Nano-scale aligning tool used in the assembly of actin filaments

Giovanie Adams

Eukaryotic cells have systems for maintaining their shape and all their movement, and for the transport of molecules within them. Intra-cellular networks of fibers assemble from actin proteins, and are an important part of these systems. This network of actin filaments maintains cell shape by forming a support structure—an important component for cell motility—and provides the paths for the transport of molecules within the cell. The intra-cellular traffic of molecules along these paths is also necessary for cell fission (the division of one parent cell into two daughter cells). Actin filaments are also a major component of the muscle fibers of animals and are essential for the contractile apparatus of muscles.[1]

Actin filaments are made up of two long, twisted chains consisting chiefly of tens to thousands of monomeric or globular actin proteins. The assembly and disassembly of actin filaments is controlled at each step by sets of actin-binding proteins.[1] The initial assembly of a short actin filament is a rate-limiting step in filament assembly,[2] and a subset of actin-binding proteins has been designed to overcome the relative instability of these short filaments.[3] This process of assembling short filaments of two or three subunits from monomeric actin is called actin nucleation.

The spire family of proteins is a family of actin-binding proteins that nucleates a pool of actin monomers and prepares the product of nucleation for elongation.[4] Spire overcomes the relative instability of short filaments made up of actin dimers and trimers, by helping to overcome the kinetic barrier to nucleation.[2] Spire also attaches the newly synthesized actin filament to a membrane and aligns the filament to a *nano-scale* machine, a dimer of formin proteins.

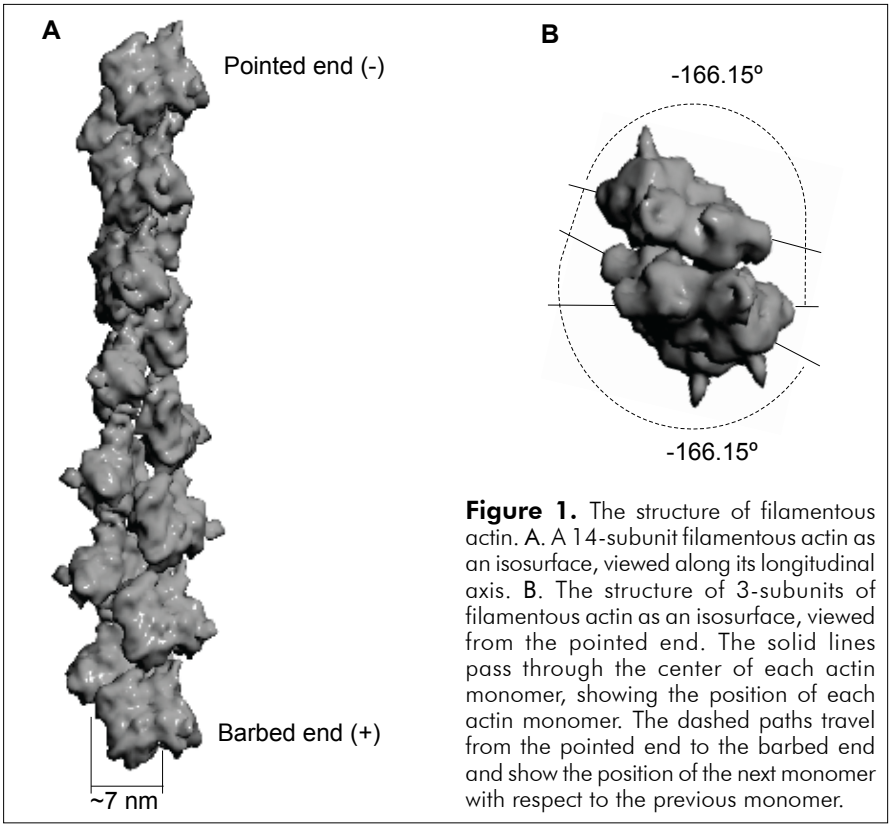

Figure 1. The structure of filamentous actin. A. A 14-subunit filamentous actin as an isosurface, viewed along its longitudinal axis. B. The structure of 3-subunits of filamentous actin as an isosurface, viewed from the pointed end. The solid lines pass through the center of each actin monomer, showing the position of each actin monomer. The dashed paths travel from the pointed end to the barbed end and show the position of the next monomer with respect to the previous monomer.

The formin dimer then proceeds to extend the filament by adding actin monomers, assembling long double-helical-twisted actin filaments. This complicated mechanism, of actin nucleation and preparation of the nucleated product for elongation by spire, suggests the work of an intelligent designer who engineered it in an incredibly intricate manner.

Structure of filamentous actin and spire proteins

Actin filaments appear in electron micrograph images as thin, dense lines of approximately 7 nm in diameter.[1] The filaments are made up of two chains of monomeric actin with each actin monomer rotated 166.15º from the other. This produces two helical chains that lie almost back to back and wind into a right-handed, double-helical structure with a very long pitch with respect to its diameter (figure 1).

Filamentous actin is polar and, therefore, the two ends of the filament are different. Because of this polarity, the filaments often fit into other cellular structures in only one orientation, in a similar manner to how many of the parts of man-made structures are assembled. Growth, the addition of monomeric actin to the filament, occurs predominantly in one direction; the plus direction. This end of the filament (where the majority of growth occurs) is known as the *barbed end* and the other end is called the *pointed end* (figure 1).

Comparison of the spire family of proteins to other proteins has identified seven domains (figure 2).

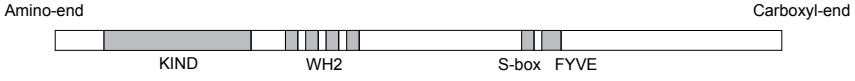

Figure 2. The domain organization of spire family of proteins. The kinase non-catalytic C-lobe domain (KIND), the four WASP-homology domains 2 (WH2), the Spir-box (S-box) and the FYVE zinc finger domain of spire.

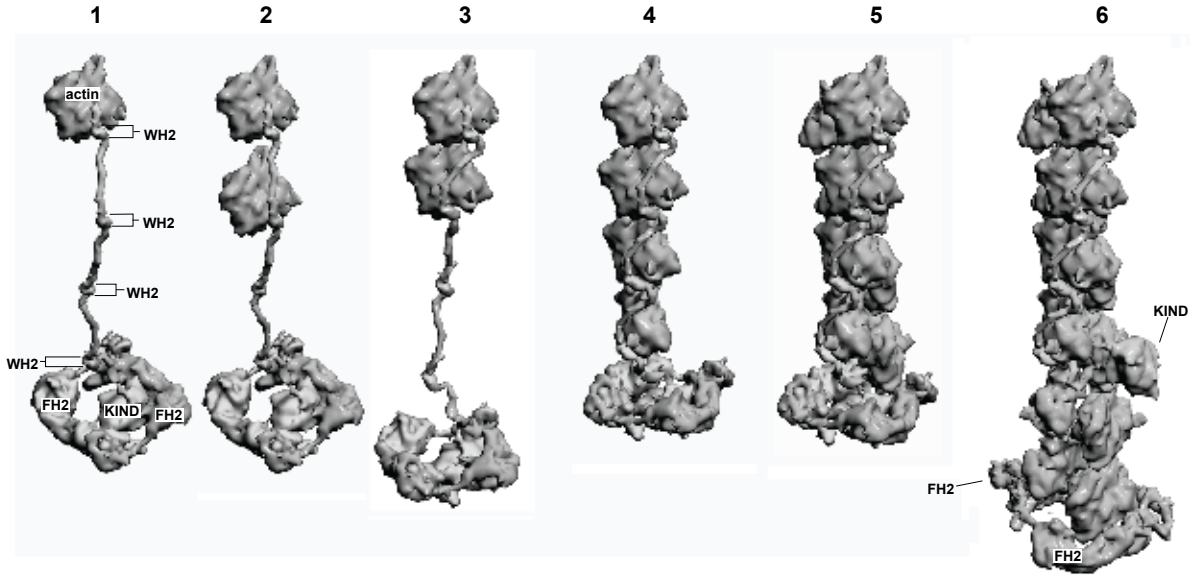

Figure 3. Proposed mechanism for actin filament nucleation by spire. From left to right the schematic shows actin nucleation by spire. Scene one shows the binding of an actin monomer to the carboxyl-end WH2 domain of spire and the binding of the FH2 domains of a formin dimer to KIND of spire. The FYVE and S-box domains of spire and the other domains of formin are not shown in these scenes. Scene two shows a second actin monomer binding to the second most carboxyl-end WH2 domain. Scene three shows the stabilization of two actin monomers by the two most carboxyl-end WH2 domains and the intervening linker region. Scene four shows the stabilization of four actin monomers into a helical structure by the WH2 domains and the intervening linker regions. Scene five shows the formation of an actin filament by four actin monomers binding to the single helical structure formed by four actin monomers. Scene six shows dissociation of KIND from the FH2 dimer and rapid polymerization of the actin filament by the FH2 dimer. All molecules are represented as isosurfaces.

At the carboxyl-end of the protein is a domain called the FYVE zinc finger,[5] and adjacent to this is a domain called the Spir-box (S-box).[6] A cluster of four, evenly spaced domains, called the Wiskott–Aldrich syndrome protein homology domain 2 (WH2), are located in the central region of the protein,[7] and a domain called the kinase non-catalytic C-lobe domain (KIND) is at the amino-end.[8] The FYVE zinc finger domain may bind to the membrane, thus, FYVE may anchor the newly assembled actin filament to a membrane.[5] The S-box is a potential binding site for Rab GTPase.[9] The four WH2 are connected by linker regions; they bind monomeric actin and are sufficient to nucleate actin[4]. Finally, KIND has a high affinity for the FH2 domain of formin.[10]

Nucleation of monomeric actin

It remains to be established exactly how a pool monomeric actin is nucleated by spire, but it may occur via the following steps (figure 3).[4,10] The process is initiated by KIND of spire binding to the FH2 domains of a formin dimer; this step inhibits formin but enhances spire activity. Next, each WH2 domain of spire binds to an actin monomer. The two WH2 domains closest to the carboxyl-end and the intervening linker region align and stabilize the bound actin monomers. Actin monomers bound to other WH2 domains are then aligned to the initial structure by the action of the other linker regions. This results in the formation of a single-stranded helical polymer of four actin monomers. Following these steps, a second helical polymer consisting of another four actin monomers binds on the other side of the helix by self-polymerization. Once an actin filament of about eight monomers has formed, KIND of spire dissociates from the FH2 domains of the formin dimer. This exposes the FH2 domains to catalyze the incorporation of actin monomers into the filament resulting in rapid polymerization of the barbed end of the actin filament by the formin dimer.

Actin filaments are highly dynamic structures that rapidly assemble and disassemble.[2] To prevent disassembly of the newly synthesized filament from the pointed end, spire caps the pointed end of the filament.[4]

Although spire performs machine-like functions during actin nucleation, the main role of spire is probably to act as a nano-scale alignment tool. A broad definition of a tool is "an entity used as an interface between two or more entities", that makes it easer for one entity to act upon the other. In the case of the function of spire in actin nucleation, spire probably acts as an interface between the actin monomers and formin dimer entities, facilitating the alignment of these entities. That is, the four WH2 domains of spire align four actin monomers, and the KIND of spire probably aligns the four monomers to the FH2 domains of a formin dimer. Therefore, spire helps to overcome the kinetic barrier to actin nucleation, stabilizing actin dimers and trimers.[2]

Conclusions

Actin filament nucleation by spire is yet another example of the

complexity of the automated systems needed to assemble the nano-scale structures and machines found within living cells. Spire is likely to be an elaborate nano-scale alignment tool with some machine-like functions. This would make spire far superior to modern tools engineered by humans. All this attests to the work of an intelligent designer who engineered these structures and machines in an incredible intricate manner.

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More confirmation for dinosaur soft tissue and protein

Carl Wieland

A brief history

Creationists were fascinated, and evolutionists mostly skeptical, when evolutionist Mary Schweitzer claimed in the 1990s that an unfossilized piece of *T. rex* bone contained red blood cells. Further, that there was immunological and spectroscopic evidence of the presence of hemoglobin, the oxygen-carrying protein that gives red blood cells their colour.[1]

Then in 2005, Schweitzer announced a further sensational discovery in a different *T. rex* bone. After the mineral matrix was dissolved,[2] what remained were structures with all the appearance of soft tissue, still soft and stretchy. Some of these appeared to be transparent branching blood vessels, with a substance inside them containing further structures looking just like nucleated red blood cells, and able to be squeezed out of the vessels (figure 1). But how could such fragile structures survive for millions of years?

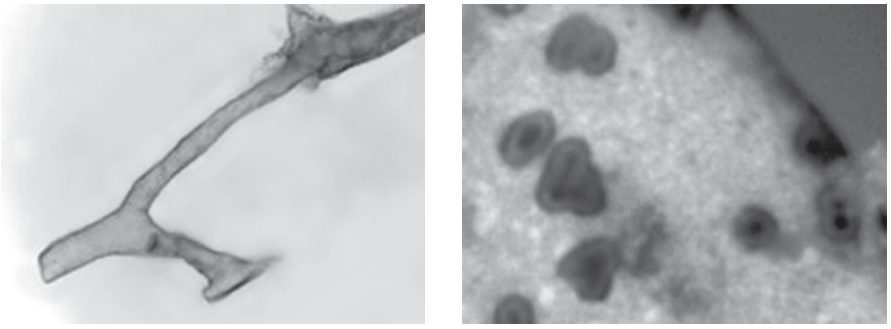
Gradually, further reports strengthened the case that Schweitzer had indeed discovered evidence of astonishing preservation of organic material in fossils. In 2007, Schweitzer and her team had performed careful tests to establish the presence of the protein collagen in the dino fossil—an important bone protein. They were even able to sequence stretches of it, which showed that it was 58% similar to collagen from a chicken, and 51% similar to that from a frog.[4]

It has been pointed out many times that fragile, complex molecules like proteins, even if hermetically sealed, should fall apart all by themselves from thermodynamic considerations alone in well under the 65 million years that evolutionists insist have passed since Schweitzer's *T. rex* specimen was entombed.[5,6] Furthermore, bones of an *Iguanodon* allegedly twice as old (dated to 120 Ma) contained enough of the protein osteocalcin to produce an immune reaction.[7]

In a seeming counter to the mounting evidence, in mid-2008 a paper claimed to have found that the transparent blood vessels, for instance, were the result of recent bacterial formation of biofilms, forming "endocasts" that followed the shape of where the original vessels lay, and that the red blood cells are actually iron-rich spheres called framboids. However, there were substantial reasons why not just creationists, but Schweitzer and other non-creationists were not at all convinced by these claims.[8]

The new findings

A recent announcement by Schweitzer and others, in the prestigious journal *Science*, has now added substantial evidence to bolster her previous findings.[9] The specimen



Photos by M. H. Schweitzer[3]

Figure 1. Left, The flexible branching structures in the *T. rex* bone were justifiably identified as "blood vessels". Right, These microscopic structures were able to be squeezed out of some of the blood vessels, and can be seen to "look like cells" in the researchers words.