

NUCLEATION OF POLAR ACTIN FILAMENT ASSEMBLY BY A POSITIVELY CHARGED SURFACE

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ABSTRACT

Polylysine-coated polystyrene beads can nucleate polar assembly of monomeric actin into filamentous form. This nucleation has been demonstrated by a combination of biochemical and structural experiments. The polylysine-coated beads accelerate the rate of actin assembly as detected by two different biochemical assays. Subsequent examination of the beads by electron microscopy reveals numerous actin filaments of similar length radiating from the beads. ATP promotes this bead-induced acceleration of assembly. Decoration of the filaments with the myosin fragment S_1 shows that these filaments all have the same polarity, with the arrowhead pattern pointing toward the bead. The relevance of the system to in vitro mechanisms and its usefulness in other studies are discussed.

KEY WORDS nonmuscle
actin · microfilament · actin polymerization

Actin assembles by nucleation and elongation reactions. Elongation occurs in a pseudo first-order reaction by addition of monomers onto filament ends (4). Elongation is polar; monomers add preferentially to one end of the filament (8, 17, 22).

Nucleation, the initial reaction(s) that give rise to filament ends, is less well understood. Studies with muscle actin (10) suggest that it is a slower reaction in which three or four actin monomers come together to make an "end."

Here we report that polystyrene-coated polylysine beads can serve as sites for nucleation and polar elongation of actin filaments. This system may prove useful in dissecting out the reactions involved in actin assembly.

MATERIALS AND METHODS

A suspension of polystyrene beads (1.01 or 0.794 μm in Diam; Dow Chemical Co., Indianapolis, Ind.; 10% beads) is incubated overnight at 4°C with 9 vol of 5 mg/ml polylysine (Type 1B, Sigma Chemical Co., St. Louis, Mo.). The beads are washed 4 times in 0.5 M KCl, 0.5 mM MgCl_2 , by resuspension in 10 times the volume of

the bead-polylysine mixture. They are then resuspended in two times the volume, using the same buffer. This gives a bead suspension containing ~ 2 mg/ml polystyrene (21) and a charge equivalent to ~ 0.15 mg/ml free polylysine, determined by titration with sodium hydroxide.

Actin is purified from *Dictyostelium discoideum* (19) and depolymerized as follows: It is pelleted for 30 min at 22 lb/in² in an Airfuge (Beckman Instruments, Fullerton, Calif.). The pellet is resuspended in 3 mM imidazole (pH 7.5), 0.2 mM DTT, and 0.1 mM ATP; sonicated twice for 15 s; and incubated for at least 1 h at 0°C.

1 vol of bead suspension is added to 4 vol of actin to give a final actin concentration of 0.2 mg/ml. This does not alter the pH of the solution. The mixture is incubated for 10 min at room temperature to allow the salt that was added with the beads to induce polymerization. These preparations are negatively stained with 1% aqueous uranyl acetate and observed in a Philips 201 electron microscope.

Protein is determined spectrophotometrically at A_{290} (6) and by the method of Hartree (7). S_1 is prepared by the method of Cooke (3), and used to decorate actin by the method of Moore et al. (13).

Two assays are used to follow the rate of actin assembly. Trace γ -labeled [³²P]ATP is added at the time of depolymerization, and [³²P]orthophosphate release is followed (2). DNase I inhibition (12) is also used; 40% sucrose is added to stabilize the actin in this assay because ATP is omitted when specified.

RESULTS AND DISCUSSION

When actin is assembled in the presence of polylysine-coated polystyrene beads, filaments of similar length are found radiating from the beads (Fig. 1). Few filaments are found in the background, i.e., not attached to beads. Decoration of the filaments with the muscle myosin fragment S_1 reveals that the polarity of these filaments is uniform; all arrowheads point toward the bead (Fig. 2). Actin has been shown to assemble preferentially at the blunt end of the arrowhead pattern on decorated filament fragments (8, 17, 22). This implies that in our system, nucleation occurs at the beads and monomers then add on to the free filament ends distal to the beads.

The following experiment (Fig. 3) suggests that the polylysine-coated beads provide nucleation sites for assembly of G-actin (monomer) and not just attachment sites for preformed F-actin (filaments): If preformed actin filaments are added to polylysine-coated beads, most of the filaments do not attach to the beads, and those that do are uneven in length and of random polarity. Some images suggest that preformed actin filaments stick to the bead along their side, rather than at one end.

If polylysine-coated beads serve as nucleation sites, they would be expected to increase the rate

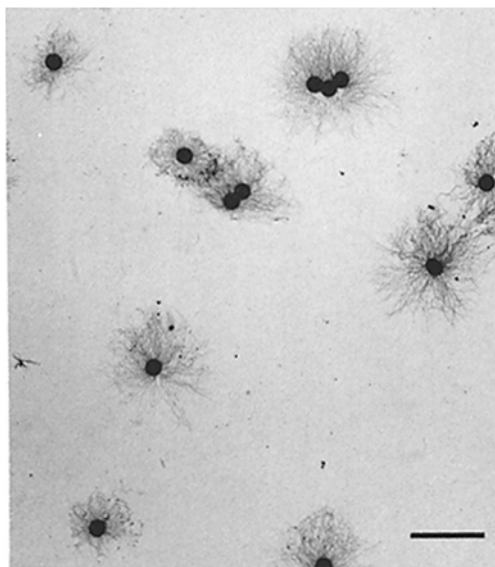


FIGURE 1 Actin assembled in the presence of polylysine-coated polystyrene beads. At this low magnification (bar, 5 μm), the actin filaments are seen as "fuzz" radiating from the beads.

of actin assembly. Because the ATP bound to *D. discoideum* G-actin is hydrolyzed upon assembly, the rate can be determined by measuring generation of [^{32}P]orthophosphate from γ -labeled [^{32}P]ATP. Rate of assembly with or without polylysine-coated beads is shown in Fig. 4. Assembly is complete at the points of inflection of the curves, when 1 mol of ATP per mol of G-actin has been cleaved. Thereafter a constant steady-state rate of turnover is seen. The rates of both assembly and turnover are enhanced by the polylysine-coated beads. Nucleation by the beads would increase the number of the filament ends, thereby increasing both rates. This should result in shorter filaments in the presence of beads, which has been confirmed qualitatively by electron microscopy. In experiments not shown, we demonstrate that polylysine coating of the beads is required for nucleation; uncoated beads do not accelerate assembly, and filaments are not associated with beads in the electron microscope. Free polylysine of a charge equivalent to that of the bead-associated polylysine results in a comparable acceleration of rate of actin-assembly. This concentration (20–40 $\mu\text{g/ml}$ free polylysine) also results in extensive side-to-side aggregation of filaments.

The role of ATP in actin assembly is somewhat obscure; ATP does not appear to be required for polar filament elongation (1, 4, 8, 9). We examined the role of ATP in nucleation of assembly by polylysine-coated beads (Fig. 5). When actin is assembled in the absence of ATP (triangles), the rate of assembly is not affected by the presence of polylysine-coated beads. ATP is then added to this same preparation of G-actin before assembly. The addition of ATP restores the nucleating effect of the beads on actin assembly shown in Fig. 4.

There are a number of situations *in vivo* where actin filaments appear to be attached by their ends, e.g., Z lines and cytoplasmic surfaces of cell membranes (5, 14, 15). If these regions served as nucleation sites, as is often assumed (18), it would be a convenient mechanism for locating filaments where they were needed. However, in all these instances, the polarity is opposite to that observed in our experiments with beads as nucleation sites; the arrowheads point away from the membrane or Z line. This does not rule out the possibility that these are nucleation sites; Woodrum et al. demonstrated that elongation can still occur in the nonpreferred direction, at a much lower frequency (22). Alternatively, elongation could be occurring

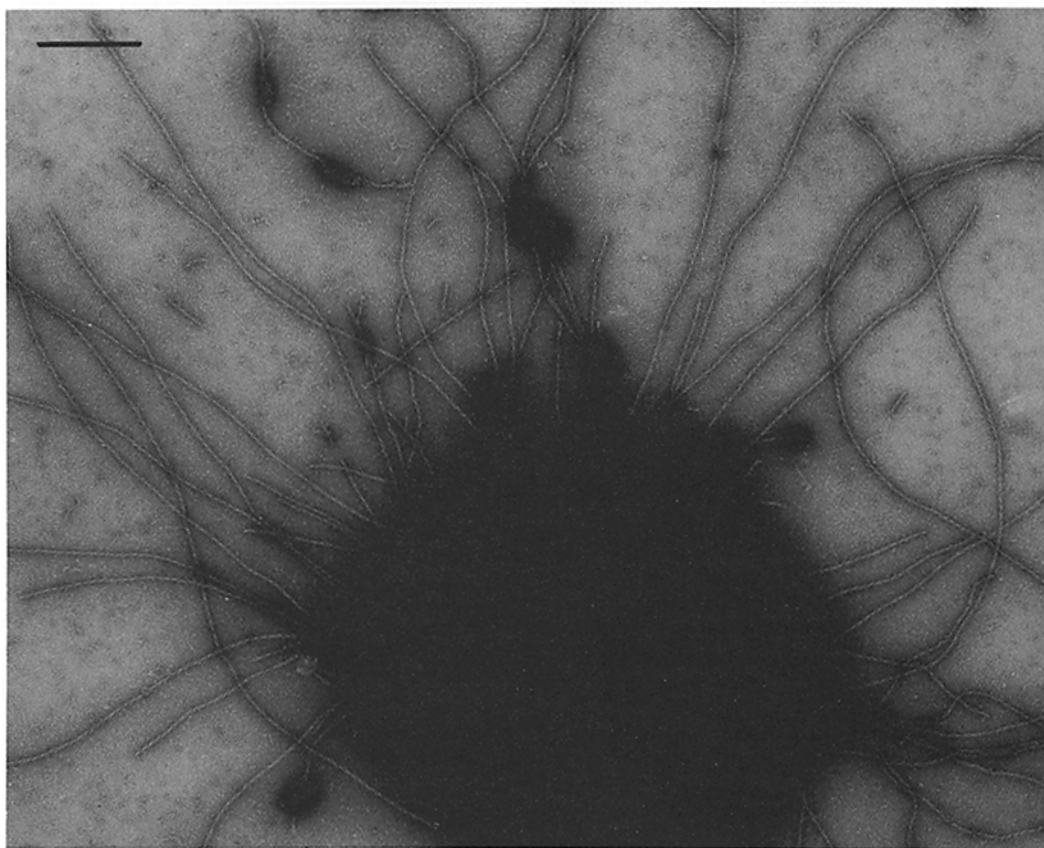
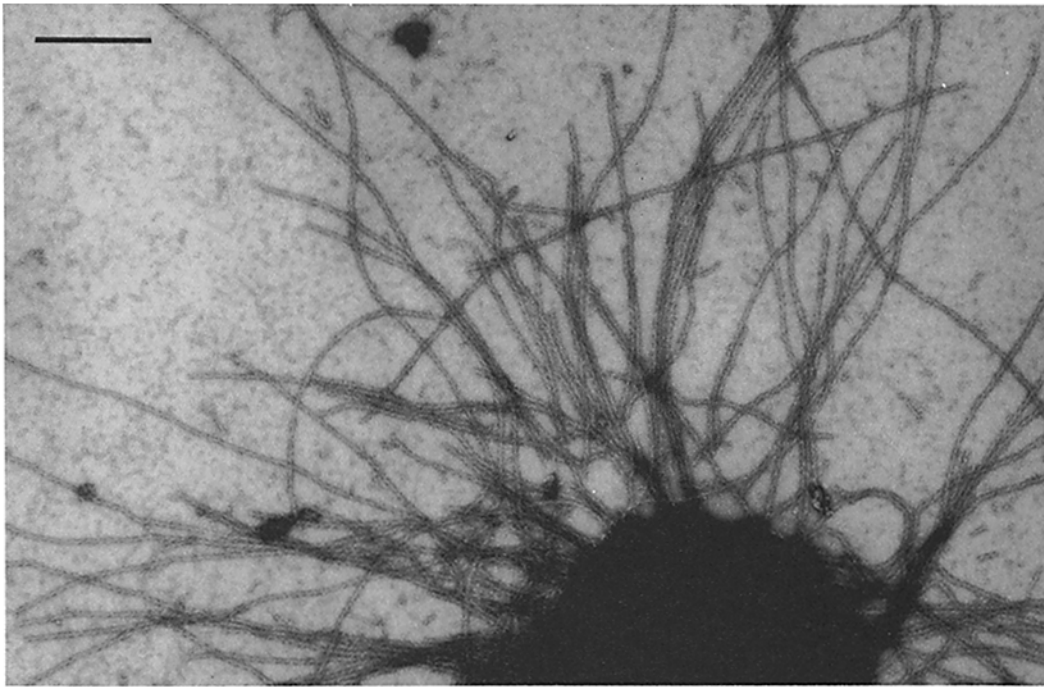


FIGURE 2 Two higher magnification examples of actin assembled onto polylysine-coated beads, decorated with S_1 to indicate the polarity of the filaments. Almost all of the arrowheads point toward the beads. Bar, $0.5 \mu\text{m}$.

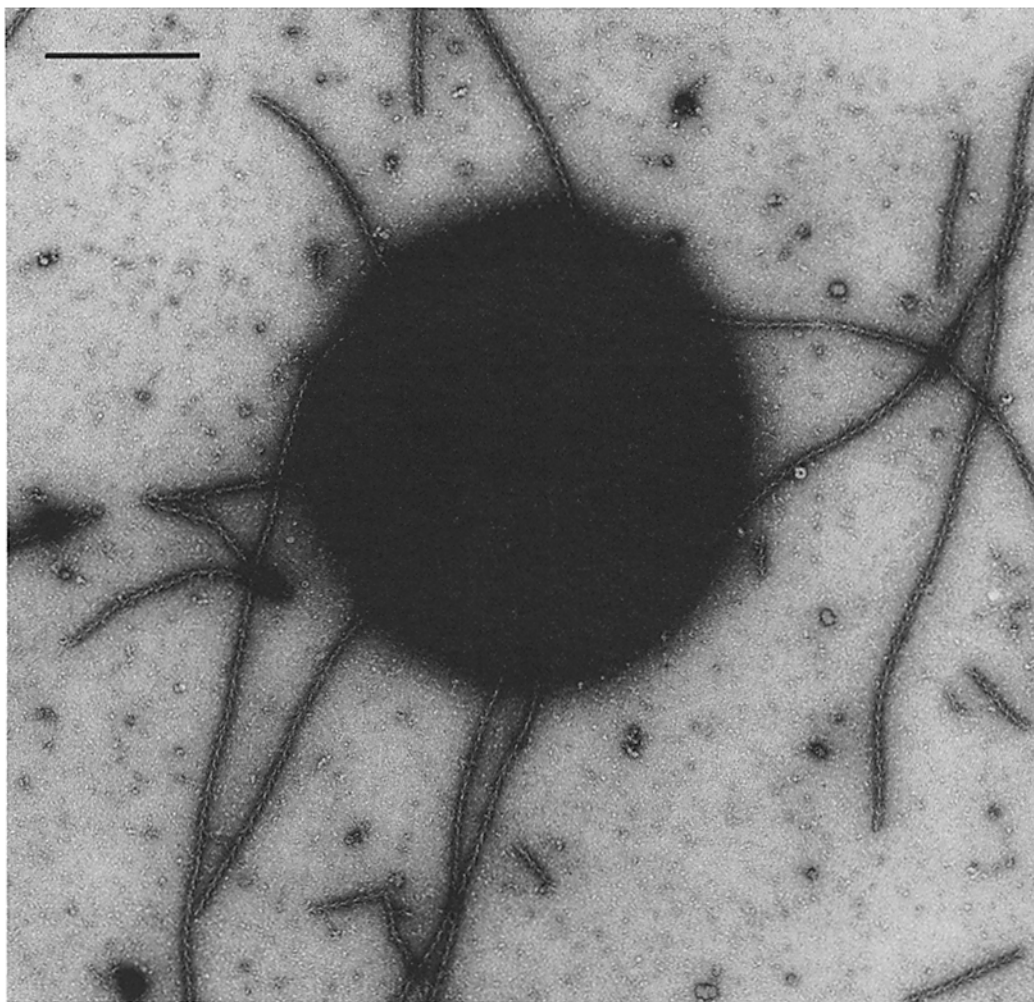


FIGURE 3 Actin assembled in the absence of polylysine-coated beads; beads are added subsequently to give the same final concentrations of actin and beads as in the previous figures. Few filaments are associated with the beads, and free filaments can be seen. The polarity of the filaments is random with respect to the beads. Bar, 0.5 μm .

somehow at the attached filament end. However, one should consider that nucleation may occur elsewhere, e.g., at a positively charged region analogous to the beads. The filaments may then grow outwards toward the Z line or cell surface and secondarily attach to Z line or membrane, perhaps via α -actinin. This mechanism would be in keeping with observations by electron microscopy in myogenic cells, in which actin filaments of the correct polarity are found in association with thick filaments before organization into myofibrils with Z lines (16). Also, the actomere in certain invertebrate sperm may be an example of this

mechanism, because associated filaments have the same polarity as we found with polylysine-coated beads (18).

Further studies on the interaction of actin with polylysine-coated beads may provide other insights into the nature of nucleation and filament elongation. We have shown here that ATP and a positively charged surface are involved in the nucleation of actin assembly by polystyrene beads; we are currently examining the effects of other variables. The assembly studies cited in the Introduction have used striated muscle actin; we have chosen instead to use a well-characterized actin

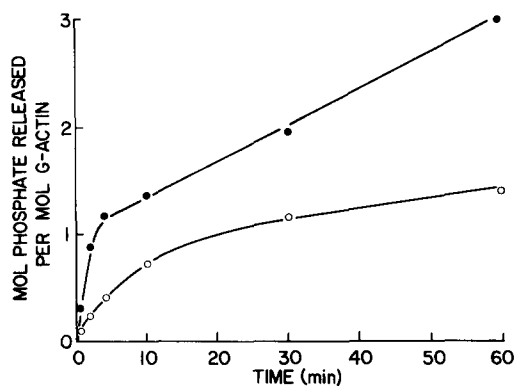


FIGURE 4 Assembly rate of 0.2 mg/ml actin with (●) or without (○) polylysine-coated beads in 3 mM imidazole, 0.2 mM DTT, 0.03 mM ATP, 0.1 mM MgCl₂, 0.1 M KCl (final pH 7.5). Assembly is monitored by cleavage of γ -labeled [³²P]ATP. A background level of orthophosphate (2.5% of the total counts) in the absence of actin has been subtracted. A similar background is obtained if the MgCl₂ and KCl which induce assembly are omitted instead of the actin, whether or not beads are present. The curves have two components: cleavage caused by assembly, complete at ~1 mol phosphate released per mol G-actin, and a constant steady-state cleavage. Both are faster in the presence of beads.

from a nonmuscle source (*D. discoideum*; 19). Nonmuscle actin is of special interest to us because the transient rearrangements of actin observed in nonmuscle cells may take place via assembly-disassembly reactions.

It is important to depolymerize the actin efficiently, so that nucleation resulting from residual filament ends is reduced. We find that muscle actin depolymerized in the same way as the *D. discoideum* actin assembles much faster, and nucleation by beads is thus partially obscured. We are investigating whether this faster rate is innate to the muscle actin, or because of some aspect of its handling before depolymerization.

Steady-state turnover of actin filaments is probably an extension of the assembly mechanism; there may be net gain of monomer on one end, and loss from the other (20). Perhaps polylysine-coated beads will block one end of the filament in analogy to podophyllotoxin and microtubules, where "opposite end assembly and disassembly" has been elegantly demonstrated (11). If so, the beads may be useful to show that exchange is occurring only at filament ends rather than throughout the filament, and also to further elucidate the properties of one end of the filament versus the other.

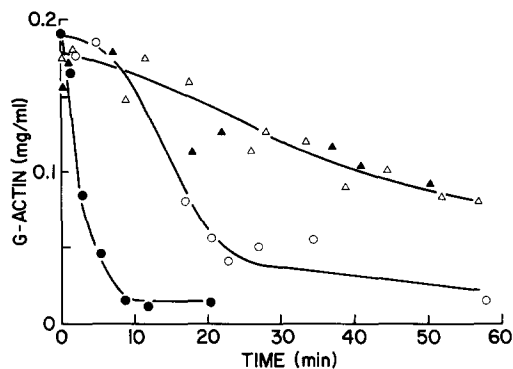


FIGURE 5 Effect of ATP on the assembly rate of actin with (closed symbols) or without (open symbols) polylysine-coated beads. At zero time, salt is added to G-actin in 3 mM imidazole, 0.2 mM DTT, 50% sucrose, \pm 0.1 mM ATP (final pH 7.5), and assembly is monitored by DNase I inhibition by G- but not F-actin (12). In the absence of ATP, the same slow assembly rate is obtained with (▲) or without (△) beads. If ATP is added to this same preparation of actin before initiation of assembly with salt, the rate of assembly with beads (●) is faster than without beads (○), as observed in Fig. 4.

Our results may prove useful to a variety of other studies. Because polylysine-coated beads provide a way of obtaining an oriented population of actin filaments, they may be helpful in structural studies in which one wishes to know the location of other molecules (such as tropomyosin) on the filament relative to its polarity. Furthermore, we may be able to construct model systems for studying actomyosin interaction or cytoplasmic streaming by using the beads in a completely defined, purified system.

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