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 (5). 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. Temperatureconditional 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 Suc6 and ACT1 sac6 single mutants do not (8). Furthermore, act1 Sac6 and ACT1 sac6

REFERENCES AND NOTES
2. P. K. Thomas and Y. Olsson, in Peripheral Nervou
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).
13. J. R. Sanes, J. L. R. Rubenstein, J.-F. Nicolas, EMBO J. 5, 3133 (1986); Sanes et al. detail the construction of the recombinant retrovirus (LZI) used here and document its use as a lineage tracer in rodent embryos.
19. Color slides of semithin plastic sections from both types of cultures were projected, and definitively labeled lacZ-positive cells were identified; cells with only a few or unevenly distributed blue granules were not considered to be lacZ-positive as there is some dispersion of reaction product.
22. Lymphocytes have been infected by a retrovirus in vitro and then introduced in vivo to study their fate [G. Keller, C. Paie, E. Gilboa, E. E. Wagner, Nature 318, 149 (1985)]. In these studies, however, populations of infected cells were identified by DNA blot analysis, whereas our methods allow histochem-
23. Supported by NIH grants NS09923 and NS22828, and the Muscular Dystrophy Association. An ab-
24. Advance by small cytoskeletons in vitro, and this has been shown to correlate with the development of a disease, whereas our methods allow histochem-
25. 5 August 1988; accepted 18 October 1988
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 antisera against ABP 67 (anti-ABP 67) described by Drubin, Miller, and Botstein (10). Three immunopositive plaques were isolated from about \(2 \times 10^5\) 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

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**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 act1-1act1-1 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 sac6-2act6-2 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 DBYS263 (ACT1′/ACT1′ SAC6′/SAC6′), DBYS217 (act1-1act1-1 SAC6′/SAC6′), DBYS264 (act1-1/ act1-1 SAC6′/sac6-2), and DBYS265 (ACT1′/ ACT1′ sac6-2sac6-2), 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.

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**Fig. 2.** Identity of the SAC6- and ABP 67-encoded 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 YC50 (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, Bsr 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).

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).

**REFERENCES AND NOTES**

18. We are grateful to K. Wettman, B. Weinstein, and K. Hennessy for helpful advice, R. Young for providing the act1-11 library, and T. Stearns for the plasmid pGB720. We thank J. Earnest and T. Stearns for comments on the manuscript. This work was supported by grants to D.B. from the National Institutes of Health (GM12183 and GM18975) and the American Cancer Society (M390). D.D. was a fellow of the Helen Hay Whitney Foundation. A.A. is a Burroughs Wellcome Fund Fellow of the Life Sciences Research Foundation.
19. July 1988; accepted 19 October 1988