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# Actin Nucleation: Spire — Actin Nucleator in a Class of Its Own

**The rate limiting step for actin filament polymerisation is nucleation, and two types of nucleator have been described: the Arp2/3 complex and the formins. A recent study has now identified in Spire a third class of actin nucleator. The four short WH2 repeats within Spire bind four consecutive actin monomers to form a novel single strand nucleus for 'barbed end' actin filament elongation.**

**Buzz Baum and Patricia Kunda**

Actin filaments help control the dynamic shape of all eukaryotic cells [1]. They grow in a polarised fashion via the addition of ATP–actin monomers to the filament 'barbed end'. As a filament ages, ATP is rapidly hydrolysed and phosphate released. Filamentous ADP–actin is then disassembled by the removal of subunits from the polymer's slow growing or 'pointed end', and the ADP moiety is exchanged for ATP to ready actin monomers for another round of polymerisation. In a eukaryotic cell, this cycle of actin filament growth and disassembly is regulated at each step by a diverse set of actin-binding proteins. As the rate-limiting step in the formation of an actin filament from purified actin–ATP monomers is the generation of dimeric and trimeric actin nuclei, actin filament nucleation is likely to be a key point of control in this process [1]. It is only recently, however, that the Arp2/3 complex and formins have been identified as distinct factors that can catalyze *de novo* actin filament formation. Now, Quinlan *et al*. [2] have identified Spire as a third class of actin nucleator.

The highly conserved, multi-subunit Arp2/3 complex was the first actin nucleation factor to be characterized [3]. When activated, its two actin-like subunits, Arp2 and Arp3, serve as a template for monomer addition by mimicking the 'barbed end' of a growing actin filament [4]. The Arp2/3 complex also interacts with the sides of existing actin filaments: this augments its nucleation activity, so that the Arp2/3 complex generates new actin filament branches at a characteristic angle of 70 degrees to the host filament. As a result of these simple biochemical properties, Arp2/3-dependent actin filament formation is auto-catalytic and generates an expanding, branched network of filaments similar to that seen in the lamellipodia of many motile cells [1].

The formins catalyze *de novo* actin filament formation via a completely different mechanism [5]. Formins dimerize to form a hoop-shaped structure that acts like a 'barbed end' filament cap to stabilize the formation of an adjacent actin dimer. Remarkably, this nascent, formin-bound actin seed is able to elongate by the insertion of ATP–actin monomers between the formin cap and the 'barbed end' of the filament.

Although it remains to be established exactly how this is achieved, an attractive model is that one subunit of the formin dimer binds an actin subunit at the tip of the filament 'barbed end', while the other subunit of the dimer catalyses addition of an ATP–actin monomer to the opposite strand of the actin filament [5,6]. As the filament elongates, the formin dimer will then step between staggered actin subunits at the filament tip as if climbing a growing spiral staircase. The 'leaky' dimeric formin cap also protects the growing filament from other 'barbed end' capping proteins. As a result, formin-induced actin nucleation generates long, unbranched bundles of actin filaments, like those used to construct actin rings during cytokinesis.

With the actin field still buzzing over the discovery of formin-dependent nucleation, Quinlan *et al.* [2] have identified a further novel mechanism of actin filament nucleation that is catalyzed by Spire.

The *Spire* gene was first identified, together with *Cappuccino*, in a *Drosophila* screen for mutations affecting oocyte polarity [7]. Although it is not clear how the oogenesis defects arise in the two mutants, aspects of this phenotype can be mirrored by loss of the actin-nucleotide exchange factor profilin, or by feeding flies with the actin poison cytochalasin D, implying that the phenotype reflects an underlying reduction in the rate of actin filament formation [8]. This inference was confirmed when the corresponding genes were cloned and *Cappuccino* was found to encode a formin [8], and *Spire* a conserved metazoan protein that has multiple copies of a well-characterised actin-binding domain, the WH2 motif [9].

WH2-like motifs are present in a wide range of actin binding

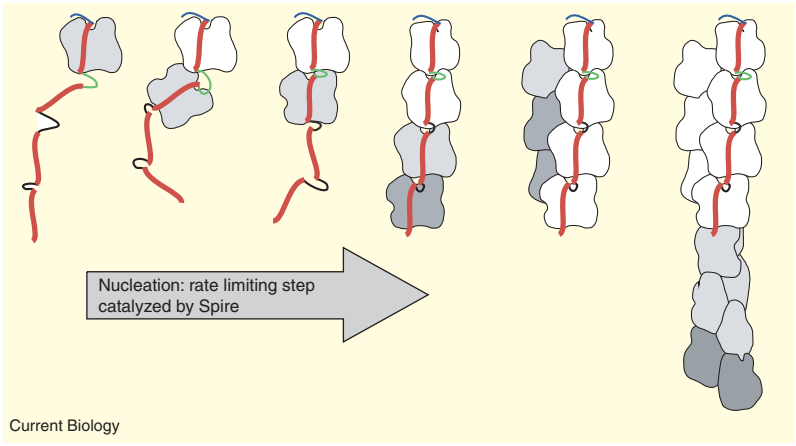


Figure 1. Proposed mechanism for the nucleation of actin filaments by Spire.

The four tandem WH2 domains (red) within Spire recruit four ATP–actin monomers to generate a novel single stranded actin tetramer that acts as a seed for new actin polymerisation. The carboxy-terminal-most WH2 domain and the intervening linker region (green loop) initiate this event and cap the 'pointed end' of the nascent filament (blue loop). Additonal ATP–actin monomers are then recruited to generate a single stranded polymer which, upon conversion to a double stranded form, becomes a suitable substrate for rapid 'barbed end' actin filament elongation. The most recently added ATP–actin monomers are shown in grey.

proteins [10]. They fall into two broad subclasses: those related to a domain present in the Arp2/3 activator WASp, and those more similar to an actin monomer sequestering protein called thymosin β4. The two types of motif are thought to make near-identical contacts with ATP–actin via a conserved stretch of six amino acids (LKKTET) that lies extended along the 'outer face' of the actin subunit [11,12] (Figure 1). In WASp family proteins, this motif promotes actin filament formation by bringing ATP–actin monomers into the proximity of the Arp2/3 complex to promote the formation of the Arp2/3–actin nucleus [1], whereas the corresponding motif in thymosin β4 inhibits actin filament elongation by sequestering actin–ATP monomers [10]. It is in this context that Quinlan *et al.* [2] set out to study the biochemical function of Spire, perhaps intrigued by its actin-related mutant phenotype in flies and its four evenly spaced WH2 domains.

Quinlan *et al*. [2] began their study by confirming that over-expression of Spire is sufficient to induce actin filament formation in mammalian cells [13]. Although similar structures are formed following the expression of constitutively active WASp, through activation of Arp2/3, Quinlan *et al.* [2] found that the filament clumps induced by Spire do not co-localise with the Arp2/3 complex. How then is Spire able to induce actin filament formation?

To answer this question, Quinlan *et al.* [2] tested the activity of Spire WH2 domains in an actin polymerisation assay. Surprisingly, they found that the amino terminus of Spire, which carries the four WH2 repeats, is sufficient to promote the formation of actin filaments from purified actin–ATP monomers, even in the absence of the Arp2/3 complex , and does so at a rate similar to that induced by the formin Cappuccino. After the new actin filament seeds are formed, they extend by rapid 'barbed end' elongation, while Spire caps the filament 'pointed end', protecting the filament from disassembly. Thus Spire represents a new class of metazoan protein that can catalyse actin filament nucleation.

Quinlan *et al.* [2] then carried out a molecular dissection of Spire to identify the region responsible for this novel nucleation activity. In the actin polymerization assay, actin filament nucleation was most profoundly affected by loss of the two most carboxy-terminal WH2 domains and by loss of a small linker region connecting the last two WH2 domains (even though these regions of the protein have been poorly conserved during metazoan evolution). In isolation, however, individual WH2 motifs were unable to induce efficient actin filament nucleation.

Thus, the novel nucleation activity exhibited by Spire appears to depend both on the sequence of the WH2 motifs involved and on their concatamerisation. These data led Quinlan *et al.* to propose a model (Figure 1) for Spire-dependent nucleation. They propose that this process is initiated by the carboxy-terminal WH2 domain binding to monomeric actin. The three other, evenly-spaced WH2 domains then bring consecutive actin monomers into alignment to promote the formation of a single-stranded polymer. This acts as a template for rapid actin polymerization, while the carboxy-terminal WH2 domain caps the 'pointed end' of the filament.

Quinlan *et al.* [2] used electron microscopy to obtain concrete evidence for hypothetical intermediates in this pathway. The structures observed were of the expected size for single filament actin tetramers and, as predicted by the model, appeared to be approximately half the length when two carboxy-terminal WH2 motifs were used in the actin nucleation assay in place of all four. Because actin nuclei rapidly elongate, it seems likely that the complexes identified represent pre-nuclei which, after the recruitment of additional actin monomers, form a two stranded polymer substrate for rapid 'barbed end' elongation.

Prior to this study [2], two other WH2 repeat proteins had been shown to regulate actin filament dynamics [11,14]. Although these proteins, Actobinin and Ciboulet, do not promote de novo actin filament formation, they facilitate 'barbed end' elongation and, like Spire, cap the 'pointed ends' of

actin filaments. Interestingly, the WH2-like motifs in Actobindin and Ciboulet are evenly spaced at approximately 30–35 residue intervals, as they are in Spire. This arrangement of closely apposed WH2-like motifs will facilitate monomer–filament and monomer–monomer interactions, lowering the critical actin concentration required for nucleation and/or polymerization. Moreover, it will severely restrict the geometry of actin–WH2 complexes, driving the formation of single polymer strands like those visualized by Quinlan *et al* [2].

Thus, in spite of their distinct biochemical functions, the tandem WH2 motifs in Actobindin, Ciboulet and Spire may have a common mode of action. In fact, flexibility of function may be a general feature of WH2 domains, as Spire, or any one of its WH2 domains, can also act to sequester actin monomers [2]. And, conversely, the classic actin monomer sequestering protein thymosin β4 can promote actin filament formation when present at very high, but physiological concentrations [15,16]. Moreover, the actin polymers formed in the presence of thymosin β4 have a tendency to form separated individual actin strands [17], reminiscent of those identified by Quinlan *et al* in mixtures of actin and Spire.

These data show that the ability of a WH2 domain to influence actin filament dynamics can be profoundly affected by its local concentration, as well as by its sequence. The residues that flank the core LKKTET motif, however, are likely to be the most important factor in determining the function of a particular WH2 domain. These residues control access to the 'barbed' and 'pointed' ends of growing filaments and they differ widely between WH2 domains that promote or inhibit actin filament formation [11,12]. It has also been suggested that interactions between the amino and carboxyl termini of adjacent WH2 domains regulate capping [11]. So the WH2-linker region identified by Quinlan *et al.* [2] may catalyse the release of the pointed end cap of the adjacent WH2 motif to promote the formation of a dimeric actin nucleus.

In conclusion, the ability of WH2-like domains to bind actin monomers and the exposed face of actin-ATP subunits within a filament gives them an unsurpassed flexibility. This enables them to be adapted during evolution for use in actin monomer sequestration, activation of the Arp2/3 complex, actin filament elongation and, as discovered by Quinlan *et al*. [2], in actin nucleation.

The Arp2/3 complex generates branched filament networks, formins give rise to single filaments that are resistant to 'barbed end' capping [5], and Spire generates unbranched filaments that are resistant to pointed end disassembly [2]. It therefore seems likely that each nucleator will give rise to a distinct set of actin-based structures, helping to generate the diversity of cell forms and behaviours observed within developing and adult animals. And given the phenotypic similarities of *Spire* and *Cappuccino* mutants [7], and the fact that the two nucleators appear to have an identical distribution in mouse embryos [18], it is possible that they function together. Although there is no evidence for biochemical synergy between these proteins [2], their active collaboration would be expected to generate relatively long and stable filaments that are protected at both barbed and pointed ends. Perhaps such filaments have a specific function during the development of the *Drosophila* oocyte.

As discussed above, however, subtle changes in the concentration and context of an individual actin binding domain can dramatically alter actin filaments dynamics in a defined biochemical system. Thus, it is difficult to extrapolate from *in vitro* experiments to predict the role of an actin regulator in a living cell, where actin filament dynamics are orchestrated by dozens of actin regulators functioning in concert. Hence the need for a combination of biochemistry, genetics and cell biology in the study of the actin cytoskeleton.

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# Senescence: Even Bacteria Get Old

**Cellular senescence, even in the presence of abundant nutrients, has now been demonstrated in several microbes, including most recently the bacterium *Escherichia coli*, suggesting that it may be a universal adaptive response to the inevitable damage to cell constituents that accumulates with time.**

**Craig Stephens**

"*Must not all things at the last be swallowed up in death?*"
Plato, *Dialogues*

The process of aging, and the apparent certainty of death, is as fascinating to scientists as to philosophers. Why do we grow old? We humans tend to view aging as a regrettable, but inevitable, process. Within the microcosm of our bodies, the vast majority of somatic cells divide infrequently and age fairly rapidly. The 'disposable soma' theory of aging postulates that senescence — the progressive, age-related decline in critical biological functions — is evolutionarily linked to the high cost of maintenance of functional cellular systems [1,2]. Only for relatively rare stem cells and the reproductive germ line are sufficient resources invested to avoid senescence while continuing to grow and divide. Unicellular microbes that reproduce solely through binary fission are analogous to the germ line of metazoans. It is perhaps surprising, therefore, that recent research [3,4] has revealed subpopulations of microbial cells that routinely age and die, even in the presence of abundant nutrients. Although mechanisms of microbial senescence are not yet clear, further investigation might yield insights relevant to aging in all life forms.

In the context of biology, the terms 'aging' and 'senescence' imply more than just the passage of time in the lifespan of an organism. With respect to microbes, the critical measure of aging would be a reduction in reproductive capacity — longer generation intervals resulting in fewer offspring produced as a function of time. Most work on microbial aging has been done with the eukaryotic budding yeast, *Saccharomyces cerevisiae* [5]. Cell division in budding yeast is asymmetric, with the larger mother cell easily distinguishable from the budded daughter cell. In 1959, Mortimer and Johnson [6] first examined the reproductive life span of individual *S. cerevisiae* cells. Using a micromanipulator, they physically removed the newly budded daughters after each division, recording the number and timing of each division event. The average mother cell underwent 24 divisions; senescence was evident during the last few divisions, which typically took considerably longer and were followed in most cases by the cells becoming granular and/or lysing (death).

Improvements in imaging technology now allow aging to be addressed experimentally in prokaryotes, which tend to be considerably smaller than yeast cells. *Caulobacter crescentus*, a bacterium studied intensively as a model for cellular development, is unusual (but not unique) among prokaryotes in that it undergoes asymmetric division [7]. One of the cells possesses a stalk, a thin, tubular extension of the cell envelope that is adhesive, and serves to attach the cell to surfaces (Figure 1A). The anchored stalked cell acts like a stem cell, producing a slightly smaller 'swarmer' cell each generation that bears a polar flagellum, rather than a stalk. The motile swarmer leaves behind the stalked cell to search for new nutrients, but eventually sheds its flagellum and grows its own stalk, allowing it to attach to a new home and continue through the cell cycle.

*Caulobacter* offers an ideal opportunity to observe senescence, as the stalk cell naturally immobilizes itself, and the swarmer swims away on its own after every division. Ackermann *et al*. [4] carried out a simple experiment in which a flow chamber was prepared on the surface of a microscope slide. Stalked cells were allowed to attach to the glass surface, where they were fed and oxygenated by a gentle flow of nutrient medium. The cells were photographed at 10 minute intervals for several days. Time intervals between cell divisions were calculated and analyzed as a function of the age of the cell, to determine whether generation time increased — so that reproductive output decreased — as the stalked cells aged.

Over the course of roughly 100 generations, Ackermann *et al*. [4] observed that the mean generation time nearly doubled, from approximately 2.6 hours per generation, to over 5 hours per generation. To control for possible deleterious effects of the lengthy observation period, stalked cells arising during the experiment — from swarmers that occasionally persisted in the chamber long enough to differentiate — were also observed. Despite