an enzymatic acylation reaction is unprecendented and requires the active site region to be capable of lowering the serine pK_a substantially, similar to the proposal for the decrease of the pK_a of creatine ($pK_a = 14.3$) by creatine kinase[34]. But the possibility of Lys 67 acting as the general base in the class A β-lactamases must also be considered. □

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Homology of a yeast actin-binding protein to signal transduction proteins and myosin-I

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In yeast, the cortical actin cytoskeleton seems to specify sites of growth of the cell surface[1,2]. Because the actin-binding protein ABP1p is associated with the cortical cytoskeleton of *Saccharomyces cerevisiae*, it might be involved in the spatial organization of cell surface growth[3]. ABP1p is localized to the cortical cytoskeleton and its overproduction causes assembly of the cortical actin cytoskeleton at inappropriate sites on the cell surface, resulting in delocalized surface growth. We have now cloned and sequenced the gene encoding ABP1p. ABP1p is a novel protein with a 50 amino-acid C-terminal domain that is very similar to the SH3 domain in the non-catalytic region of nonreceptor tyrosine kinases (including those encoded by the proto-oncogenes c-*src* and c-*abl*), in phopholipase Cγ and in α-spectrin. We also identified an SH3-related motif in the actin-binding tail domain of myosin-I. The identification of SH3 domains in a family of otherwise unrelated proteins that associate with the membrane cytoskeleton indicates that this domain might serve to bring together signal transduction proteins and their targets or regulators, or both, in the membrane cytoskeleton.

Genetic mapping of the gene encoding ABP1p (*ABP1*) to a previously unassigned locus on chromosome III between *thr4* and *cdc39* (12 centiMorgans from *cdc39*), indicates that *ABP1* is a novel yeast gene[4].

The *ABP1* nucleotide sequence and deduced amino-acid sequence are shown in Fig. 1. ABP1p has a relative molecular mass of 65,500 (M_r 65.5K), is very hydrophilic, very acidic (pI = 4.6, net charge = −45), and has a high proline content (11.2%). The overestimation of the size of ABP1p (M_r of 85K instead of 65.5K) when deduced from SDS-PAGE mobility is characteristic of proline-rich proteins[5]. We identified two short repeated amino-acid motifs, indicating that there is a genetic duplication in *ABP1* (Fig. 2). Each unit of the first repeat (Fig. 2*a*) seems itself to have resulted from a duplication.

By searching the protein-sequence data base, we identified a 50-amino-acid region at the C terminus of ABP1p that is strikingly homologous to the C terminus of myosin-I[6,7] and to the SH3 domains in the noncatalytic regions of nonreceptor tyrosine kinases and phospholipase Cγ[8,9], as well as in α-spectrin[10] and the v-*crk* transforming protein[9] (Fig. 3). The importance of the SH3 domain in c-*src* and c-*abl* proteins is indicated by the observation that deletion and insertion mutations in it can lead to oncogenic activation[11–13].

On the basis of sequence conservation, the myosin-I SH3 region seems to be the closest relative of the corresponding ABP1p sequence. Moreover, whereas SH3 regions can be located anywhere in proteins, the ABP1p and myosin-I SH3 regions are located at the C terminus. Also, the 137 amino acids immediately upstream of the SH3 regions have limited similarity between ABP1p and *Dictyostelium* myosin-I; both regions are rich in alanine (12%) and proline (18% and 21% for ABP1p and myosin-I, respectively). But the myosin-I upstream region is 21% glycine and is basic, whereas the ABP1p region is 2% glycine and is acidic. The identification of SH3-related regions in non-metazoan cells indicates that the domain arose early in evolution.

A feature common to proteins with SH3 regions is physical or functional association, or both, with the cortical actin cytoskeleton, a submembranous protein network important for the regulation of cell shape, cell adherence and cell movement. ABP1p, myosin-I, spectrin, $p60^{v\text{-}src}$, $p120^{v\text{-}gag\text{-}abl}$, and $p90^{v\text{-}gag\text{-}yes}$, $p80^{v\text{-}gag\text{-}yes}$ are all associated with the membrane cytoskeleton[3,14–17]. Other indications of cytoskeletal associations are that, when overexpressed, type IV c-*abl* protein is seen to associate with actin stress fibres (as well as with the nucleus)[18], and that v-*src* protein associates with a detergent-insoluble cytoplasmic matrix[19]. Also, the cadherins, which colocalize with apical actin bundles in living and detergent-extracted cells[20], contain in their cytoplasmic domains[21] a region related, although remotely, to SH3 regions (a homology noted by D. Woods, personal communication).

Functionally, SH3-containing proteins affect cell properties through action on the cortical cytoskeleton, as illustrated by the two examples below. First, changes in actin cytoskeleton organization and cell shape are observed within 15 min or v-*src* activation[22]. Many substrates of the v-*src* tyrosine kinase ($p60^{v\text{-}src}$) are components of the cortical cytoskeleton[17]. It is

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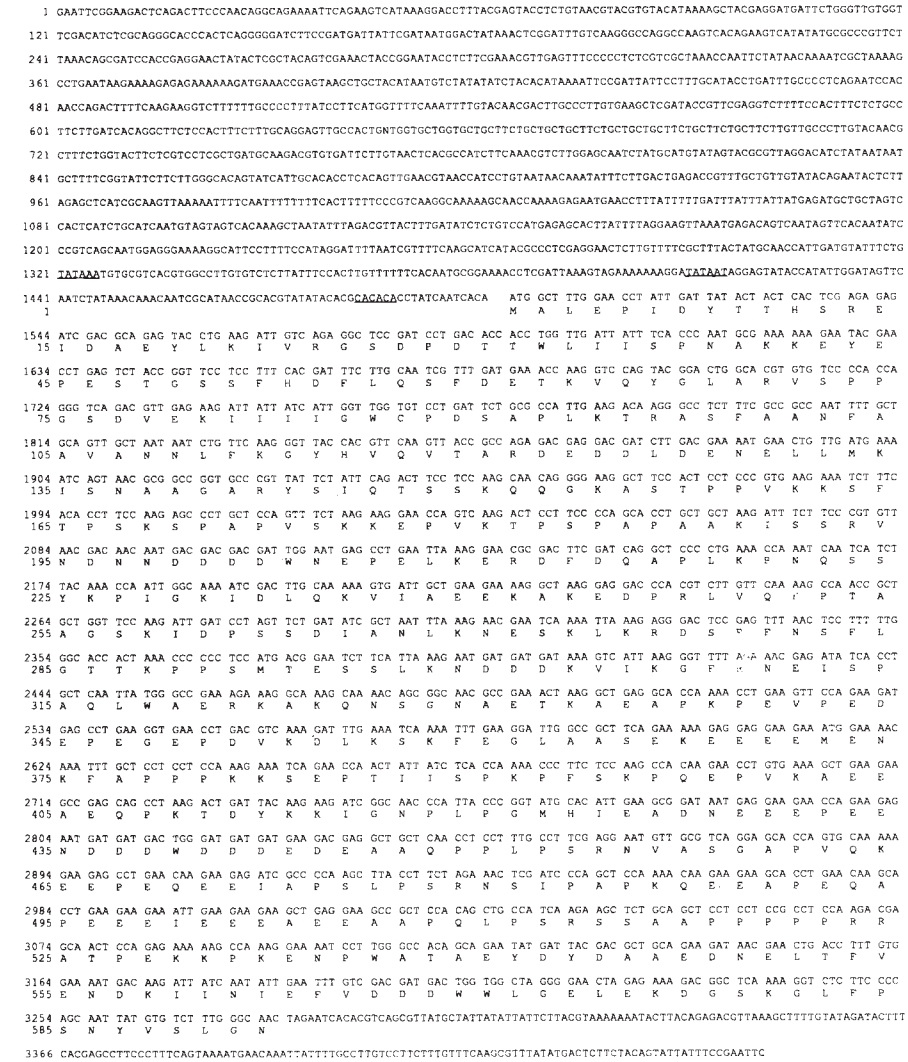

FIG. 1 Nucleotide and predicted amino-acid sequence of *ABP1* (single-letter code). Presumptive consensus TATAAA and CAYACA hexamer sequences are underlined (Y is an unspecified pyrimidine nucleotide).
METHODS. To isolate *ABP1*, 2×10^6 plaques from a λgt11 library (provided by R. Young) were screened using a polyclonal antiserum, and 38 positive plaques were identified. After two rounds of plaque purification, 21 plaques were obtained that were still positive. Restriction mapping and DNA hybridization indicated that all of the DNA fragments isolated were related. The smallest common DNA fragment (3.4 kilobases (kb)) was subcloned for further characterization and sequencing. Three lines of evidence indicated the correct identity of the fragment: (1) when the fragment was introduced into yeast at a high copy number, ABP1p was overproduced about fivefold; (2) bacteria transformed with this fragment expressed a full-size (apparent M_r of 85K) immunoreactive polypeptide; and (3) immunoblots showed that gene disruptions in yeast using the 3.4-kb fragment result in elimination of ABP1p (these mutants are viable and are now being characterized). The region between residues 1,294 and 3,439, containing the entire coding region, was sequenced at least twice and on both strands. The remaining regions were sequenced once and on the first strand only.

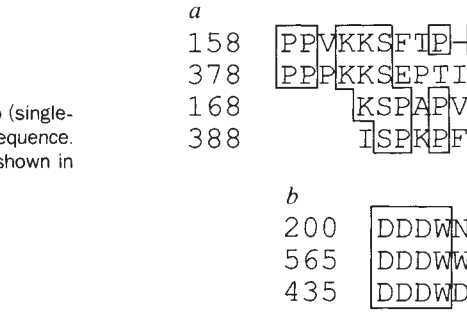

FIG. 2 Internal repeated amino-acid sequences identified in ABP1p (single-letter code). The numbers refer to the initial amino acid in the sequence. The second sequence shown in *b* lies in the SH3-related region shown in Fig. 3.

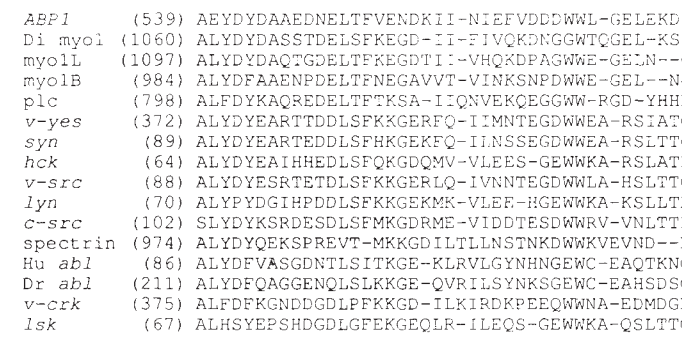

FIG. 3 Amino-acid (single-letter code) alignment of homologous regions of ABP1p, myosin-I, α-spectrin, phospholipase Cγ, nonreceptor tyrosine kinases, and v-*crk* protein. Numbers on the left are positions of the initial amino acid of the homology box. Numbers on the right indicate the number of identities with the 50-amino-acid ABP1p region shown on the first line. References to amino-acid sequences: *Dictyostelium* myosin-1 and *Acanthamoeba* myosin-IL, ref. 7; *Acanthamoeba* myosin-IB, ref. 6; phospholipase Cγ, ref. 8; a-spectrin, ref. 10; human *abl* ref. 29; *Drosophila abl*, ref. 30; and v-*crk*, ref. 9. References for the following protein sequences can be found in ref. 8: v-*yes*, *syn*, *hck*, v-*src*, *lyn*, c-*src*, *lsk*. Abbreviations: Di, *Dictyostelium*; Hu, human; Dr, *Drosophila*; myo, myosin; Plc, phospholipase Cγ.

significant that mutations in the SH3 region of $p60^{v\text{-}src}$ can affect the shape of a transformed cell, indicating a direct role for this domain in the cell shape changes (reviewed in ref. 23). Second, rapid tyrosine phosphorylation of phospholipase Cγ and the rapid stimulation of inositol 1,4,5-trisphosphate formation in cells treated with epidermal growth factor are associated with rapid changes in cell morphology, cytoskeleton organization and phosphorylation[24,25]. Inositol 1,4,5-trisphosphate has been proposed to regulate the actin-binding proteins gelsolin, profilin and myosin-I[14,26]. The SH3 domain might bring together signalling proteins and their targets or regulators, or both, in the membrane cytoskeleton by interaction with a common cellular ligand.

Because several of the proteins that contain SH3 domains bind to actin, it is possible that actin is an SH3 ligand. This possibility is supported by the location of the SH3 domain of myosin-I within a 250-amino-acid proteolytic fragment that binds to actin in an ATP-insensitive manner[14]. It is significant that brush-border myosin-I[27] lacks an SH3 domain and does not contain the ATP-insensitive actin-binding site. Also, biochemical studies indicate that actin can bind to $p60^{v\text{-}src}$ and inhibit its tyrosine kinase activity[28]. Although spectrin binds to actin, the actin-binding site is believed to be in a region other than the SH3 domain[16]. Other proteins should also be considered as candidates for SH3-binding ligands. For example, calpactin-I is a candidate because it binds to spectrin and is a substrate for $p60^{v\text{-}src}$ (ref. 10). Another candidate is ezrin, because like spectrin and phospholipase Cγ, it is phosphorylated rapidly when cells are treated with epidermal growth factor[25].

The identification of SH3-binding ligands and determination of whether the various SH3 domains have the same function are now required. The discovery of a protein with an SH3 domain in yeast should allow the use of classical and molecular genetic approaches to identify interacting proteins and to test the functional equivalence of different SH3 regions. The identification of new proteins with SH3 domains reported here indicates that more members of this protein family are likely to be discovered. □

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