AFP. However, NMR studies at temperatures near or below the equilibrium freezing point of water do not indicate any intermolecular associations or significant structural changes (*14*).

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Identification of a Ten–Amino Acid Proline-Rich SH3 Binding Site

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The Src homology 3 (SH3) region is a small protein domain present in a very large group of proteins, including cytoskeletal elements and signaling proteins. It is believed that SH3 domains serve as modules that mediate protein-protein associations and, along with Src homology 2 (SH2) domains, regulate cytoplasmic signaling. The SH3 binding sites of two SH3 binding proteins were localized to a nine– or ten–amino acid stretch very rich in proline residues. Similar SH3 binding motifs exist in the formins, proteins that function in pattern formation in embryonic limbs of the mouse, and one subtype of the muscarinic acetylcholine receptor. Identification of the SH3 binding site provides a basis for understanding the interaction between the SH3 domains and their targets.

The SH3 domain, which contains approximately 60 amino acids, is found in a wide variety of proteins. It exists in association with catalytic domains, as in the nonreceptor protein-tyrosine kinases and phospholipase C-γ, within structural proteins such as spectrin or myosin, and in small adapter proteins such as Crk or sem-5 (*1*). SH3 domains are often accompanied by SH2 domains of 100 amino acids that bind to tyrosine-phosphorylated regions of target proteins, frequently linking activated growth factor receptors to putative signal transduction proteins (*1*). The function of the SH3 domain is less well defined. Its presence in a variety of proteins associated with the cytoskeleton implies that it may participate in regulating the cytoskeleton. Deletion or mutation of the SH3 domain generally activates the transforming potential of nonreceptor tyrosine kinases, suggesting that SH3 mediates negative regulation of an intrinsic transforming activity in such proteins (*2*). Studies of vulval development in the nematode, *Caenorhabditis elegans* showed that an SH3-containing protein, Sem5, is central to that process and that the SH3 domains are crucial for the function of Sem5 (*3*).

Two proteins that bind specifically to the SH3 domain of the product of the *abl* proto-oncogene were isolated by screening a λgt11 cDNA expression library with a fusion protein containing glutathione-S-transferase (GST) fused to the SH3 domain of Abl (*4*). One of the proteins, termed 3BP1, contains a region of similarity to GAP-Rho, the guanosine triphosphatase–activating protein for the Ras-related protein Rho. The high-affinity binding site of 3BP1 for the Abl SH3 domain was localized to a 28–amino acid region outside of the domain with similarity to GAP-Rho. We have now localized the SH3 binding sites of 3BP1 and the other Abl SH3 binding protein, termed 3BP2, to a nine– or ten–amino acid stretch very rich in proline residues. The presence of SH3 binding motifs in other known proteins was suggested by a search of the protein sequence data bases and tested in the cases of the formins and a subtype of the muscarinic acetylcholine receptor.

The binding sites for the Abl SH3 do-

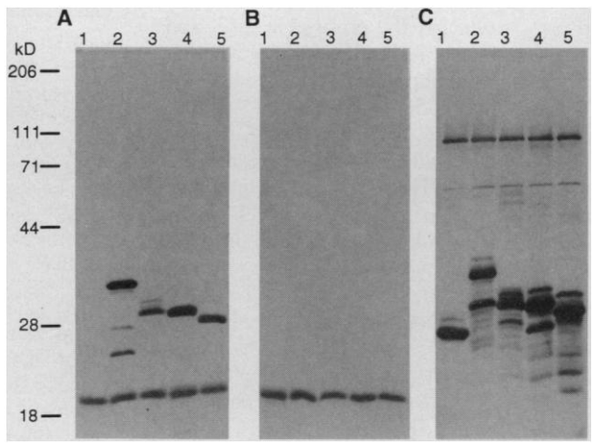

Fig. 1. Mapping of the SH3 binding site on 3BP1. Proteins from lysates of bacteria that expressed the GST-3BP1 peptide fusion proteins were probed with biotinylated GST–Abl SH3 fusion protein (0.2 μg/ml) (**A**), biotinylated GST (0.2 μg/ml) (**B**), or antibody to GST (0.5 μg/ml) (**C**). Lane 1, GST (*13*); lane 2, GST-3BP1-64; lane 3, GST-3BP1-28; lane 4, GST-3BP1-22; lane 5, GST-3BP1-10. Numbers on left represent the molecular size in kilodaltons. The antiserum used for detecting GST fusion proteins contains some antibodies to bacterial proteins of large molecular size.

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main were determined by ligating various DNA fragments in-frame in a position COOH-terminal to the segment of pGEX vectors that encodes GST. The GST fusion proteins were expressed in *Escherichia coli* (*5*). GST fusion proteins in *E. coli* extracts were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transfered to a filter (*6*). SH3 binding was examined by incubating the filter with biotinylated probe containing the SH3 domain of Abl (GST–Abl SH3), as described (*7*). The relative amount of GST fusion protein on the filter was determined by probing with an antibody to GST or by Coomassie blue staining (*8*). All of the constructs discussed in this report were confirmed by DNA sequence analysis (*9*). The amino acid sequences of various peptides that were tested for SH3 binding are shown in Table 1.

The SH3 binding site of 3BP1 was localized. A 64–amino acid segment bound most strongly (Fig. 1A) whereas segments of 28 (*4*), 22, and 10 amino acids all bound less well. GST itself did not bind detectably to these peptides (Fig. 1B). Each lane contained approximately the same amount of GST fusion protein (Fig. 1C). The lower apparent binding affinity of the shorter peptides may arise either because of an effect of an altered structure relative to the longer peptide or because the larger peptide contains a second binding site that binds SH3 cooperatively with the binding site in the shorter peptide. There is a known low-affinity binding site NH_2-terminal to the 3BP1-28 peptide (*4*).

To determine which residues in 3BP1-10 are important for SH3 binding, individual amino acids were changed to alanine (*5*). Changing T3 (the number represents the position of the amino acid in 3BP1-10), M4, P5, P6, and L8 to alanine did not affect SH3 binding (Fig. 2A). A P9A mutation reduced the binding affinity whereas A1G (*10*), P2A, P7A, and P10A mutations abolished binding. Thus certain positions, particularly the prolines at 2, 7, and 10, seem crucial to binding affinity whereas others are less important.

The SH3 binding site of 3BP2 was also determined (Fig. 3). Sequence analysis showed no overall similarity between 3BP2 and 3BP1 (*10*). However, 3BP2 contains a region similar in sequence to a 40–amino acid region containing the SH3 binding site in 3BP1. The sequence of this region, termed 3BP2-40, is shown in Table 1. 3BP2-40 was bound specifically by the GST–Abl SH3 probe (Fig. 3A). The COOH-terminal ten amino acids of 3BP2-40, termed 3BP2-C10, has some similarity to the minimal SH3 binding site of 3BP1, 3BP1-10. However, 3BP2-C10 and a COOH-terminal 21–amino acid region bound only weakly to the Abl SH3 domain. Strong SH3 binding was found in the NH_2-terminal 22 amino acids of 3BP2. This peptide bound well when trimmed to 12 amino acids (3BP2-12). By sequential deletion analysis of 3BP2-12, we mapped the core SH3 binding site to a nine–amino acid region. As we observed for 3BP1, the SH3 binding affinity of the minimal binding site of 3BP2 was lower than that of 3BP2-40. The relative binding affinities of various peptides for the Abl SH3 domain are summarized in Table 1.

Because the SH3 binding sites of both 3BP1 and 3BP2 contain multiple proline residues, we examined whether a peptide composed of ten prolines expressed as a GST fusion protein would bind to GST–Abl SH3. Little binding was detected (Fig. 3A). Thus, amino acids other than proline are critical to binding.

If, as we expect, SH3 domains take part in signal transduction then there must be specificity in the interaction of particular SH3 regions with their ligands. 3BP1 binds with different affinities to different SH3 domains (*4*). The relative binding affinity of various SH3 domains to the high-affinity SH3 binding site of 3BP2, 3BP2-40, was evaluated (*11*) with biotinylated GST fusion proteins containing either no insert, or the SH3 domains of Abl, Src or neural Src (N-Src) or the entire proteins of GRB2 (*12*) or Nck (*13*). The probes were standardized for protein and biotin content as

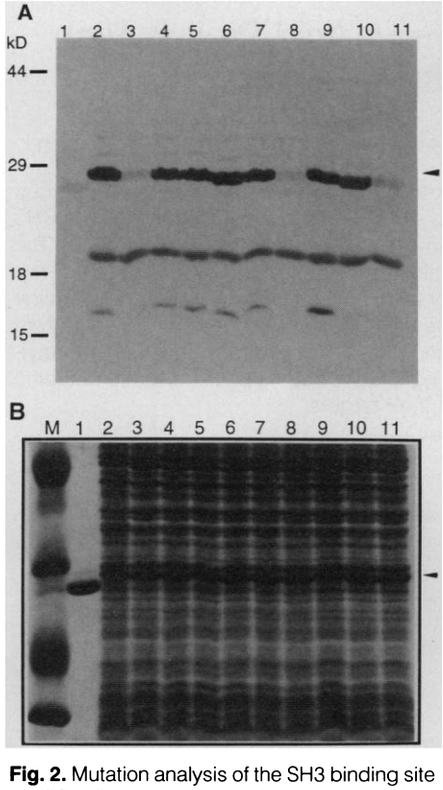

Fig. 2. Mutation analysis of the SH3 binding site of 3BP1. Proteins from lysates of bacteria that expressed the GST-3BP1 peptide fusion proteins were transferred to a filter and probed with biotinylated GST–Abl SH3 fusion protein (1 μg/ml) (**A**) or stained with Coomassie blue (**B**) after electrophoretic separation. Lane 1, purified GST; lane 2, GST–3BP1-10; lane 3, GST–3BP1-10P2A; lane 4, GST–3BP1-10T3A; lane 5, GST–3BP1-10M4A; lane 6, GST–3BP1-10P5A; lane 7, GST–3BP1-10P6A; lane 8, GST–3BP1-10P7A; lane 9, GST–3BP1-10L8A; lane 10, GST–3BP1-10P9A; lane 11, GST–3BP1-10P10A. Lane M in (B), prestained protein molecular size marker (Gibco BRL). In (A), numbers on left represent the molecular size in kilodaltons. Arrow shows the position of wild-type and mutant GST-3BP1-10 fusion proteins on the gel.

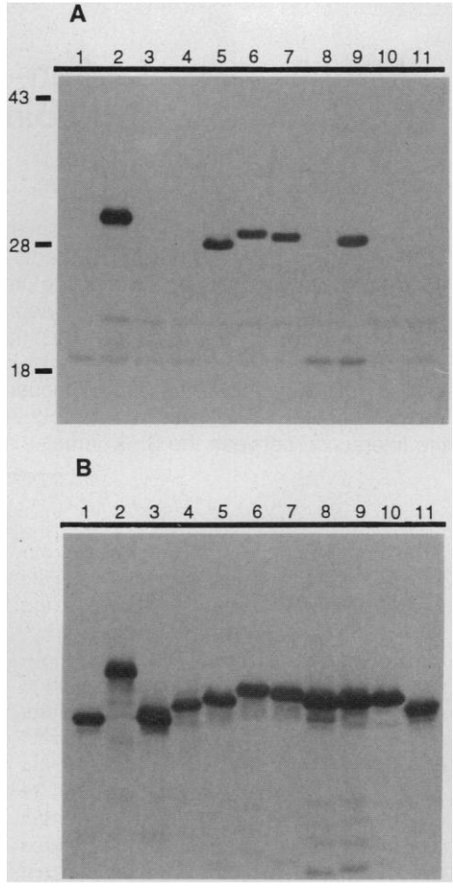

Fig. 3. Mapping of the SH3 binding site on 3BP2. Proteins from lysates of bacteria that expressed the GST-3BP2 peptide fusion proteins were probed with biotinylated GST–Abl SH3 fusion protein (0.2 μg/ml) (**A**), or antibody to GST (0.5 μg/ml) (**B**). Lane 1, GST; lane 2, GST–3BP2-40; lane 3, GST–3BP2-C10; lane 4, GST–3BP2-C21; lane 5, GST–3BP2-22; lane 6, GST–3BP2-12; lane 7, GST–3BP2-10-1; lane 8, GST–3BP2-10-2; lane 9, GST–3BP2-9; lane 10, GST–3BP2-8; lane 11, GST–P-10. Some GST fusion proteins (such as GST–3BP2-12) run anomylously on SDS-PAGE. The constructs have been confirmed by DNA sequence analysis. Numbers on left represent the molecular size in kilodaltons.

described (*4*). 3BP2-40 bound most strongly to the SH3 domain from Abl, more weakly to those from Src and GRB2, and poorly to the SH3 domains of Nck. No apparent binding to the neural Src (N-Src) SH3 domain was detected (Fig. 4). These data demonstrate that there is specificity in the binding strengths of the different SH3 domains for a given binding site. The low binding affinity of SH3 domains from Nck and N-Src for 3BP1 (*10*) and 3BP2 suggest that the SH3 binding sites described in this report represent a class of motifs that bind well to SH3 domains from Abl, Src, and GRB2, and that the SH3 domains from Nck, N-Src, and Crk (*4*) may interact with different targets.

A comparative search of protein sequence data bases with 3BP1-10 and 3BP2-12 revealed cellular and viral proteins containing sequences with similarity to the proline-rich SH3 binding motifs. The greatest similarity (eight of ten amino acids identical) found by searching the GenBank data base with 3BP1-10 was the APPTPPPLPP sequence in the murine *limb deformity* (*ld*) gene products, the formins (*14*). The greatest similarity (10 of 12 amino acids identical) found by searching the GenBank data base with 3BP2-12 was the sequence PPALPPPPRPVP in the rat m4 muscarinic acetylcholine receptor (mAChR) (*15*). The murine formins also contain the region PPAPPIPPVCPV, the second most similar sequence to 3BP2-12 (9 of 12 amino acids identical). To examine the binding of the first two regions to SH3 domains, we prepared GST fusion proteins containing the peptides (see Table 1) and expressed them in bacteria. Both regions bound specifically to the GST–Abl SH3 probe, and a dimer of the putative SH3 binding region from the mAChR bound even more efficiently (Fig. 5A), suggesting cooperativity of binding. These peptides had lower apparent affinity for GST–Abl SH3 than that of 3BP1-10 and 3BP2-12 but the amount of the formin and mAChR peptides bound was greater than that of GST–poly-proline or GST alone. The amounts of protein loaded in this experiment were approximately equal (Fig. 5B). Like the high-affinity SH3 binding domains, 3BP1-64 and 3BP2-40, the formin SH3 binding site, formin-33, also bound to the GRB2 protein and Src SH3 probes with lower apparent affinity than it did to Abl SH3 (*10*). These data demonstrate that murine formins and rat m4 mAChR contain SH3 binding sites. The low apparent binding affinity of Abl SH3 to the SH3 binding region of formins and rat m4 mAChR may indicate that formins and m4 mAChR interact with different but related SH3-containing proteins.

Aligning the four binding sites that have been identified shows that the conservation of the three proline sites identified as critical in the alanine scanning experiment is absolute (Fig. 5C). All of the binding sites share the property of being very hydrophobic and rich in prolines. Although proline residues have a hydrophobic side chain they are generally found on the surface of proteins exposed to solvent, and because of their constrained shape, multiple prolines often adopt the helical configuration found in collagen (*16*).

The crystal structure of the SH3 domain for spectrin has been solved to 1.8 Å resolution and the solution structure of the SH3 domain of the tyrosine kinase Src has been determined by multidimensional nuclear magnetic resonance methods (*17*). The SH3 domain is composed of two antiparallel β sheets packed together at approximately right angles. Studies of the Src SH3 domain bound to proline-rich peptides similar to the SH3 binding peptides described in this report revealed a hydrophobic binding site on the surface of the protein that is rich in the side chains of conserved aromatic amino acids (*17*).

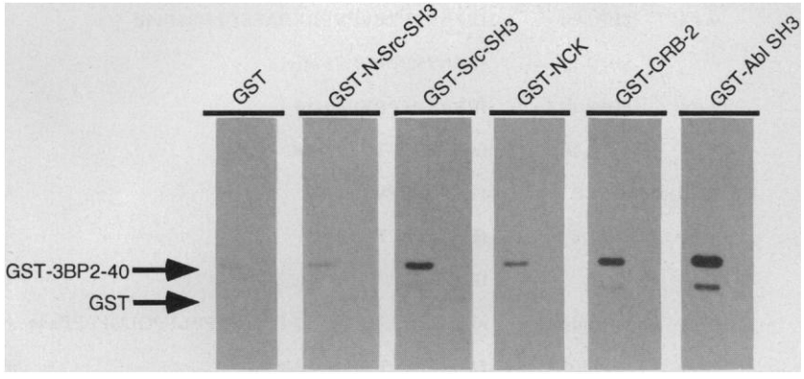

Fig. 4. Differential binding of the 3BP2 SH3 binding domain to various SH3 regions. Purified GST–3BP2-40 and GST were probed with the biotinylated proteins indicated above each pair of lanes. The positions of GST–3BP2-40 and GST on filters are indicated on left.

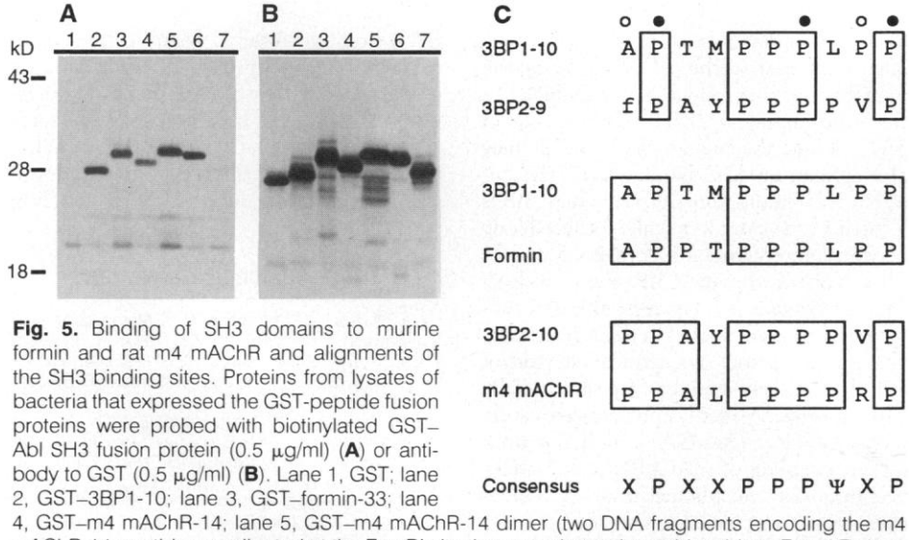

Fig. 5. Binding of SH3 domains to murine formin and rat m4 mAChR and alignments of the SH3 binding sites. Proteins from lysates of bacteria that expressed the GST-peptide fusion proteins were probed with biotinylated GST–Abl SH3 fusion protein (0.5 μg/ml) (**A**) or antibody to GST (0.5 μg/ml) (**B**). Lane 1, GST; lane 2, GST–3BP1-10; lane 3, GST–formin-33; lane 4, GST–m4 mAChR-14; lane 5, GST–m4 mAChR-14 dimer (two DNA fragments encoding the m4 mAChR-14 peptide were ligated at the Eco RI site that encodes amino acid residues E and F); lane 6, GST–3BP2-12; lane 7, GST–P-10. Molecular size markers are indicated to the left in kilodaltons. (**C**) Alignments of the SH3 binding sites of 3BP1-10 and 3BP2-9, 3BP1-10 and formin, and 3BP2-9 and m4 mAChR. Amino acids that are shared by each pair of sequences are boxed. The closed circles mark the amino acid residues that are both essential for the SH3 binding to 3BP1-10 and conserved among four SH3 binding proteins. Open circles mark the amino acid residues that affect the SH3 binding to 3BP1-10 when changed to certain amino acid residues but are not highly conserved among four SH3 binding proteins. Consensus amino acid residues among these sites are shown. Amino acid residues are represented by single letters. Capital letters represent amino acid residues in the SH3 binding sites. Lowercase letters represent flanking amino acids in the GST protein. ψ represents hydrophobic amino acid residues. Amino acid residues that are not conserved among four SH3 binding proteins are represented with X in consensus.

Table 1. Summary of the Abl SH3 binding to the peptides expressed as GST fusion proteins. Amino acid residues are represented by single letters. Capital letters represent the amino acid sequences of the peptides tested for the SH3 binding. Lowercase letters represent the flanking amino acids in GST protein. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Peptides	Sequences	SH3 binding*	Peptides	Sequences	SH3 binding*
3BP1-64	PTMPPPQPSSTRSSPPAPSLPPGSVSPGTPQAL	++++	3BP1-10L8A	sAPTMPPPAPPnss	+++
	PRRLVGTSLRAPTMPPPLPPVPPQPARRQSR †		3BP1-10P9A	sAPTMPPPLAPnss	+
3BP2-40	fPPAYPPPPVPVPRKPAFSDLPRAHSFTSKSPS	++++	3BP1-10P10A	sAPTMPPPLPAnss	–
	PLLPPPPPefivtd		3BP2-C10	sSPSPLLPPPPnss	+/–
3BP1-28	SPGTPQALPRRLVGTSLRAPTMPPPLPP †	+++	3BP2-C21	sLPRAHSFTSKSPSPLLPPPPPnss	+/–
3BP1-22	sALPRRLVGTSLRAPTMPPPLPPnss	+++	3BP2-22	fPPAYPPPPVPVPRKPAFSDLPRefivtd	+++
3BP1-N12	sALPRRLVGTSLRnss	–	3BP2-12	fPPAYPPPPVPVPefivtd	+++
3BP1-10	sAPTMPPPLPPnss	+++	3BP2-10-1	fPPAYPPPPVPefivtd	+++
3BP1-10A1G	sGPTMPPPLPPnss	–	3BP2-10-2	fAYPPPPVPVPefivtd	–
3BP1-10P2A	sAATMPPPLPPnss	–	3BP2-9	fPAYPPPPVPefivtd	+++
3BP1-10T3A	sAPAMPPPLPPnss	+++	3BP2-8	fPPAYPPPPefivtd	–
3BP1-10M4A	sAPTAPPPLPPnss	+++	P-10	fPPPPPPPPPPefivtd	+/–
3BP1-10P5A	sAPTMAPPLPPnss	+++	formin-33	sKISPPAPPTPPPLPPPLIPPPPPLPPGLGPLPPnss	++
3BP1-10P6A	sAPTMPAPLPPnss	+++	m4 mAChR-14	fAPPPALPPPPRPVPefivtd	++
3BP1-10P7A	sAPTMPPALPPnss	–			

*The relative SH3 binding affinity of the peptides shown in the figures are presented by ++++, very strong binding, +++, strong binding; ++, binding; +, weak binding, +/–, very weak binding; and –; no binding. †The constructs were described in (4). In 3BP1-64, the sequence that shows similarity to the sequence of 3BP2-40 is underlined.

The formins are a set of protein isoforms encoded by the alternatively spliced transcripts arising from the *ld* locus of the mouse (*14*). Mutations in this locus disrupt pattern formation of embryonic limbs and result in smaller size and fusion of the distal bones and digits of all limbs as well as variable incidence of renal aplasia (*18*). Certain isoforms of formin might act through interactions with SH3-containing proteins. The gene encoding 3BP1 has been mapped on mouse chromosome 15 in a region syntenic with human chromosome 22, and the gene encoding 3BP2 is located in the proximal region of mouse chromosome 5, which is syntenic with human chromosomes 7 and 4p (*19*). The gene encoding 3BP2 is located close to the *Hm* and *Hx* genes, which, like *ld*, take part in limb morphogenesis (*20*). The *3BP2* gene, however, was not altered in structure as analyzed by Southern (DNA) blots with several enzymes in DNA from mice with mutations in either *Hm* or *Hx* (*10*) and therefore the possible relationship of *3BP2* and these genes remains conjectural.

An SH3 binding region is also present in human m4 mAChR, the human sequence being APPPALPPPPRPVA (*21*). The COOH-terminal alanine residue of this sequence differs from the proline of the rat m4 mAChR but this residue is not included in the nine–amino acid SH3 binding site of 3BP2. Thus, the human m4 mAChR may also contain an SH3 binding site. The putative SH3 binding site in the m4 mAChR is located in the cytoplasmically localized loop between transmembrane domains 5 and 6. This loop of the mAChR shows a high degree of divergence between mAChR subtypes that may be related to their differential coupling to distinct biochemical effectors or ion channels via distinct G proteins (*22*). The guanosine triphosphatase–activating protein for Ras (Ras-GAP), and Ras itself inhibit coupling of mAChR's to K^+ channels in isolated membranes from atrial myocytes of guinea pig (*23*). The SH3 domain and an SH2 domain of Ras-GAP are sufficient for the inhibition of muscarinic atrial K^+ channel currents (*24*). Although the m4 mAChR is not the major subtype of mAChR in human heart (*21*), it may take part in signal transduction mediated by an SH3-containing protein.

Various SH3 domains may have different specific target proteins as suggested by the differential binding of SH3 domains to the known 3BP's, and there may be many SH3 binding proteins. Many cellular proteins bind to SH3 probes in a filter binding assay, and different SH3 probes bind different sets of proteins in individual cell lysates (*25*). The identification of the SH3 binding motifs described in this report may facilitate the identification of other SH3 binding proteins.

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5. The DNA fragment encoding 3BP2-40 was constructed with synthetic oligonucleotide-directed polymerase chain reaction (PCR) of the 3BP2 cDNA under conditions recommended by Perkin-Elmer Cetus. The PCR reaction was continued for 30 cycles at 95°C for 30 s, 55°C for 1 min, and 72°C for 2 min The oligonucleotides contained 22 bp of identical sequence for hybridization and an

entire Eco RI site with four extra base pairs at the 5′ end for stability and recutting efficiency. The PCR fragment was cloned in-frame into the Eco RI site of the pGEX-1λT vector (Pharmacia) The rest of the DNA fragments encoding the peptides listed in Table 1 were derived from synthetic oligonucleotides. The oligonucleotides, which were cloned into the Bam HI and Eco RI sites of the pGEX2T vector, contained coding sequences and the sequence 5′-GATCC-3′ at the 5′ end of the sense strand oligos and the sequence 5′-AATT-3′ at the 5′ end of the antisense strand oligos. The 5′ end of the DNA fragments derived from these oligos were in frame with the GST of pGEX2T and the 3′ ends were out of frame, resulting in amino acid residues NSS at the COOH-terminus of the GST-peptide fusion proteins. The oligonucleotides that were cloned into the Eco RI site of the pGEX1λT vector contained coding sequences and the sequence 5′-AATTC-3′ at the 5′ end of both sense and antisense strand oligos. The 5′ end of these oligos were in frame with the GST of pGEX1λT vector The synthetic oligos were phosphorylated with T4 polynucleotide kinase under conditions recommended by the manufacturer (New England Biolabs). The sense and antisense oligos were phosphorylated and annealed by boiling for 2 min and then cooling slowly to room temperature The DNA fragments were cloned into pGEX vectors The recombinant DNA was transformed into either *Escherichia coli* DH5α or *E. coli* NB42 as described (*26*)

6. Filter binding assay. Transformants were grown into log phase and induced by 0.1 mM isopropyl-β-D-thiogalactopyranosid galactoside (IPTG) for 1 hour at 37°C Cells were lysed on ice in Triton lysis buffer (phosphate-buffered saline, 100 mM EDTA, 1% Triton X-100, 1% aprotinin, and 1 mM phenylmethylsulfonyl fluoride) by sonication. The cell extract was boiled in Laemmli sample buffer, and proteins were separated by SDS-PAGE (12 5% gel) and transferred to nitrocellulose in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (pH 11), 20% (v/v) methanol Filters were blocked in 50 mM tris (pH 8.0), 150 mM NaCl, 0.1% Tween 20 (TBST buffer) containing 0.2% gelatin or 2% nonfat dry milk. Biotinylated probes were added in the same buffer at 0.2 to 1 μg/ml, incubated at 4°C for 2 hours, and washed extensively in TBST. Filters were incubated with streptavidin-conjugated alkaline phosphatase (Boehringer Mannheim Biochemicals) at a dilution of 1:5000 in TBST-gelatin buffer at 4°C for 1 hour, washed, and developed with Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega Biotec), as described by the manufacturer.
7. The Abl SH3 probe contains amino acids 84 through 138 of murine type IV c-Abl (*27*) fused in-frame at the Bam HI site of pGEX-2T. Biotinylation was done as described (*28*).
8. For immunoblotting with affinity-purified polyclonal antibody to GST (0.5 μg/ml), filters were treated as described at room temperature (*6*). The bound antibody was detected with goat antibody to rabbit immunoglobulin conjugated to alkaline phosphatase (Promega Biotec).
9. Sequencing of the constructs to confirm their identity was done with Sequenase according to the manufacturer's directions (U.S. Biochemicals).
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11. The Src and N-Src SH3 probes were prepared as described (*4*). The GST-GRB2 and GST-Nck fusion proteins were from J Schlessinger Biotinylation of these proteins was done as described (*28*). GST and GST–3BP2-40 were purified as described (*28*).
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Mineralocorticoid and Glucocorticoid Receptor Activities Distinguished by Nonreceptor Factors at a Composite Response Element

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Mineralocorticoid and glucocorticoid hormones elicit distinct physiologic responses, yet the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) bind to and activate transcription similarly from a consensus simple hormone response element (HRE). The activities of GR and MR at plfG, a 25–base pair composite response element to which both the steroid receptors and transcription factor AP1 can bind, are analyzed here. Under conditions in which GR represses AP1-stimulated transcription from plfG, MR was inactive. With the use of MR-GR chimeras, a segment of the NH_2-terminal region of GR (amino acids 105 to 440) was shown to be required for this repression. Thus, the distinct physiologic effects mediated by MR and GR may be determined by differential interactions of nonreceptor factors with specific receptor domains at composite response elements.

Mineralocorticoids and glucocorticoids, two classes of adrenal steroid hormones, differ greatly in their physiologic effects. They can elicit opposing effects on ion transport within a single tissue (*1*), a single cell type (*2*), or within an individual cell (*3*). However, molecular biological studies of mineralocorticoid and glucocorticoid action suggest a paradox: The varied effects of these hormones are mediated by receptor proteins that are closely related (*4*) and that bind to and enhance transcription from a common consensus DNA sequence, designated originally as a glucocorticoid response element (GRE) (*5*). Receptors for two other classes of steroid hormones, progestins and androgens, also recognize and function from this element (*6*). Because receptor binding alone is sufficient to confer hormone-mediated transcriptional enhancement from promoters linked to the GRE, this consensus sequence has been denoted a simple hormone response element (HRE) (*7, 8*). Thus, the specificity and complexity of hormone effects must be conferred by other means such as differential actions of receptors that are contingent upon communication between receptor and nonreceptor factors (*9–11*).

Interactions of steroid receptors with other transcription factors may explain responses from at least three types of regulatory regions (*9–16*). (i) When binding sites for various regulatory factors are placed in proximity to simple steroid response elements, a mutual increase in the stimulatory activity of both factors is commonly observed (*16*), suggesting that they may interact with each other or with a common target. (ii) Transcriptional activation mediated by sequence-specific binding of AP1 transcription factors can be repressed by the glucocorticoid recep-

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