other cell populations, either in vitro or in vivo (*22*).

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10. For example, perineurial cells resemble SCs but not Fbs in bearing a basal lamina; they are like Fbs but unlike SCs in their capacity to synthesize fibronectin; and they resemble neither Fbs nor SCs in their abundance of caveolae and their interconnections by tight junctions (*2, 12*).
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A Yeast Actin-Binding Protein Is Encoded by *SAC6*, a Gene Found by Suppression of an Actin Mutation

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The protein encoded by *SAC6*, a gene that can mutate to suppress a temperature-sensitive defect in the yeast actin gene, has been identified as a 67-kilodalton actin-binding protein (ABP 67) that associates with all identifiable actin structures. This finding demonstrates the in vivo functional importance of the actin–ABP 67 interaction previously established in vitro and illustrates the use of suppressor analysis to identify physically interacting proteins.

THE EUKARYOTIC CYTOSKELETON IS a dynamic structure characterized by complexity in function, organization, and in the number of protein components. Although these features make understanding the cytoskeleton a fascinating problem, they also provide a considerable challenge. Whereas it has been possible to identify many cytoskeletal constituents, and to determine the functional capacity of these proteins in vitro, it has proven much more difficult to demonstrate functional association of the proteins and the relevance of their biochemical activities in vivo.

Drugs and microinjected antibodies have been useful for probing function in the cytoskeleton, but each of these approaches has limitations. Drug action can be complex (*1*), making interpretation of effects difficult, and although drugs acting on tubulin and actin are known, no drugs targeting associated proteins have been identified. Antibody microinjection has established a role for myosin in cytokinesis (*2*); however, general applicability of this approach is limited by problems of antibody accessibility, and by the difficulty of obtaining inactivating antisera and antisera specific for protein subdomains.

An alternative way to probe functional interactions in the cytoskeleton is through genetics (*3*). Mutants defective in genes encoding cytoskeletal proteins can be isolated and the effects of these mutations can be studied both in vivo and in vitro. A variety of genetic approaches can then be used to identify genes encoding interacting components, and the effects of mutations in these can in turn be studied both in vivo and in vitro.

An important advantage of identifying genes by mutant phenotypes is that the mutant phenotype implicates the biological role of the protein encoded by the gene. Difficulty, however, is usually encountered in determining how the gene product functions on a biochemical level. Conversely, when proteins are identified on the basis of physical interactions and biochemical activities in vitro, difficulty is often encountered in establishing the in vivo function of the protein. We report here a biochemical and genetic study on the actin cytoskeleton of yeast that demonstrates the value of the combined approach.

Saccharomyces cerevisiae has a single essential actin gene (*ACT1*), which has been cloned and sequenced (*4*) and found to encode a protein 90% identical to vertebrate actins. Temperature-conditional lethal (Ts^-) mutations in this gene have been isolated and characterized (*5, 6*), and six genes (*SAC1, 2, 3, 4, 5*, and *6*) that can mutate to suppress the Ts^- defect due to the *act1-1* mutation have been identified (*7, 8*). Genetic evidence suggests that these genes encode components of the actin cytoskeleton. For example, mutations in *SAC6* and *ACT1* can suppress each other's defects. Thus, whereas *act1 sac6* double mutants grow well at all temperatures, *act1 SAC6*$^+$ and *ACT1*$^+$ *sac6* single mutants do not (*8*). Furthermore, *act1 SAC6*$^+$ and *ACT1*$^+$ *sac6*

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single mutants both have grossly disorganized actin cytoskeletons and cell morphologies, whereas the actin cytoskeletons and cell morphologies of *act1 sac6* double mutants resemble those of wild-type cells (*9*) (Fig. 1). These observations make it likely that suppression is due to compensatory changes in two interacting proteins, and is not due to some more global mechanism; a suppressor mutation that, for example, altered the intracellular milieu, should not cause a growth defect and disorganization of the actin cytoskeleton on separation from the original mutation (Fig. 1).

In parallel to these genetic studies, a number of protein components of the yeast actin cytoskeleton have been identified biochemically. Two actin-binding proteins, ABP 67 and ABP 85, were isolated by their ability to bind to actin in vitro and have been demonstrated by immunofluorescence microscopy to colocalize with actin in vivo (*10*). Here we describe the isolation of the gene encoding the yeast actin-binding protein ABP 67, and we present evidence demonstrating that this protein is encoded by the *SAC6* gene (*8*).

The gene encoding ABP 67 was isolated by immunoscreening a λgt11 expression library (*11*) with the antiserum against ABP 67 (anti-ABP 67) described by Drubin, Miller, and Botstein (*10*). Three immunopositive plaques were isolated from about 2×10^5 plaques screened. The three isolated λgt11 recombinants contained overlapping inserts, as shown by restriction analysis; the largest is shown in Fig. 2D. Bacteria containing various putative ABP 67 clones were all found to express a 46-kD polypeptide that was recognized by anti–ABP 67 (Fig. 3, lane 3). Peptide mapping (*12*) was used to demonstrate that this is a truncated ABP 67 protein (Fig. 4). Thus, partial proteolysis of ABP 67 by chymotrypsin or *Staphylococcus aureus* protease generated six

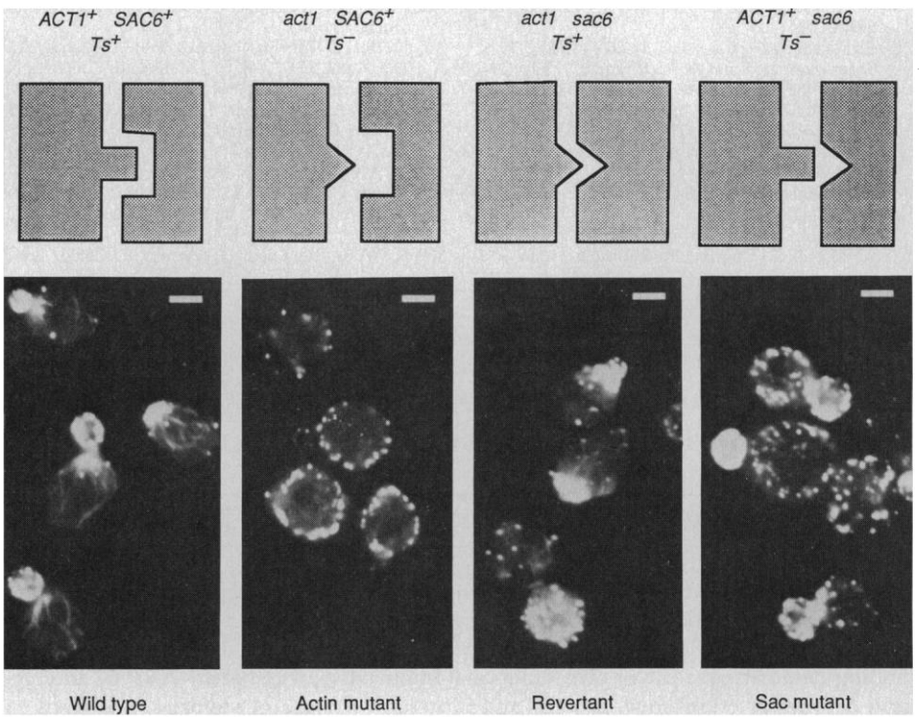

Fig. 1. Illustration (top) of interactions between various combinations of wild-type and mutant *ACT1* and *SAC6* gene products, and anti-actin immunofluorescence micrographs (bottom) of the corresponding strains. As reported previously, the distribution of actin is asymmetric in wild-type cells (*9, 10*), and randomized in *act1-1/act1-1* mutant cells (*5*). In the revertant, the dominant *sac6-2* mutation restores the actin cables and asymmetry typical of wild-type cells, although the cables are fainter. In *sac6-2/sac6-2* mutant cells the asymmetry is largely retained, although there are no detectable cables, and the dots are also found in the mother cells. Cultures of strains DBY5263 (*ACT1*$^+$/*ACT1*$^+$ *SAC6*$^+$/*SAC6*$^+$), DBY5217 (*act1-1/act1-1 SAC6*$^+$/*SAC6*$^+$), DBY5264 (*act1-1/act1-1 SAC6*$^+$/*sac6-2*), and DBY5265 (*ACT1*$^+$/*ACT1*$^+$ *sac6-2/sac6-2*), growing exponentially in rich medium at the nominally permissive temperature of 26°C, were prepared for indirect immunofluorescence microscopy with an affinity-purified anti-actin as described previously (*10*). Bars, 3.5 μm.

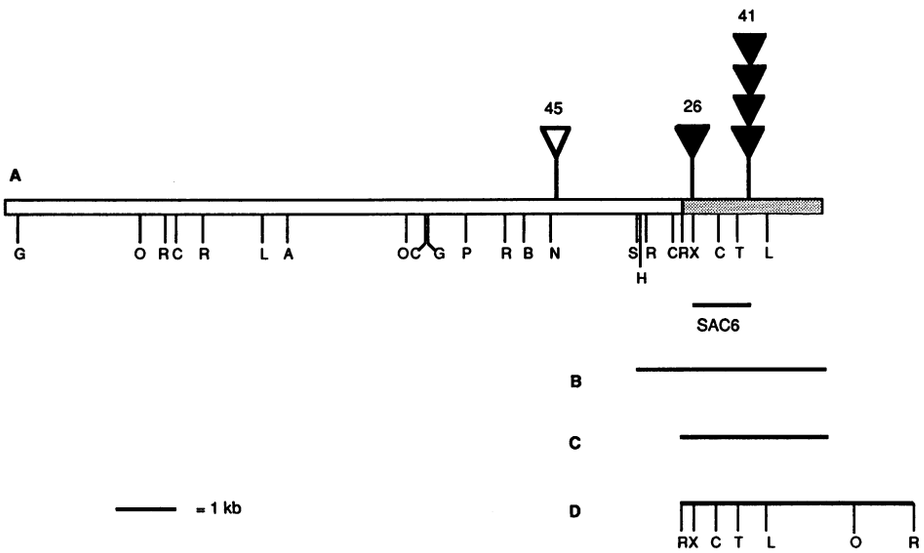

Fig. 2. Indentity of the *SAC6*- and ABP 67–encoding genes. (**A**) The *sac6* gene was localized on the cloned insert (*8*) by insertion mutagenesis with a Tn10-derived transposon as described by Huisman *et al.* (*15*). Thus, plasmids containing insertions were screened for those unable to suppress the Ts$^-$ defect of an *act1-1* mutant strain because of an insertion within *sac6*. Five (of 55) such plasmids were identified and the sites of insertion were determined by restriction analysis. Filled triangles indicate transposition events within *sac6*; the open triangle indicates one (of 50) events outside *sac6*. Numbers above the triangles identify particular transposition events referred to below and in Fig. 3. The analysis indicates that the *sac6* gene flanks the sites defined by insertions 26 and 41, but does not extend as far as that defined by insertion 45 (A). (**B** and **C**) The location of the *sac6* gene was confirmed by subcloning. Restriction fragments derived from the 14.5-kb insert were subcloned into the Eco RI–Sph I or Sac I–Sph I sites of a yeast centromere-containing plasmid YCp50 (*16*) or pRB720 (*17*), respectively, and tested for ability to suppress when used to transform an *act1-1* mutant strain. A fragment extending leftwards as far as the Sac I site was able to suppress (B), whereas a fragment extending only as far as the rightward-most Eco RI site was not (C). (**D**) Restriction map of the ABP 67–encoding gene. Restriction enzymes: A, Aat II; B, Bam HI; C, Cla I; G, Bgl II; H, Xho I; L, Sal I; N, Nru I; O, Nco I; P, Sph I; R, Eco RI; S, Sac I; T, Bst EII; X, Xba I.

or three immunoreactive proteolytic fragments, respectively. Partial proteolysis of the 46-kD protein with chymotrypsin generated five peptides that each comigrated with an ABP 67 fragment, whereas *S. aureus* protease generated two peptides that each comigrated with an ABP 67 fragment (Fig. 4).

To determine whether any of the *SAC* genes identified genetically encode any of the actin-binding proteins identified biochemically, DNA hybridization experiments were carried out. This was possible in those cases where the gene had been cloned [*SAC1, SAC2, SAC3* (*7*), *SAC6* (*8*), and the genes encoding ABP 67 (this study), and ABP 85 (*13*)]. Hybridization between *sac6* and the gene encoding ABP 67 was strongly positive. The region of homology, with the Eco RI fragment of the gene encoding ABP 67 as probe (Fig. 2D), covered the entire functional *sac6* gene as determined by insertion mutagenesis (Fig. 2A) and subcloning (Fig. 2, B and C), and is indicated by stippling in Fig. 2A. The identity of these sequences was confirmed by restriction analysis of the plasmids (compare Fig. 2, A and D), strongly suggesting that *sac6* encodes ABP 67.

Since the ABP 67 clone described above expresses immunoreactive sequences in bacteria, we expected that bacteria transformed with *sac6* should also express a protein immunoreactive with anti–ABP 67. Strains carrying either the ABP 67–encoding gene or *sac6* produce a 46-kD protein that reacts with affinity-purified anti–ABP 67 (Fig. 3, lanes 3 and 4). This 46-kD protein was absent from strains carrying insertion mutations in *sac6* (Fig. 2A and Fig. 3, lanes 5 and 6), whereas it remained in strains carrying an insertion adjacent to, but not in, the *sac6* gene (Fig. 2A and Fig. 3, lane 7).

The demonstration that *sac6* encodes ABP 67 provides evidence that ABP 67, a protein identified on the basis of its association with actin in vitro, interacts functionally with actin in vivo. In addition, this finding demonstrates the feasibility of identifying interacting components of the actin cytoskeleton by suppressor analysis, an approach that has long been recognized as a useful means to elucidate biological interactions (*14*).

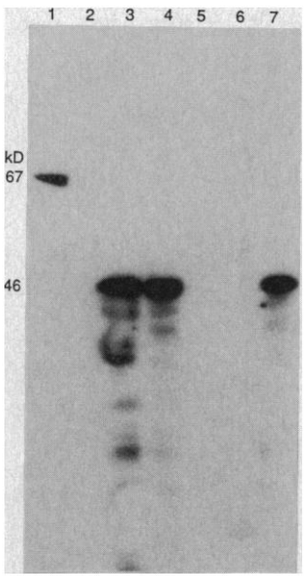

Fig. 3. Demonstration that *sac6* and the gene encoding ABP 67 produce the same immunoreactive protein. Extracts were obtained from yeast (lane 1) or bacteria (lanes 2 to 7), and probed with anti-ABP 67 as described in the legend to Fig. 4. ABP 67 is seen as a 67-kD protein in extracts from yeast cells (strain DBY877; lane 1). Bacterial extracts from *Escherichia coli* strain HB101 contain no endogenous immunoreactive protein (lane 2), but cells transformed with either the ABP67–encoding gene (lane 3) or the *sac6* gene (lane 4) express a 46-kD protein that is recognized by the antiserum. Extracts from HB101 containing *sac6* disrupted by either insertion 26 or 41 (Fig. 2A) contain no detectable immunoreactive protein (lanes 5 and 6), whereas extract from HB101 containing *sac6* plasmid with the nondisrupting insertion (Fig. 2A, insertion 45) contains the 46-kD protein (lane 7).

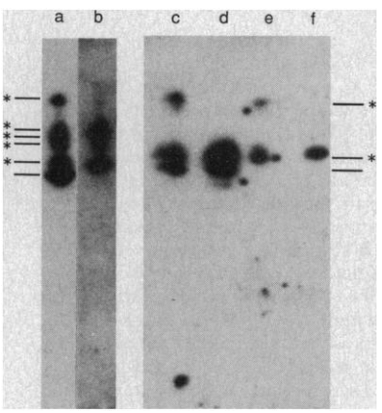

Fig. 4. Comparison of peptide maps generated by partial proteolysis of yeast ABP 67 (lanes a, c, and d) or the bacterially expressed 46-kD protein (lanes b, e, and f) with 2 μg of chymotrypsin (lanes a and b) or *S. aureus* protease (2.5 μg, lanes c and e; 20 μg, lanes d and f). The lines on the left and right of the figure represent ABP 67 proteolytic fragments generated by chymotrypsin and *S. aureus* protease, respectively. The asterisks mark the proteolytic fragments also generated from the bacterially expressed 46-kD protein. Purified ABP 67 (*10*) and an extract from bacteria infected with λgt11 containing the 4.1-kb insert (Fig. 2D) were fractionated on an 8.5% polyacrylamide-SDS gel. After Coomassie staining, ABP 67 and the region from 43 to 49 kD of the lane containing bacterial extract were excised. The gel slices were placed in wells of a 15% polyacrylamide-SDS gel, and overlayed with chymotrypsin of *S. aureus* protease, as described by Cleveland *et al.* (*12*). After electrophoresis, peptides were electrotransferred to nitrocellulose and visualized with a 1:500 dilution of affinity-purified anti-ABP 67 and ^{125}I-labeled protein A, followed by autoradiography.

The relation between *SAC6* and ABP 67 is the only case of identity we have observed among the six *SAC* genes and the several actin-binding proteins identified so far. The genetic and biochemical approaches therefore appear to have identified overlapping, but not identical, sets of cytoskeletal components. This is not surprising, as it is likely that some constituents will be more readily identified by one method than the other.

Mutations in *SAC6* suppress the pleiotropic effects of the *act1-1* mutation on actin organization and multiple cellular functions. This demonstrates that the actin–ABP 67 interaction is important for normal actin organization and for a variety of cellular processes. This is consistent with the observation that ABP 67 colocalizes with all identifiable actin structures during all phases of the cell cycle (*10*).

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