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MOLECULAR GENETIC ANALYSIS OF THE YEAST ACTIN CYTOSKELETON

DAVID DRUBIN and DAVID BOTSTEIN
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

INTRODUCTION

The actin cytoskeleton has been implicated in many structural and motile functions in eukaryotic cells. Understanding the formation and function of actin filaments is fundamental to understanding many aspects of spatial organization and motion in living cells. However, actin cytoskeleton function is understood well only in muscle cells. Study of the nonmuscle cell actin cytoskeleton is complicated by the relative lack of actin filament order, the tendency of the filaments to rearrange dynamically, and the large number of proteins that interact with actin.

Many of the proteins that comprise the actin cytoskeleton have been identified and studied *in vitro*. Of central importance is the 42 kd actin molecule that by itself is capable of assembling into actin filaments. Each actin molecule binds one ATP, and hydrolysis of this ATP after association of actin subunits causes conformational changes that can give dynamic assembly/disassembly properties to the filaments; ATP binding and hydrolysis can potentially affect interactions with actin binding proteins (ABPs) (1).

ABPs can affect actin filaments in many ways, and are thought to be important for providing functional, dynamic and structural diversity to these otherwise highly invarient polymers. ABPs can exert forces on actin filaments, they can cross-link or sever filaments, they can modulate actin subunit exchange by binding to filament ends, and they can block assembly by binding to actin monomers (1).

In the test tube ABPs and purified actin can interact in ways that seem to mimic complex *in vivo* processes. For example, one combination of purified proteins can form a contractile network responding to calcium gradients in an ATP-dependent manner (2). Alternatively, actin cables can guide membrane vesicle translocation in the presence of ATP (3). These and other experiments demonstrate some of the functional capacity of the actin cytoskeleton. However, it

is not possible to determine from these *in vitro* experiments which interactions are relevant *in vivo*.

Actin and ABP function *in vivo* have been probed primarily by the use of drugs and by microinjection of antibodies. These approaches, while implicating the actin cytoskeleton in a number of cellular processes, have serious limitations. Genetics should provide an alternate way to address functional and mechanistic questions in an *in vivo* context. We have chosen to study the actin cytoskeleton in the yeast *Saccharomyces cerevisiae* primarily because this organism has the most well-developed genetics available in a eukaryote. A significant additional advantage is the apparent actin cytoskeleton simplicity in this nonmotile organism. For example, most organisms have 6-20 actin genes, but *Saccharomyces cerevisiae* has only one (4,5). Indeed, *Dictyostelium* has a single myosin gene, and a lesion in this gene has helped to better define the scope of *in vivo* processes requiring myosin function (6,7).

A major uncertainty concerns the degree to which any understanding gained by cytoskeletal studies using genetics in yeast might apply to higher cell cytoskeletons as well. Our initial hopes for this were greatly encouraged by the observation of extreme conservation in the amino acid sequence of yeast actin which has about 90% identity to actin of higher animals (4,5). The best evidence for meaningful conservation is functional interchangeability. Indeed, rabbit muscle myosin on glass slides can move yeast actin filaments in an ATP-dependent manner (Kron, S., Drubin, D., Spudich, J., and Botstein, D., unpublished). Thus, there is reason to believe that what is learned through the application of the entire panoply of molecular genetic methods about the yeast cytoskeleton will have direct application in understanding the cytoskelton of higher eukaryotic cells.

YEAST MORPHOGENESIS AND ACTIN ORGANIZATION

Saccharomyces cerevisiae cells grow in a highly polarized manner, as depicted schematically in Figure 1. Early in the cell cycle a new cell begins to form as a bud on the mother cell. Growth and secretion are largely restricted spacially to the budded portion of the yeast cell. Also, the site of bud emergence is nonrandom; in successive cell cycles, buds emerge from the same pole in haploid cells, or from one of two opposing poles in dipoid cells (8).

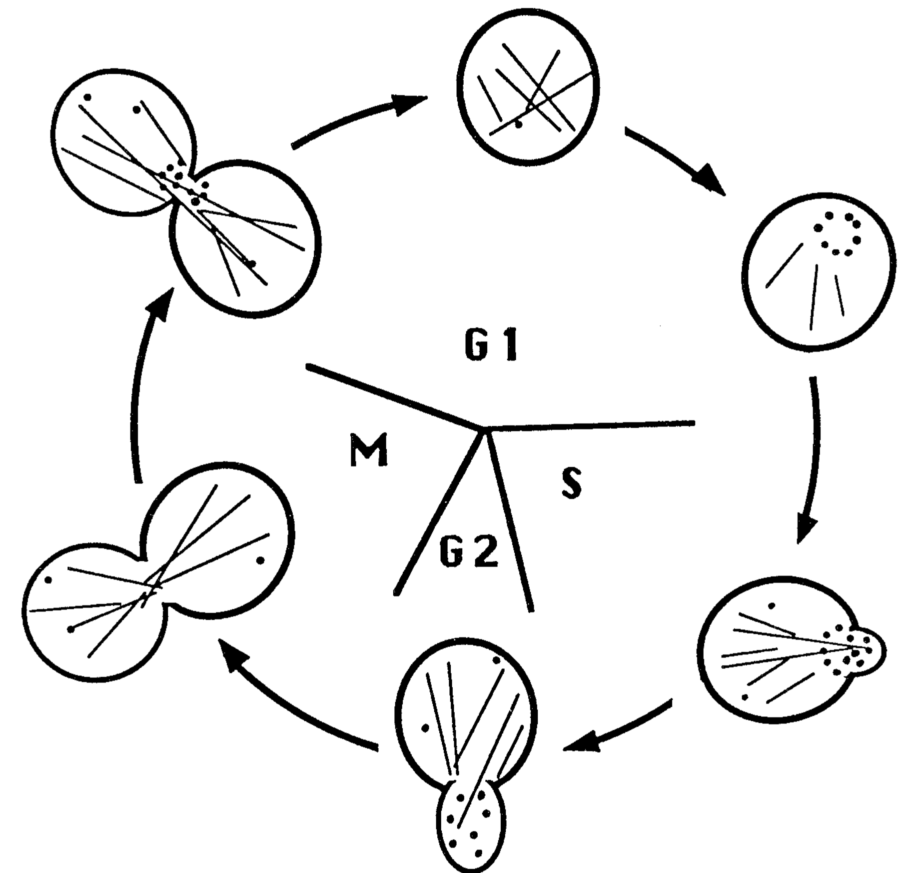

Figure 1. Cell growth pattern and actin organization through the *Saccharomyces cerevisiae* cell cycle. Thick lines represent the cell margin, thin lines represent actin cables, and dots represent cortical actin patches.

How is the initial polar axis of cell growth chosen? How is subsequent growth spatially restricted to a defined portion of the yeast cell? The answers to these questions are not known, but several lines of evidence suggest that the actin cytoskeleton functions to organize the spatial pattern of yeast cell growth.

Two kinds of actin structure can be observed in yeast by immunofluorescence or by staining with a fluorescent derivative of the actin-binding drug phalloidin (9,10). These structures are depicted in Figure 1. Cortical actin patches are associated closely with growing regions of yeast cells, and these structures rearrange coincident with programed changes in sites of growth. They first appear early in the cell cycle in a ring on the unbudded cell that is the first known indicator of the precise site of bud emergence. These patches are concentrated in the growing bud for most of the cell cycle, and then rearrange to the region of septum growth at the time of cytokinesis. Additionally, cytoplasmic cables are aligned along the growth axis, perhaps to direct vesicle transport to growth sites. This complex and dynamic organization suggests that actin may mediate secretory vesicle targeting and act to determine yeast cell morphology. Phenotypes of actin mutants support these conclusions (see below).

PHENOTYPES OF ACTIN MUTANTS

The finding that the actin gene is present in only a single copy per haploid genome immediately presented an opportunity to make mutations in this gene and to then observe, for the first time, the phenotypes associated with lesions in this central cytoskeletal protein. The peculiar ability of yeast cells to integrate DNA by homologous recombination was used to perform integrative gene disruption experiments that showed that the null phenotype of mutations in the actin (*ACT1*) gene is recessive lethality (11). A variation on the integrative gene disruption theme allowed production of mutations *in vitro*, followed by direct integration of the mutant actin genes into the genome, so that recessive conditional-lethal *ACT1* mutations could be recognized (12). These mutants were then studied in detail with the expectation that something about the role of actin in the cell would be learned.

Indeed, the phenotypes of the actin mutants are quite informative (13). At their nonpermissive temperature, *act1* mutants show

aberrant or absent actin cables and complete loss of normal asymetry of cortical actin patches. Coincident with the loss of normal actin organization, the mutants show a clear but incomplete invertase secretion defect, accumulation of vesicles, aberrant distribution of chitin on the cell surface, and unusual sensitvity to high osmotic pressure in the medium. Although they failed to show a uniform defect in the progress through the cell division cycle, they showed a clearly aberrant distribution of cell types. More than 70% of the mutant cells accumulated as single unbudded cells, suggesting a difficulty in bud emergence. These genetic findings suggest a primary function of actin in controlling biosynthesis and morphogenesis of the cell surface, entirely consistant with functions suggested by the organization of the yeast actin cytoskeleton (see discussion above).

Further study of the mechanisms underlying actin organization and dynamics *in vivo*, and the role of actin in cytoplasmic organization and cellular morphogenesis, now requires extension of this genetic analysis to other components of the yeast actin cytoskeleton. Two approaches have been used to expand the repetoire of identified yeast actin cytoskeletal elements. One is the genetic psuedoreversion approach, and the other is the biochemical affinity chromatography approach.

PSEUDOREVERSION OF ACTIN MUTATIONS

Psuedoreversion (14,15) has been used to identify genes encoding proteins essential for proper actin cytoskeleton function. Phenotypic revertants of mutations in one gene (in this case Ts+ revertants of *act1-ts*) that simultaneously acquire a second phenotype (in this case cold sensitivity) were sought. These are called Sup/Cs mutants to denote their double properties: suppression of the ts-actin mutation and the new cold sensitivity. These are commonly extragenic suppressors that define a new gene. The virtue of this is that the new genes can be studied in their own right directly through the new (Cs, in this case) phenotypes.

There are serious potential problems with the pseudoreversion scheme as outlined thus far: The suppressor mutations may in fact be quite nonspecific, not actually changing an interacting protein but changing instead the intracellular environment in a more general way. For example, a lowering of the overall growth rate might allow a marginally defective cytoskeleton to function adequately.

In order to minimize the effects of these difficulties, only suppressors that pass two tests are studied. First, the secondary phenotype (i.e., the Cs of a Sup/Cs) should resemble in some way the phenotype of the original mutation that was suppressed (i.e., the original actin mutation), even in the absence of the original mutation (i.e., in an *ACT1*+ background). Second, the suppressors should show allele specificity (i.e., we study suppressors that suppress one or more, but not all, actin mutations), to eliminate suppressor mutations that might somehow bypass the function of the actin cytoskeleton altogether.

Six new genes (called *SAC* for *s*uppressor of *ac*tin) have been identified as suppressors of the actin mutations (P. Novick, B. Osmond, and D. Botstein, unpublished; and A. Adams and D. Botstein, unpublished). All are unlinked to the *ACT1* locus, they are allele specific, and *SAC1* through *SAC5* have Sup/Cs phenotypes. The mutant phenotypes of these suppressors closely resemble the original actin mutant phenotypes, including the loss of normal actin organization, defects in chitin deposition, and, in the case of *sac2*, the partial secretion defect (P. Novick, and D. Botstein, unpublished).

ACTIN FILAMENT AFFINITY CHROMATOGRAPHY

Chromatography of yeast extracts on columns containing immobilized yeast actin filaments (stabilized with the actin-binding drug phalloidin) allowed the identification of proteins of 200 kd, 85 kd, and 67 kd (D. Drubin, K. Miller, and D. Botstein, unpublished). This is far fewer proteins than are isolated from other organisms under similar conditions (K. Miller and B. Alberts, unpublished), and may reflect the relative simplicity of the yeast cytoskeleton with respect to more complex higher cells. All three proteins appear to be *bona fide* actin binding proteins. The 200 kd protein is myosin-like based on biochemical properties and on its ability to react with antibodies directed against *Dictyostelium* myosin heavy chain. Furthermore, immunofluorescence studies using affintity-purified antibodies directed against the 85 kd and 67 kd proteins shows that both colocalize with actin in yeast cells (D. Drubin and D. Botstein, unpublished). The genes encoding the 67 kd and 85 kd proteins have been cloned and genetic analysis is in progress. We have preliminary evidence that our aim of unifying the biochemical and genetic

approaches, so that biochemical properties can be associated genetically with *in vivo* functions, is being met.

CONCLUSIONS

Yeast is rapidly proving to be a valuble organism with which to study the function of the actin cytoskeleton. The combination of biochemistry, genetics, and DNA manipulation methods is particularly powerful in yeast, and the cell biology is beginning to catch up. When these experimental strengths are coupled with the inherent simplicity of the yeast actin cytoskeleton, in terms of the relative lack of protein complexity and reiteration of genes, and with the conservation of protein structure and function, it seems likely that the most primative and perhaps universal actin functions will be uncovered.

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