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Bacterial actin homolog ParM – arguments for an apolar, antiparallel double helix

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Abstract

The bacterial actin homolog ParM has always been modeled as a polar filament, comprising two parallel helical strands, like actin itself. I present arguments here that ParM may be an apolar filament, in which the two helical strands are antiparallel.

The structure of eukaryotic actin filaments is well characterized as a polar, two-stranded helix with all subunits facing in the same direction relative to the filament axis. This was first demonstrated by decorating actin with heavy meromyosin, which formed spiraling arrowheads along the filament[1]. The two ends are called barbed and pointed based on these arrowheads. The polar structure was confirmed at higher resolution by fitting the crystal structure of monomeric actin to fiber diffraction patterns[2]. Consistent with this polarity, the growth and shrinking rates are quite different at the two ends. Also, proteins such as capping protein and formins bind only to the barbed end, whereas Arp2/3 binds and nucleates at the pointed end; myosins walk toward the barbed end.

Bacteria and archaea have several divergent families of actin homologues, including ParM/ALP, MreB, FtsA, MamK and crenactin (see[3] for a phylogenetic tree of these families with actin and HSP70). ParM is structurally and biochemically the best characterized bacterial actin. ParM is encoded by several low-copy plasmids, the most studied being that from plasmid R1, which I am discussing here. ParM operates to separate the plasmids by assembling two-stranded helical filaments that bind a plasmid at each end. The plasmids bind ParM through a helical, lock-washer structure of the protein ParR, stabilized by short repeats of plasmid DNA called ParC[4]. The ParM filament inserts into the hole in the center of the ParRC lock washer. The ParRC tracks to the two ends of the ParM filament, perhaps by a more favorable binding to GTP vs GDP subunits. Binding of ParRC stabilizes the filament ends and enhances growth, which pushes the two plasmids apart as they continue to track the ends[67]. For reviews of the ParMRC system see.

Several studies have imaged ParM filaments by negative stain, and have have used Fourier image reconstruction to obtain a 3-D helical structure and to fit the crystal structure of the ParM monomer into the reconstruction. The reconstruction methods generally start with a Fourier analysis of the images, which gives the average helical parameters, angular rotation and axial shift. A provisional 3-D model structure is constructed, typically based on the x-ray structure of the subunit and the helical parameters from the Fourier analysis. A series of

projection images is calculated for increments of 4 degree rotations of this model. A large set of EM images is then analyzed by first comparing each image to the set of model projection images. The best match determines the rotation and translation of that EM image. Helical parameters (rotation and rise) are adjusted to fit the EM image. A new 3-D model is reconstructed by back-projection from all of the matched EM images. The process is repeated until the structure converges.

The reconstruction of Popp et al[11] presumed a single azimuthal rotation and axial rise for all images. The “iterative helical real-space reconstruction” method of Galkin et al[12] assumed that the azimuthal rotation and axial rise can vary along the filament, and reconstructed these separately for short segments of the filament. In their final model a filament has internal flexibility and includes variations in helical parameters over different segments.

The assumption of polarity entered the reconstructions at the beginning and at the end. Both reconstructions started with the assumption that the ParM filament is polar, like actin, i.e., subunits on each of the long-pitch helices face the same direction. Popp et al stated “Only filaments with clear polarity and clear correlation pattern … were selected”[11]. Apparently images with no preferred polarity were discarded. Similarly the first step in Galkin's analysis was to fit each EM image to the best match of polarity.

Polarity was imposed again in the final stage of the reconstruction, fitting the x-ray structure into the 3-D model reconstructed from the EM images. The actin subunit is thin in one direction, and a flat square, with a modest arrowhead structure, in the other two. In previous reconstructions of actin, the point of one subunit fits into the barb of the next one, forming a thin helical ribbon. The same was true for ParM. The main parameters in the fit are to slide and rotate the two ribbons to get the best overall match.

ParM differs from actin in two important structural features related to polarity. First, the growth rate is identical at the two ends[13]. Usually polar filaments have distinctly different kinetics at the two ends, although there is no thermodynamic requirement for this. The identical growth rates at the two ends of ParM could be a remarkable coincidence. Alternatively, it could be because the growth rates measured by total internal reflectance microscopy were actually growth of filament bundles, rather than single filaments. Popp et al[14] showed that in the presence of crowding agents ParM filaments associate into bundles, with apparently random polarity. The measurements of Garner et al[13] were done in the presence of methylcellulose, a crowding agent. Some images definitely give the appearance of bundles – they are brighter in the middle than at the ends, and increase in brightness over time. However, most of the “bundles” were very thin, some perhaps single filaments. If growth were occuring only at one end, polar filaments this would likely have been observed.

The second feature related to polarity, is that the ParRC lock washers appear to bind identically to each end, to stabilize each end and favor growth, and to track equally to the two ends, avoiding the middle. This is much more difficult to explain for polar filaments. Indeed, Salje and Lowe[15] have remarked “Therefore, unlike all known actin-binding proteins, the ParRC complex appears able, at least in vitro, to bind simultaneously to two non-identical ends of the ParM filament.” ParRC is itself a polarized structure, so one would have to question whether it faces opposite directions on the plus and minus end, or faces the same direction but has some mechanism to track towards each of the ends, in spite of their opposite polarity.

If one were not biased by the well-established polar structure of actin, there would be a simple hypothesis to explain the apparent lack of polarity – that the ParM filament itself is apolar. This could easily be achieved by having the two strands of the long-pitch helix be antiparallel instead of parallel. One might think that this is too large a departure from the

actin structure, but I would suggest that it is really no greater than the switch from right-handed helix of actin to left handed helix if ParM, which is well established.

There is actually little or no evidence supporting the model of a parallel, polar structure for the double-helical ParM filaments. The existing EM reconstructions imposed an assumption of polarity, rather than discovering it. One might suggest repeating the EM reconstruction with an assumption of antiparallel symmetry, both in the initial model and in the final fitting. However, it is important to recognize the limited resolution of the EM reconstruction. Although information to 1/2.3 nm^{-1} was included in the reconstruction[11], the power spectrum showed strong layer lines only to 1/4.5 nm^{-1} in both reconstructions. At this resolution the thin ribbon representing one helix should be very similar upside down. An illustration of the limitations of the technique is that the initial reconstruction of one group had substantial errors in corrections for the contrast transfer function, yet still arrived at a fit that was judged satisfactory (although it needed an “open” conformation of the subunit)[12].

In the absence of convincing structural evidence for a polar, parallel structure of the ParM filament, I would suggest that thinking should tilt toward the apolar, antiparallel structure. This would explain the otherwise enigmatic evidence for symmetrical growth rates, and especially the equal binding and tracking of ParM to the opposite ends of a filament.

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- Bacterial actin homolog ParM has previously been modeled as a polar filament.
- I argue here that ParM may be apolar, with two antiparallel strands.
- This would explain that the growth kinetics are identical at each end;
- and that the ParRC complex binds and tracks identically to the two ends.