A fruitful tree: developing the dendritic nucleation model of actin-based cell motility

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ABSTRACT **A fundamental question in cell biology concerns how cells move, and this has been the subject of intense research for decades. In the 1990s, a major leap forward was made in our understanding of cell motility, with the proposal of the dendritic nucleation model. This essay describes the events leading to the development of the model, including findings from many laboratories and scientific disciplines. The story is an excellent example of the scientific process in action, with the combination of multiple perspectives leading to robust conclusions.**

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To those involved in it, scientific research is high drama. When working efficiently, research is conducted simultaneously by many small labs that are alternately competing, cooperating, arguing, and negotiating amongst themselves. Why would this seemingly chaotic system be efficient? The fact is that any individual researcher will frequently be a little "off" in his or her findings. The competitive nature of science makes for an outstandingly self-correcting system, weeding out errors on the fly.

This story is about a period of rapid advance in cell biology, covering roughly the years 1997–2001. Those years happen to encompass my time as a postdoctoral fellow in Tom Pollard's lab, but my role was that of an observer for much of this time. I observed a very rich story, spanning many people, places, and ways of thinking. Above all, it is a triumphant story, showing how our seemingly fragmented and discordant research system results in robust solutions to complex problems. Similar stories can be told for other fields. I am telling this one and I am telling it from my perspective. I appreciate that others might have different perspectives.

The story concerns the mechanism by which cells move when put on a glass slide, sometimes called crawling motility. Many cells do this: amoebae, immune cells, fibroblasts, and keratocytes from fish scales. There has been a long-standing appreciation that this admittedly artificial system clearly uses elements involved in more "natural" cell motility, and also shares mechanistic elements used in other processes (e.g., endocytosis). Thus, explaining cell motility has been a fundamental goal in cell biology.

In 1997, there was a basic understanding of the process. It was reasonably clear that actin filament polymerization powered the initial motility step—protrusion of the leading-edge plasma membrane (Figure 1A). Actin filaments were known to be abundant at the leading edge, in a region of relatively uniform width called the lamellipodium (Figure 1B). Biochemically, there was a good understanding of how actin polymerizes (Pollard and Cooper, 1986). It was known that actin monomers assemble into two-stranded helical filaments of uniform polarity, with a "barbed" and a "pointed" end (Figure 1C). Seminal work showed that, in motile cells, filament elongation occurs from barbed ends, and these barbed ends face the leading-edge plasma membrane (Wang, 1985; Theriot and Mitchison, 1991). Lamellipodial actin filaments turn over rapidly as the cell moves.

But that was where most of the clarity ended and the questions began. How can so many lamellipodial actin filaments be generated so quickly? How can these filaments drive leading-edge protrusion? How is filament turnover coordinated so exquisitely to maintain the lamellipodium even as the cell advances? Several other proteins were clearly needed (Pollard and Cooper, 1986). *First*, something had to enhance assembly (nucleation) of new filaments, since nucleation of actin alone is unfavorable. *Next*, something had to cross-link these filaments into a network capable of directing a protrusive force. *Finally*, a system was needed to recycle the actin monomers so that protrusion could happen again and again. As so often happens in nature, the solution turned out to be extremely elegant, requiring a surprisingly small number of proteins.

At that time, quite a few actin-binding proteins had been identified and characterized biochemically, including profilin, cofilin,

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Abbreviations used: Arp, actin-related protein; EM, electron microscopy; TIRF, total internal reflection; WASp, Wiskott–Aldrich syndrome protein.

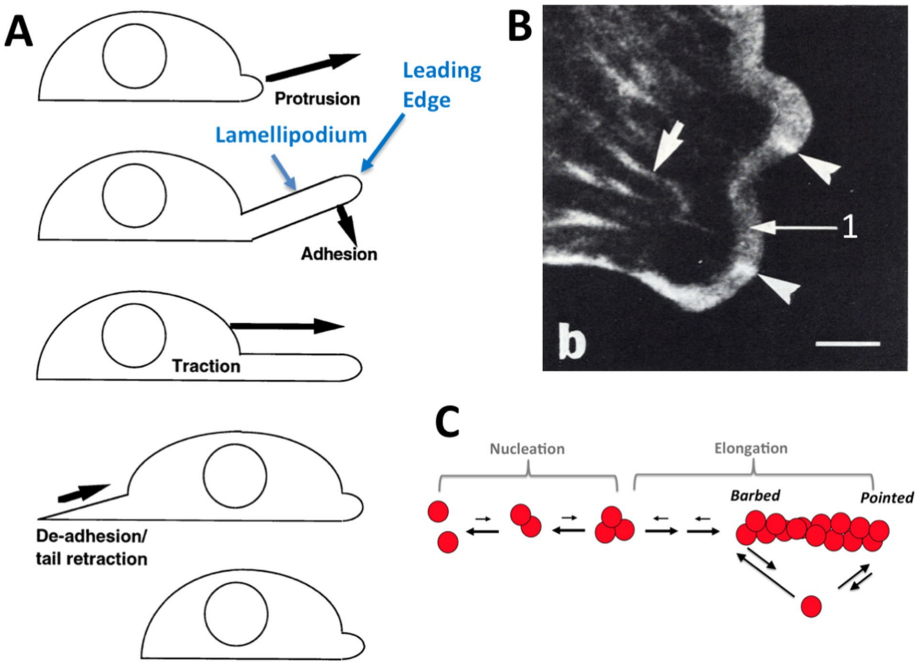

FIGURE 1: Actin and cell motility. (A) Crawling cell motility schematic, with emphasis on the initial protrusion step. The figure is modified from Mitchison and Cramer (1996) with permission. (B) Mammalian culture cell injected with fluorescent actin, showing enrichment in lamellipodium (arrow 1). Scale bar, 5 μm. The figure is modified from Wang (1985) with permission. (C) Actin polymerization from monomers (red), consisting of unfavorable nucleation steps and more favorable elongation. Elongation occurs more readily at the filament barbed end.

capping protein, gelsolin, alpha-actinin, VASP, and filamin. It was unclear, however, how these proteins could affect leading-edge protrusion, either separately or together. Perhaps the most glaring deficiency was lack of a mechanism to nucleate new filaments that could elongate at their barbed ends. Many laboratories were very interested in this nucleation mechanism.

Such was the situation in 1997.

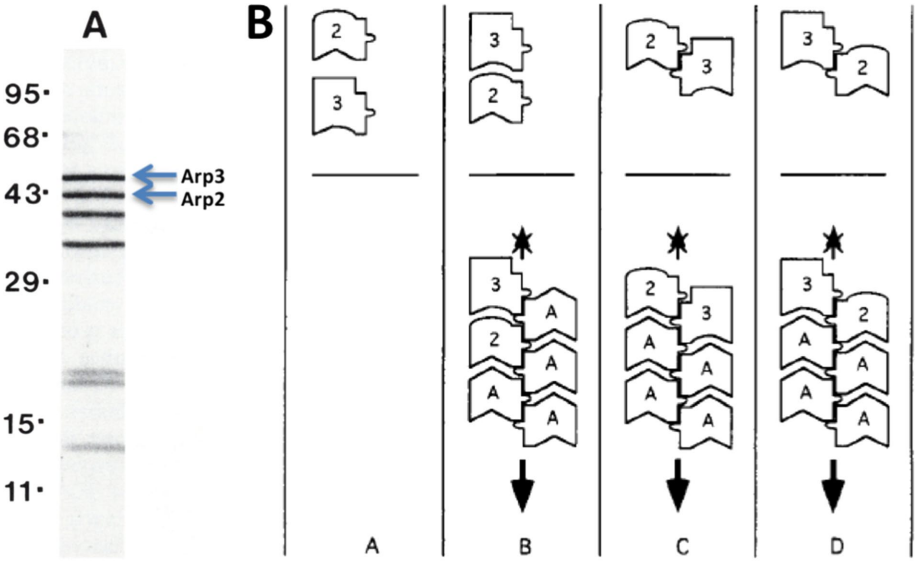

FIGURE 2: Arp2/3 complex as a potential nucleation factor. (A) Protein gel of purified complex. The figure is modified from Machesky *et al.* (1994) with permission. (B) Schematic of possible viable dimers for Arp2 and Arp3, and potential for elongation at the barbed end (down) or pointed end (up), based on structural models. From Kelleher *et al.* (1995) with permission.

THE LEAD-UP

I joined Tom's lab at the Salk Institute in June 1997. I was looking for a postdoctoral mentor who was firmly rooted in chemistry, but with a clear eye to the bigger cellular picture. I certainly found that in Tom. Tom is the first character in this story, and is a constant throughout. Indeed, there is very little that is variable about Tom.

A big draw to Tom's lab was the Arp2/3 complex, not because of its clear central role in the cellular actin economy (as yet unknown) but because of how Tom's lab had acquired the little knowledge they had at the time. Enter the second character in the story: Laura Machesky. Laura would provide not one but *two* fundamentally transformative findings, and epitomizes out-of-the-box thinking. In this case, Laura decided to do affinity chromatography using a profilin column. She poured extract from the amoeba *Acanthamoeba castellanii* over the column, and took a look at what stuck. One obvious answer—*actin*. In addition, she identified seven other proteins that seemed to be part of a complex (Machesky *et al.*, 1994). Identification of the proteins by peptide sequencing (not straightforward in those days) showed that two of these were actin-related proteins (Arp2 and Arp3) and the other five were, well, nothing with any recognizable function (Figure 2A). The Arp2/3 complex had been discovered.

I found this paper to be amazing, but equally so was a follow-up paper from Tom's lab, using molecular modeling to predict that Arp2/3 complex might be a barbed-end nucleator (Kelleher *et al.*, 1995). Molecular modeling was comparatively crude in those days, and they had no idea how the complex was put together. Nonetheless, they determined that a dimer of Arp2 and Arp3 could, in principle, mimic a barbed end (Figure 2B). When I arrived at the Salk, those publications made up the bulk of the published knowledge on Arp2/3 complex.

CELL MOTILITY FROM A DIFFERENT PERSPECTIVE—*LISTERIA* HYSTERIA

Model systems have always played a major role in cell biology, and the study of cell motility is no exception. In the late 1980s, an interesting and perhaps unexpected model system arose in the form of intracellular pathogenic bacteria, particularly *Listeria monocytogenes* and *Shigella flexneri*. These bacteria can invade a eukaryotic cell and hijack the host actin cytoskeleton, causing polymerization of an actin filament "comet tail" that serves to propel the bacterium around the cytoplasm (Figure 3, A and B). After the seminal work of Lew Tilney and Dan Portnoy introduced this phenomenon (Tilney and Portnoy, 1989), several people, including Julie Theriot in Tim Mitchison's lab, were instrumental in

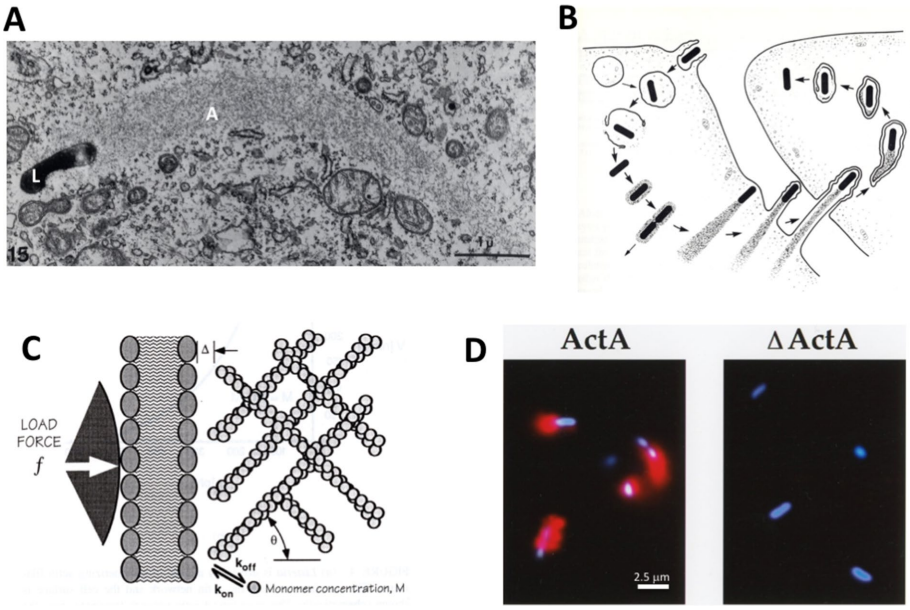

FIGURE 3: *Listeria*, actin, and force. (A) Electron micrograph of *Listeria* (L) associated with an actin comet tail (A) in an infected macrophage. The figure is adapted from Tilney and Portnoy (1989) with permission. (B) Model of *Listeria* entry into host cell, use of actin tail for motility within cytoplasm, and transfer to adjacent cell. From Tilney and Portnoy (1989) with permission. (C) Schematic of elastic Brownian Ratchet model for a lamellipodium. From Mogilner and Oster (1996) with permission. (D) *Listeria* (blue) mixed with Arp2/3 complex and actin (red). At left is WT *Listeria*. At right is an ActA deletion strain. Contributed by Matt Welch.

showing that it was barbed-end actin polymerization at the bacteria/comet tail interface that was responsible for the motility (Theriot *et al.*, 1992). A conceptual leap was that the comet tail was very similar to leading-edge actin polymerization during cell motility. By this route, *Listeria* and other pathogenic microbes became tractable and powerful model systems for cell motility. We shall hear from them again.

MOTILITY FROM A BIOPHYSICAL PERSPECTIVE—MAY THE FORCE BE WITH YOU

Thus, actin polymerization supplies the force both for leading-edge protrusion during cell motility and for pathogenic bacterium motility within cells. A major question, though, was exactly how an actin network could be used to generate protrusive force. Two computational biologists at Berkeley, Alex Mogilner and George Oster, had been working on this issue, building off previous work in the Oster lab (Peskin *et al.*, 1993). Their model demonstrated that a network of actin filaments abutting a surface at an angle could exert force on that surface by an "elastic Brownian Ratchet" mechanism (Mogilner and Oster, 1996), in which thermal fluctuations of the filament cause filament bending away from the surface, allowing actin monomer addition to the barbed ends. When the filament bends back and contacts the surface, force is generated (Figure 3C). The two surfaces modeled were *Listeria* and the leading edge.

There are several key points in this model. First, a certain threshold number of filaments must be present, and they must be linked together, or else the force would push the filaments back instead of pushing the surface forward. Second, the length between the filament's barbed end and the link to the network must be short enough to prevent the filament from buckling when it pushes against the surface. In keeping with electron microscopy (EM) studies that had been conducted by several groups (Small *et al.*, 1978, 1995; Hoglund *et al.*, 1980; Svitkina *et al.*, 1986), the actin network was depicted as long filaments cross-linked side to side, with the barbed ends contacting the surface at an approximately 45° angle.

The elastic Brownian Ratchet model would have a strong influence on cell motility studies, providing a plausible mechanistic explanation of how actin polymerization could push a membrane forward.

LINKING ARP2/3 COMPLEX TO *LISTERIA* MOTILITY

Elsewhere in the Bay Area, other important events were occurring—specifically, in the lab of Tim Mitchison at the University of California, San Francisco (UCSF), who, in addition to his many other discoveries, had already made key findings on actin dynamics at the leading edge and in *Listeria* actin comets (Theriot and Mitchison, 1991; Theriot *et al.*, 1992). A postdoc in the Mitchison lab, Matt Welch, was hard at work trying to figure out what might nucleate the actin filaments that make up the *Listeria* comet tail. It was known that a single bacterial membrane protein, ActA, was sufficient for comet tail assembly but that purified ActA itself did not nucleate actin.

In what ranks as one of the most beautiful pieces of work that I have ever seen, Matt purified Arp2/3 complex as the relevant nucleation factor, using a cell-free assay to test chromatographic column fractions for their ability to enable ActA-mediated actin polymerization (Figure 3D; Welch *et al.*, 1997). In a subsequent study, Welch and Mitchison also identified the first direct Arp2/3 complex activating protein (ActA), using the purified proteins (Welch *et al.*, 1998). It was quickly shown that *Shigella*, vaccinia virus, and other pathogens also activate Arp2/3 complex, albeit through different proteins (Suzuki *et al.*, 1998; Frischknecht *et al.*, 1999a,b; Gouin *et al.*, 1999).

ARP2/3 COMPLEX AND BRANCHING NUCLEATION—"THE DAMNEDEST THING"

The words "Where's Dyche?" say two things about the man: 1) Dyche is a difficult man to find; and 2) Dyche is a man well worth finding. Enter Dyche Mullins, a postdoc in the Pollard lab and an interesting combination of experimentalist, mathematician, mystic, and pragmatist. Frequently, though, Dyche was nowhere to be found. Perhaps he was down on the beach reading a volume of Russian poetry, or hidden in the library poring over old papers on *Limulus* sperm, or in any number of places but the lab. Despite appearances, he would put in huge hours, many of them at night, testing fundamental things about Arp2/3 complex. Before the events described below, Dyche had established the basic subunit interactions of the complex, found that it could cross-link actin filaments, and shown that it could cap filament pointed ends (Mullins *et al.*, 1998b).

I will never forget a day in 1997 that I feel was transformative for cell motility. Dyche was sitting on the edge of his desk, examining the latest issue of the *Journal of Cell Biology* with rapt attention. I asked him what was so interesting. He replied, "the damnedest thing." It was an article by Tatyana Svitkina, Gary Borisy, and others, in which they used EM to examine the arrangement of actin and myosin II in fish keratocytes (Svitkina *et al.*, 1997). While the paper was mainly focused on myosin, they noticed that the actin filaments at the

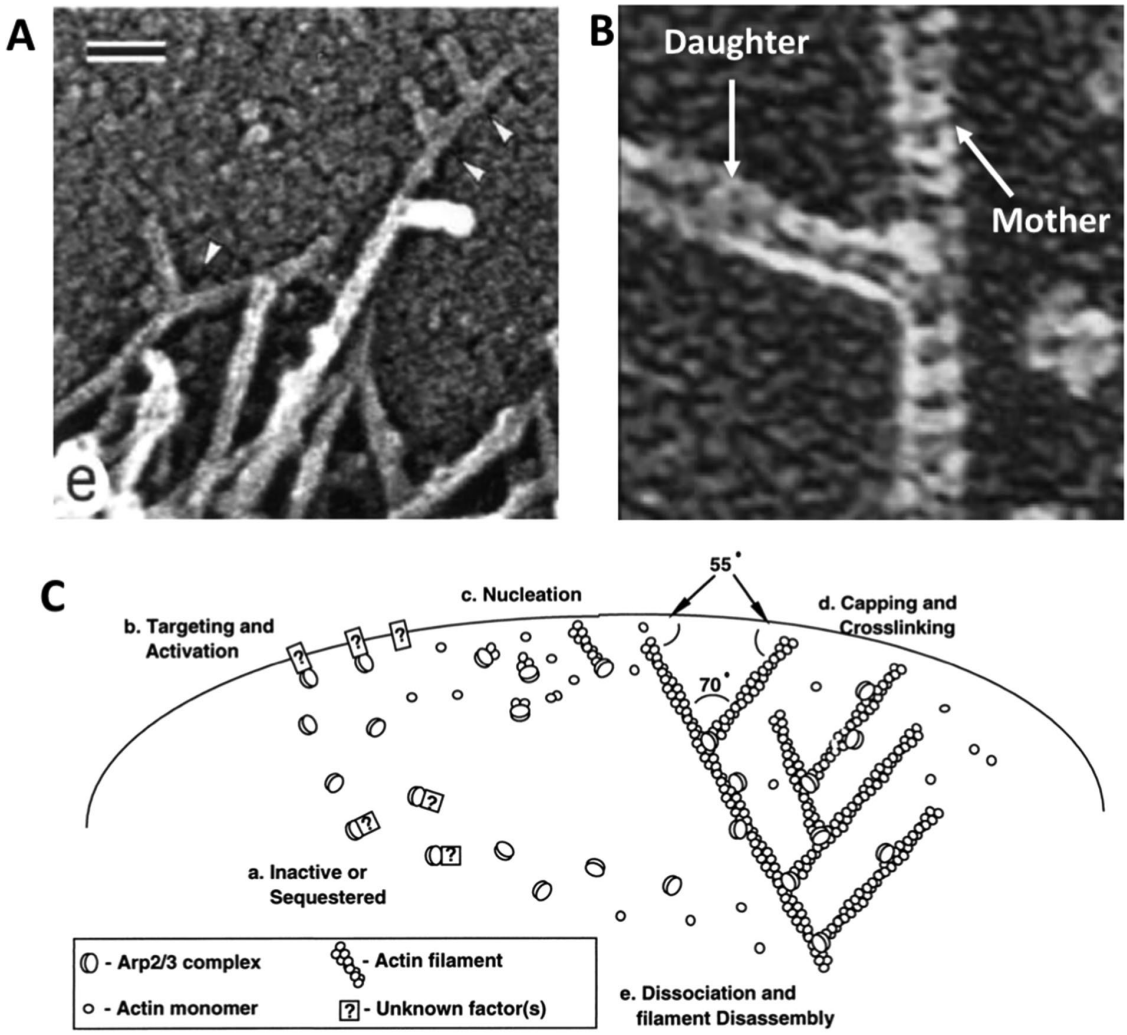

FIGURE 4: Dendritic nucleation by Arp2/3 complex. (A) Example of dendritic branches in fish keratocytes. Bar, 50 nm. From Svitkina *et al.* (1997) with permission. (B) Example of dendritic branch from purified Arp2/3 complex and actin. From Mullins *et al.* (1998a) with permission. (C) Dendritic nucleation model from Mullins *et al.* (1998a) with permission.

extreme leading edge were branched in characteristic "Y junctions," with the pointed end of one filament (the "daughter") binding to the side of another (the "mother"; Figure 4A).

These branched filaments caught the attention of Tom and Dyche, making a connection to their findings that Arp2/3 complex can both cross-link actin filaments and bind pointed ends. Could the complex be causing these branched filament structures? Over the next few months, amazingly rapid progress was made after years of diligent work. Immediately on seeing this paper, Tom contacted John Heuser at Washington University, sent him purified Arp2/3 complex and actin, and received back EM micrographs showing that the purified complex caused filament branching with angles indistinguishable from those observed by Svitkina and Borisy (Figure 4B). From these results, a model came forth in which Arp2/3 complex nucleates a new filament and then links it to the side of an existing filament to form a branch (Figure 4C). Repeated branching results in a pattern uncannily similar to tree branches, hence "dendritic nucleation" (Mullins *et al.*, 1998a). Svitkina and Borisy directly identified Arp2/3 complex at branch points in lamellipodia a year later (Svitkina and Borisy, 1999).

One might ask why these branched filaments were not identified earlier at the leading edge. Thin-section EM studies from several laboratories suggested that the leading-edge filaments were long and cross-linked in a side-to-side manner in a variety of cell types (Small *et al.*, 1978, 1995; Hoglund *et al.*, 1980; Svitkina *et al.*, 1986). The EM technique employed by Svitkina and Borisy (platinum replica) provided another way of looking at these filaments, which gave a different answer. Another factor may have been that the short, labile Arp2/3 complex–nucleated filaments were better preserved in this procedure. As will be mentioned at the end of the essay, cellular dendritic nucleation was not universally accepted for many years.

ARP2/3 COMPLEX REGULATION—MACHESKY ACTIVATES THINGS, AGAIN!

The pace of discovery at that time was extremely rapid. In little more than one year, Arp2/3 complex had been established as a bona fide nucleation factor, the concept of dendritic nucleation had been launched, and the first Arp2/3 complex activator had been identified. But there was still a pretty big hole in the story. What eukaryotic protein activates Arp2/3 complex during cell motility? The nucleation activity Dyche found with purified *Acanthamoeba* Arp2/3 complex was very modest. Matt Welch had found a potent bacterial activator in ActA, but there were no good leads as to what the endogenous mammalian activator could be.

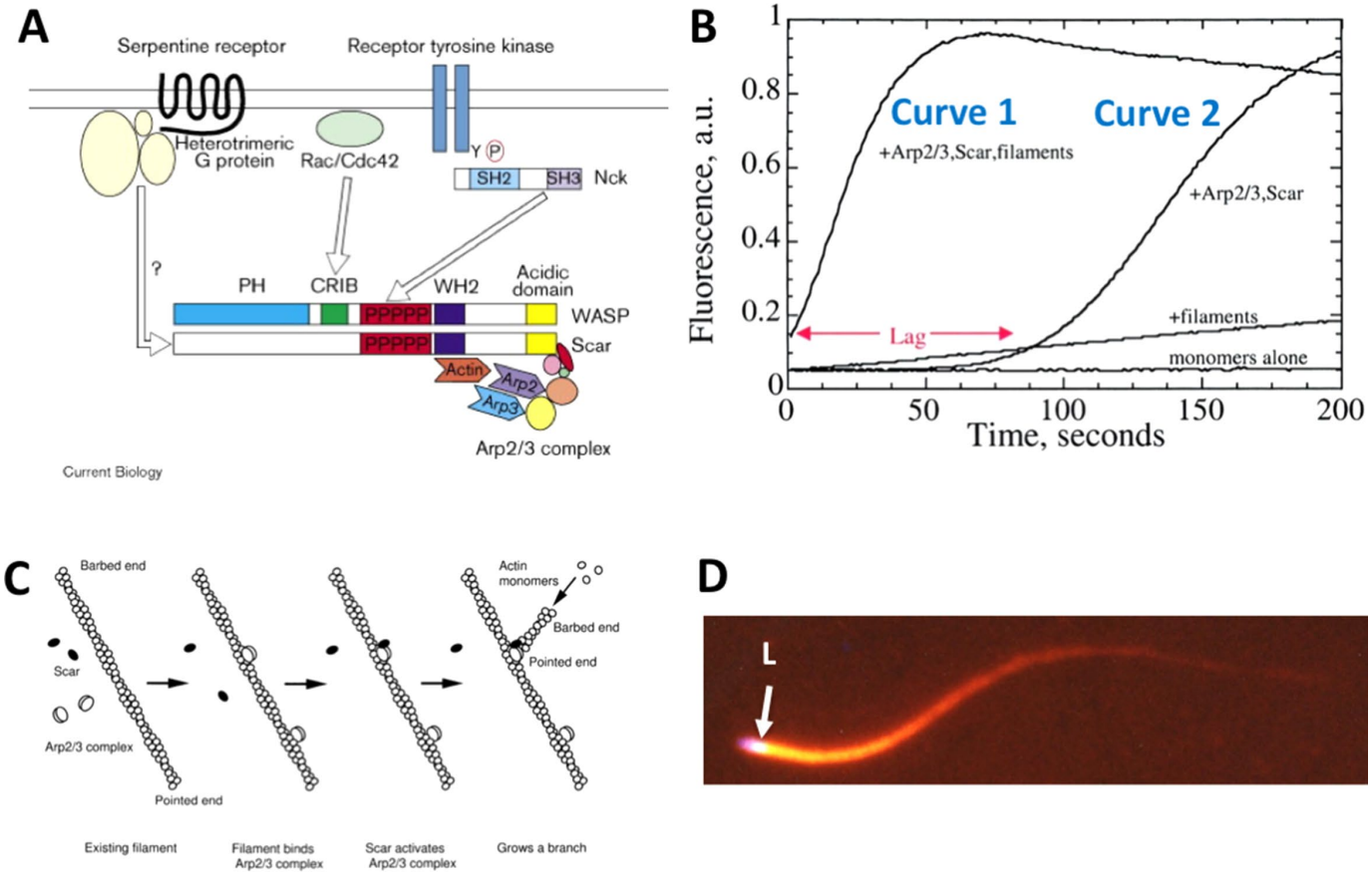

FIGURE 5: Arp2/3 complex activation and cell-free reconstitution. (A) Model of WASP and Scar interaction with activators and Arp2/3 complex. The WA region consists of the WH2 and the acidic domain. From Machesky and Insall (1998) with permission. (B) Actin polymerization assay showing that addition of filaments (curve 1) eliminates the polymerization lag that occurs with Arp2/3 complex activated by Scar alone (curve 2). The figure is modified from Higgs and Pollard (1999) with permission. (C) Model showing Arp2/3 complex activation by Scar and filament side-binding. From Machesky *et al.* (1999) with permission. (D) Actin comet tail (red) behind *Listeria* (L) in an extract from *Xenopus* oocytes. Contributed by Matt Welch.

Reenter Laura Machesky, who had now started her own lab at the University of Birmingham, United Kingdom. Ever the maverick, she embarked on a seemingly hairbrained scheme to identify Arp2/3 complex interacting proteins: conducting yeast 2-hybrid screens using individual Arp2/3 complex subunits as bait. Why was this hairbrained? Because the Arp2/3 complex was very clearly … a *complex*! There was little indication that any of the subunits existed in a free pool, strongly suggesting all would be pretty unhappy if expressed alone, leading to artifactual interactions.

Undeterred by any of this seemingly airtight logic, Laura went ahead and did the experiment anyway. It worked. Laura identified the C-terminal regions of WASp (Wiskott–Aldrich syndrome protein) and Scar (now more commonly called WAVE) as interacting with the 21-kDa subunit of the complex (Machesky and Insall, 1998). The relevant regions of these proteins, termed "WA" by Laura, bound both actin monomers and Arp2/3 complex from tissue extract (Figure 5A). When expressed in culture cells, the WA region potently perturbed a variety of actin-based structures and caused Arp2/3 complex to mislocalize. Presumably, the freely cytosolic WA region was competing with endogenous Scar or WASp that was properly localized.

A crucial question remained: could the purified WA region directly activate purified Arp2/3 complex? Laura enlisted Tom's help in testing this biochemically and, since Dyche had already left for UCSF to start his lab, WA peptide was tested at both UCSF (using amoeba Arp2/3 complex) and Salk (using human Arp2/3 complex).

I will never forget the day we first did these biochemical tests. In my own lab now, I tell my lab members that, most of the time, a long-anticipated experiment ends up being a dud on the first attempt. By the time it finally works, the burning ardor that once existed has cooled significantly. This was not one of those times. I mixed Scar WA, Arp2/3 complex, and actin together and put them in the fluorimeter, and several of us watched the progress of the polymerization reaction on the computer screen. At first, for maybe 100 s, nothing happened. That was a long 100 s! Then, all of a sudden, the polymerization rate shot up impossibly quickly (Figure 5B; Machesky *et al.*, 1999). It was better than I could even imagine—a moment of excitement matched by few others. We were all gathered around the fluorimeter (Laurent Blanchoin, Don Kaiser, Magdalena Bezanilla, Wei Li Lee, Kirsi Turbedsky, and probably others), cheering. More or less simultaneously, Dyche was having a similar experience up the coast from us using amoeba Arp2/3 complex. A true moment of scientific elation.

This was a huge result. Mutations in WASp were known to result in Wiskott–Aldrich syndrome, a hematopoietic disorder, and WASp also interacted with Rho GTPases important for cell motility, Cdc42 and Rac. Scar/WAVE was a component of cAMP-mediated

chemotaxis in *Dictyostelium*. Thus, Arp2/3 complex could now be connected with signaling pathways controlling specific actin polymerization events (Figure 5A).

Work from other laboratories came out shortly thereafter, showing the direct activation of Arp2/3 complex by other proteins and adding key insights. Rajat Rohatgi in Marc Kirschner's lab showed that N-WASP activated Arp2/3 complex, and that full-length N-WASP was activated to do so by Cdc42 and PIP_2 (Rohatgi *et al.*, 1999), building on previous work by Le Ma, Rohatgi, and Kirschner investigating Cdc42-induced actin polymerization in *Xenopus* extracts (Ma *et al.*, 1998). Defne Yarar in Matt Welch's recently started lab at Berkeley showed that WASP activated Arp2/3 complex, both directly and in bovine brain extracts (Yarar *et al.*, 1999). Dirk Winter in Rong Li's lab showed that the WA region from the budding yeast protein Bee1p/Las17p could activate Arp2/3 complex (Winter *et al.*, 1999), building on their work on Arp2/3 complex–dependent actin patch assembly (Winter *et al.*, 1997). Interestingly, deletion of the WA region from Las17p did not abolish actin patch assembly in budding yeast, leading to dissection of the intricate Arp2/3 complex regulation pathways in yeast endocytosis (Kaksonen *et al.*, 2003).

A common feature of all of these activators was the WA region, also variously called the WCA or VCA region. This polypeptide contains three sequences: an actin monomer-binding WASP homology 2 (WH2) motif (also called a Verprolin homology motif), followed by an Arp2/3 complex–activating region (originally called a "cofilin-homology" or "central" region), followed by an acidic region that binds Arp2/3 complex (also containing an important aromatic amino acid, most often a tryptophan). Generally, the WA region is at the C-terminus of an Arp2/3 activator, but in LatA, it is at the N-terminus. A large number of additional WA-containing Arp2/3 complex activators have been identified over the years, including WASH, DIP/WISH, WHAMM, and JMY proteins (Alekhina *et al.*, 2017).

TAKING CARE OF A LOOSE END—WHY THE LAG?

But why the 100-s lag? Who cares, it worked!?! At least that was my attitude at the time. Thankfully, not everybody was so unconcerned—namely, Laurent Blanchoin. Laurent was a postdoc in Tom's lab and had done a lot of seminal work on another actin-binding protein, cofilin. But Arp2/3 complex was my project, so despite having an idea of what was going on, he tread carefully. In a lab full of postdocs and grad students, there is a certain air of competition, with everybody trying to make important findings. Stepping on another person's turf can be a problem. Eventually, Laurent asked me for some Arp2/3 complex, to try his idea. I had to think about whether this was good for me. We had a good working relationship and I trusted him. I gave him the protein.

I'm very glad I did. Laurent's idea was that Arp2/3 complex had *two* activators: the WA sequence and the actin filaments themselves. Maybe Arp2/3 complex was forced to make branches, because it needed to bind the side of the mother filament in order to nucleate. A few experiments showed this to be the case, with addition of a small amount of prepolymerized filaments eliminating the nucleation lag (Figure 5, B and C) (Machesky *et al.*, 1999). In the end, supplying Laurent with the protein helped both of us, making me look good by being a part of the discovery. I have learned and unlearned the lesson of sharing reagents many times since in my career. It is best just to give things out and see what happens.

A year or so after this, Laurent pioneered fluorescence microscopy techniques to observe dendritic nucleation in vitro (Blanchoin *et al.*, 2000), leading to total internal reflection (TIRF) systems that have benefited many aspects of cytoskeletal research (Amann and Pollard, 2001; Kovar *et al.*, 2006; Reymann *et al.*, 2012; Smith *et al.*, 2013, Bridges *et al.*, 2014; Schaedel *et al.*, 2015). Laurent has been a steady force in the field ever since.

RECONSTITUTING A MOTILITY SYSTEM—MADAME ACTINE

We now knew a lot: Arp2/3 complex activation by both WA motifs and preexisting actin filaments causes dendritic network assembly similar to that observed at the leading edge. The filament barbed ends in this network push on the leading edge membrane. How, then, does the network keep up with the moving cell edge to cause continuous motility? Studies of fast-moving fish keratocytes had shown that the width of the actin-rich lamellipodium stayed exquisitely constant over large distances even as this cell moved at a body length per minute. Similar motility and actin network formation had been observed in cell-free systems containing *Listeria* (Theriot *et al.*, 1994), showing that all the proteins necessary were present in cytosol (Figure 5D). How could the branched actin filament network turn over in such a coordinated manner, and what was the minimal set of proteins? Matt Welch had shown that addition of Arp2/3 complex alone to *Listeria* resulted in actin clouds, but not the full comet tails capable of motility.

Years of work by many laboratories had characterized three abundant cytosolic proteins in great detail: profilin, cofilin, and barbed end-capping protein. Owing to their high cytoplasmic concentrations, these three proteins played important roles in most actin-based processes. Exactly where they participated in cell motility, however, was unclear.

By this point in our story, 1999, Marie-France Carlier at the Laboratoire d/Enzymologie et Biochimie Structurales (LEBS), Gif-sur-Yvette, France, had been a central figure in the actin world for many years. Known for her meticulously thorough biochemical analysis, "Madame Actine" had worked extensively on many actin-binding proteins, including profilin and cofilin. The scientific interplay between the Carlier lab, the Pollard lab, and other labs is another beautiful example of the self-correcting research system at work. Two of Marie-France's grad students, Laurent Blanchoin and Jean-Baptiste Marchand, became postdocs in Tom's lab, illustrating the mutual respect between the two groups.

With the newly discovered nucleation activity of Arp2/3 complex, Marie-France was ready to try a bold move: reconstitution of a full motility system capable of continuous regeneration. Building off of the *Listeria* cell-free motility system she had been optimizing for several years (Marchand *et al.*, 1995; Laurent *et al.*, 1999), she was able to reconstitute continuous-motility *Listeria* by adding a very minimal set of purified proteins: actin, Arp2/3 complex, capping protein, and cofilin, with profilin being stimulatory to the process (Loisel *et al.*, 1999). Marie-France showed a similar dependence for another bacterial motility protein, IcsA from *Shigella flexneri*. IcsA is an N-WASP activator (Suzuki *et al.*, 1998), so N-WASP was also required in this case. Typical of Marie France's careful experimental approach, she showed that each of the essential proteins had a narrow concentration window that promoted motility, emphasizing the delicate balance of factors required. This work marked a watershed in cell motility research—the ability to reconstitute a motility process from purified proteins.

PUTTING IT ALL TOGETHER—THE FULL DENDRITIC NUCLEATION MODEL

While many individual eureka moments occurred during this period, for me the crowning achievement was assembling a cohesive and parsimonious model that explained the entire cycle of dendritic nucleation, comprising assembly and disassembly of the network

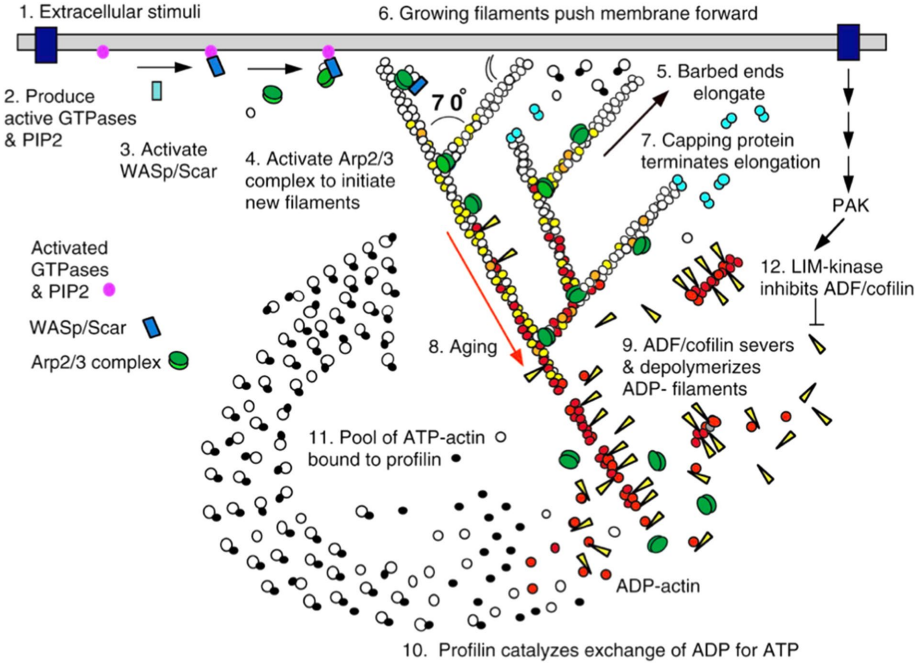

FIGURE 6: The complete dendritic nucleation model. Includes actin nucleation, capping, severing, and recycling. The figure is adapted from Pollard and Borisy (2003) with permission. Based on the original model from Pollard *et al.* (2000).

along with recycling of monomers so that it could be done again and again. This model was described in detail in a review article from Tom, Laurent, and Dyche (Pollard *et al.*, 2000). The full model is beautiful in its elegance and simplicity, while profound in its implications (Figure 6).

Important steps in the model are as follows:

Arp2/3 complex activation by membrane-bound activator and binding the side of an existing filament, resulting in a new filament as a branch from the existing filament (step 4 in Figure 6).

Barbed-end elongation (step 5) causing membrane advance by the elastic Brownian Ratchet mechanism (step 6).

Termination of barbed-end elongation by capping protein before the filament buckles, resulting in a network of short filaments (step 7).

ATP hydrolysis and phosphate release in actin filament subunits, causing older filament segments to be ADP-bound (step 8).

Cofilin binding to ADP-filament segments, with three effects: 1) enhancing phosphate release on neighboring subunits, allowing more cofilin binding; 2) severing of cofilin-bound regions; and 3) accelerating Arp2/3 complex release from the mother filament. The end result is production of more ADP-actin–containing filament ends for depolymerization (step 9).

Profilin-binding ADP-actin monomers and accelerating nucleotide exchange, resulting in ATP-actin monomers that can be used for further rounds of nucleation/elongation (steps 10 and 11).

Many aspects of the model were supported by subsequent mathematical modelling (Mogilner and Edelstein-Keshet, 2002). A key feature of the model is that only *one* event is needed to start the entire system: Arp2/3 complex activation. Everything else follows from the other proteins simply steering actin in a concerted manner to do what actin does anyway. Once Arp2/3 complex activation ends, the system stops on its own.

EPILOGUE—DENDRITIC NUCLEATION 20 YEARS ON

It is difficult to put time boundaries on discoveries of this nature. When did the discovery period start, and when did it end? I define the dendritic nucleation discovery as starting with the first model in 1998 (Mullins *et al.*, 1998a) and ending with a more refined model in 2000 (Pollard *et al.*, 2000). I discussed some of the key discoveries leading up to the model, but I could have gone farther back to early cell motility studies or the discovery of actin itself. As an incomplete list of people not yet mentioned, prior work by Bruno Straub, Albert Szent-Gyorgyi, Fumio Oosawa, Albrecht Wegner, Ed Korn, Karl Frieden, Jim Estes, Jurgen Wieland, Vic Small, Yu-Li Wang, Annemarie Weber, Sally Zigmond, Tom Stossel, Mike Bubb, Gerhard Isenberg, Mark Mooseker, John Cooper, David Botstein, David Drubin, Barbara Windsor, Jim Bamberg, Uno Lindberg, Ken Holmes, and many others was important in leading to the model. Here, I point out some of the many sequelae from the model.

Insights into the biochemical mechanism of Arp2/3 complex activation have come from a combination of an x-ray crystal structure (Robinson *et al.*, 2001), electron microscopy (Rodal *et al.*, 2005; Rouiller *et al.*, 2008; Xu *et al.*, 2012; Espinoza-Sanchez *et al.*, 2018), dynamic microscopy systems (Smith *et al.*, 2013), biochemical studies (Goley *et al.*, 2004; Padrick *et al.*, 2011; Ti *et al.*, 2011; Espinoza-Sanchez *et al.*, 2018), and inhibitor development (Nolen *et al.*, 2009). There is a greater appreciation of mammalian Arp2/3 complex heterogeneity through isoforms and substitutions of specific subunits (Chorev *et al.*, 2014; Abella *et al.*, 2016). Additional Arp2/3 complex activators have been identified (Campellone and Welch, 2010; Alekhina *et al.*, 2017), including DIP/WISH proteins that activate without the need for branch assembly (Wagner *et al.*, 2013) and two proteins that increase branch stability, cortactin (Weed *et al.*, 2000; Weaver *et al.*, 2002) and Abp1 (Goode *et al.*, 2001; Guo *et al.*, 2018). Two new mechanisms of actin nucleation have been identified, through formin proteins and tandem WH2 proteins (Chesarone *et al.*, 2010; Dominguez, 2016).

The list of cellular processes requiring Arp2/3 complex has also grown substantially. Elegant work defining the endocytic process (Kaksonen *et al.*, 2003) has strongly suggested that an Arp2/3 complex–dependent dendritic network powers key steps during invagination and has led to elucidation of a complex system of Arp2/3 complex regulation in multiple systems (Merrifield *et al.*, 2004; Sirotkin *et al.*, 2005; Sun *et al.*, 2006; Galletta *et al.*, 2008). Similarly, Arp2/3 complex plays a role in phagocytosis (May *et al.*, 2000), although its role and requirement differ depending on the specific phagocytic mechanism (Rotty *et al.*, 2017). Multiple cell–cell and cell–substratum adhesion mechanisms use Arp2/3 complex, with two notable examples being podosomes and invadopodia (Linder *et al.*, 2000; Yamaguchi *et al.*, 2005). For neurons, in addition to contributing to growth cone motility (Mongiu *et al.*, 2007; Korobova and Svitkina, 2008; Yang *et al.*, 2012), Arp2/3 complex is required for multiple aspects of dendritic spine structure and function (Hotulainen *et al.*, 2009; Korobova and Svitkina, 2010; Kim *et al.*, 2013), as well as for dendrite branching (Zou *et al.*, 2018). In case you had not

caught this, dendritic actin branching leads to neuronal dendrite branching! An area of active growth is Arp2/3 complex roles in organelle dynamics, including: endosomes/lysosomes (Derivery *et al.*, 2009; Gomez and Billadeau, 2009; Duleh and Welch, 2010; Carnell *et al.*, 2011), endoplasmic reticulum/Golgi (Fucini *et al.*, 2002; Campellone *et al.*, 2008), autophagosomes (Coutts and La Thangue, 2015; Kast *et al.*, 2015), and mitochondria (Moore *et al.*, 2016). There is even evidence for Arp2/3 complex–mediated nucleation in the nucleus (Caridi *et al.*, 2018; Schrank *et al.*, 2018). Many intracellular pathogens are now known to engage actin by both Arp2/3 complex-dependent and independent mechanisms (Welch and Way, 2013; Lamason and Welch, 2017).

There have also been modifications to the model, particularly in the recycling steps. While cofilin is a key recycling protein, additional proteins accelerate cofilin-mediated recycling significantly, including Aip1, coronin, and Srv2/CAP (Balcer *et al.*, 2003; Mohri and Ono, 2003; Nadkarni and Brieher, 2014). Although cofilin can accelerate debranching through actin-filament side-binding (Chan *et al.*, 2009), additional debranching mechanisms through GMF (Gandhi *et al.*, 2010; Haynes *et al.*, 2015) and coronin 1B (Cai *et al.*, 2008) have also been shown. The arpin protein is another Arp2/3 complex inhibitor (Dang *et al.*, 2013). The role of profilin in nucleotide exchange may not be universal, since profilin from plants does not accelerate nucleotide exchange measurably (Perelroizen *et al.*, 1996) and budding yeast profilin catalyzes nucleotide exchange significantly more slowly than human profilin 1 (Eads *et al.*, 1998; Wen *et al.*, 2008). Another nucleotide exchange protein, Srv2/CAP, might mediate nucleotide exchange in certain situations (Balcer *et al.*, 2003; Mattila *et al.*, 2004). Finally, it is clear that, in some systems, other filament populations exist at/near the lamellipodial leading edge (Ponti *et al.*, 2004; Giannone *et al.*, 2007; Kage *et al.*, 2017).

Two aspects of the dendritic nucleation model have contributed to these subsequent discoveries. First, the model has served as a very well-defined scaffold on which to predict and test new hypotheses. Second, it has served as a conceptual framework for discovery in the actin field and beyond. From personal experience, the work conducted on formin-mediated actin polymerization has used the prior Arp2/3 complex studies as a blueprint for elucidating the activities and functional partners of these actin-binding proteins.

Finally, the dendritic nucleation model has not been without controversy, with the fundamental disagreement being about whether branched actin filaments are actually present in cells (Small, 2010). While feelings were very strong during this debate, it was highly appropriate as a means of cross-checking the model, in the true spirit of scientific research. In an event rarely seen in science, the debate ended abruptly with a very gracious acknowledgement of cellular branched filaments by the principal opponent of the model (Small *et al.*, 2011). All in all, a fitting ending to the story of an extraordinary discovery.

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