Reference: *Biol. Bull.* **194:** 406–408. (June, 1998)

Two Distinct Mechanisms of Actin Bundle Formation

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Actin filaments (F-actin)—one of the major types of cytoskeletal filament—can be induced to form bundles by the addition of any of a number of polycations, including divalent metal ions, trivalent hexaminecobalt, or basic polypeptides. The general features of bundle formation, as detected by light scattering, centrifugation, optical and electron microscopy, are largely independent of the specific structure of the bundling agent used. The formation of lateral aggregates of actin filaments in response to polycations begins at a threshold concentration that varies strongly with the valence of the cation and increases with the ionic strength of the solution. Polyanions, such as nucleoside phosphates and acidic polypeptides, disperse actin bundles into single filaments. These features are similar to those associated with DNA condensation and can be explained analogously by polyelectrolyte theory (Tang and Janmey, 1996; Tang *et al.,* 1996). The general behavior is dictated by the polyelectrolyte nature of F-actin, which causes a class of nonspecific binding by ligands that carry several net, opposite charges. Such a bundling mechanism can be applied to a class of cationic actin-bundling proteins, including smooth muscle calponin and the microtubule-associated proteins tau and Map2c.

One direct consequence of this model of bundling is that neither dual binding sites nor dimerization of a protein with a single binding site is required to bundle F-actin (Tang *et al.,* 1997b). This alternative and somewhat counterintuitive concept may help explain why some actin-bundling and cross-linking proteins have only a single identifiable actin-binding site, and the purified proteins exist in solution as monomers. Smooth muscle calponin and a 25-amino-acid actin-binding peptide (aa 151–175) derived from the myristoylated, alanine-rich, C kinase substrate (MARCKS) are two examples among polycations that appear to induce actin-bundle formation mainly by an electrostatic mechanism (Tang and Janmey, 1996; Tang *et al.,* 1997b).

A different type of actin bundle is formed by solution crowdedness, the thermodynamic basis of which is to maximize the entropy of the solution, including all solute and solvent molecules. Two equivalent terms—the excluded volume effect (Onsager, 1949) and steric exclusion (Arakawa and Timasheff, 1985)—have been used in the literature to describe the restriction that two macromolecules cannot overlap their positions in solution. At high concentrations, such a constraint may lead to various forms of self-assembly or other aggregation phenomena (Herzfeld, 1996). For the case of an F-actin solution, high concentrations of noninteracting proteins like ovalbumin, and inert polymers like polyethylene glycol, facilitate lateral aggregation of F-actin (Suzuki *et al.,* 1989). This type of actin-bundle formation has different features than that induced by polycations, including an opposite dependence on the ionic strength of the solution (Tang *et al.,* 1997a) and an opposite dependence on the concentration of actin (Suzuki *et al.,* 1989; Tang *et al.,* 1997a; Tang and Janmey, 1996), which by itself contributes to the solution crowdedness.

The optical images in Figure 1 show examples of actin bundles formed by the two different mechanisms. Panel A shows bundling induced by 50 m*M* $MgCl_2$, representative of excess polycation concentration. Panel B shows bundles induced by 8% polyethylene glycol [molecular

This paper was originally presented at a workshop titled *The Cytoskeleton: Mechanical, Physical, and Biological Interactions.* The workshop, which was held at the Marine Biological Laboratory, Woods Hole, Massachusetts, from 15–17 November 1996, was sponsored by the Center for Advanced Studies in the Space Life Sciences at MBL and funded by the National Aeronautics and Space Administration under Cooperative Agreement NCC 2-896.

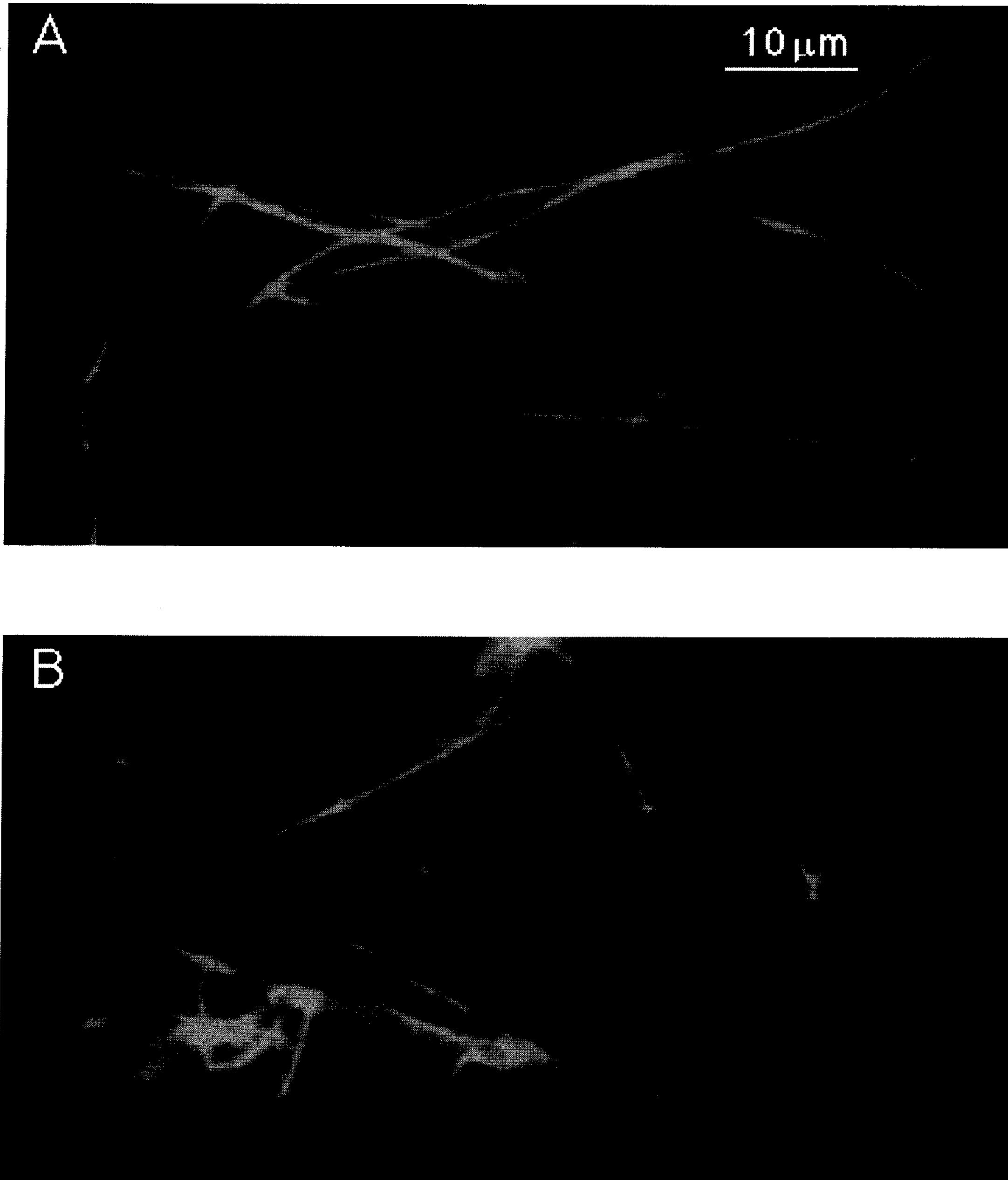

Figure 1. Comparison of actin bundles formed by excess polycations (A) and by solution crowdedness (B). Panel A: large bundles of F-actin induced by 50 m*M* $MgCl_2$. Panel B: actin bundles formed by 8% (wt) polyethylene glycol of average molecular weight 8000 dalton (PEG-8000).

weight approximately 8000 (PEG-8000)], illustrating the effect of solution crowding. Bundles of both types were visualized by the fluorescent labeling of F-actin with rhodamine phalloidin. The pair of images illustrates the general observation that the two types of bundles are virtually identical in appearance at this level of resolution. The only subtle difference is that the Mg-actin bundles tend to reach a larger size than PEG-actin bundles, and the difference is more apparent by phase-contrast microscopy (data not shown). Both types of actin bundles have also been examined by electron microscopy, and the general morphology is indistinguishable. In cells, various poly-

cations and cationic proteins are abundant, and the cytoplasm is also crowded with other macromolecules that may or may not directly interact with F-actin. Therefore, the two distinct mechanisms may jointly play pivotal roles in forming the functional arrays of bundled actin filaments found in many cell types.

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