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it will be important to analyze the effects of the large-scale genomic rearrangements on global regulation of the transcriptome. These questions can be addressed, for instance, by using the latest sequencing technologies. Moreover, the molecular mechanisms of this PGR phenomenon in lampreys need to be studied, including the developmental timing and molecular components regulating both DNA recognition and removal.

Taken together, we are just starting to unravel the biological significance of PGR, with the most fundamental questions remaining to be answered: what could this mechanism, which seems to be more widespread than initially anticipated, be used for and how conserved is this process in all living organisms? If PGR is indeed understood as an irreversible mechanism of gene silencing, it might be pertinent to compare and contrast PGR with known reversible mechanisms of gene silencing, including epigenetic modifications of chromatin and DNA.

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Actin Cytoskeleton: A Team Effort during Actin Assembly

Two recent studies highlight how tandems of previously described actin nucleators collaborate to produce new actin filaments. One key player in these collaborations is formin, which appears to function as a modulator of filament elongation.

Laurent Blanchoin and Alphée Michelot

The actin cytoskeleton of eukaryotic cells is characterized by numerous different structures, each composed of dynamic assemblies of actin filaments. These structures with their different geometric and mechanical properties are each tuned to perform particular cellular functions [1]. The first critical step towards the generation of a new actin structure is the targeted nucleation of individual actin filaments from a cytoplasmic pool of actin monomers. In the cytoplasm, nucleators are essential for generating new filaments because actin monomers are buffered by profilin to inhibit spontaneous actin assembly. After nucleation, additional factors are required to spatially and temporally control the elongation of actin filaments [2].

Because our knowledge of the proteins involved in the nucleation of actin filaments has been limited for many years, it was naively believed that each nucleator is uniquely implicated in the generation of a particular type of actin-filament structure. The first actin nucleator to be discovered was the Arp2/3 complex. This complex has relatively similar biochemical properties in a variety of experimental systems tested so far, and its constituent proteins are conserved across a wide range of organisms [3]. For this reason, the Arp2/3 complex alone was often considered as the only contributor to all branched actin networks in cells, such as those found in lamellipodia or at sites of clathrin-mediated endocytosis. Formin was the second actin nucleator to be discovered. Formin assembles unbranched actin filaments, and typically remains processively associated with the fast-growing (barbed) end of the actin filament [3]. Formins are implicated in the regulation of linear bundles of actin filaments, such as yeast cables, filopodial structures or the contractile ring during cytokinesis.

Two important recent discoveries [4,5] now challenge the concept that a distinct structure of actin filaments

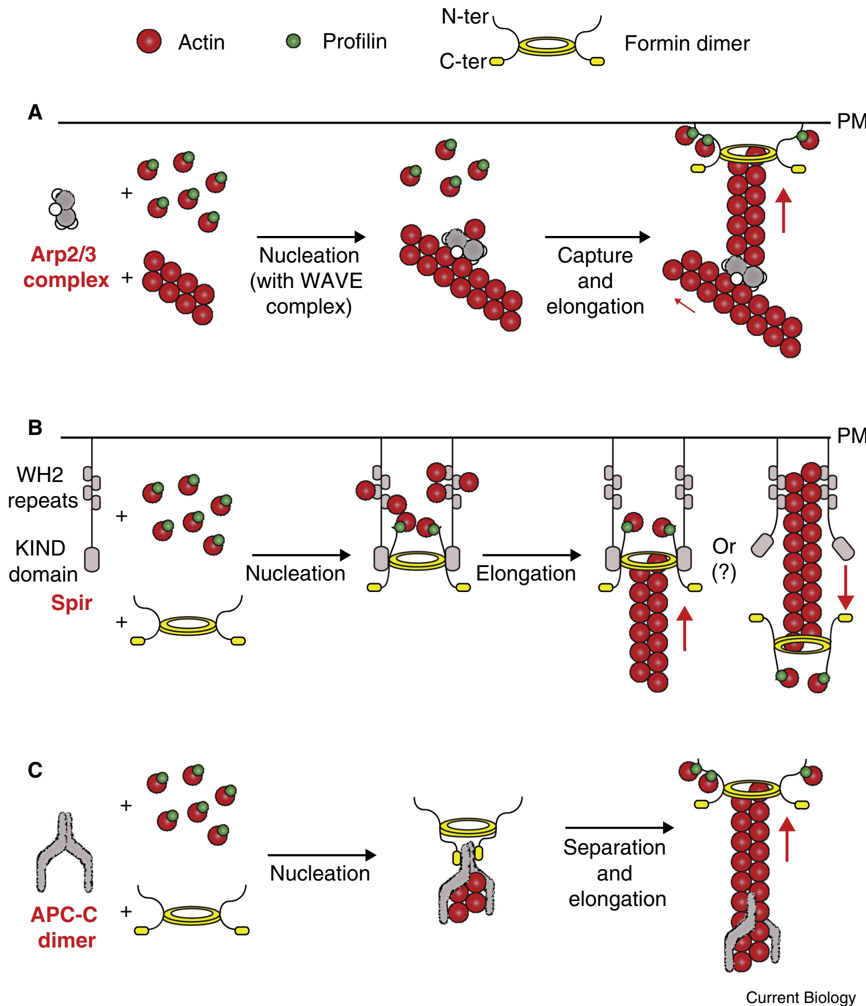

Figure 1. Mechanisms for collaborative actin assembly.

(A) Model of the collaboration between FMNL2 and Arp2/3 complex during lamellipodium protrusion. The Arp2/3 complex nucleates a branched network of actin filaments. FMNL2 targeted at the plasma membrane (PM) captures the barbed ends to favor rapid elongation in the presence of profilin–actin complexes and lamellipodium extension. (B) Model for Spir and Cappuccino collaboration during actin assembly. Spir WH2 repeats capture actin monomers to generate a nucleation site that is captured and elongated by Cappuccino: whether or not the Spir–Cappuccino complex is released during elongation has yet to be demonstrated. (C) Model of the synergy between APC and formin. The APC–mDia1 complex generates an actin nucleation site that is elongated by mDia1 after dissociation of the APC–mDia1 complex.

in cells is established by a single type of actin nucleator, and suggest instead that the collaborative action of nucleators might be a more general mechanism than previously expected.

A striking feature of the formin family is that its members can have very different biochemical properties. Some formins bundle actin filaments [6,7], whereas others do not, and a plant formin has been found to be non-processive [8]. Almost all formins differ greatly in their nucleation efficiencies [7,9] and in the rates by which they processively elongate actin filaments [10]. In addition, the presence of profilin greatly reduces the nucleation activity of formins in favor of their elongation activity [9]. What, then, would be the *in vivo* functions of formins that have a low actin nucleation efficiency?

A paper published by Block *et al.* [5] in a recent issue of *Current Biology* provides an explanation. This study examined the formin FMNL2, which is highly expressed in a variety of motile cell types. The authors found that FMNL2 accumulates not only at the tips of filopodia but also at the tips of lamellipodia. Moreover, RNAi-mediated silencing of FMNL2 markedly reduced the rate of lamellipodial protrusion, providing additional evidence supporting the role of formins in regulating the formation of actin arrays that promote membrane protrusions [11,12]. Interestingly, FMNL2 displayed minimal nucleation activity in actin assembly assays *in vitro*, whereas it significantly increased the rate of filament elongation in the presence of profilin. Therefore, this study suggests a synergistic role between FMNL2 and the Arp2/3 complex during lamellipodium assembly. In this model, the Arp2/3 complex, but not FMNL2, nucleates actin filaments. The Arp2/3 complex also regulates actin-filament branching. FMNL2 serves only to control the elongation of the actin filaments after capturing newly formed filament barbed ends. FMLN2 is proposed to function by increasing the rate of filament elongation, by protecting the growing end of the filament from capping proteins (proteins that bind filament ends and terminate elongation), and by attaching the filament ends to the plasma membrane (Figure 1A).

Numerous additional proteins involved in the nucleation of new actin filaments have been discovered in recent years and their interactions suggest a higher degree of complexity in the regulation of actin-filament structures than previously appreciated [13]. One of the first characterized collaborations was the protein Spir and the formin Cappuccino, which were jointly implicated in a wide range of cellular processes in oocytes [14–16]. Spir contains a cluster of four actin-binding WH2 domains, which bring actin monomers into close proximity and favor the nucleation of a new filament (Figure 1B) [17]. Spir directly interacts with Cappuccino, but more work needs to be carried out to understand how these molecules cooperate as a complex during nucleation and elongation of new actin filaments (Figure 1B).

A recent study from Breitsprecher *et al.* [4], published in *Science*, now provides new insights into how the collaboration between formin and another actin nucleator works. These authors examined the formin mDia1 and one of its binding partners, adenomatous polyposis coli (APC), using state-of-the-art triple-color total internal reflection fluorescence microscopy single-molecule imaging

techniques. They had previously shown that mDia1 and APC have a synergistically positive effect on actin filament assembly in bulk assays [18]. In the new study, they found that the APC dimer alone nucleates actin filaments, and that these filaments subsequently elongate with a free barbed end, i.e. away from the site of interaction of APC with the actin filament (Figure 1C). APC and mDia1 were required to form a complex in the early stages of nucleation. However, during the polymerization phase, APC and mDia1 dissociated, with APC remaining in close proximity to the non-growing end of the filament (nucleation site) while mDia1 remained processively attached to the rapidly elongating end of the filament.

What is striking is how the distinct properties of the different nucleators contribute to the assembly of a new filament. On the one hand, APC is an efficient nucleator, and ensures that new filaments are generated even at high profilin levels, where formins are less efficient. On the other hand, mDia1 has an independent role in protecting filament elongation from capping proteins and in increasing polymerization rates. Therefore, formin's most important role appears to be regulating elongation rather than nucleation. However, this study also does not rule out the possibility that formin acts in both steps. In addition, the demonstration that formin has nucleation activity when associated with the APC complex would require careful measurement.

A lot of work remains to be carried out to understand the collaborative interplay between nucleators. It will be critical to understand the similarities and differences in the mechanisms of different dual collaborations of actin nucleators, such as between the Spir–formin and APC–formin complexes, and to investigate the cooperative mechanisms of other actin regulators that have not been discussed here, such as VASP, JMY or Cordon-bleu. Hence, these and future studies may build upon the concept that the dual collaboration of different permutations of actin nucleators provides a further degree of complexity and subtlety to the regulation of the actin cytoskeleton.

These recent studies bring a new perspective on the role of formins as elongation factors. Given that the concentration of elongating filament ends in actin-filament structures in the cytoplasm is very low in comparison with the concentration of actin and other actin-binding proteins, then presumably the concentration of formin required to promote elongation of these ends is very low. Therefore, it would not be surprising to learn that formins operate in many other cellular locations where they have not yet been detected by normal fluorescence imaging techniques.

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Plant Development: Should I Stop or Should I Grow?

Plant growth is tightly controlled through the integration of environmental cues with the physiological status of the seedling. A recent study now proposes a model explaining how the plant hormone ethylene triggers opposite growth responses depending on the light environment.

Séverine Lorrain and Christian Fankhauser

Being sessile, plants adapt to their surrounding environment by changing their shape and their development. Different environmental cues such as light quantity, quality or temperature are integrated with the physiological and hormonal status of the plant to trigger appropriate organ-and tissue-specific responses [1]. The embryonic stem (hypocotyl) of *Arabidopsis thaliana* is a good model