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Bacterial Actin-Like Proteins: Purification and Characterization of Self-Assembly Properties

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Abstract

The sequence, structure, and assembly dynamics of eukaryotic actins are conserved across phyla. In contrast, actin-like proteins (ALPs) from eubacteria share little sequence homology, form polymers with different architectures, and assemble with different kinetics. The structural and functional diversity of the bacterial ALPs appears to arise from their (i) high degree of functional specialization and (ii) small number of regulatory factors. To understand the molecular mechanism by which a given ALP carries out its biological function, we must, therefore, understand its unique architecture and assembly dynamics. In this chapter, we provide a basic toolbox for studying the self-assembly of uncharacterized ALPs, including methods for characterizing the architecture and stability of polymers, specifying the mechanism of their nucleation, and measuring their rate of growth. Determining these basic properties provides a stable base for more complex reconstitutions of biological function.

1. INTRODUCTION

Actin filaments are everywhere. They (and their relatives) are found in every domain of life. They exist in every known eukaryotic cell as well as in many eubacterial and archaeal cells. Muscle actin was first discovered in the 1940s (Straub, 1943); nonmuscle actins were discovered in the 1960s (Hatano & Oosawa, 1966); and bacterial actin-like proteins (ALPs) were discovered in the early 2000s (Jones, Carballido-López, & Errington, 2001). As a consequence of this order of discovery, we know much more about the biochemical properties and cellular functions of eukaryotic actins than pro-karyotic ALPs. Cell biological functions of these ALPs include coordinating cell division machinery (FtsA) (Pichoff & Lutkenhaus, 2005), controlling cell wall synthesis (MreB) (Dominguez-Escobar et al., 2011; Garner et al., 2011; van Teeffelen et al., 2011), segregating DNA (ParM, AlfA, Alp7A) (Becker et al., 2006; Campbell & Mullins, 2007; Derman et al., 2009), and aligning organelles (MamK) (Komeili, Li, Newman, & Jensen, 2006). Unlike the actin cytoskeleton in eukaryotes, whose architecture and function are determined by a multitude of regulatory

proteins, each bacterial actin appears to be adapted to a specific function and to possess specific biochemical properties that reduce its need for accessory factors.

A current frontier in prokaryotic cell biology is to understand the assembly dynamics and regulation of these ALPs and the mechanisms by which they help to establish order in eubacterial and archaeal cytoplasm. Although they participate in analogous processes, prokaryotic and eukaryotic actins differ in many ways. For example, they (1) share low sequence homology (Bork, Sander, & Valencia, 1992; Derman et al., 2009), (2) assemble with different dynamics (Garner, Campbell, & Mullins, 2004; Polka, Kollman, Agard, & Mullins, 2009; Rivera, Kollman, Polka, Agard, & Mullins, 2011), (3) form filaments with different architectures (Polka et al., 2009; van den Ent, Møller-Jensen, Amos, Gerdes, & Löwe, 2002), and (4) are controlled by different regulatory factors (Draper et al., 2011). Sequence analysis (Derman et al., 2009) also reveals a low level of sequence conservation among members of the 40 families of ALPs in eubacteria and archaea (ALP1–ALP41; 30% sequence similarity). As might be expected from this low sequence similarity, the architecture and dynamics of ALPs *in vitro* differ significantly from one other as well as from conventional actin.

The most well-studied bacterial actin, ParM, promotes inheritance of plasmids in Gram-negative enteric pathogens. ParM filaments are simple, polymerization-based motors that push plasmids to the poles of rod-shaped cells (Campbell & Mullins, 2007). Four basic biochemical properties of ParM, none of which are true of eukaryotic actin, appear to contribute to this cellular function: (1) dynamic instability (Garner et al., 2004), (2) rapid spontaneous nucleation (Campbell & Mullins, 2007), (3) symmetrical, bidirectional elongation (Garner, Campbell, Weibel, & Mullins, 2007), and (iv) weak antiparallel self-association (Gayathri et al., 2012). Arguably, the most surprising property of ParM is its dynamic instability, an energy-dependent switch from filament growth to catastrophic shortening. This inherent instability of ParM filaments produces a high concentration of monomeric ParM that is available to do polymerization-coupled work. Interestingly, another DNA-segregating ALP, AlfA, has a remarkably different set of assembly properties from ParM: (1) it is not inherently dynamically unstable (Polka et al., 2009), (2) its nucleation is controlled by accessory factors (Polka, Kollman, Mullins, 2014), (3) its elongation is polarized (Polka, Kollman, Mullins, 2014), and (4) it strongly self-associates and forms large filament bundles (Polka et al., 2009).

The goal of this chapter is to provide a set of techniques for characterizing the basic assembly properties of a prokaryotic actin-like protein. We will begin by discussing general strategies for expressing and purifying bacterial ALPs. We will then describe how to use three basic techniques—right-angle light scattering, electron microscopy, and total internal reflection fluorescence microscopy—to characterize polymer assembly. We will describe how to use right-angle light scattering to (1) measure the critical concentration for polymerization; (2) determine whether the polymer is dynamically unstable; (3) estimate the size of the filament nucleus; and (4) determine the rate of spontaneous nucleation. We will explain how to use electron microscopy to characterize polymer architecture and determine whether filament assembly is symmetrical or polarized. Finally, we will describe how to use TIRF microscopy to directly observe filament elongation. Determining the assembly

properties of ALPs by these techniques is an essential step in understanding the molecular mechanisms by which they carry out their cellular functions.

1.1. Protein expression and purification

1.1.1 Expression—Unlike eukaryotic actins, which do not fold properly in bacteria, high expression of properly folded ALPs is relatively easy to achieve by conventional bacterial expression methods. We typically use expression strains of *E. coli* and induce protein expression overnight at 18 °C with 0.2 mM IPTG. For proteins not native to *E. coli*, say an ALP normally expressed in Gram-positive organisms, efficient expression may require the gene to be codon optimized (Polka et al., 2009). We generally avoid affinity tags and purify wild-type (or nearly wild-type) proteins using a combination of selective precipitation and conventional chromatography. Polyhistidine affinity tags, in particular, have been shown to alter the solubility and assembly properties of actin-like polymers (unpublished). For labeling the protein with maleimide derivatives (e.g., fluorescent dyes or biotin), we sometimes append a tripeptide sequence (lysine-cysteine-lysine) to the C-terminus with a short linker. In the cases we have tested, (R1 ParM, pB171 ParM, AlfA), this does not alter filament architecture or assembly dynamics.

1.1.2 Protein purification—To purify native, bacterial ALPs, we rely on three general properties: (1) high expression level; (2) relatively low solubility in ammonium sulfate; and (3) ATP-dependent polymerization. A 2 L culture of *E. coli* (~40 g packed cells) often yields 50–150 mg of purified protein. Briefly (Fig. 2.1), we lyse cells (emulsiflex or microfluidizer) and then clarify the lysate with a high-speed spin (60 min at 140,000×g) to remove cellular debris and insoluble protein. We then fractionate the extract using ammonium sulfate. Optimum ammonium sulfate concentrations for selective precipitation should be determined for each protein (30–50% for AlfA, 10% for ParM). After resuspending the protein, we dialyze it overnight into polymerization buffer without ATP. We then warm the protein to room temperature and induce polymerization by adding ATP and MgCl₂ to a final concentration of 5–10 mM each. We pellet the polymer by high-speed centrifugation (30 min at 140,000×g) and then resuspend the pellet in 1/10 volume of depolymerization buffer. We typically dialyze the resuspended protein overnight to remove residual ATP and ensure complete depolymerization. Finally, we gel filter the protein on Superdex S-200 resin, pool the purest protein fractions and dialyze them into storage buffer containing a cryoprotectant, typically 20% glycerol. We divide the protein into small aliquots and snap freeze them in liquid nitrogen. The ALPs that we have studied are all stable at –80 °C and, unlike eukaryotic actin, they do not require the presence of nucleotide in the storage buffer.

1.1.3 Buffers

Lysis buffer

25 mM Tris (pH 7.5–8), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF

Depolymerization Buffer

25 mM Tris (pH 7.5–8), 300 mM KCl, 5 mM EDTA, 1 mM DTT

Note: EDTA concentration should be equivalent to MgCl_2 added for polymerization.

Storage buffer

25 mM Tris (pH 7.5–8), 100 mM KCl, 1 mM MgCl_2 , 1 mM DTT, 20% glycerol

Polymerization buffer

25 mM Tris (pH 7.5–8), 100 mM KCl, 1 mM MgCl_2 , 1 mM DTT

TIRF buffer

25 mM Tris-HCl (pH 7.5), 100 mM KCl, 1 mM MgCl_2 , 1 mM DTT, 0.8% methylcellulose, 0.5% BSA

Cross-linking buffer

15 mM Imidazole (pH 7) (no primary amines, i.e., Tris), 100 mM KCl, 1 mM MgCl_2

Note: No reducing agent in this buffer.

1.2. Bulk measurements

1.2.1 Light scattering versus FRET—We use bulk assays, such as FRET or light scattering, to determine various polymerizations, such as the critical concentration versus the steady-state monomer concentration, kinetics of polymerization, and nucleation rates. Light scattering is a powerful technique as it enables observation of kinetic properties in the absence of protein dyes, which have been shown to affect the kinetics of polymerization for actins and ALPs. Light scattering, however, can be quite noisy, making it sometimes difficult to obtain data with an interpretable signal-to-noise. In such cases, we turn to FRET as an alternative. FRET is also linear over a greater range of protein concentrations and filament lengths. Protein should always be buffer exchanged to remove glycerol and hard spun to remove any aggregate formed as a result of freeze/thaw.

For optimal results, the fluorimeter settings and assay conditions should be optimized for a particular system. Of particular concern is making sure that there is a sufficiently high intensity of incident light to minimize signal-to-noise, while still remaining in the linear range of the detector across the range of conditions for a particular experiment. The intensity of the incident beam can be modulated either with slits that physically obstruct light or dialing the diaphragm appropriately. If necessary, the gain on the PMT can be tuned to obtain a proper signal. For comparison of reactions, it is crucial that the fluorimeter settings remain identical for all conditions tested. For light scattering, particular care must be taken with respect to cleanliness of the quartz cuvette. Dirt or protein aggregate on the inside of the cuvette can scatter light or nucleate small bubbles, both of which will severely diminish the quality of data. Likewise, dirt in or on the window can contribute to scattered light. For this reason, we recommend soaking cuvettes in nitric acid for 30 min prior to use.

1.2.2 Light scattering

1. Set up fluorimeter. Optimize settings, including excitation intensity and detector sensitivity.

2. The excitation wavelength should be set at 320 nm (wavelength should be sufficiently high to avoid buffer scattering and absorbance by aromatic residues, but low enough to observe the smallest polymers possible).
3. Make a 2× solution of ALP protein in polymerization buffer and a 2× solution of equimolar ATP/Mg⁺⁺. ATP concentration should be saturating and will need to be experimentally determined.
4. To begin the polymerization reaction, place quartz cuvette containing 50 μL of 2× protein solution in the fluorimeter and start the program (should set collection time for 60–2000+s depending on the kinetics of polymerization).
5. Once the program is running, manually close the shutter and add saturating amount of ATP/Mg⁺⁺ to initiate polymerization. Mix rapidly by carefully pipetting up and down three times. Be extremely careful to not to generate bubbles. Reopen the shutter and observe polymerization.

Note: This step should be done as quickly as possible.

6. Polymerization is observed as an increase in light scattered at 320 nm.

1.2.3 FRET—The basic strategy is to polymerize a combination of unlabeled protein with protein labeled with two different fluorophores (a FRET pair). Copolymerization of these constructs will result in a FRET signal proportional to the amount of polymer in solution.

1. Fluorimeter settings should be such that the excitation monochromator is set to an appropriate wavelength for your donor FRET dye. The emission monochromator should be set to as near peak emission of your acceptor dye as possible (avoid overlap with donor dye emission spectrum).
2. Polymerization reactions should proceed as with light scattering except that your 2× protein mix will be composed of a mixture of unlabeled monomer, ~15% donor-labeled monomer, and ~15% acceptor-labeled monomer (actual % labeled monomer will need to be experimentally determined). Polymerization is then observed as an increase in intensity at the appropriate acceptor wavelength.

1.2.3.1 Critical concentration versus steady-state monomer concentration: Using these techniques, valuable parameters of polymer assembly can be extracted, such as the steady-state monomer concentration and the critical concentration. The maximum intensity of a polymerization curve scales with protein concentration linearly. Therefore, the steady-state monomer concentration can be extracted from these data by plotting the steady-state intensities (maximum intensity) from a protein titration versus protein concentration as shown in Fig. 2.2A. A line fit to the data will intersect the *x*-axis at the steady-state monomer concentration. The critical concentration can be determined by the same type of experiment in the presence of a non-hydrolyzable ATP analog or with a mutant deficient in hydrolyzing ATP (i.e., ParM_E148A; Fig. 2.2A) (Garner et al., 2004).

1.2.3.2 Nucleation: Spontaneous assembly of actin and actin-like filaments is a nucleation condensation reaction; it occurs in two distinct steps (Oosawa & Kasai, 1962). Nucleation is

typically a slow process; once a critical nucleus size is exceeded, rapid elongation is favored. Bacterial ALPs have variable rates of nucleation and a variety of critical nucleus sizes. It is therefore necessary to experimentally determine the nucleus size and nucleation kinetics for uncharacterized bacterial actins.

Using a rapid mixer attachment for the fluorimeter with either FRET or light scattering, it is possible to measure the lag phase for nucleation, as well as to determine the number of kinetically resolvable nucleation steps (and therefore an estimate of nucleus size). This analysis is described in detail by Nishida and Sakai (1983) and involves a log/log plot of the ratio of maximum polymerization velocity to maximum intensity versus protein concentration (Fig. 2.2B). Twice the slope ($2n$) of this line represents the number of kinetically resolvable steps involved in nucleating the particular filament. Therefore, $2n+1$ estimates the size of the nucleus, which is defined as the number of monomers that need to assemble for elongation to be favored over disassembly (Fig. 2.2B). Additionally, putative regulatory factors can be tested as potential nucleators using this technique.

1.2.3.3 Dynamic instability: Dynamic instability can be inferred from light scattering or FRET data by the following approaches. In limiting ATP, a characteristic polymerization curve is generated by dynamically unstable proteins. The initial elongation phase is followed by a rapid decrease in intensity, then a slow increase to equilibrium or a return to baseline (Fig. 2.2C). This overshoot and rapid decline does not occur in saturating ATP, or in mutants incapable of ATP hydrolysis, indicating that the rapid decrease is the result of dynamic instability-driven depolymerization. Likewise, the dynamic instability can be repressed by addition of excess phosphate analog (BeF_3), which stabilizes filaments by maintaining monomers within the filament in an ADP-Pi state (Fig. 2.2C).

Note: It is critical to verify that the increase in light scattering is due to polymerization and not protein aggregation. Light scattering and FRET experiments should be accompanied by negative stain EM using identical conditions for polymerization. Light scattering without EM is like driving in the dark without your headlights on.

1.2.4 Negative stain electron microscopy—Interestingly, the bacterial ALPs form a variety of filamentous structures. ParM forms two stranded filaments with a left-handed helical twist (Fig. 2.3A, left), while AlfA forms bundles of filaments (Fig. 2.3A, center). Under high-salt conditions, AlfA bundles can be broken into two stranded filaments (Fig. 2.3A, right). Filament reconstructions of ParM (van den Ent et al., 2002) and AlfA (Polka et al., 2009) show that both proteins form two stranded, helical filaments, but with distinct architectures. Both form helical filaments opposite in handedness to eukaryotic actin; additionally, AlfA filaments are highly twisted, which enhances lateral filament interactions and bundling. Explicit staining procedures are described by Huxley (1963).

Briefly, grids are stained by

1. Applying 5 μL of polymerized ALPs to glow discharged grids and incubating for 30 s.
2. Washing grids 3 $\times$ in polymerization buffer, blotting between washes.

3. Staining 3× with 0.75% uranyl formate, third stain step is done for 30 s.

1.3. TIRF: Visualizing filament dynamics

Filament dynamics are best characterized by direct visualization using TIRF microscopy (Fig. 2.4). Using this approach, one can directly measure the rate at which single filaments assemble, whether catastrophic disassembly occurs (dynamic instability), whether filaments undergo treadmilling, and how any accessory factors may modulate these dynamic behaviors.

1.3.1 Labeling strategies—Labeling of bacterial actins can be done by purifying the bacterial actin of choice with a c-terminal GSKCK amino acid sequence added to the construct for covalent cross-linking to maleimide dyes or biotin. The two positively charged lysines cause the flanked cysteine to be hyperreactive, enabling efficient and rapid labeling. If possible, endogenous cysteines should be mutated to alanines to create a cys-lite mutant. This will ensure that maleimide labeling will yield a protein with only a single dye attached. If this is not possible, stoichiometry of dye:protein can be adjusted to favor a single-labeling event of the highly reactive c-terminal cysteine.

1.3.2 Surface chemistry: APTES-PEG-biotin glass

1.3.2.1 Silanized glass

1. Place coverslips (mark with diamond pen) in a 500–1000 mL glass beaker.
2. Sonicate in 200 mL of 3 M NaOH for 30 min. Swirl every 10 min.
3. Wash thoroughly with MilliQ water.
4. Wash with 100% isopropanol.
5. Make 200–300 mL of 5% APTES (3-aminopropyltriethoxysilane—Sigma Aldrich) solution in isopropanol.
6. Next, remove coverglass individually, rinse with isopropanol, and then drop into 5% APTES solution made in isopropanol.
7. Sonicate for 20 min.
8. Rinse all glass 3× with 100% isopropanol.
9. Remove coverslips one-by-one. Dip into a series of 2–3 beakers containing isopropanol. Rack coverslips in ceramic holders.
10. Bake for 2 h at 90 °C.
11. Place coverslips in beaker containing 100% EtOH, sonicate for 15 min.
12. Wash 2× in 100% EtOH.

1.3.2.2 Pegylation

1. Rinse coverslips in MilliQ water. Oven or spin dry.

2. Weigh ~27 mg of CH₃O-PEG-NHS and 2–3 mg Biotin-PEG-NHS (JenKem Technology, USA). Add 1 mL dimethylformamide. 30 mg/mL final concentration.
3. Place coverslip in glass weigh jars.
4. Spot 75 μ L of solution on coverslip.
5. Place second coverslip on top of first to make sandwich.
6. Incubate at 75 °C for 1–2 h.
7. Remove glass and place in a large beaker of MilliQ water. Wash multiple times with water.
8. Remove coverslips one-by-one. Dip into a series of 2–3 beakers containing MilliQ water.
9. Rack and dry glass. Store in covered container at 4–25 °C.

1.3.3 TIRF microscopy

1. Clean the counter glass with ethanol.
2. Assemble functionalize glass with double-sided tape.
3. Wash with TIRF buffer.
4. Incubate for 1 min.
5. Flow 40 μ L of 50 nM streptavidin in TIRF buffer. Incubate for 30 s.
6. Wash with 40 μ L TIRF buffer.
7. Begin polymerization reaction by adding 20 μ L of 2 $\times$ ATP/Mg⁺⁺ in TIRF buffer to 20 μ L of 2 $\times$ protein in TIRF buffer, mix well and flow in all 40 μ L. Final concentrations of protein in this reaction should be composed of 0.1–0.5% biotinylated monomer and 10–25% fluorescently labeled monomer.

Note: Oxygen scavengers should be added fresh to each polymerization reaction to a final concentration of

- 200–320 μ g/mL glucose oxidase (Biophoretics/Serva Electrophoresis #22778.01)
- 30–60 μ g/mL catalase (Sigma C40-100MG; from Bovine Liver)
- 20 mM glucose
- 20 mM β -mercaptoethanol

1.3.4 Polarity

1.3.4.1 Making cross-linked stable seeds

1. Preincubate your KCK-hydrolysis dead mutant with 5 mM TCEP.

Note: Mutants capable of binding ATP, but hydrolysis deficient will form stable polymers that disassemble extremely slowly (i.e., ParM_E148A). If unable to

obtain mutant, polymerize wild-type protein with a nonhydrolyzable nucleotide analog (i.e., AMP-PNP).

2. Buffer exchange your KCK-hydrolysis dead into labeling buffer.
3. Resuspend NHS-PEG-biotin (JenKem Technology, USA) reagent in DMSO to 20 mM.
4. Add biotin in a 5:1 molar ratio to your protein (if endogenous cysteines present, use 1:1 ratio).
5. Incubate at RT for 30 min.
6. Quench by adding DTT to 10 mM final concentration.
7. Buffer exchange into cross-linking buffer (no primary amines)+DTT to remove excess biotin.
8. Hard spin to remove any aggregate (20 min, 175,000×g, 4 °C).

Note: Very important step, this is the only opportunity to remove any aggregate that may have formed during biotin labeling. Aggregate = bad for EM and your assays.

9. Polymerize protein with excess nucleotide in the presence of equimolar (or slightly excess) cross-linking reagent for 30 min (e.g., DSS or BS3).

Note: This polymerization mixture will be a mix of about 15% bio-tinytated and 15% dye-labeled protein if being used for TIRF, or 100% biotinylated hydrolysis dead mutant if for EM. This will need to be optimized.

10. Quench reaction by adding excess 1 M Tris (pH 8).

Note: Do not pipette up and down to mix, this will sheer filaments. Flick gently.

11. Pellet seeds at 175,000×g, 20 min, 4 °C.
12. Resuspend in reaction buffer *very gently* with a p1000 pipetteman with the pipette tip cut at end.
13. Aliquot and freeze if using for TIRF, store at 4 °C and use within a few days if for EM.
14. Check seeds by SDS-PAGE gel to verify cross-linking and visualize by negative stain to verify that most are cross-linked single filaments.

Note: Too much cross-linker can result in cross-linked bundles which is bad as it will convolute interpretation of experiments. Optimization of cross-linker:protein may be necessary to yield mostly single cross-linked filaments.

For EM

1. Incubate seeds with equimolar streptavidin for 10 min.
2. Start polymerization reaction by mixing 2× protein solution (seeds+monomeric protein+any additional factors of interest) with 2× nucleotide/Mg⁺⁺.
3. Make grids and image.

Analysis: Measure the filament length distribution on either side of the seed in the presence and absence of any factor that may affect bidirectional growth.

For TIRF

1. Seeds can be attached to surface-functionalized glass (described above) using streptavidin–biotin linkage.
2. Polymerization mixture can then be added to the seeds. Importantly, the assembling monomeric protein must be of a different color so that seeds can be distinguished from assembling protein.
3. Observe dynamics by time-lapse TIRF microscopy.

1.4. Other considerations

1.4.1 Nucleotide affinity and preference—Bacterial actins are unique among cytoskeletal proteins in that they are general NTPases as opposed to strict ATPases (actin) or GTPases (eukaryotic and bacterial tubulin). While polymerization kinetics can vary for each nucleotide, the affinity of a bacterial actin, taken together with the relative abundance of nucleotides within the cell, is the best metric for nucleotide preference. An approach for directly measuring nucleotide affinity is described by Rivera et al. (2011).

2. CONCLUSION

The study of bacterial ALPs is in its infancy but it is rapidly expanding and promises to reveal new biology and new principles of self-organization. The ALPs share very little sequence identity (as low as 11%) (Derman et al., 2009) and their filament structures and assembly properties can vary significantly. Therefore, to understand the molecular mechanism by which any ALP carries out its biological function, it is essential to characterize its basic structure and assembly dynamics. On a wider scale, such studies may help us to better understand the functions and the family history of one of the most conserved and abundant eukaryotic proteins: actin.

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References

- Becker E, Herrera NC, Gunderson FQ, Derman AI, Dance AL, Sims J, et al. DNA segregation by the bacterial actin AlfA during *Bacillus subtilis* growth and development. *EMBO Journal*. 2006; 25:5919–5931. [PubMed: 17139259]
- Bork P, Sander C, Valencia A. An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; 89:7290–7294. [PubMed: 1323828]
- Campbell CS, Mullins RD. In vivo visualization of type II plasmid segregation: Bacterial actin filaments pushing plasmids. *Journal of Cell Biology*. 2007; 179:1059–1066. [PubMed: 18039937]
- Derman AI, Becker EC, Truong BD, Fujioka A, Tucey TM, Erb ML, et al. Phylogenetic analysis identifies many uncharacterized actin-like proteins (Alps) in bacteria: Regulated polymerization,

- dynamic instability and treadmilling in Alp7A. *Molecular Microbiology*. 2009; 73:534–552. [PubMed: 19602153]
- Dominguez-Escobar J, Chastanet A, Crevenna AH, Fromion V, Wedlich-Soldner R, Carballido-López R. Processive movement of MreB-associated cell wall bio-synthetic complexes in bacteria. *Science*. 2011; 333:225–228. [PubMed: 21636744]
- Draper O, Byrne ME, Li Z, Keyhani S, Barrozo JC, Jensen G, et al. MamK, a bacterial actin, forms dynamic filaments in vivo that are regulated by the acidic proteins MamJ and LimJ. *Molecular Microbiology*. 2011; 82:342–354. [PubMed: 21883528]
- Garner EC, Bernard R, Wang W, Zhuang X, Rudner DZ, Mitchison T. Coupled circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science*. 2011; 333:222–225. [PubMed: 21636745]
- Garner EC, Campbell CS, Mullins RD. Dynamic instability in a DNA-segregating prokaryotic actin homolog. *Science*. 2004; 306:1021–1025. [PubMed: 15528442]
- Garner EC, Campbell CS, Weibel DB, Mullins RD. Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. *Science*. 2007; 315:1270–1274. [PubMed: 17332412]
- Gayathri P, Fujii T, Møller-Jensen J, van den Ent F, Namba K, Löwe J. A bipolar spindle of antiparallel ParM filaments drives bacterial plasmid segregation. *Science*. 2012; 338:1334–1337. [PubMed: 23112295]
- Hatano S, Oosawa F. Extraction of an actin-like protein from the plasmodium of a myxomycete and its interaction with myosin a from rabbit striated muscle. *Journal of Cellular Physiology*. 1966; 68:197–202. [PubMed: 6007202]
- Huxley HE. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *Journal of Molecular Biology*. 1963; 7:281–308. [PubMed: 14064165]
- Jones LJ, Carballido-López R, Errington J. Control of cell shape in bacteria: Helical, actin-like filaments in *Bacillus subtilis*. *Cell*. 2001; 104:913–922. [PubMed: 11290328]
- Komeili A, Li Z, Newman DK, Jensen GJ. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science*. 2006; 311:242–245. [PubMed: 16373532]
- Nishida E, Sakai H. Kinetic analysis of actin polymerization. *Journal of Biochemistry*. 1983; 93:1011–1020. [PubMed: 6863231]
- Oosawa F, Kasai M. A theory of linear and helical aggregations of macromolecules. *Journal of Molecular Biology*. 1962; 4:10–21. [PubMed: 14482095]
- Pichoff S, Lutkenhaus J. Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Molecular Microbiology*. 2005; 55:1722–1734. [PubMed: 15752196]
- Polka JK, Kollman JM, Agard DA, Mullins RD. The structure and assembly dynamics of plasmid actin AlfA imply a novel mechanism of DNA segregation. *Journal of Bacteriology*. 2009; 191:6219–6230. [PubMed: 19666709]
- Polka JK, Kollman JM, Mullins RD. Accessory factors promote AlfA-dependent plasmid segregation by regulating filament nucleation, disassembly, and bundling. *Proceedings of the National Academy of Sciences of the United States of America*. 2014 In Press.
- Rivera CR, Kollman JM, Polka JK, Agard DA, Mullins RD. Architecture and assembly of a divergent member of the ParM family of bacterial actin-like proteins. *Journal of Biological Chemistry*. 2011; 286:14282–14290. [PubMed: 21339292]
- Straub FB. Actin, II. Studies of the Institute of Medical Chemistry University of Szeged. 1943; 3:23–37.
- van den Ent F, Møller-Jensen J, Amos LA, Gerdes K, Löwe J. F-actin-like filaments formed by plasmid segregation protein ParM. *EMBO Journal*. 2002; 21:6935–6943. [PubMed: 12486014]
- van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, et al. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:15822–15827. [PubMed: 21903929]
- Woodrum DT, Rich SA, Pollard TD. Evidence for biased bidirectional polymerization of actin filaments using heavy meromyosin prepared by an improved method. *Journal of Cell Biology*. 1975; 67:231–237. [PubMed: 240859]

Figure 2.1.
(A) Schematic of a generalized purification scheme. (B) Gel of purification scheme for R1 ParM.

Figure 2.2.

Assembly dynamics as determined by bulk measurements. Steady-state monomer concentration of ParM (closed squares), ATP critical concentration (closed circles), and BeF_3 critical concentration (open circles) indicating that ATP hydrolysis is required for filament turnover and that filaments are stabilized in the ATP or ADP-Pi (Garner et al., 2004). (B) Nucleation kinetics of AlfA in the presence (closed circles) and absence (open circles) of DNA-binding protein AlfB. Inset shows that nucleation occurs by a different mechanism in the presence of AlfB as indicated by the slope of the log/log plot. (C) Characteristics of dynamic instability. In saturating ATP (solid line) or ATP supplemented with BeF_3 (solid circles), filaments are stabilized over long periods. In limiting ATP concentrations (open circles) and in ADP supplemented with BeF_3 (dashed line), polymerization is either highly ephemeral or nonexistent, respectively (Garner et al., 2004). (B) Adapted from Polka et al. (2009).

Figure 2.3.

Filament architecture and polarity. (A) Filament architecture of ParM and AlfA. ParM forms two stranded helical filaments (left). AlfA forms bundles of filaments (center), which can be broken up by high salt (right). (B) Polymerization of wild-type polymer from a stabilized, labeled seed to determine filament polarity. As an example, wild-type actin elongates bidirectionally from meromyosin-labeled stable seeds (left) (Woodrum, Rich, & Pollard, 1975). AlfA (right) seeds with wild-type protein growing from a streptavidin-labeled seed (Polka, Kollman, Mullins, 2014). (A) *AlfA* images from Polka et al. (2009).

Figure 2.4.

Single-filament dynamics by TIRF. (A) A typical TIRF field with GFP-ParM filaments polymerized in AMP-PNP. Scale bar 5 μm. (B) A montage of a single filament over time shows bidirectional growth (white arrow) and catastrophic disassembly (red arrows). Scale bar 2 μm (Garner et al., 2004). (C) Schematic of two-color seeded filament assembly by TIRF microscopy. (D) A representative filament shows bidirectional growth. (E) Kymograph analysis shows bidirectional growth and catastrophic disassembly over many cycles.