The Diverse Family of Arp2/3 Complexes.

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Abstract

The Arp2/3 complex has so far been considered to be a single seven-subunit protein complex required for actin nucleation and actin filament polymerization in diverse critical cellular functions including phagocytosis, vesicular trafficking, lamellipodia extension and cytokinesis. The Arp2/3 complex is also exploited by bacterial pathogens and viruses during cellular infectious processes. Three recent studies suggest that some subunits of the complex are dispensable in specific cellular contexts, pointing to the existence of alternative Arp2/3 complexes containing other components such as vinculin or α-actinin, as well as different isoforms or phosphorylation variants of canonical Arp2/3 subunits. This diversity should be considered when assigning specific Arp2/3 assemblies to different actin-dependent cellular processes.

Main Text

Introduction

The actin cytoskeleton is one of the main components of eukaryotic cells, not only providing the molecular basis for cellular morphogenesis and migration, but also participating dynamically in mechanical resistance to deformation, uptake of extracellular material, intracellular vesicular transport, cytokinesis and cell adhesion. The actin cytoskeleton also participates in the organization of complex cellular structures such as filopodia, lamellipodia and podosomes [1,2].

Polymerization of actin monomers into actin filaments requires the activity of cellular actin nucleators. The Arp2/3 complex, the first nucleator identified in eukaryotic cells, plays a central role in many cellular processes and is highly conserved from trypanosomes to the fission yeast and humans [3-5]. Other nucleators include formins, Spire, Cordon-bleu (COBL) and Leiomodins [6].

The Arp2/3 complex is composed of seven subunits [7] and it has been traditionally considered as a single entity, associated with the vast majority of cellular processes in which its function is required and has been studied. Three recent studies [8-10] in the same mammalian cell system reveal that diverse Arp2/3 complexes may regulate different cellular and pathogen-associated functions, raising the interesting possibility that Arp2/3 complex compositions may have been overlooked, paving the way for the identification of novel complexes associated to different actin polymerization-mediated processes.

Discovery and functions of the classical Arp2/3 complex

The Arp2/3 complex was first isolated from *Acanthamoeba castellanii* during a search for ligands of the actin-binding protein profilin [11]. It contained seven proteins: the actin-related proteins Arp2 (44-kD) and Arp3 (47-kD) considered as 'unconventional actins', together with a 40-kD protein similar to a WD40 ß-propeller protein from *Dictyostelium discoideum,* and four additional proteins of 35-, 19-, 18- and 13-kD [11]. Subsequently, the Arp2/3 complex was also identified and associated with actin-rich structures in the fission yeast *Schizosaccharomyces pombe* [12] and in the budding yeast *Saccharomyces cerevisiae* [13]. In human cells, the Arp2/3 complex consists of Arp2 and Arp3, together with the Arp complex subunits ARPC1, ARPC2, ARPC3, ARPC4 and

ARPC5 [14]. While consensus exists concerning the Arp2 and Arp3 nomenclature, different names have been used in the literature concerning the other Arp2/3 complex subunits: a nomenclature proposal across species is presented in **Table 1**.

The function of the Arp2/3 complex was shown for the first time to be critical in triggering actin polymerization when it was isolated from a subcellular fraction of human platelets that sustained actin assembly by the bacterial pathogen *Listeria monocytogenes* [15]. The *L. monocytogenes* surface protein ActA activates the Arp2/3 complex to initiate actin polymerization, and was the first actin nucleation promoting factor (NPF) to be identified [16,17]. Several mammalian NPFs were subsequently identified, including WASP [18], N-WASP [19], Scar/WAVE [20], and cortactin [21] (see **Text Box 1**). The purified *A. castellanii* Arp2/3 complex was shown to nucleate the formation of actin filaments at 70° from other filaments [22]. A series of elegant microscopy and biochemistry investigations then definitively established the key role of Arp2/3 in actin polymerization and the formation of branched structures [23,24] (see **Text Box 2**).

As mentioned above, the function of the Arp2/3 complex is subverted by bacterial pathogens at different stages of their infectious processes [25]. The Gram-positive pathogen *L. monocytogenes* uses Arp2/3 not only to mediate intra- and inter cellular movements but also to trigger cellular invasion [26-28]. The Gram-negative pathogen *Shigella flexneri* also requires Arp2/3 function for actin-based motility [29] and for bacterial internalization within host cells [30]. Interestingly *S. flexneri* does not express an ActA-like protein but instead recruits on its surface, via the protein IcsA/VirG, the NPF N-WASP which in turn activates Arp2/3 to mediate actin-based motility [29,31]. The Gram-negative bacteria *Rickettsia parkerii* and *R. conorii* activate Arp2/3 during early stages of bacterial intracellular motility via a protein called RickA [32-34]. Moreover, *R. parkerii* requires Arp2/3 activity to invade diverse host cells [35]. Vaccinia virus is able to move at the surface of cells on actin-based structures [36], which requires the function of the Arp2/3 complex [37]. Other bacteria including Mycobacteria [38] and *Burkholderia thailandensis* also move via an actin-based motility requiring Arp2/3 functions [39-42].

In *S. pombe* and *S. cerevisiae,* deletions of genes encoding each of the subunits of the Arp2/3 complex cause severe growth defects or lethality [43,44], suggesting a major role for all subunits *in vivo*. In particular, Arp2/3 had been shown to be important for the formation and function of cortical actin patches where clahtrin-mediated endocytosis takes

place [13]. Mammalian Arp2/3 complex was localized to regions of lamellipodial protrusion [14,45] and together with cofilin and other actin-binding proteins, was shown to control the organization and tread-milling of actin filaments in lamellipodia [23]. The Arp2/3 complex has been associated to other cellular functions requiring actin polymerization including phagocytosis [46], trafficking within and from the Golgi apparatus [47] as well as formation of focal adhesions [48]. The critical role of Arp2/3 in humans is highlighted by the Wiskott-Aldrich syndrome (WAS), a recessive X-linked genetic disorder characterized by mutations in the WAS protein (WASP), which is characterized by defects in the actin-rich immunological synapse between T cells and antigen presenting cells, leading to severe defects in immunological responses [49,50].

Initial detailed analysis of the contribution of each Arp2/3 complex subunit to actin polymerization, using *L. monocytogenes* ActA as a NPF in a baculovirus expression system in insect cells, indicated that only Arp2 and Arp3 are directly involved in actin polymerization, the role of the other subunits being less clear [51]. More recent structural evidence [52] confirms an initial prediction that ARPC2 and ARPC4 provide the main surface for interaction of the complex with the mother actin filament [51]. ARPC3 is proposed to form a bridge between Arp3 and the mother actin filament [52] but complexes lacking ARPC3 display minor functional defects [44,51]. While ARPC1 is supposed to make only minor contacts with the mother actin filament [52], complexes lacking this subunit are far less effective in actin nucleation, suggesting additional roles for ARPC1 including binding of NPFs [53]. ARPC5 was proposed to tether Arp2 to the rest of the complex [52].

Several reports also suggest a functional role played by phosphorylation of different subunits of the Arp2/3 complex. ARPC1 phosphorylation by p21-activated kinase (Pak1) was reported to be crucial for mammalian cell motility [54]. It has been suggested that Arp2 phosphorylation is required and critical for Arp2/3 complex binding to the pointed end of actin filaments and actin nucleation in cultured *Drosophila* cells [55], but mutation of the phosphorylated residues had only subtle effects on motility in *Dictyostelium* [56]. As shown recently, phosphorylation of Arp3 by the *Legionella pneumophila* kinase LegK2 inhibits actin polymerization at the surface of bacterial-containing phagosomes [57].

Several subunits of the Arp2/3 complex (i.e. Arp3, ARPC1 and ARPC5) display more than one isoform [14], but the functional significance of these variants had not been

investigated in detail. While the major subunit Arp3 is detected in all tissues, a gene encoding the isoform ARP3β was detected predominantly in brain neuronal cells and was proposed to play a role in the development and/or maintenance of nerve cells [58]. Two variants of ARPC1 presenting 70% homology had been known for long [12,45] and a mutation in the gene *ARPC1A* was shown to impact cell migration and invasion in pancreatic cancer [59]. ARPC5 was also found to display a second isoform, named ARPC5B, which exhibited a regular expression in many tissues but with the highest levels in the brain, while the original ARPC5A was found highly enriched in the spleen and thymus [60].

Diversity of Arp2/3 complexes

Focal adhesions. Association of the Arp2/3 complex to focal adhesions in human skin cells had previously been shown to require interactions with vinculin [48]. A recent native mass spectrometry analysis of proteins extracted from the dense plaques (focal adhesion homologous structures) of chicken smooth muscle revealed surprisingly that Arp2/3 complexes present in these structures, as inferred from mass spectrometry results, are actually 'hybrid complexes', consisting of a core composed of Arp2, Arp3 and ARPC2, together with α-actinin and vinculin, or Arp2, Arp3, ARPC2, ARPC3 and vinculin [8]. This study therefore supported, for the first time, the notion that alternative Arp2/3 complexes that do not consist of the seven classical subunits are involved in specific cellular processes. Notably, these alternative complexes contain vinculin that can mediate the recruitment of the complex to focal adhesions and compete with ARPC1B in HeLa cells; knock-down of ARPC1B has therefore a positive effect on focal adhesion and stress fiber formation, as the equilibrium is shifted towards Arp2/3-vinculin hybrid complexes formation [8].

***Listeria monocytogenes* infection.** Specific roles for ARPC1A and ARPC1B were recently identified in human genome-wide RNA interference (RNAi) screens investigating HeLa cell infection by *L. monocytogenes* [9]. Knock-down of ARPC1B but not of ARPC1A significantly diminished bacterial entry, highlighting a critical contribution for ARPC1B function in this context. Moreover, it was observed that ARPC4 and ARPC5 subunits do not contribute to *L. monocytogenes* cellular invasion. The contribution of the different Arp2/3 subunits to bacterial actin-tail formation was also studied, identifying a major contribution of Arp2, Arp3, ARPC1A, ARPC2, ARPC3 and ARPC4 in *L. monocytogenes*

actin-based motility, but no role for ARPC1B nor ARPC5 was found [9]. These results therefore show not only that ARPC5 is dispensable for both bacterial entry and actin-tail formation but also that ARPC1 isoforms contribute to different cellular processes during *L. monocytogenes* infection, at least in HeLa cells. ARPC4 was found dispensable for bacterial entry, but taking into account the central place of this subunit in Arp2/3 complex function according to previous functional and structural results [51,52], it is possible that residual ARPC4 upon RNAi treatment suffices for partial complex function.

Vaccinia virus mobility. In a recent study of actin polymerization by Vaccinia virus, specific roles for ARPC1B and ARPC5B have been found [10]. Indeed, it has been observed that Arp2/3 complexes containing ARPC1B and ARPC5B (named ARPC5L in this work) are significantly more efficient at promoting actin assembly than those containing ARPC1A and ARPC5A. Actin networks induced by complexes containing the subunits ARPC1B and ARPC5B were found more stable since in the presence of these specific subunits, cortactin stabilizes the Arp2/3 complexes against coronin-mediated disassembly [10].

Overall, these three reports indicate that only Arp2, Arp3 are directly partaking in the actin nucleating activity of the complex, together defining an actin nucleation core module, whereas the other subunits serve alternative roles such as determining the efficiency of actin nucleation, localization of the complex, as well as serving as an auto-inhibitory mechanism [8,51,52]. Together, the other subunits thus define the regulatory module of the Arp2/3 complex. In the case of the *L. monocytogenes* model, it is interesting to mention that vinculin inactivation by RNAi did not perturb bacterial cellular invasion nor actin-based motility [9], raising the possibility that other cellular molecule(s) not yet identified may participate to the localization/modulation of the Arp2/3 complex during *L. monocytogenes* infection-related processes.

Concluding Remarks

While the Arp2/3 complex has been classically considered as a single molecular entity for 20 years since its discovery, an emerging possibility from recent research suggests that multiple versions of the Arp2/3 complex may co-exist in cells (**Figure 1** presents a summary of currently described complexes and their mode of regulation).

Indeed, the subunits ARPC1, ARPC3, ARPC4 and ARPC5 can be replaced by vinculin and α-actinin in focal adhesions [8].

In the case of the *L. monocytogenes* system, even if dispensable, ARPC5 can be detected at both bacterial entry sites and actin comet tails by fluorescence microscopy, indicating that Arp2/3 complexes containing this subunit, while not required, may still be recruited during both processes [9]. It is possible that Arp2/3 complexes of different composition have overlapping functions during *L. monocytogenes* infection, but current data suggests that the precise composition of different Arp2/3 complexes plays a role in fine-tuning actin rearrangements in both instances. This hypothesis is supported by the observation that ARPC4 can be found predominantly early during bacterial actin comet tail formation and that knock down of ARPC4 affects initial actin polymerization at the bacterial surface rather than actin tail elongation indicating that different Arp2/3 complexes may be required in a sequential manner. The fine tuning of Arp2/3 complex actin polymerization activity depending on the subunit composition is also supported by results on the Vaccinia virus system [10].

Overall, the reports discussed herein point to the possibility that Arp2/3 is a natural modular nano-machine, capable of regulating its activity via replacement of its subunits.

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Figure

Figure 1: Diversity of Arp2/3 complexes. Central circle (gray): the canonical 7-subunit form. Top left (blue): Arp2/3 complex variants used by Vaccina virus (alternative subunits are enclosed by a red line). Many combinations of Arp2/3 subunits are recruited by the virus. Bottom left: two “hybrid complexes”, containing the actin nucleation core and vinculin, or vinculin plus α-actinin, which presumably localize the complex to focal adhesions. Bottom right: Arp2/3 complexes hijacked by *L. monocytogenes* during cellular infection (alternative subunits are enclosed by a red bold line, dispensable subunits are enclosed by a red pointed line). Top right: variations in Arp2/3 complexes caused by phosphorylation of specific subunits. The effect of the subunit substitution on the actin nucleation activity is color coded (red: reduce; green: enhance).

Table

Table 1: Arp2/3 complex nomenclature

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