Sequence of cDNAs Encoding Components of Vascular Actin Single-stranded DNA-binding Factor 2 Establish Identity to Purα and Purβ

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Robert J. Kelm, Jr.‡§, Paula K. Elder‡, Arthur R. Strauch¶, and Michael J. Getz∥∥

From the Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, Minnesota 55905 and †The Biochemistry Program and Department of Cell Biology, Neurobiology, and Anatomy, The Ohio State University College of Medicine, Columbus, Ohio 43210-1239

Transcriptional repression of the mouse vascular smooth muscle α-actin gene in fibroblasts and myoblasts is mediated, in part, by the interaction of two single-stranded DNA binding activities with opposite strands of an essential transcription enhancer factor-1 recognition element (Sun, S., Stoflet, E. S., Cogan, J. G., Strauch, A. R., and Getz, M. J. (1995) Mol. Cell. Biol. 15, 2429–2436). One of these activities, previously designated vascular actin single-stranded DNA-binding factor 2 includes two distinct polypeptides (p44 and p46) which specifically interact with the purine-rich strand of both the enhancer and a related element in a protein coding exon of the gene (Kelm, R. J., Jr., Sun, S., Strauch, A. R., and Getz, M. J. (1996) J. Biol. Chem. 271, 24278–24285). Expression screening of a mouse lung cDNA library with a vascular actin single-stranded DNA-binding factor 2 recognition element has now resulted in the isolation of two distinct cDNA clones that encode p46 and p44. One of these proteins is identical to Purα, a retinoblastoma-binding protein previously implicated in both transcriptional activation and DNA replication. The other is a related family member, presumably Purβ. Comparative band shift and Southwestern blot analyses conducted with cellular p46, p44, and cloned Pur proteins synthesized in vitro and in vivo, establish identity of p46 with Purα and p44 with Purβ. This study implicates Purα and/or Purβ in the control of vascular smooth muscle α-actin gene transcription.

Transcriptional activation or repression of RNA polymerase II-dependent genes is often mediated by sequence-specific DNA-binding proteins (i.e. transcription factors) that respond to extracellular signals by interacting with critical cis-acting regulatory elements. Activator or repressor proteins generally bind to double-stranded DNA recognition sites distal to the TATA box and influence promoter activity by interacting, either directly or through an adaptor protein, with factor(s) comprising the RNA polymerase II basal transcription complex (1, 2). Although most transcription factors demonstrate preferential binding to double-stranded DNA, a number of recent studies have associated sequence-specific, single-stranded DNA (ssDNA)-binding factors with both transcriptional activating (3–6) and repressing elements (7–12). In particular, some ssDNA-binding proteins, such as the far upstream element-binding protein and heterogeneous nuclear ribonucleoprotein K, have been shown to utilize cis elements, prone to forming non-B-DNA structures, as docking sites to effect gene expression (13–15).

In support of a role for ssDNA-binding proteins as transcription factors, our study of the vascular smooth muscle (VSM) α-actin gene has revealed the integral involvement of several ssDNA-binding proteins in the control of promoter activity (16–18). Activation and repression of the mouse VSM α-actin promoter in both fibroblasts and undifferentiated myoblasts is mediated, in part, by positive and negative trans-acting factors that bind to a 30-base pair polypurine-polypyrimidine tract containing an inverted, muscle-specific M-CAT consensus motif (AGGAATG) (16, 17). While activation likely requires the M-CAT-binding protein, transcription enhancer factor-1 (TEF-1) (17), repression appears to result from the interaction of two, tissue-restricted, ssDNA binding activities which putatively function by stabilizing local alterations in DNA secondary structure that preclude TEF-1 binding to double-stranded DNA (16). We have recently reported that one of these ssDNA binding activities, designated vascular actin single-stranded DNA-binding factor 2 (VACssf2), also interacts with a conserved 30-base coding sequence element (CE) which borders the 3’ end of intron 2 in the mouse VSM α-actin gene (19). Interestingly, this CE sequence was found to repress promoter activity when positioned 5’ and adjacent to either a TEF-1 or activator protein-1 enhancer element (18), further confirming the ability of VACssf2 to function as a transcriptional repressor.

Our initial efforts to biochemically characterize VACssf2 prompted us to search the literature for known ssDNA-binding proteins that exhibited a similar preference for purine-rich sequences and possessed a molecular weight comparable to the p46 and p44 components of VACssf2. Our search revealed a protein known as Puro (19). This ssDNA-binding protein was originally identified as a HeLa cell nuclear protein that interacted with a region of stably-bent DNA in the 5’-region of the human c-myc gene, designated the PUR (for purine-rich) element (20). This sequence element is present in several eukary...
otic zones of initiation of DNA replication as well as in the flanking regions of a number of different genes (20). Although the exact role of Pur in mammalian DNA replication is still unknown, recent studies have implicated Pur in the transcriptional activation of several different genes (5, 21–23). Moreover, the identification and partial cloning of a DNA encoding the C-terminal region of a homologous protein, termed Purβ (19), suggested that a family of Pur proteins may exist in higher eukaryotes. In this study, we have utilized both biochemical and molecular cloning techniques to reveal the identity of the p46 and p44 components of VACssBF2 as mouse Pur and Purβ, respectively.

**EXPERIMENTAL PROCEDURES**

**Oligonucleotide Probes—**Oligonucleotide CE-PrM9, a multimer of the VACssBF2 exon-binding site (18) (GGAGAATGGTGGGAAGGCGCAAAAAAGA)4 was synthesized on a Applied Biosystems model 394 DNA/RNA synthesizer and gel filtered over a NAP-25 column (Pharmacia) in distilled water. The full-length 120-base oligonucleotide was purified from smaller synthesis products by polyacrylamide gel electrophoresis. CE-PrM9, end-labeled with γ-32PATP and T4 polynucleotide kinase was used as the primary probe in expression library screening and Northern blot analysis. A 30-base fragment of the VACssBF2 exon-binding site, designated CE-PrMmu2 (18) or CE-Fmu2, was used as a nonspecific competitor. Oligonucleotides corresponding to the forward and reverse strands of the human c-myc PUR element (20), PUR-F, GAGAGTGGTGGAGGACAAAG, and PUR-R, CTTTGTCTCTCCACACCTCCT, were synthesized as described above. Biotinylated oligonucleotides corresponding to the PUR element and the VSM α-actin promoter element (16, 18) were also prepared by chemical synthesis using a biotin phosphoramidite containing a mixed polarity triethylene glycol spacer (BioTEG, Glen Research, Sterling, VA).

**Preparation AKR-2B Fibroblast Nuclear Extract—**Nuclear extract was prepared according to the method of Sealey and Chalkley (24) with some minor modifications. AKR-2B fibroblasts were harvested from monolayer cultures by scraping in the presence of ice-cold phosphate-buffered saline and then collected by centrifugation. Following two additional washes with phosphate-buffered saline, the cell pellet was resuspended in 10 volumes of 10 mM HEPES pH 8, 50 mM NaCl, 0.5 mM sucrose, 1.0 mM EDTA, 0.5 mM spermidine, 0.15 mM spermine, 0.2% (v/v) Triton X-100, 7 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml aprotinin. After a 20-min incubation on ice, the cell extract was vortexed (every 5 min) vortexing, nuclei were collected by centrifugation. The nuclear pellet was suspended by vigorous vortexing in 6 volumes of extraction buffer (10 mM HEPES pH 8, 350 mM NaCl, 0.1 mM EDTA, 0.5 mM spermine, 0.15 mM spermidine, 25% (v/v) glycerol, and protease inhibitors). After centrifugation at 12,000 × g for 10 min, the supernatant was saved and the pellet was resuspended a second time with 6 volumes of extraction buffer containing 530 mM NaCl. Following centrifugation, the 350 and 530 mM NaCl supernatants were pooled, assayed for total protein, then aliquoted, and stored at –80 °C.

**Fractionation of AKR-2B Nuclear Proteins—**AKR-2B nuclear protein (~10 mg) was diluted to 0.1 mM NaCl and applied to 3 ml of heparin-agarose resin (Sigma) equilibrated in 25 mM Tris-HCl, pH 7.5, 1.0 mM EDTA, 0.1 mM NaCl, 1 mM EDTA, TNE) for 30 min at room temperature. Under these conditions, all oligonucleotides tested exhibited a coupling efficiency of 100% as assessed by absorbance measurement at 280 nm. Parallel reaction mixtures containing AKR-2B nuclear protein (150 μg) and 12.5 pmol equivalents (50 μl) of ssDNA-coupled particles in 0.5 ml of 1 × electrophoretic mobility shift assay buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 μM spermidine, 15 μM spermine, 2.5% (v/v) glycerol containing 0.01% (v/v) Tween 20) were incubated for 30 min at room temperature. The particles were then captured with a magnet and washed sequentially three times with 0.5 ml buffer containing 0.01% (v/v) Tween 20, 10 μg/ml poly(dI-dC), and 0.01% (v/v) Tween 20 and 10 μg/ml poly(dI-dC). Bound protein was eluted from the ssDNA-coupled particles by adding 40 μl of 30 mM Tris-HCl, pH 6.8, 10% glycerol, 1% (v/v) SDS and incubating at 65 °C for 3 min. Eluates were supplemented with 5% (v/v) β-mercaptoethanol, heated for 5 min at 90 °C, then assayed for VACssBF2 components by Southwestern blotting.

**Screening of a Mouse Lung Expression Library for cDNAs Encoding VACssBF2 p46 and p44 and Sequencing of Binding-site Clones—**A mouse lung cDNA expression library (Stratagene) was plated in Escherichia coli host strain, XLI-Blue, on 10 150-mm plates at a density of 25,000 plaque forming units per plate. After a 4 h incubation at 42 °C, the plates were overlaid with nitrocellulose filters saturated with 10 mM isopropyl-β-D-thiogalactopyranoside. Following an additional 16-h incubation at 37 °C, filters were lifted from the plates, placed in a blocking buffer of 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA (TNE) containing 5% Carnation nonfat dry milk, and gently agitated for 1 h at room temperature. After a 15-min equilibration in binding buffer (1% nonfat dry milk-TNE), filters were then incubated for 3 h with 1–2 × 106 cpm/ml 32P-labeled DNA oligonucleotides in the presence of a 160-fold molar excess of CE-PrMmu2 diluted in binding buffer containing 10 μg/ml poly(dI-dC). Filters were washed three times with TNE containing 0.05% Tween 20 and one time with TNE alone. Filters were then air dried and autoradiographed for 1–2 h at –80 °C. Potential positive plaques were selected, titered, replicated on 100-mm dishes, and re-screened as above. Two clones, designated 9-5 and 10-1, were positive in the secondary screen. After a final tertiary screen to ensure clonal homogeneity, single plaques were selected and cDNA inserts were excised as pBluescript phagemids by co-infection with helper phage. The upper and lower strands of clones 9-5 and 10-1 were sequenced by automated dideoxy termination using an Applied Biosystems Model 377A DNA sequencer and the appropriate T3, T7, and internal primers.

**In Vitro Synthesis of Mouse Purα and Purβ—**Proteins encoded by clones 9-5 (Purα) and 10-1 (Purβ) were synthesized in vitro using a T3-dependent, transcription/translation-coupled wheat germ extract system (TnT, Promega). Cesium chloride-purified pBluescript plasmid (empty vector or vector containing cDNA insert), 1 μg, was combined with 100 μg of wheat germ extract and incubated as follows: 1 h of linearization, 20 min of incubation with 200 units of ribonuclease inhibitor, and 10 units of T3 RNA polymerase in a final volume of 50 μl. After a 1-h incubation at 30 °C, an 8-μl aliquot from each TnT reaction was removed, combined with an equal volume of 2 × SDS sample preparation buffer with 5% (v/v) β-mercaptoethanol and heated at 90 °C for 5 min. TnT products were analyzed for VACssBF2 DNA binding activity by Southwestern blotting.

**Construction and Transfection of Mouse Purα (9-5) and Purβ (10-1) Expression Vectors—**The cDNAs encoding mouse Purα (clone 9-5) and mouse Purβ (clone 10-1) were cut from pBluescript by EcoRI and KpnI. Agarose gel purified cDNAs were then subcloned into a mammalian expression vector, pcI (Promega). Ligated plasmids were transformed into E. coli HB101 cells using standard CaCl2 techniques. All expression vectors used in transfection experiments were purified by double cesium chloride gradient centrifugation and sequenced by semi-automated dideoxy DNA sequencing to confirm the orientation and fidelity of the cDNA insert. Transient expression of the mouse-derived AKR-2B fibroblasts were performed using 10 μg of total plasmid DNA per 106 cells as described previously (18). After a 16–18 h recovery period, the transfected cells were rendered quiescent by incubating 48 h in serum-free MCDB402 medium (JRH Biosciences, Lenexa, KS). Cells were restimulated for 6 h with fresh MCDB402 medium supplemented with 20% fetal bovine serum (HyClone, Logan, UT). Cells were then washed three times with phosphate-buffered saline and embryo-derived by adding 1 ml of hypotonic lysis buffer (10 mM MOPS, pH 6.5, 10 mM NaCl, 1.5 mM MgCl2, 1% (v/v) Triton X-100) supplemented with 0.5 mM phenylmethylsulfonyl fluoride and 0.5 μg/ml each leupeptin, pepstatin, and aprotinin. Following centrifugation at 14,000 × g, cell extracts were assayed for total protein by BCA dye-binding assay (Pierce) using bovine serum albumin as a standard. Equivalent amounts of cellular protein were evaluated for p46 (Purα) and p44 (Purβ) ssDNA binding activity by Southwestern blotting and band shift assay.
Identification of VACssBF2 p46 and p44 as Purα and Purβ

**RESULTS**

VACssBF2 Is Composed of Three, Electrophoretically Distinct, ssDNA-binding Proteins—To facilitate the molecular characterization of VACssBF2, we performed experiments aimed at defining the polypeptide composition of VACssBF2. Nuclear proteins from AKR-2B fibroblasts were applied to heparin-agarose fractions (5 μl) of AKR-2B nuclear protein were assayed for VACssBF2 by band shift assay using a 32P-ssDNA probe corresponding to the purine-rich sense strand of the CE (CE-PrMss). Arrows indicate band-shifted complexes characteristic of VACssBF2 (16, 18). Lower panel, fractions enriched in VACssBF2 (numbers 33–38, 0.6 M NaCl) were pooled and ~4 μg of protein was analyzed by Southwestern blotting. Arrows (from top to bottom) show p115 (single arrow), and p46/p44 (double arrows) protein-ssDNA complexes. Abbreviations used are: SM, starting material; PT, flow through; FP, free probe.

*Fig. 1. VACssBF2 resolves as three distinct ssDNA-binding polypeptides of $M_r \sim 115,000, 46,000,$ and 44,000. Upper panel, heparin-agarose fractions (5 μl) of AKR-2B nuclear protein were assayed for VACssBF2 by band shift assay using a 32P-ssDNA probe corresponding to the purine-rich sense strand of the CE (CE-PrMss). Arrows indicate band-shifted complexes characteristic of VACssBF2 (16, 18). Lower panel, fractions enriched in VACssBF2 (numbers 33–38, 0.6 M NaCl) were pooled and ~4 μg of protein was analyzed by Southwestern blotting. Arrows (from top to bottom) show p115 (single arrow), and p46/p44 (double arrows) protein-ssDNA complexes. Abbreviations used are: SM, starting material; PT, flow through; FP, free probe.*

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*Fig. 2. VACssBF2 p46 and p44 interact with the purine-rich sense strand of the human c-myc PUR element. AKR-2B nuclear protein (3 μg) was incubated with 32P-oligonucleotide corresponding to the sense strand of the VSM α-actin coding element (CE-PrMss, ~40 fmol, 20,000 cpm) in the presence of the indicated molar excess of unlabeled oligonucleotide corresponding to either the coding element sense strand (CE-F, lanes 1–4), coding element mutant sense strand (CE-Fmu2, lanes 17–20), promoter element sense strand (PE-F, lanes 13–16), promoter element sense strand (PUR-F, lanes 5–8), or PUR element antisense strand (PUR-R, lanes 9–12). Protein-ssDNA complexes were resolved by band shift assay. The 24-base PUR element oligonucleotides correspond to base pairs ~1648 to ~1625 upstream of the P1 transcription start site of the human c-myc gene. Arrows (from top to bottom) show p115 (single arrow), and p46/p44 (double arrows) protein-ssDNA complexes. FP, free probe.*

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Identification of VACssBF2 p46 and p44 as Pura and Purβ

FIG. 3. VACssBF2 p46 and p44 are “captured” from AKR-2B nuclear extract using ssDNA affinity beads coupled with the sense strand of the human c-myc PUR element. Parallel reaction mixtures containing equivalent amounts of AKR-2B nuclear protein (150 µg) and ssDNA-coupled magnetic particles (12.5 pmol of ssDNA) corresponding to the PUR element sense strand (PUR-F, lane 1), the VSM o-actin promoter element sense strand (PE-F, lane 2), or the promoter element antisense strand (PE-R, lane 3) were incubated under conditions which mimicked a band shift assay. After magnetically coupling and washing the particles with buffer containing excess poly(dI-dC), bound protein was eluted with 1% SDS and analyzed by Southern blotting. Lane 4 is an additional negative control in which oligonucleotide-free particles were used. Each lane represents the amount of VACssBF2 captured from 75 µg of starting AKR-2B nuclear protein.

coupled particles were used in each instance, these results were not attributable to a loading artifact but represent actual differences in ssDNA-binding affinity. The absence of p115 in this experiment is indicative of its weak ssDNA-binding affinity and specificity (16, 18). The stringency of the binding and washing conditions and/or competition by p46/p44 probably contributed to the failure of p115 to bind the DNA-affinity particles. Collectively, these results indicated that p46 and p44 bind to purine-rich ssDNA with substantially greater affinity and specificity than p115. Furthermore, the data suggested that VACssBF2 p44 and p46 might be related to the Pur family of ssDNA-binding proteins.

Isolation and Sequence Analysis of VACssBF2 cDNA Clones—Owing to the enrichment of VACssBF2 in the lung (17), a mouse lung λ cDNA expression library was selected as a source for isolating cDNA clones encoding p44 and p46 using a ssDNA-binding site screening strategy. Initially, 250,000 plaques were screened with a 32P-end labeled oligonucleotide corresponding to the PUR element sense strand (PUR-F, lane 1), the VSM o-actin promoter element sense strand (PE-F, lane 2), or the promoter element antisense strand (PE-R, lane 3) were incubated under conditions which mimicked a band shift assay. After magnetically coupling and washing the particles with buffer containing excess poly(dI-dC), bound protein was eluted with 1% SDS and analyzed by Southern blotting. Lane 4 is an additional negative control in which oligonucleotide-free particles were used. Each lane represents the amount of VACssBF2 captured from 75 µg of starting AKR-2B nuclear protein.

FIG. 4. Nucleotide and deduced amino acid sequences of VACssBF2 recognition element-binding proteins. Expression screening of a mouse lung λ cDNA library using a tetramer of the VACssBF2 exon recognition element as a probe resulted in the isolation of two clones, designated 9-5 and 10-1. The upper and lower stands of each clone were sequenced independently. The amino acid sequences derived from the corresponding upper strand nucleotide sequence are represented using single letter code. Clone 9-5 encodes a 321-amino acid protein that is identical to mouse Purβ (26). Clone 10-1 encodes a related 324-amino acid protein, presumably Purγ. Stop codons are indicated by a "*" symbol.
Identification of VACssBF2 p46 and p44 as Purα and Purβ

protein of 42 (28) or 45–47 kDa (27). In at least one study, the anomalous electrophoretic mobility of Purα was attributed to post-translational processing (27) although another report ruled out N-glycosylation and phosphorylation as contributing to its unusual migration in SDS gels (28). To confirm the identity of cellular p46 and p44 as Purα and Purβ, respectively, the electrophoretic mobility of both in vitro and in vivo translation products of clones 9-5/purα and 10-1/purβ were compared with cellular p46 and p44. As shown in Fig. 6c, the in vitro transcription/translation products of clones 9-5 and 10-1 migrated similarly to cellular p46 and p44, respectively, when analyzed by Southwestern blotting. Moreover, transient transfection of cytomegalovirus enhancer driven-expression vectors harboring clones 9-5 (Purα) and 10-1 (Purβ) into AKR-2B fibroblasts resulted in the enhanced expression of p46 in cells transfected with pCI 9-5a (Fig. 6A, lanes 2–4), while p44 was elevated in fibroblasts transfected with pCI 10-1b (Fig. 6A, lanes 6–8). Cotransfection of both pCI 9-5a and pCI 10-1b resulted in the enhanced expression of the p46/p44 bands relative to endogenous p46 and p44 (Fig. 6B, compare lanes 2 and 5). Corroborative results were obtained when selected transfectants were analyzed by band shift assay. As shown in Fig. 7 (lanes 5–8), cotransfection of pCI 9-5a and pCI 10-1b resulted in the enhanced expression of the p46/p44 gel shift doublet. Importantly, only the faster migrating band of the doublet was augmented in cells transfected with pCI 10-1b alone (Fig. 7, lanes 1–4). These data establish the identity of slower migrating band as the Purα/p46 ssDNA complex and the faster migrating species as the Purβ/p44 ssDNA complex. Interestingly, the overall expression of transfected Purβ/p44 was consistently greater than that of Purα/p46 (Fig. 6, A and B, and Fig. 7). This was probably due to some inherent difference in mRNA or protein stability, since both expression constructs utilized the same parent vector. In any case, these transfection results support the conclusion that p46 is Purα and p44 is Purβ.
Protein-ssDNA complexes were resolved by band shift assay. Arrows (top) show free probe.

Recent experiments conducted with chimeric promoters have critically important in determining the operative regulatory elements and DNA-binding proteins that mediate transcriptional activation and repression (36–39). Our previous analyses of the mouse VSM α-actin promoter pointed to a novel molecular model in which sequence-specific VACssBFs repress promoter activity in cells of both myogenic and fibroblastic lineage by interacting with opposing strands of an essential TEF-1 enhancer element (16–18). One of these factors, VACssBF1, has been cloned and is identical to the mouse Y-box protein MSY1 (40). Interestingly, the human homolog, YB-1, has also been reported to function as a transcriptional repressor within the context of the major histocompatibility class II DRA (41) and grp78 (42) promoters. In the mouse VSM α-actin promoter, MSY1 interacts with the pyrimidine-rich, antisense strand of the TEF-1 enhancer. The other factor, VACssBF2, binds to the purine-rich sense strand of the TEF-1 enhancer and a related element in a protein-coding exon of the gene (18). While initial data suggested that VACssBFs function in a cooperative manner to disrupt TEF-1 enhancer function (16), recent experiments conducted with chimeric promoters have indicated that VACssBF2 can repress enhancer activity in the absence of detectable VACssBF1/MSY1-binding to the opposing strand (18).

Owing to the relative paucity of reports documenting a role for ssDNA-binding proteins in transcriptional repression, we sought to characterize VACssBF2 using biochemical and molecular cloning techniques. Southwestern blotting of heparin-agarose fractions enriched in VACssBF2 revealed three prominent bands of Mr ~115,000, 46,000, and 44,000 (Fig. 1).

However, only the p46 and p44 species demonstrated high affinity, purine-rich ssDNA-binding specificity (Figs. 2 and 3). Expression screening of a mouse lung cDNA library with a multimer of VACssBF2 exon recognition element yielded two cDNA clones (9-5 and 10-1) that encode related ssDNA-binding proteins (Figs. 4 and 5). Clone 9-5 encodes Purα, a purine-rich ssDNA-binding protein previously shown to interact with (GGN)n repeats in sequence elements functionally linked to both DNA replication and transcription (5, 19, 20, 23, 26, 28, 43). Based upon homology to a partial cDNA clone of human Purβ previously reported (19), clone 10-1 encodes mouse Purβ. These results were not surprising given the prior finding that cellular p46 and p44 could also bind to an authentic Purα ssDNA-recognition element in vitro (Figs. 2 and 3). However, clones 9-5 and 10-1 encode full-length proteins with predicted molecular masses of 34.9 and 33.9 kDa, respectively. While these values differ substantially from the apparent molecular weights of p46 and p44, both in vitro and in vivo synthesized Purα and Purβ co-migrate with cellular p46 and p44 in SDS-polyacrylamide gels (Fig. 6). Thus, the apparent discrepancies in molecular weight are a result of anomalous electrophoretic migration.

Because the cloning and detection strategies employed in these studies were based on affinity for a VACssBF2 ssDNA recognition element, it might be argued that cellular p46 and p44 are not identical to the Pur proteins but simply exhibit similar ssDNA-binding properties. We believe this is highly unlikely for the following reasons. First, with the exception of p115, no other bands are detectable in band shift, Southwestern blot, or biotinylated ssDNA capture experiments using fibroblast nuclear extracts and either VACssBF2 or human c-myc Purα ssDNA-oligonucleotide probes (Figs. 1–3, and data not shown). Second, and most importantly, Purα and Purβ exactly co-migrate with cellular p46 and p44 when overexpressed in fibroblasts and subjected to SDS-polyacrylamide gel electrophoresis (Fig. 6). Thus, any non-Pur proteins which exhibit Pur-like ssDNA binding activity would have to exactly co-migrate with authentic Purα and Purβ. Based on these considerations, we conclude that the p46 and p44 components of the VACssBF2 complex correspond to Purα and Purβ, respectively.

With the molecular cloning of the p46 and p44 components of VACssBF2, we have now identified 4 of the 5 proteins previously shown to interact with single- or double-stranded forms of an essential VSM α-actin TEF-1 enhancer element (16, 17). These include a TEF-1 related protein (17), MSY1 (or VACssBF1), and now Purα and Purβ (or VACssBF2 p46 and p44). Only the relatively weak and nonspecific ssDNA-binding p115 component of VACssBF2 remains to be cloned and identified. Curiously, both cellular Purα and YB-1 (the human homolog of MSY-1) have also been implicated in the activation of early (Purα) and late (YB-1) gene transcription of human JC polyomavirus in glial cells of the central nervous system (22).

In this context, Purα and YB-1 were found to function cooperatively in that each protein was capable of modulating the binding of the other to its respective viral promoter ssDNA-binding site (23). Since certain astroglial cells are known to also express VSM α-actin (44, 45), the functional association of Y-box and Purα proteins may be common to VSM α-actin expressing cell types.

It is interesting that human Purα and YB-1 function as activators of JC polyomavirus gene transcription in glial cells while mouse Purα and Purβ, and MSY1 apparently function as repressors of VSM α-actin gene transcription in myoblasts and fibroblasts. While it is not uncommon for a transcription factor, such as Purα or YB-1, to act as either an activator or as...
a repressor depending upon the influence of other cell and/or promoter-specific gene regulatory proteins, the structural differences between mouse Pur and Purβ are nevertheless provocative (Fig. 5). Because glutamine-rich sequences have been associated with transcriptional activation domains (1), the absence of a Q7 sequence and other differences in the COOH-terminal region of Purβ may be of functional significance in terms of repression. Perhaps Purβ antagonizes activation by Pur via competitive ssDNA binding. Elucidation of the individual functional roles of MSY1, Pur, and Purβ, in repression of the VSM α-actin gene promoter in myoblasts and fibroblasts will obviously require further investigation. Several questions to be addressed include 1) do these proteins possess the ability to modulate TEF-1 enhancer topology, 2) do they interact with the enhancer in a mutually-dependent or independent manner, 3) what are the consequences of such interactions in terms of enhancer function, 4) are these proteins differentially expressed or post-translationally modified in a cell- or tissue-specific manner, and 5) does Purβ, like Pur (27), interact with the retinoblastoma protein? Answers to such questions should lead to an improved understanding of the mechanisms that regulate VSM α-actin gene expression during smooth muscle myogenesis, cutaneous wound healing, vascular injury, fibrocontractive disease, and stromal responses to neoplasia.

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