Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 91–95, January 1996
Biochemistry

Yeast actin: Polymerization kinetic studies of wild type and a poorly polymerizing mutant

(nucleation/elongation/gelsolin/fluorescence)

JENNY M. BUZAN AND CARL FRIEDEN*

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110

Contributed by Carl Frieden, October 2, 1995

ABSTRACT **Wild-type actin and a mutant actin were isolated from yeast (*Saccharomyces cerevisiae*) and the polymerization properties were examined at pH 8.0 and 20°C. The polymerization reaction was followed either by an increase in pyrene-labeled actin fluorescence or by a decrease in intrinsic fluorescence in the absence of pyrene-labeled actin. While similar to the properties of skeletal muscle actin, there are several important differences between the wild-type yeast and muscle actins. First, yeast actin polymerizes more rapidly than muscle actin under the same experimental conditions. The difference in rates may result from a difference in the steps involving formation of the nucleating species. Second, as measured with pyrene-labeled yeast actin, but not with intrinsic fluorescence, there is an overshoot in the fluorescence that has not been observed with skeletal muscle actin under the same conditions. Third, in order to simulate the polymerization process of wild-type yeast actin it is necessary to assume some fragmentation of the filaments. Finally, gelsolin inhibits polymerization of yeast actin but is known to accelerate the polymerization of muscle actin. A mutant actin (R177A/D179A) has also been isolated and studied. The mutations are at a region of contact between monomers across the long axis of the actin filament. This mutant polymerizes more slowly than wild type and filaments do not appear to fragment during polymerization. Elongation rates of the wild type and the mutant differ by only about 3-fold, and the slower polymerization of the mutant appears to result primarily from poorer nucleation.**

The budding yeast *Saccharomyces cerevisiae* expresses a single actin, encoded from the *ACT1* gene (1, 2), that is 87–90% identical to muscle and cytoplasmic actins. This actin represents an excellent model to study, not only because of its conserved nature but because the *ACT1* gene can be mutated to give rise to a large number of site-directed mutants whose function can be studied both *in vivo* and *in vitro* (3–5). Because of the high degree of homology between actins from different sources, yeast actin can be and has been used as an analog of muscle actins. Rubenstein and coworkers and others, for example, have investigated the role of various amino acid residues in yeast actin alone (6, 7) and its interactions with muscle myosin (8, 9).

Yeast actin was first purified by Greer and Schekman (10), who reported some unusual properties with respect to the Ca^{2+} dependence of the polymerization. These properties and others were reexamined by Nefsky and Bretscher (11), who concluded that yeast actin was relatively well behaved and suggested that the unusual polymerization properties seen earlier might have been due to a minor contaminant.

In spite of the fact that yeast actin represents an excellent system to use for understanding the molecular basis of actin polymerization and interactions with actin-binding proteins, the kinetics of the polymerization process have not been well studied. There is a need for such studies because of the number of investigators who use the yeast system to examine phenotypic changes that occur as a consequence of mutations (4, 5, 12). It appears to be implicitly assumed that the polymerization characteristics of yeast actin are the same as those of muscle actin. Although there is a high degree of sequence identity among all actins, comparison of yeast and muscle actins shows that there are clusters of amino acid residues that do differ and that at least two of these clusters occur in regions of interaction between monomers in the actin filament as proposed by Holmes *et al.* (13).

This paper examines the kinetics of the polymerization of yeast actin and a yeast actin mutant at a constant pH and temperature. We find that while the kinetics of yeast actin polymerization are qualitatively similar to those of muscle actin, there are some important differences in describing the process. The mutant actin examined is one in which residues proposed to be in close contact in the filament have been altered. This actin, in which residues at positions 177 (arginine) and 179 (aspartate) are changed to alanine, is strikingly different from the wild type with respect to both nucleation and fragmentation of the filaments.

MATERIALS AND METHODS

Materials. Mutant yeast strains were from a collection made by Wertman *et al.* (3). DNase I was obtained from Boehringer Mannheim. The DNase I column was prepared by linking the DNase I to Affi-Gel 10 (Bio-Rad) according to the published procedure (14). Gelsolin was from Sigma. Spectrin/F-actin seeds were the gift of Dorothy Schafer (Washington University) and had been prepared according to a method adapted from procedures in the literature (15, 16). Pyrene-labeled muscle actin (chicken skeletal muscle pyrene-actin) was a gift from Christopher Hug (Washington University). All other chemicals were reagent grade.

Yeast Growth. The mutant yeast strain was grown to saturation at permissive temperature in 6-liter batches of YPD medium (17) for 48–60 hr with one or two feedings of glucose (2%, wt/vol) and adenine (35 μg/ml). Cells were harvested by centrifugation, washed with cold distilled water, and stored frozen at −75°C.

Actin Purification. Actin purification, as described by Cook *et al.* (7), was carried out at 4°C unless otherwise specified. For wild-type actin, 75–100 g of wet-packed yeast cells, or one-fourth of a commercially available bakers' yeast cake (Red Star), were thawed and lysed by beating with glass beads in G buffer (10 mM Tris Cl, pH 7.5/0.2 mM $CaCl_2$/0.2 mM ATP) containing protease inhibitors (pepstatin, phenylmethylsulfonyl fluoride, leupeptin, aprotinin, and antipain). The lysate was spun 45 min at 40,000 rpm in a Beckman model 45Ti rotor to remove unlysed cells. Large fat particles were removed by filtering through a coarse filter (Zeta-Plus 30LP; Cuno). This

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed.

filtrate was applied directly to a 25-ml DNase I–Affi-Gel column and the column was washed in G buffer including the protease inhibitors, G buffer plus 0.4 M NH_4Cl, and G buffer alone, and the actin was eluted with 50% formamide in G buffer onto a 1-ml DE52 (Whatman) column saturated in ATP and equilibrated with G buffer. After extensive washing with G buffer, the actin was eluted from the DE52 column with 0.3 M KCl in G buffer. Fractions containing protein were identified by spotting onto Whatman filter paper and staining with Coomassie brilliant blue. These fractions were pooled and dialyzed against G buffer to remove salt and excess ATP. The actin was then polymerized for 1 hr at room temperature by addition of 50 mM KCl and 2 mM $MgSO_4$ and collected by centrifugation for 1 hr at 48,000 rpm in a Beckman 50Ti rotor. The polymerized actin was depolymerized by redissolving in buffer containing 2 mM Tris Cl (pH 8.0), 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM dithiothreitol, and 0.01% NaN_3 and dialyzed against this buffer for 2–3 days. After centrifugation to remove any remaining filamentous actin, the monomeric actin was frozen in aliquots in liquid nitrogen and stored at −75°C. A typical yield of pure actin was 4.5–5 mg. The preparation of mutant actin followed the same procedure as for the wild type except that 5 mM Mg^{2+} at 25°C was required for complete polymerization. Actin protein concentration was determined by Coomassie blue dye assay relative to rabbit skeletal muscle actin, using a bovine serum albumin standard solution.

Polymerization Assays. Actin polymerization was assayed by fluorescence with trace amounts of pyrene-labeled muscle actin (excitation at 365 nm, emission at 386 nm) or by intrinsic fluorescence changes of the actin itself (excitation at 300 nm, emission at 335 nm). Fluorescence measurements were made with a PTI (Alphascan) spectrofluorometer. The final volume of the assay was 0.2 ml. All experiments were at 20°C.

Kinetic Simulation. Simulation of the time course of actin polymerization (as in Fig. 3) was done with KINSIM (18) and the mechanism

$$(i)\ A + A \rightleftharpoons A_2$$

$$(ii)\ A_2 + A \rightleftharpoons A_3$$

$$(iii)\ A_3 + A \rightleftharpoons A_4$$

$$(iv)\ A_4 + A \rightleftharpoons A_5$$

$$(v)\ A_5 + A \rightleftharpoons A_6$$

$$(vi)\ A_6 + A \rightleftharpoons A_n$$

$$(vii)\ A_n + A \rightleftharpoons A_n \text{ (elongation)}$$

$$(viii)\quad A_n \rightleftharpoons 2A_n \text{ (fragmentation)}$$

as described elsewhere (19).

RESULTS

Pyrene-labeled actin has frequently been used in tracer amounts to measure the time course of polymerization (20, 21), since there is about a 20-fold increase in fluorescence on incorporation of the labeled monomer into polymer. Fig. 1 shows such a time course for the polymerization of yeast wild-type actin, muscle actin and a yeast mutant (R177A/D179A) actin. It can be seen for both yeast actins that the fluorescence reaches a maximum value and then begins to decrease and eventually levels off. This decrease does not appear to be a change in the extent of polymerization (see below, Fig. 2) but rather some other process and makes the time course of the reaction difficult to describe quantitatively. The data in this figure are still useful, however, in showing that the mutant actin polymerizes more slowly than wild type. Fig. 1 also shows that skeletal muscle actin polymerizes much more slowly than wild-type yeast actin under the same conditions but does not show the same fluorescence decrease. Selden *et al.* (22) have observed that a decrease in the intrinsic fluorescence of actin (excitation at 300 nm, emission at 335 nm) is also a measure of the polymerization process. Fig. 2 shows data for wild-type yeast actin, comparing, after normalization, the change in the intrinsic fluorescence to that observed with pyrene-labeled actin. In contrast to the measurement with pyrene-labeled actin, no decrease is observed in the intrinsic fluorescence measurement at the end of the reaction and the two curves appear otherwise to follow a very similar time course. Since Selden *et al.* (22) noted that a smaller change in intrinsic fluorescence also was observed on exchange of the tightly bound Ca^{2+} with Mg^{2+}, the data in Fig. 2 were collected after EGTA and a small amount of Mg^{2+} were added and the

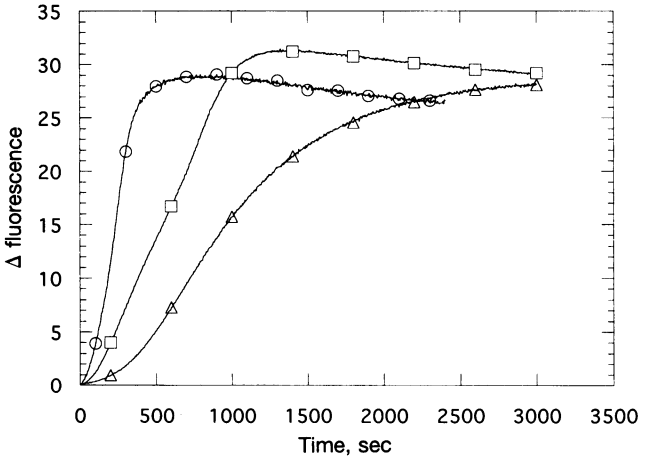

FIG. 1. Comparison of the time courses of polymerization of yeast wild-type (○), yeast R177A/D179A mutant (□), and skeletal muscle (△) actins. The experiment was performed at pH 8 in 2 mM Tris Cl buffer containing 200 μM ATP, 200 μM Ca^{2+}, and a trace amount of pyrene-labeled muscle actin at 20°C. The actin concentration used was 0.5 mg/ml in all experiments. Polymerization was initiated by the addition of 2 mM Mg^{2+}. The change in fluorescence is given in arbitrary units.

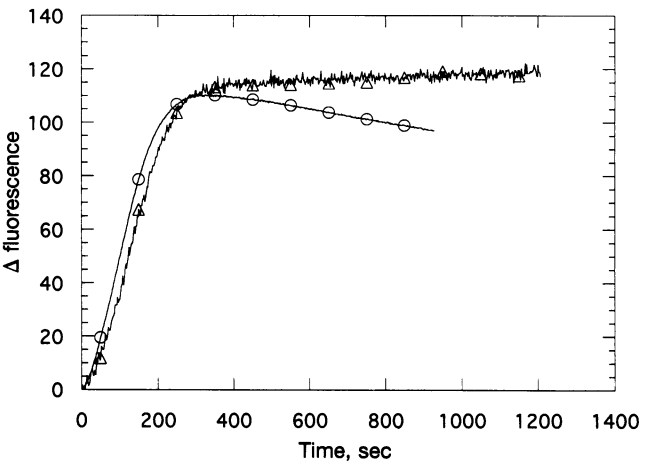

FIG. 2. Comparison of the time course of polymerization of wild-type yeast actin using either pyrene-labeled actin (○) or the intrinsic fluorescence (△). Experimental conditions were as in the legend to Fig. 1 except that the actin was incubated for 5 min with 475 μM Mg^{2+} and 2 mM EGTA prior to addition of Mg^{2+} to a final concentration of 2.4 mM to initiate the polymerization. The actin concentration was 0.25 mg/ml and the time courses were normalized since the intrinsic fluorescence decreases while the pyrene fluorescence increases on polymerization.

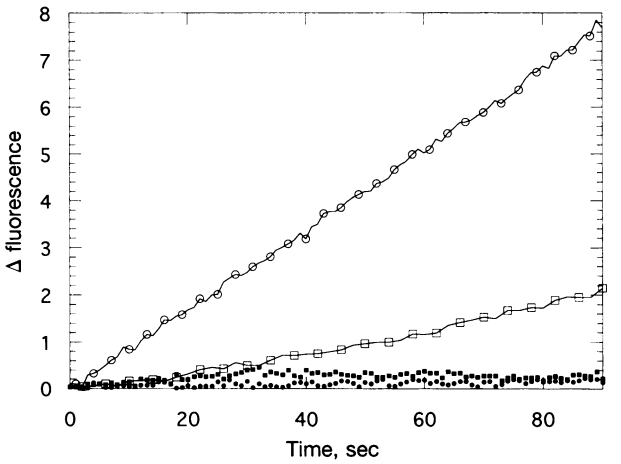

FIG. 3. Elongation as measured by the increase in fluorescence of pyrene-labeled actin in the presence or absence of nucleating spectrin/F-actin seeds. The concentration of either wild-type (○) or mutant (□) actin was 0.1 mg/ml. The solid symbols are for the polymerization of either wild-type or mutant actin in the absence of seeds. In the presence or absence of spectrin/F-actin seeds, the reaction was initiated by addition of 2 mM Mg^{2+}.

mixture was allowed to incubate for several minutes. No polymerization occurred during this incubation period. These conditions, incubation with EGTA and Mg^{2+}, and following intrinsic fluorescence were used for the concentration-dependence studies described later.

Relative Rates of Elongation of Wild-Type and Mutant Actin. Spectrin/F-actin seeds were used as nuclei to determine elongation rates of wild-type and mutant yeast actins. Fig. 3 shows the results of these experiments. With actin at 0.1 mg/ml in the presence of 2 mM Mg^{2+}, where there was no polymerization of either the wild-type or the mutant actin for at least 100 sec, the seeds promoted incorporation of monomer into filaments as measured by the increase in fluorescence of a trace amount of pyrene-labeled actin. While the concentration of the nuclei is unknown, the data of Fig. 3 provide relative rates of elongation. The difference in the slope between wild-type and mutant actin is 3-fold, indicating that the elongation rate for wild-type actin is 3 times faster than that for the R177A/D179A mutant. This 3-fold difference was incorporated into the simulations as discussed below.

Simulation of the Time Course of Polymerization. Fig. 4 shows the concentration dependence of wild-type and mutant actin polymerization measured by intrinsic fluorescence changes. Fits to the data, shown in Fig. 4, were simulated with the mechanism shown in *Materials and Methods* by KINSIM (18). For fitting the wild-type data we assumed that each association rate constant used for steps *i–vii* was 2×10^6 M^{-1}·sec^{-1}, a value within the range of a diffusion-controlled reaction. The data were best fit by assuming the trimer as the effective nucleus with an overall dissociation constant for trimer formation of 4.5×10^7 μM^{-2}. Reverse rate constants for steps *iii* and *iv* were set to 1 sec^{-1} and that for step *v* was set to 0 (19). To fit the data it was necessary to assume a fragmentation rate constant (step *viii*) of 0.004 sec^{-1}. To fit the time course of the mutant actin, the association rate constants used were 0.7×10^6 M^{-1}·sec^{-1}. It was found necessary to assume a tetrameric nucleus with an overall dissociation constant of 2.1×10^{10} μM^{-3}, indicating a poorer nucleation process. It was not necessary to include a fragmentation step in describing the time course of the polymerization of the mutant actin.

Effect of Gelsolin. The actin-binding protein gelsolin has several effects on muscle actin: it forms a 2:1 G-actin/gelsolin complex that can nucleate the polymerization (23), it caps the fast growing (barbed) ends of actin filaments and it severs actin filaments (reviewed in refs. 24 and 25). As a consequence of the formation of the G-actin/gelsolin complex, small amounts of gelsolin markedly increase the rate of skeletal muscle actin polymerization (23). A similar effect is not observed with yeast actin. Instead, gelsolin increases the lag time of the polymerization and decreases the rate and extent (Fig. 5). The decreased rate of polymerization and lower extent suggest that gelsolin cannot nucleate the polymerization but can cap yeast actin filaments, presumably at the fast-growing end. The lower extent of polymerization suggests a higher critical concentration for the slow-growing end of the yeast actin filament, much as has been observed for muscle actin.

DISCUSSION

Attempts to express actin in bacteria or other systems usually results in low yields (26, 27) or in material expressed in inclusion bodies from which it is not possible to reconstitute native actin (unpublished observations). Yeast appears to be an excellent source of actin for examining the effects of site-directed amino acid changes either *in vivo* or *in vitro* (4, 5, 28–30), since the one actin gene can be easily mutated. Yet

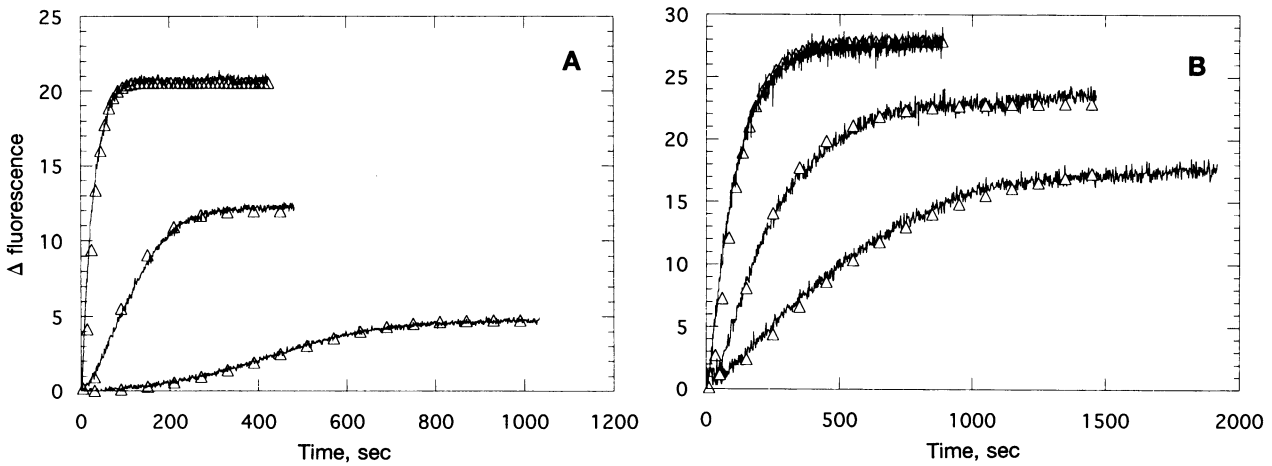

FIG. 4. Polymerization of wild-type (*A*) and mutant (*B*) actin as a function of actin concentration. Polymerization was monitored by changes in intrinsic fluorescence. The actin was incubated for 5 min with 95 μM Mg^{2+} and 2 mM EGTA prior to addition of Mg^{2+} to a final concentration of 2 mM to initiate the polymerization. The wild-type actin concentrations (*A*), top to bottom, were 0.61, 0.3, and 0.12 mg/ml. The mutant actin concentrations (*B*), top to bottom, were 1, 0.75, and 0.5 mg/ml. The triangles (△) represent the data fitted by use of the mechanism shown in *Materials and Methods* and the rate and overall dissociation constants given in the text. Other experimental conditions were as in the legend to Fig. 1.

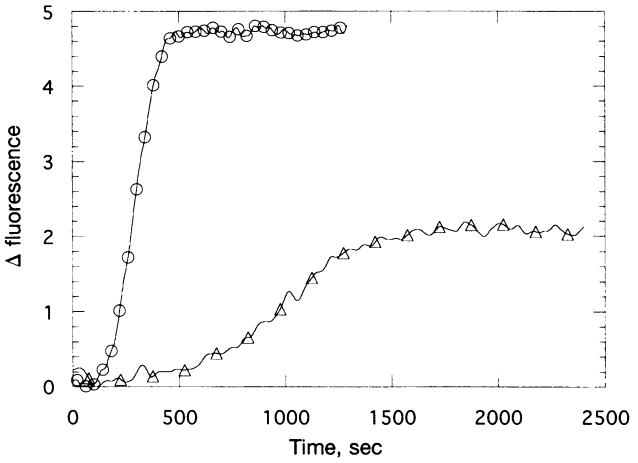

FIG. 5. Effect of gelsolin on the polymerization of wild-type actin. The polymerization was monitored by changes in intrinsic fluorescence in the absence (○) or presence (△) of gelsolin (0.5 μg/ml, a 400:1 actin/gelsolin molar ratio). The actin concentration was 0.1 mg/ml and it was preincubated with 250 μM Mg^{2+} for 5 min prior to addition of Mg^{2+} to a final concentration of 2 mM to initiate the polymerization. Other experimental conditions were as in the legend to Fig. 1.

very few extensive kinetic studies have been carried out with yeast actin, and available data suggest that its properties are similar to those of muscle actin (11). The present study shows that while there are similarities between muscle and yeast actin with respect to polymerization properties, there are also striking differences. First, we observed that yeast actin polymerized much more rapidly than muscle actin under the same experimental conditions. Simulation of the time course of polymerization suggests that this difference is a consequence of more effective nucleation and that a trimer can serve as an effective nucleus for wild-type yeast actin polymerization. Previous simulations suggested that a nucleus size close to a tetramer was needed to describe the polymerization of muscle actin (19). Second, in order to fit the time course of yeast actin polymerization it is necessary to include fragmentation of the filaments during polymerization, a process that is not normally seen in polymerization of muscle actin (19). This implies that some contacts between monomers along the axis of the double-stranded structure may be weaker for yeast than for muscle actin. Third, there is an overshoot in polymerization as measured by pyrene fluorescence—a result that differs from that obtained with muscle actin except under conditions of rapid polymerization by, for example, continuous sonication (31, 32). For yeast actin, this overshoot occurs over a wide range of polymerization rates and must represent some other process. The overshoot is not observed when intrinsic fluorescence changes are monitored, suggesting that the environment of the four tryptophans in the molecule may be different from the one pyrene moiety attached to Cys-374.

Finally, gelsolin inhibits yeast actin polymerization. While the structure of yeast actin has not been determined, the amino acid sequence of yeast actin is about 90% identical to that of skeletal muscle actin. Comparisons of the two sequences show that amino acid differences can occur at points of contact between subunits in the filament and these differences must be responsible for the different kinetic properties. In view of the high degree of identity, the inhibition of the polymerization of yeast actin by gelsolin, compared with its activation of muscle actin, is quite surprising. One possibility is that gelsolin cannot form a complex with yeast monomeric actin as it does with muscle actin. The inhibition of the yeast actin polymerization does suggest, however, that gelsolin can cap actin filaments. Way *et al.* (33) have dissected the gelsolin molecule and shown that one domain consisting of segments 1–3 can cap and sever filaments but cannot nucleate polymerization. McLaughlin *et al.* (34, 35) have characterized the interaction of gelsolin segment 1 with muscle actin and postulated several amino acid residues involved in the capping and severing. According to those investigators, the important feature of the interaction is the insertion of Ile-103 of gelsolin between Leu-346 and Leu-349 of subdomain 1 and Tyr-143 of subdomain 3 of actin (35). Differences in sequence between yeast and muscle actins do occur near some of these residues but they are not large, so that capping may still occur with yeast actin. Crosslinking experiments have suggested that the N-terminal 12 amino acid residues of actin are involved in the interaction of monomeric actin with gelsolin (36). Comparison of the N-terminal regions of yeast and muscle actin shows some differences in sequence—particularly at position 10, which is cysteine in muscle but isoleucine in yeast.

The properties of the mutant actin (R177A/D179A) are strikingly different from wild type in that the polymerization process is much slower and there appears to be no need to include a fragmentation step to simulate the time course of the reaction (Fig. 4*B*). The data obtained when nucleating seeds were used indicates that the elongation rates between wild-type and mutant actin differ by only about 3-fold, so that the difference in overall polymerization rates is associated with the nucleation process rather than with elongation. This is perhaps not surprising, since the mutation does not directly involve either the fast- or slow-growing end of the molecule but rather the interactions that occur between monomers across the long axis of the filament. Examination of the filament structure (13) shows close interactions of Arg-177 and Asp-179 with residues 199–201 (Ser-Phe-Val) of a neighboring subunit. In the absence of a yeast actin structure we must assume that these interactions are important in the formation of nuclei. As noted by Drubin *et al.* (37), there are *in vivo* consequences of this mutation, since yeast strains expressing this mutation cannot grow at 37°C and have moderate defects in actin organization even when grown at 25°C. Preliminary data show a similar temperature dependence of polymerization of wild-type and mutant actin (data not shown). The assembled actin cables and patches seen in yeast cells also could not be labeled by rhodamine-phalloidin for this mutant. Drubin *et al.* (37) suggested that phalloidin may interact with two or three monomers via residues 177/179 as a mechanism for stabilizing the normal filament. The loss of this interaction with phalloidin may be consistent with the poorer nucleation observed.

Phenotypic effects of a number of actin mutants of yeast strains have been observed (6, 7, 29, 30, 38). In order to relate these effects to the properties of actin, it is essential to determine the characteristics of the polymerization process and also of the interactions of actin with actin-binding proteins. The present results represent a step in that direction.

We thank Mr. Erwin Yu for technical assistance and Dr. John Cooper for helpful discussions. This work was supported in part by Grant R37DK13332 from the National Institutes of Health.

1. Ng, R. & Abelson, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3912–3916.
2. Gallwitz, D. & Seidel, R. (1980) *Nucleic Acids Res.* **8,** 1043–1059.
3. Wertman, K. F., Drubin, D. G. & Botstein, D. (1992) *Genetics* **132,** 337–350.
4. Wertman, K. F. & Drubin, D. G. (1992) *Science* **258,** 759–760.
5. Hennessey, E. S., Drummond, D. R. & Sparrow, J. C. (1993) *Biochem. J.* **291,** 657–671.
6. Chen, X., Cook, R. K. & Rubenstein, P. A. (1993) *J. Cell Biol.* **123,** 1185–1195.
7. Cook, R. K., Blake, W. T. & Rubenstein, P. A. (1992) *J. Biol. Chem.* **267,** 9430–9436.
8. Cook, R. K., Root, D., Miller, C., Reisler, E. & Rubenstein, P. A. (1993) *J. Biol. Chem.* **268,** 2410–2415.
9. Miller, C. J. & Reisler, E. (1995) *Biochemistry* **34,** 2694–2700.

10. Greer, C. & Schekman, R. (1982) *Mol. Cell. Biol.* **2,** 1270–1278.
11. Nefsky, B. & Bretscher, A. (1992) *Eur. J. Biochem.* **206,** 949–955.
12. Welch, M. D., Holtzman, D. A. & Drubin, D. G. (1994) *Curr. Opin. Cell Biol.* **6,** 110–119.
13. Holmes, K. C., Popp, D., Gebhard, W. & Kabsch, W. (1990) *Nature (London)* **347,** 44–49.
14. Cook, R. K. & Rubenstein, P. A. (1992) in *The Cytoskeleton: A Practical Approach*, eds. Carraway, K. L. & Carraway, C. A. C. (Oxford Univ. Press, Oxford), pp. 99–121.
15. Lin, D. C. & Lin, S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2345–2349.
16. Casella, J. F., Maack, D. J. & Lin, S. (1986) *J. Biol. Chem.* **261,** 10915–10921.
17. Sherman, F. (1991) *Methods Enzymol.* **194,** 3–21.
18. Barshop, B. A., Wrenn, R. F. & Frieden, C. (1983) *Anal. Biochem.* **130,** 134–145.
19. Frieden, C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6513–6517.
20. Tellam, R. & Frieden, C. (1982) *Biochemistry* **21,** 3207–3214.
21. Cooper, J. A., Walker, S. B. & Pollard, T. D. (1983) *J. Muscle Res. Cell Motil.* **4,** 253–262.
22. Selden, L. A., Kinosian, H. J., Estes, J. E. & Gershman, L. C. (1994) *Adv. Exp. Med. Biol.* **358,** 51–57.
23. Doi, Y. & Frieden, C. (1984) *J. Biol. Chem.* **259,** 11868–11875.
24. Yin, H. L. (1987) *BioEssays* **7,** 176–179.
25. Weeds, A. & Maciver, S. (1993) *Curr. Opin. Cell Biol.* **5,** 63–69.
26. Frankel, S., Condeelis, J. & Leinwand, L. (1990) *J. Biol. Chem.* **265,** 17980–17987.
27. Karlsson, R. (1988) *Gene* **68,** 249–257.
28. Drubin, D. G. (1990) *Cell Motil. Cytoskeleton* **15,** 7–11.
29. Chen, X. & Rubenstein, P. A. (1995) *J. Biol. Chem.* **270,** 11406–11414.
30. Chen, X., Peng, J. M., Pedram, M., Swenson, C. A. & Rubenstein, P. A. (1995) *J. Biol. Chem.* **270,** 11415–11423.
31. Carlier, M. F. & Pantaloni, D. (1988) *J. Biol. Chem.* **263,** 817–825.
32. Carlier, M. F., Pantaloni, D. & Korn, E. D. (1985) *J. Biol. Chem.* **260,** 6565–6571.
33. Way, M., Gooch, J., Pope, B. & Weeds, A. G. (1989) *J. Cell Biol.* **109,** 593–605.
34. McLaughlin, P. J., Gooch, J. T., Mannherz, H. G. & Weeds, A. G. (1993) *Nature (London)* **364,** 685–692.
35. McLaughlin, P. & Weeds, A. (1995) *Annu. Rev. Biophys. Biomol. Struct.* **24,** 643–675.
36. Doi, Y., Higashida, M. & Kido, S. (1987) *Eur. J. Biochem.* **164,** 89–94.
37. Drubin, D. G., Jones, H. D. & Wertman, K. F. (1993) *Mol. Biol. Cell.* **4,** 1277–1294.
38. Cook, R. K., Blake, W. & Rubenstein, P. A. (1991) *J. Cell Biol.* **115,** 158a (abstr.).