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Discussing the morphology of actin filaments in lamellipodia

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Biologists working on cellular motility are in disagreement as to the morphology of actin filaments at the leading edge. Electron microscopy (EM) studies in the 1980s and 1990s suggested a crosslinked network of long filaments attached in a side-to-side manner¹. Subsequent EM studies suggested that cells fixed while moving have a network of short branched filaments linked in an end-to-side manner, forming a dendritic branched network² (Figure 1). The *in vitro* discovery that Arp2/3 complex nucleated filaments into a dendritic network with strikingly similar branch angles as in cells (reviewed in ³) provided mechanistic explanation for these branches. Many biochemical, cell-free, and cellular studies have supported the dendritic network³, but recent EM work challenged the existence of a dendritic network at the leading edge of motile cells⁴.

Both sides of this debate worry that the methods used to prepare cells create artifacts, either in the appearance or elimination of branches. An Opinion in this issue by Vic Small argues against the branching model. In this letter, I will explain why I think the branching model is correct, highlighting the extensive biochemical and cellular evidence supporting the model and listing my concerns with the recent evidence against branching. However, I also think it is clearly time for a third (or even fourth) party to examine this important issue independently.

Before having that discussion, there are several points on which, to my knowledge, both camps agree: i) Arp2/3 is highly enriched at the leading edge; ii) Arp2/3 is a major actin nucleation factor at the leading edge; iii) Arp2/3 is at the pointed (minus) end of filaments it nucleates; and iv) WAVE, a critical Arp2/3 activator, localizes to the leading edge plasma membrane (PM)³⁻⁵.

Biochemical evidence from many laboratories strongly suggests that Arp2/3 must bind to the side of an existing actin filament to nucleate a new filament⁶. This side-binding requirement causes Arp2/3 to assemble a branch as an integral part of the nucleation process. The structure of this branch, determined by 3D-tomography⁷, agrees extremely well with mutagenesis studies of a key Arp2/3 subunit involved in branching⁶. In fluorescence microscopy assays from multiple groups using purified proteins or extracts, Arp2/3 activators cause branched filament assembly⁸. Thus, any model of an unbranched actin network generated by Arp2/3 must provide a mechanism by which Arp2/3-nucleated filaments can be de-branched. Two proteins, cofilin and coronin, can de-branch Arp2/3-nucleated filaments^{9, 10}, but their abundance towards the rear of the leading edge and their biochemical mechanisms suggest that branches do not disassemble immediately. A recently

identified cofilin homologue, GMF, also de-branches¹¹, but its localization in the lamellipodium has not been determined.

Many groups have provided examples of branched networks in multiple cell types, as well as behind intracellular pathogens such as *Listeria monocytogenes* and intracellular organelles such as yeast actin patches (reviewed in ³). These studies use techniques that have raised concerns by some ⁵, prompting use of cryo-EM techniques in which few branches were found⁴. However, an earlier cryo-EM tomography study of Dictyostelium shows branched actin filaments [¹²].

An important point is that the results described in ⁴ are not without potential problems of their own. A major concern is that this labile branched network may disassemble in some preparative techniques. It is known that Arp2/3-mediated branches are highly labile, owing to sensitivity to the nucleotide state of actin as well as to the action of cofilin⁹. Over 50% of the actin filaments can be lost during detergent extraction¹. The majority of the cells examined in ⁴ were processed by detergent extraction in low concentrations of the fixative glutaraldehyde before full fixation, and I am not convinced that this procedure preserves structure adequately. To circumvent this problem, cryo-EM is also used, without extraction/fixation. However, this procedure is only used on fibroblasts, since keratocytes respond poorly to the blotting procedure required prior to freezing⁴. This sensitivity suggests that this technique is not without adverse effects. Other cryo-EM tomography studies have had difficulties detecting short actin filaments^[13], for a variety of reasons. I also think it is important to show the ultra-structural location of Arp2/3 in micrographs such as those in ⁴, since actin filament structure is beautifully revealed and Arp2/3 is retained at the leading edge. To me, the positive identification of Arp2/3 at branches in cells trumps negative results.

Discussions about the EM techniques could go on indefinitely. The discussion may be more complex than simply “branches versus no branches”, since coronin can remodel Arp2/3-generated branches into branches of more varying angles, shown both *in vitro* and in cells¹⁰. Importantly, coronin must start with Arp2/3-branched filaments, not unbranched filaments. It is time for additional techniques, including high-resolution fluorescence microscopy and genetic manipulation of Arp2/3, to be used in resolving this issue. I understand that such studies are ongoing, and eagerly await these results. Ultimately, I think this debate is healthy for the field, and is challenging many to consider and re-test models carefully.

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