How distinct Arp2/3 complex variants regulate actin filament assembly

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The heptameric Arp2/3 complex generates branched actin filament networks driving lamellipodium protrusion, vesicle trafficking and pathogen motility. Distinct variants of the Arp2/3 complex are now shown to have deviating activities in tuning actin assembly and disassembly, in concert with the prominent actin regulators, cortactin and coronin.

Since its discovery more than two decades ago[1], the Arp2/3 complex has been a central focus in the field of cytoskeleton biology, and has revolutionized our views on how actin filament nucleation and turnover is controlled in cells. It was the nucleating agent employed for the first time in *in vitro* reconstitution of actin-based motility[2], and constitutes one of the most prominent actin assembly factors usurped by bacterial or viral pathogens. In analogy to pathogenic bacteria such as *Listeria* and *Shigella* or to baculovirus inside infected cells, Vaccinia virus can employ Arp2/3 complex to form actin tails at the plasma membrane, a process that is essential for viral spread. In both physiological and pathogenic processes, the Arp2/3 complex must be activated by nucleation promoting factors (NPFs), such as members of the Wiskott Aldrich Syndrome protein (WASp) and Scar/WAVE families. Whereas *Listeria* or baculovirus express their own, pathogen-derived NPFs, *Shigella* and Vaccinia virus are among those that employ the ubiquitously expressed, host-derived N-WASP (neural WASP)[3-5]. In the case of Vaccinia virus, N-WASP is recruited downstream of a signalling cascade that mimics receptor tyrosine kinases by recruiting src-homology 2 (SH2)- and SH3-domain-containing proteins such as Grb2, Nck1 and Nck2 downstream of the viral SH2-ligand A36R[6]. This type of constitutive, virus-induced, actin-based motility contains only a few essential components, including N-WASP[5] and the Arp2/3 complex[7] (which likely accounts for the robustness of using this system to quantitate actin-based motility), but has the potential to be further modulated by additional actin-binding proteins in the host. Way and colleagues now report that the actin-based motility of Vaccinia virus is tuned by the isoform composition of the Arp2/3 complex, in concert with additional modulatory factors[7].

In general, Arp2/3 complex-dependent actin networks such as lamellipodia or pathogen tails are not static structures. Instead, both actin filaments in the network and Arp2/3 complexes residing at branches undergo continuous turnover within the structure, which can be considered as a cycle powered by specific protein activities (Fig. 1a). In this cycle, NPF-

mediated Arp2/3 complex activation is followed by branch formation, its stabilization by cortactin, and branch destabilization and filament disassembly by coronin and cofilin. In contrast to the activities exerted by NPFs and Arp2/3, functions arising from cortactin and coronin family members are not essential[7, 8], but enable cells to tune the efficiency of formation and turnover of a given Arp2/3-dependent structure. Actin disassembly, mediated for instance by cofilin family members, is expected to be essential both *in vitro*[2] and *in vivo*, but the relative functions of these proteins compared to other filament severing or disassembly factors remains to be established.

The cycle of branched actin network assembly and disassembly that has emerged over time from the work of numerous laboratories has been developed assuming that the Arp2/3 complex exists as single functional species, prone to activation (mentioned above) and also inhibition[9]. The work of Way and colleagues now fundamentally changes this view, by demonstrating that Arp2/3 should be considered as a set of distinct complexes (in theory numbering up to 8 in mammalian cells), each of which may exert fundamentally divergent activities *in vivo*. The current study focuses on the ArpC1 and ArpC5 subunits of the complex, each of which exists as two isoforms with high and low activity, respectively. All four combinations of complexes with distinct ArpC1 and ArpC5 subunit composition were found to be expressed in cells, and to varying relative extents in different cell types. Generation of recombinant complexes *in vitro* and assessment of their respective activities in pyrene-actin assembly assays proved that subunit composition alone, independent of interaction with additional actin binding proteins, can determine actin assembly efficiency. As a consequence, knockdown of high and low activity subunits (for simplicity from now on referred to as Arp2/3^{high} and Arp2/3^{low}) in cells generates slow and fast Vaccinia virus motility, correlating with short and long tails, respectively (Fig. 1b), whereas forced expression of mixed complexes generated no significant phenotype. Surprisingly, however, Arp2/3^{high} complexes

displayed low intrinsic stability of branches *in vitro*, whereas Arp2/3^{low}-generated branches were more stable, stimulating the authors to explore the *in vivo* effects of Arp2/3 complex composition on the activities of branch stability regulators, such as cortactin and coronin.

The authors tested Vaccinia motility, actin tail length and velocity by depleting Arp2/3^{high} and Arp2/3^{low} complex components in multiple distinct combinations, in the presence or absence of cortactin and coronin. Among their many remarkable results, the authors observed that knocking down coronin 1B or 1C (which are found to operate in a complex) increased actin tail length, consistent with an established function in promoting tail disassembly through debranching and/or disassembly. However, no such increase was observed if tails were already long due to their generation by Arp2/3^{high} complexes. Hence, the presence of coronin does not seem to affect this type of branches (Fig. 1c). The long-tail effect of coronin knockdown was reverted to control levels when cortactin was also removed, indicating that the coronin loss of function phenotype required cortactin. This and additional results led the authors to propose that cortactin directly impacts on coronin function at Arp2/3 complex branches (compare Fig. 1a).

The complexity is increased further when focusing on the contribution of cortactin to both tail length and velocity. In brief, cortactin knockdown had no effect on tail lengths when looking at wild type cells expressing mixtures of Arp2/3 complex variants (Fig. 1c), reminiscent of the lack of evident changes in lamellipodial dimension and structure in cortactin-deficient fibroblasts[10]. However, cortactin knockdown in cells depleted of Arp2/3^{high} and Arp2/3^{low} complexes converted the short tails of the former and long tails of the latter to a regular length (Fig. 1c). These findings indicate that the relevance of cortactin for Arp2/3-mediated actin structures depends on Arp2/3 complex composition. But how can these effects be explained? Aside from its function in regulating coronin's debranching activity, the authors

propose that cortactin selectively stabilizes Arp2/3^{high} complex branches over Arp2/3^{low} ones. This is an attractive hypothesis, which would be worth confirming with additional experiments.

Finally, combination of Arp2/3 complex variant and cortactin depletions revealed that tail length and velocity do not always correlate. Specifically, cortactin knockdown increases tail velocity (but not length) in wild-type cells, but has no further effect on increased tail velocities induced by Arp2/3^{low} complex removal or reduced tail velocities caused by Arp2/3^{high} complex removal (Fig. 1c). This is even more remarkable given the multitude of potential activities ascribed to cortactin including (aside from branch stabilization and coronin regulation) the promotion of N-WASP dissociation from the Arp2/3 complex[11]. Interestingly, increased actin assembly rates and Arp2/3 complex flux were previously also observed in cortactin-deficient lamellipodia[10], which are Arp2/3-[12, 13] but not N-WASP-dependent[4, 5], indicating that those effects are independent of the NPF at play. Inspecting those velocity data in isolation, it is tempting to speculate that cortactin specifically selects for the preferred usage of Arp2/3^{high} and Arp2/3^{low} complexes over mixed ones, but independent experiments would be needed to solidify such ideas.

Cortactin was thought for years to be essential for processes involving Arp2/3 complex function, such as lamellipodia formation or receptor-mediated endocytosis[14], and the finding that it was dispensable for these processes[10] was quite surprising, albeit consistent with earlier observations e.g. using *Drosophila* genetics[15]. The work by Way and colleagues now sheds new light on seemingly conflicting previous reports concerning the mechanistic functions of this branched actin network regulator, and on coronin. The authors also reveal that combined action, or combined loss of simple biochemical activities established *in vitro* can rapidly lead to complex outcomes *in vivo*, a phenomenon often not adequately considered in current cell biology. Moreover, the present data indicate that cortactin and coronin are able to 'distinguish' between Arp2/3 complex variants, at least indirectly - meaning that it is the Arp2/3 complex

6

composition in a cell type and/or tissue that determines whether or not coronin and cortactin affect the efficiency of a given Arp2/3-driven process. This insight will be of utmost significance for efforts aimed at dissecting the precise functions of these proteins in Arp2/3-dependent actin networks.

In conclusion, the present work represents a landmark in our understanding of the Arp2/3 complex and its interplay with its modulators. Future results on Arp2/3 complex regulators will have to consider the potential impact of Arp2/3 complex composition, irrespective of the process studied.

COMPETING FINANCIAL INTERESTS

The authors declare no financial interest.

FIGURE LEGENDS

Fig. 1: Arp2/3 complex composition determines efficiency of actin-based Vaccinia virus motility

(a) Cycle of Arp2/3-mediated branch formation and turnover. Arp2/3 complex-mediated branching of actin filaments and their turnover is initiated by an active NPF, such as N-WASP, e.g. acting at the membrane below Vaccinia virus particles, and modulated by cortactin and coronin by various means, as indicated. Green and red arrow indicates association and dissociation to the cycle of respective actin regulator, respectively.

(b) Cells express mixtures of Arp2/3 complex variants, and their respective levels and activities drive the formation of Vaccinia virus actin tails with distinct efficiencies, as established by Way and colleagues. *In vitro* assembly rates of Arp2/3 complex variants and resulting branch stabilities are also provided for comparison (right panel).

(c) Summary of the effects of Arp2/3 complex variant, cortactin and coronin knockdown combinations performed by Way and colleagues on actin tail velocities and lengths. Colour code is given on the right; Arp2/3^{high} and Arp2/3^{low} denotes knockdown of respective Arp2/3 complex variant; Cttn: cortactin, Coro: coronin. Note that combined knockdown of Arp2/3^{high} and Arp2/3^{low} complex variants is deduced to abolish tails (0), in analogy to single-gene subunit knockdown[7].

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