mid-logarithmic phase, and whole-cell extracts of protein and nucleic acid were prepared (15). Proteins were revealed by immunoblot analysis with antibodies against PAB (15) and ^{125}I-protein A. RNA was revealed by S1 nuclease analysis with nucleotides 1 to 1689 of the PAB gene as the hybridization probe (15). Ratios of levels of wild-type and mutant gene products were determined.

Expression and purification of PABs. *E. coli* AR120 transformed with the pAS1 derivatives described above (24 l) was grown to the mid-logarithmic phase and induced for 4 h with 40 mg of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) per ml (14). Cells were harvested, washed once with 6 liters of STE (150 mM NaCl, 30 mM Tris hydrochloride [pH 8.0], 5 mM EDTA, 15 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1.4 μg of pepstatin per ml, 400 ng of leupeptin per ml), resuspended in 300 ml of STE, cooled to 4°C, stirred with 400 μg of lysozyme (Sigma) per ml, frozen in liquid nitrogen, quickly thawed, sonicated, and centrifuged for 30 min at 12,000 rpm in a Beckman JA14 rotor. The supernatant (containing approximately 6 to 7 g of protein) was loaded at 300 ml/h on a 350-ml Affi-Gel blue column (5.5 by 15 cm) equilibrated in STE. The column was washed at this rate with 900 ml of STE, 3,500 ml of 2 M NaCl in STE, and 300 ml of STE. Protein was eluted with 1.3 M guanidine hydrochloride in STE, and peak protein fractions (containing approximately 600 mg of protein) were pooled, dialyzed three times against 4 liters of LS (50 mM NaCl, 5 mM Tris hydrochloride [pH 8.0], 0.5 mM EDTA, 15 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl floride), and centrifuged at 11,000 rpm in a Sorvall SS34 rotor. The supernatant was loaded at 60 ml/h on either a 60-ml DEAE-Sephacryl (Pharmacia, Uppsala, Sweden) column (2.5 by 15 cm) for p55, p66, and p68 or a 60-ml carboxymethyl cellulose column (Whatman, Maidstone, Kent) for p35 and F364. The columns were washed with 180 ml of LS buffer at this rate, and protein was eluted with a 500-ml linear gradient of 0 to 333 mM NaCl in LS. Peak protein fractions (eluting between 70 and 120 mM NaCl) were pooled and either precipitated with ammonium sulfate (45, 70, and 55% of saturation for p55, p66, and p68, respectively), dialyzed against LS, and stored at 10 mg/ml at −70°C or simply stored at −70°C (p35 and F364). All steps were carried out at 4°C.

Final yields of protein were approximately 20 mg for p35 and F364 and 100 mg for p55, p66, and p68. Purity was estimated to be between 90 and 95% from Coomassie blue-stained sodium dodecyl sulfate–10% or 12.5% polyacrylamide gels (8). Protein concentrations were determined from the A_{257}, using the calculated molar extinction coefficients (M^{-1} cm^{-1}) for tryptophan and tyrosine residues (p68, 36,420; p66, 33,740; p55, 29,720; p35 and F364, 17,420).

Oligo(A) and poly(A). Poly(A), poly(1,N^6-ethenoadenylic acid) [poly(EA)], and poly(C) (P-L Biochemicals, Inc., Madison, Wis.) were dialyzed for 48 h against water. Poly(A) with a degree of polymerization of greater than 800, obtained from the void volume of a Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) column (9), was used in all experiments.

Oligo(A)s 8 to 12 residues in length were purchased from P-L Biochemicals. Oligo(A)s of 13 to 23 residues were prepared by heating poly(A) (1.5 mg/ml) in 0.2 N NaOH for 50 min at 37°C, followed by neutralization, dialysis against water, lyophilization, and electrophoresis in a 1.5-mm-thick 15% polyacrylamide gel containing 7.5 M urea and half-strength TBE (10). Gel slices were crushed and eluted with 3 ml of TE (10 mM Tris hydrochloride [pH 8.0], 0.1 mM EDTA) for 20 h. The eluates were centrifuged, desalted by filtration through a Sephadex G-25 (Pharmacia) column (1.5 by 6 cm), lyophilized, and suspended in water at a final concentration of 100 μM.

Fluorescence. Fluorescence measurements were made in duplicate or triplicate at 23°C with an SLM 8000 fluorimeter (SLM Instruments, Urbana, Ill.). Protein fluorescence was

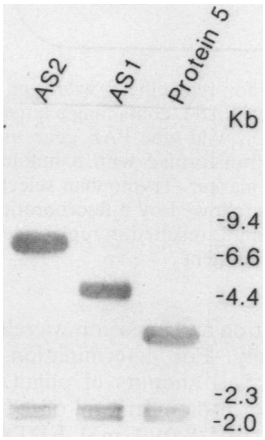

FIG. 1. PAB genes in mutant strains revealed by blot hybridization. DNA from yeast strain AS1, AS2, or AS2 with the plasmid-borne wild-type PAB gene replaced with a plasmid-borne mutant gene (construction 5 of Table 1, encoding protein 5 of Fig. 3) was digested with *Sal*I and *Pst*I, fractionated in a 0.8% agarose gel, transferred to Gene Screen, and hybridized with an *Hha*I-*Eco*RI fragment (nucleotides 524 to 3435) of the PAB gene as a probe. Bands correspond to the wild-type chromosomal PAB gene (6.6 kb), the largely deleted chromosomal gene (2.0-kb doublet), the wild-type gene on a *URA3* plasmid (4.4 kb), and a mutant gene on a *TRP1* plasmid (3.1 kb).

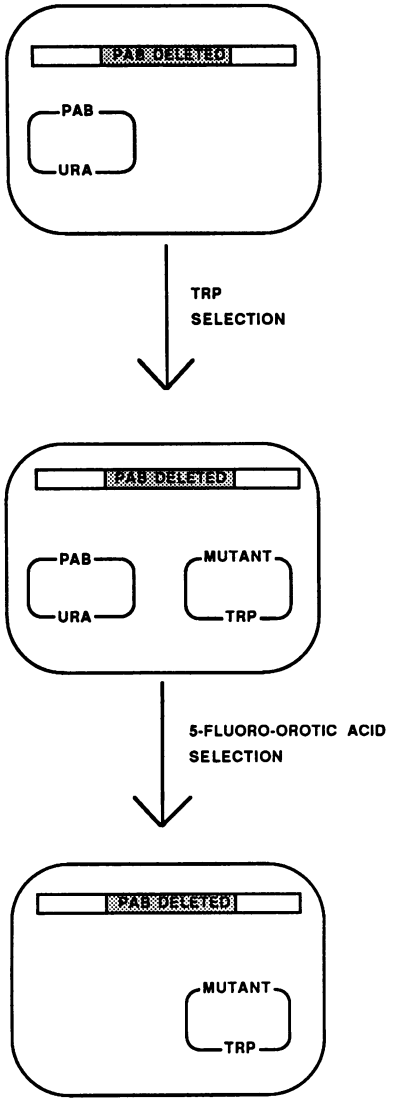

FIG. 2. Procedure for replacing a wild-type PAB gene with a mutant PAB gene. Strain AS2, containing a largely deleted chromosomal PAB gene and a wild-type PAB gene on a plasmid with a *URA3* marker, was transformed with a mutant PAB gene on a plasmid with a *TRP1* marker. Tryptophan selection for acquisition of the *TRP1* plasmid, followed by 5-fluoroorotic acid selection for loss of the *URA3* plasmid, resulted in replacement of the wild-type PAB gene with a mutant gene.

monitored at excitation and emission wavelengths of 277 and 306 nm, respectively. For determination of binding constants, a series of 5-μl aliquots of oligo(A) solution were added to 0.5 nmol of protein in 1 ml of 0.1 M NaCl–10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA. Ionic strength was varied by adjusting the NaCl concentration. The maximum quenching of protein fluorescence depended on the length of oligo(A) [increasing approximately 30% from $(A)_8$ to $(A)_{21}$] and on the ionic strength (decreasing approximately 50% from 50 to 250 mM NaCl). All data were therefore expressed as percentages of maximum quenching. Binding constants were derived by the use of a curve-fitting program for the single-site binding isotherm $K_D = [A][B]/[AB]$ (6).

Poly(EA) fluorescence was measured at excitation and emission wavelengths of 310 and 396 nm, respectively. Dissociation of poly(EA)-protein complexes was monitored after the addition of 1.5 nmol of poly(EA) and 0.1 nmol of protein in 0.2 ml of 0.1 M NaCl–10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA to 0.8 ml of the same buffer containing the indicated amounts of poly(A).

RESULTS

PAB gene is essential for cell growth. The essential nature of the PAB gene was demonstrated by the lethal effect of a large deletion and by the rescue of a deletion mutant with a plasmid bearing a wild-type copy of the gene. A diploid yeast strain heterozygous for a deletion in the PAB gene (strain AS1) was constructed by transplacement of *his3*$^-$ cells with nucleotide residues 1 to 884 and 2438 to 3435 of the PAB gene separated by the entire *HIS3* gene (Fig. 1, AS1). Sporulation of strain AS1 yielded up to two viable spores in each of 21 tetrads (2:0 segregation), showing that the largely deleted PAB gene would not support cell growth. Transformation of AS1 with a plasmid carrying an intact PAB gene and a *URA3* marker gave some tetrads with four viable spores. All viable spores containing the largely deleted PAB gene also carried the plasmid (Fig. 1, AS2), indicating that introduction of the intact PAB gene was responsible for viability. The requirement for the PAB gene on the plasmid was further revealed by a lack of growth of strain AS2 on medium containing 5-fluoroorotic acid, which selects for *ura3*$^-$ cells and therefore loss of the plasmid (J. D. Boeke, J. Trueheart, G. Natsoulis, and G. R. Fink, Methods Enzymol., in press). Spontaneous inactivation of the *URA3* gene was not observed in this or in subsequent experiments, owing to the small number of cells examined.

Half of one N-terminal domain is sufficient for cell viability. The plasmid in strain AS2 bearing an intact PAB gene and a *URA3* marker was replaced by plasmids bearing mutant PAB genes and a *TRP1* marker (5). This was accomplished

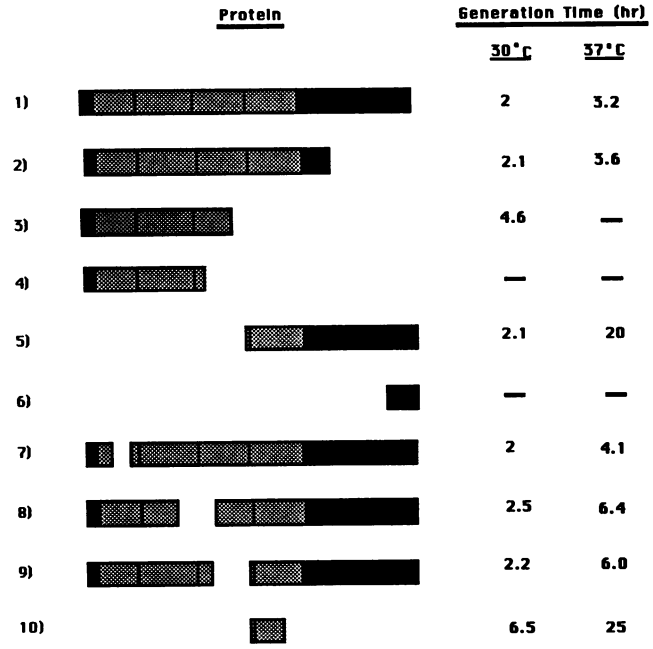

FIG. 3. Effects of PAB mutations on cell growth. PAB genes are represented schematically, with stippled areas corresponding to N-terminal domains, dark areas to other coding regions, and blank areas to deleted regions. Numbers of mutant genes correspond to those of constructions in Table 1.

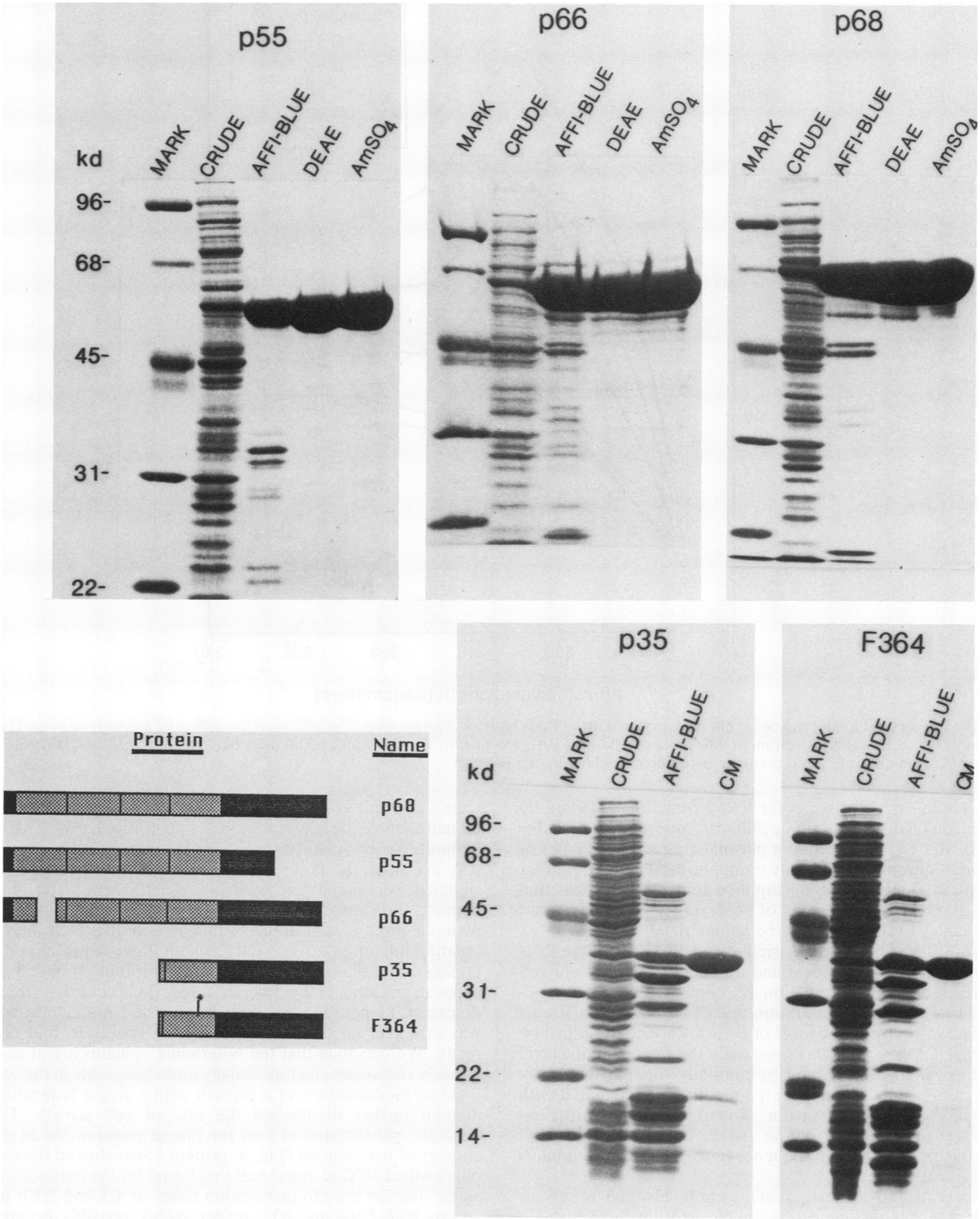

FIG. 4. Purification of wild-type and mutant PABs. Inset: Schematic representation of the various PABs as in Fig. 2, with the approximate location of the Phe-364 to Leu substitution indicated by an asterisk. Crude extracts were chromatographed on Affi-Gel blue and either DEAE-Sephacryl or carboxymethyl cellulose (CM). Coomassie blue-stained gels of the proteins at various stages of purification, with Bio-Rad low-molecular-weight markers (MARK), are shown. kd, Kilodaltons.

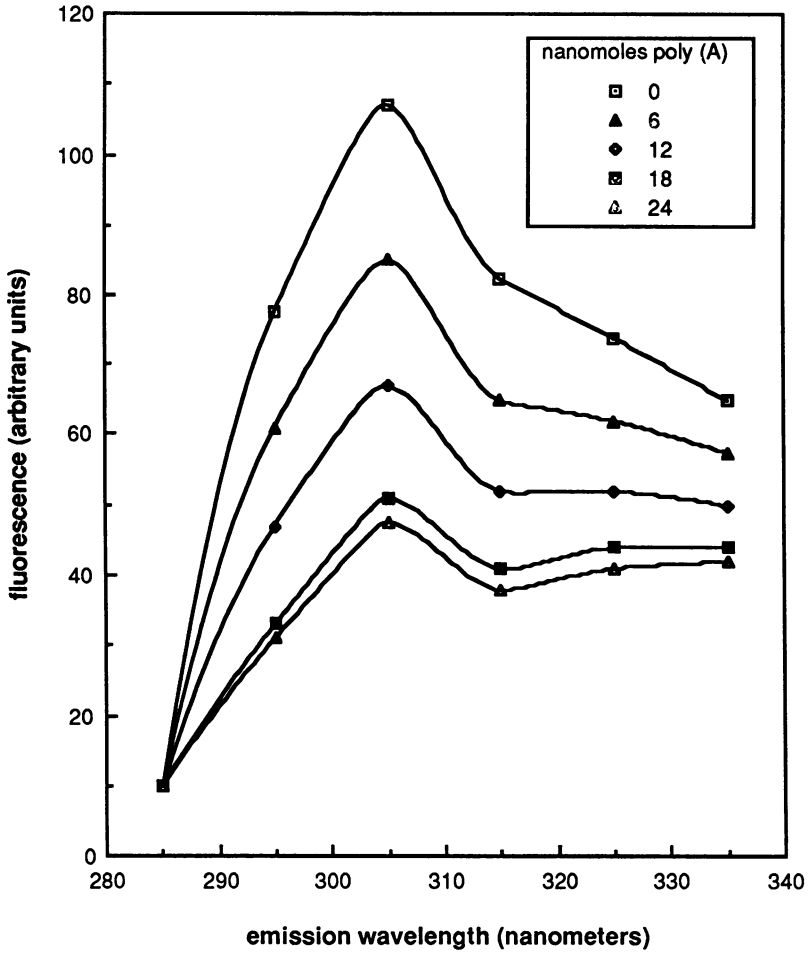

FIG. 5. Poly(A) binding can be monitored by quenching of PAB tyrosine fluorescence. The effects of the indicated amounts of poly(A) on the fluorescence emission spectrum of 0.62 nmol of wild-type PAB are shown. The difference in intensity between the spectra obtained with 18 and 24 nmol of poly(A) represents nonspecific quenching by the polymer.

by selecting transformants for *TRP1* function, followed by plating on medium containing 5-fluoroorotic acid to select for loss of the *URA3*-containing plasmid (Fig. 2). Survivors of the procedure contained only a mutant PAB gene (e.g., Fig. 1, protein 5), and this gene supplied sufficient PAB function for cell growth. The results of analyzing a series of mutant PAB genes in this way may be summarized as follows.

Removal of most of the A-rich region upstream of the PAB gene (39 of 56 nucleotides) had no effect on the rate of cell growth (Table 1, construction 12, compared with construction 11 as a control; generation times of 2 h for both strains at 30°C). Moreover, the level of PAB mRNA (determined by S1 nuclease mapping) was essentially the same, and the level of the protein (determined by antibody binding) was only 20% greater in cells carrying the modified as compared with the wild-type gene (data not shown). Apparently, the remaining small portion of the A-rich upstream region still contains autoregulatory sequences, or else this region plays no major role.

The entire C-terminal domain could also be removed, leaving a protein similar to the nuclear form (Fig. 3, protein 2), without affecting cell growth. The function of the C-terminal domain and conversion of cytoplasmic to nuclear forms are evidently dispensable.

Only alterations of the N-terminal domains had a discernable effect on cell growth, and even here extensive changes were tolerated. For example, any one of the four N-terminal domains could be altered by deletion (proteins 3, 7, 8, 9) without loss of viability, showing that no particular domain was essential. In fact, a single domain, the fourth, was sufficient for viability (protein 5). Deletions of N-terminal domains did slow the rate of cell growth, especially at 37°C, with deletion of the fourth domain and the C terminus creating a temperature-sensitive phenotype. A protein containing only the first two domains (protein 4) failed to support growth at any temperature, either because these domains are nonfunctional or because the mutant protein was unstable, improperly processed, or unable to fold correctly. We conclude that the N-terminal domains are at least partially redundant and functionally interchangeable in the cell.

Some modifications of a protein with a single N-terminal domain further diminished the rate of cell growth. For example, substitution of Leu for Phe at position 364 in the conserved octapeptide (Fig. 4, protein F364) slowed the rate of growth at 30°C to about half that found for the unmodified, single-domain protein (generation times of 4.1 and 2.1 h for strains with F364 and p35, respectively), possibly because the level of the substituted protein was only 35% that of the unmodified one. The substituted protein failed to support growth at 37°C, and thus conferred a temperature-sensitive phenotype.

Remarkably, a deletion removing the entire conserved

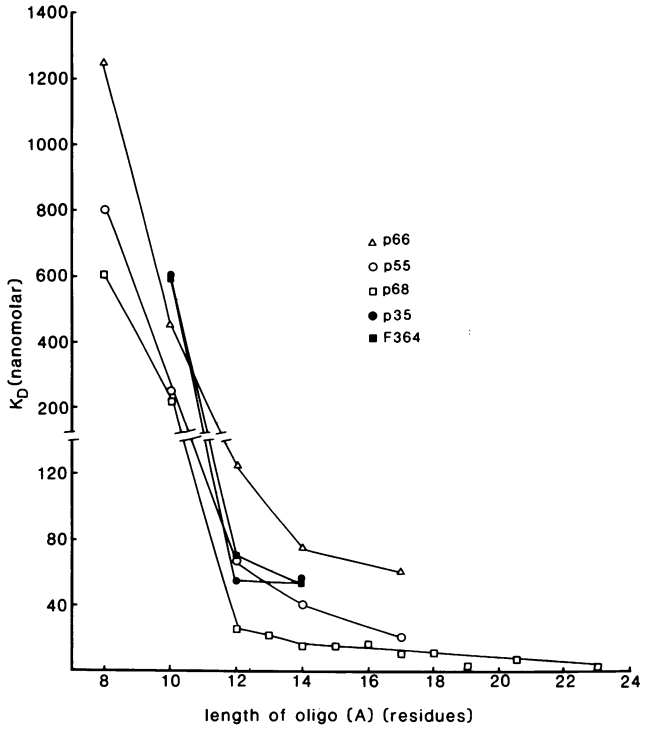

FIG. 6. Determination of the poly(A)-binding site size of wild-type and mutant PABs. Binding constants for various lengths of oligo(A) were determined as described in the text. The standard error for each determination is approximately 20%.

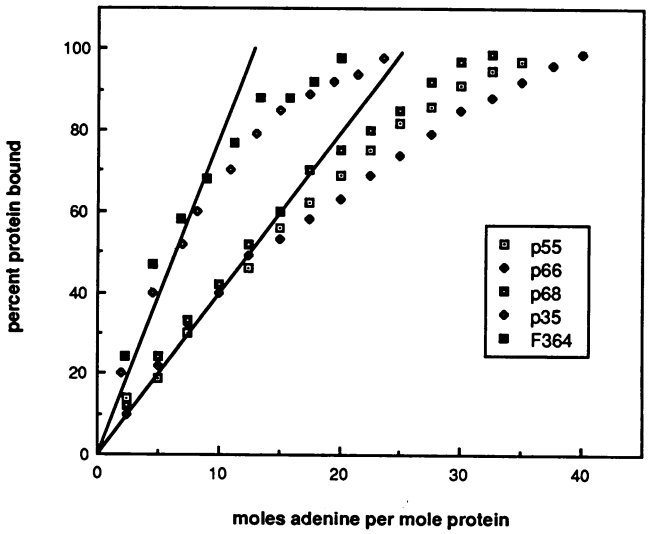

FIG. 8. Determination of packing density of wild-type and mutant PABs. The indicated amounts of poly(A) were added to 0.56 nmol of protein. The fraction of the protein bound was assessed from the fluorescence quenching, and the stoichiometry at saturation was determined by extrapolating the linear portion of the curve to 100% binding.

octapeptide and leaving only half of an N-terminal domain (protein 10) was tolerated, although the rate of growth at 30°C was 30% of that found with the unmodified, single-domain protein. A 66-amino acid polypeptide could therefore supply the essential function of the entire 577-residue PAB.

N-terminal domain binds poly(A). A number of the mutations whose physiologic effects are described above were investigated in regard to poly(A) binding in vitro. Wild-type and mutant proteins were prepared for this purpose by expression in *E. coli* and purification by a version of the procedure reported previously (15) (Fig. 4). Protein-poly(A) interaction was monitored by fluorescence measurements. All proteins studied showed a maximum of fluorescence emission at 306 nm (Fig. 5), characteristic of tyrosine fluorescence, which was quenched upon interaction with poly(A). The proportion of bound protein, and thus affinity for poly(A), was determined from the extent of fluorescence quenching.

Wild-type PAB (p68) showed a striking increase in affinity (decrease in dissociation constant, K_D) with increasing length of oligo(A) up to 12 residues, but little dependence on length beyond 12 residues (Fig. 6), indicative of a binding site size of about 12 residues. [Longer oligo(A) molecules had slightly higher affinities owing to statistical effects (6).] Deletion of part of the C-terminal domain (p55), deletion of part of one or of three N-terminal domains (p66, p35), or even substitution of Leu for Phe in the conserved octapeptide of the protein with a single N-terminal domain (F364) caused no significant change in the binding site size or affinity for oligo(A) (Fig. 6). The only effect of the mutations studied upon affinity for oligo(A) was on the ionic strength dependence of the binding (Fig. 7). The wild-type protein showed a slight ionic strength dependence, indicative of the release of a single counterion upon interaction with oligo(A), whereas the mutant proteins showed no dependence (12).

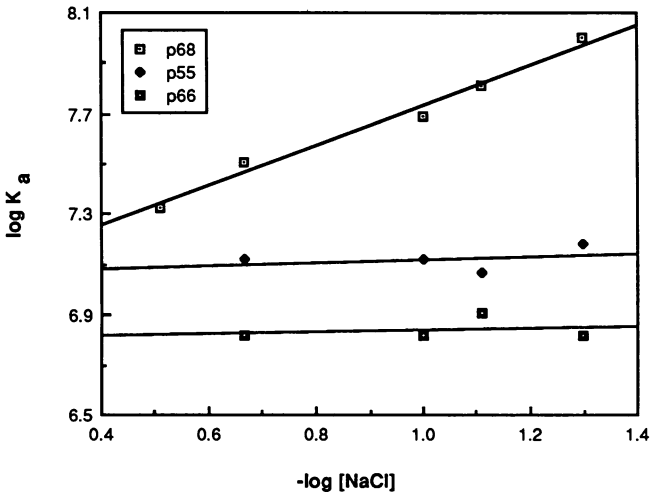

FIG. 7. Ionic strength dependence of oligo(A) binding. Binding constants (K_a) for oligo(A)$_{12}$ are shown.

Although one or more domains of PAB could be deleted without effect on the binding site size, large deletions did alter the packing density of the protein on poly(A) (the occluded site size of the protein [7]). The packing density was obtained from the stoichiometry of poly(A) saturated with protein. Saturation occurred at a ratio of approximately 25 A residues per molecule of wild-type protein (Fig. 8). The stoichiometry was similar for proteins lacking the C-terminal or one N-terminal domain, but it fell to 13 to 15 A residues per molecule for proteins lacking three N-terminal domains, indicative of closer packing along the poly(A) of these much smaller proteins. Stoichiometries were determined over a

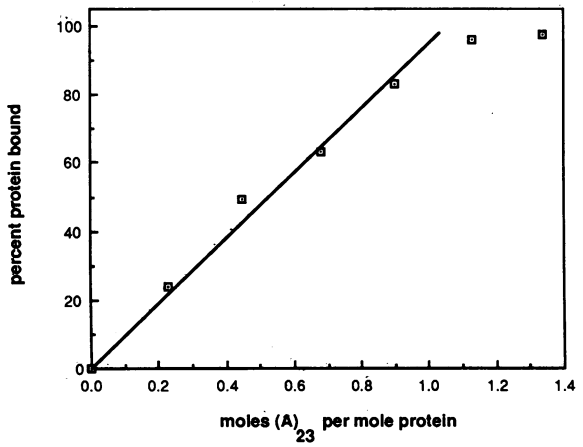

FIG. 9. Titration of wild-type PAB with oligo(A). The indicated amounts of oligo$(A)_{23}$ were added to 0.6 nmol of PAB. The stoichiometry at saturation was determined by extrapolating the linear portion of the curve to 100% binding.

10-fold range of protein concentration by titrating poly(A) with protein or protein with poly(A), giving essentially the same results.

Role of multiple N-terminal domains. The capacity of various deleted forms of PAB to support cell growth revealed more than one functional N-terminal domain, yet studies of binding in vitro revealed only a single high-affinity binding site for oligo(A) (Fig. 9). A possible resolution of this paradox was suggested by results with the fluorescent molecule poly(EA) (18). Wild-type PAB bound poly(EA) with an affinity about 100-fold lower than that of poly(A) and caused an increase in poly(EA) fluorescence (Fig. 10A). Dissociation of the poly(EA)-PAB complex was stimulated by poly(A); dissociation was a first-order process (Fig. 10B) whose rate depended on the concentration of poly(A) (Fig. 10C). These data may be explained by the binding of poly(A) at a low-affinity second site in the poly(EA)-PAB complex, promoting dissociation of poly(EA) from the first site (Fig. 11). The rate-limiting step at high concentrations of poly(A), which saturate the second site, is the dissociation of poly(EA). Consistent with this, the dissociation of a complex formed between poly(EA) and a protein with a single N-terminal domain was unaffected by poly(A) (Fig. 10C).

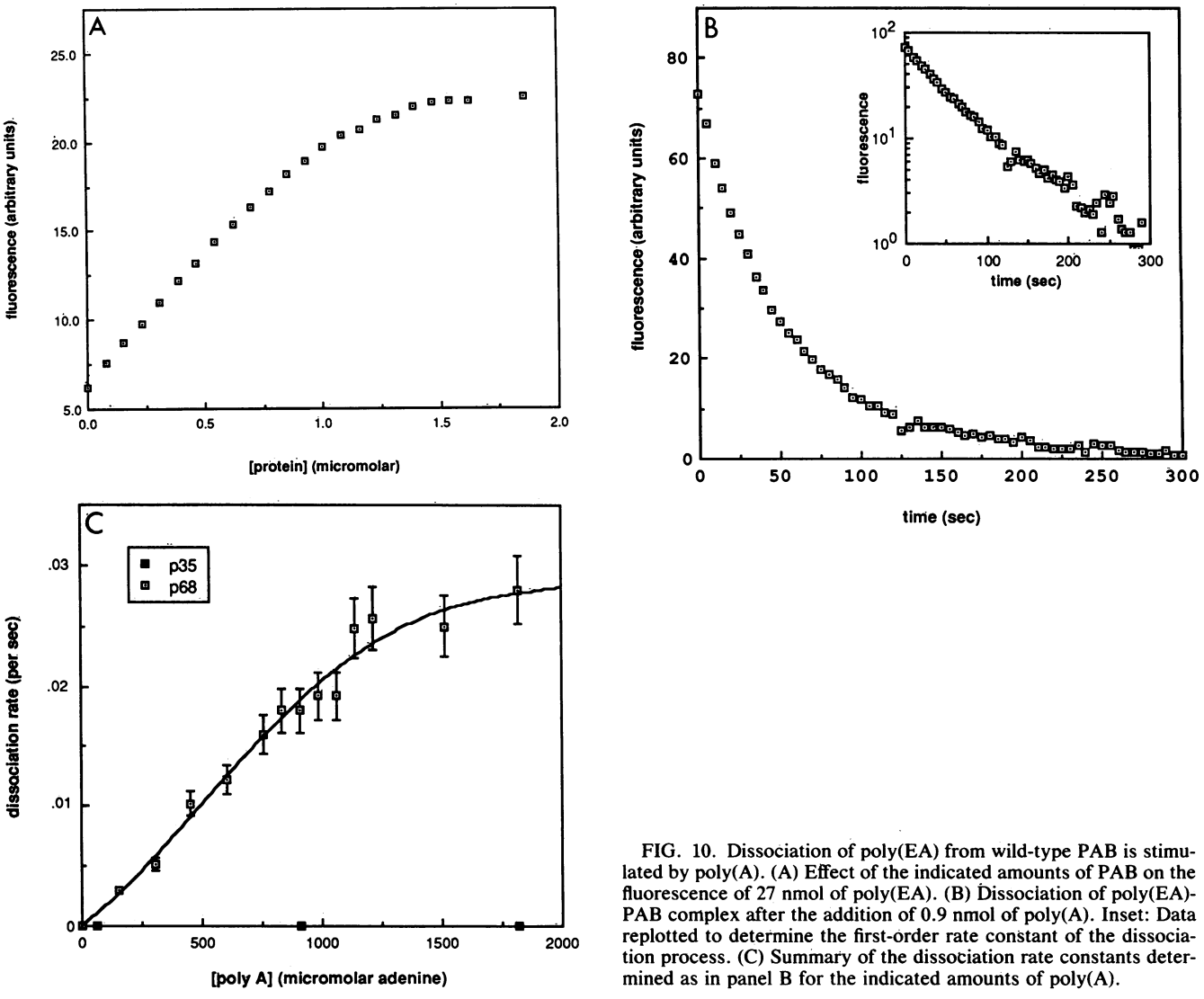

FIG. 10. Dissociation of poly(EA) from wild-type PAB is stimulated by poly(A). (A) Effect of the indicated amounts of PAB on the fluorescence of 27 nmol of poly(EA). (B) Dissociation of poly(EA)-PAB complex after the addition of 0.9 nmol of poly(A). Inset: Data replotted to determine the first-order rate constant of the dissociation process. (C) Summary of the dissociation rate constants determined as in panel B for the indicated amounts of poly(A).

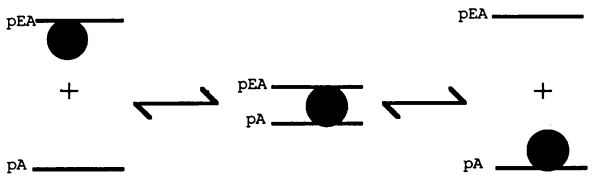

FIG. 11. Possible mechanism of poly(A)-stimulated dissociation of poly(EA)-PAB complex. Two N-terminal domains of PAB are represented by the filled circles.

Similar results were obtained by nitrocellulose filter binding with ^{32}P-labeled poly(A) rather than poly(EA) (15). High concentrations of poly(C) had no effect on the dissociation rate of the complex with wild-type protein, further pointing to a role of specific poly(A)-binding in the dissociation process.

DISCUSSION

The data presented here suggest that poly(A) binding is an essential feature of PAB in vivo. The key findings in this regard are that a single N-terminal domain can replace the entire protein in supporting cell growth and that a single-domain protein binds poly(A) in a similar fashion to the entire protein in vitro. The size and affinity of the binding site in the two proteins are similar, and only one molecule of poly(A) is bound with high affinity, despite the occurrence of multiple binding domains in the entire protein.

The location of the essential region of PAB was narrowed to half an N-terminal domain (37 amino acids) by the finding that a 66-amino acid polypeptide would support cell growth. This polypeptide contained half of one domain and a small part of another. It lacked the octapeptide sequence conserved among RNA-binding proteins, showing that this sequence is not essential for function and probably not required for poly(A) binding.

Proteins containing a single N-terminal domain differ from those with multiple domains in regard to the density of packing on poly(A) and the mechanism of dissociation from poly(A). The packing density of the wild-type yeast protein, approximately 1 molecule per 25 A residues, is in striking agreement with the periodicity of mammalian poly(A) RNP, determined by nuclease digestion to be about 27 residues (2). The spacing of PABs along a poly(A) molecule seems not to be determined by the C-terminal domain, as previously suggested (15), since it is unaffected by removal of this domain. The spacing is diminished, however, by deletion of three N-terminal domains. The spacing is not an essential feature, inasmuch as the deleted proteins support cell growth. Evolutionary conservation of the spacing may be fortuitous, or it may reflect some ancillary role.

The dissociation of multidomain proteins from poly(A) is stimulated by additional poly(A). Apparently, the binding of poly(A) to one domain promotes dissociation from another. This raises the possibility of PAB transfer within a single poly(A) tail (with formation of a transient loop structure), between poly(A) tails of different mRNAs, or even between the A-rich region at the 5′ end and the poly(A) tail of PAB mRNA. Such a transfer, although not essential for function, may nonetheless enhance the rate of some process (4).

The results of our genetic and biochemical analysis of yeast PAB may be applicable to other members of the eucaryotic RNA-binding protein family. A1 heterogeneous nuclear ribonucleoprotein contains two N-terminal domains, and as for PAB, one of these domains binds RNA more tightly than the other (Merrill et al., submitted). It remains to be determined whether one domain predominates in RNA binding by other multidomain proteins and whether this affords a mechanism of interstrand or intrastrand transfer. It also remains to be determined whether the RNA-binding site of the other proteins lies in the region homologous to the 37 amino acids implicated in poly(A) binding by yeast PAB.

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