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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 and lamellipodia extension. The Arp2/3 complex is also exploited by bacterial pathogens and viruses during cellular infectious processes. Recent studies suggest that some subunits of the complex are dispensable in specific cellular contexts, pointing to the existence of alternative 'hybrid 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. Therefore, this diversity should be now considered when assigning specific Arp2/3 assemblies to different actin-dependent cellular processes.

Introduction

The actin cytoskeleton is a main component of eukaryotic cells, that not only provides the molecular basis for cellular morphogenesis and migration, but also participates dynamically in mechanical resistance to deformation, uptake of extracellular material, intracellular vesicular transport 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].

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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. 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 possibility that Arp2/3 complex compositions may have been overlooked, and 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 β-propeller protein from *Dictyostelium discoideum*, and four additional proteins of 35 kD, 19 kD, 18 kD 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 other Arp2/3 complex subunits: a nomenclature update 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*** (see Glossary) [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], and Scar/WAVE [20] (see Box 1). The purified *A. castellanii* Arp2/3 complex was shown to nucleate the formation of actin filaments at 70° from other filaments [21]. 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 [21–23] (see Box 2).

As mentioned above, the function of the Arp2/3 complex is subverted by bacterial pathogens at different stages of their infectious processes [24]. The Gram-positive pathogen *L. monocytogenes* uses Arp2/3 not only to mediate intracellular and intercellular movements but also to trigger cellular invasion [25–27]. The Gram-negative pathogen *Shigella flexneri* also requires Arp2/3 function for actin-based motility [28] and for bacterial internalization within host cells [29]. 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 [28,30]. The Gram-negative bacteria *Rickettsia conorii* and *Rickettsia parkerii* activate Arp2/3 during early stages of bacterial intracellular motility via a protein called RickA [31–33]. Moreover, *R. conorii* and *R. parkerii* require Arp2/3 activity to invade diverse host cells [34,35]. **Vaccinia virus** is able to

move at the surface of cells on actin-based structures that resembles pedestals [36], which requires the function of the Arp2/3 complex [37]. Other bacteria including *Mycobacterium marinum* [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 clathrin-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 treadmilling of actin filaments in lamellipodia [22]. 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 cryo-electron microscopy [51], X-ray crystallography [52] and biochemical reconstitution [53] studies identified major roles played by each subunit of the Arp2/3 complex, which have been complemented by electron tomography of the branch junction [54]. Arp2 and Arp3 are folded like actin and form the first two subunits of the daughter filament [51,52]. A dimer of ARPC2 and ARPC4 forms the structural backbone of the complex [52,54] which provides the main surface for interaction of the complex with the mother actin filament [53]. ARPC3 is proposed to form a bridge between Arp3 and the mother actin filament [54] and increases the efficiency of nucleation [53], but complexes lacking ARPC3 display minor functional defects [44,53]. While ARPC1 is supposed to make only minor contacts with the mother actin filament [54], complexes lacking this subunit are far less effective in actin nucleation, suggesting additional roles for ARPC1 including binding of NPFs [55]. ARPC5 was proposed to tether Arp2 to the rest of the complex [54].

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 [56]. 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 [57], but mutation of the phosphorylated residues had only subtle effects on motility in *Dictyostelium* [58]. As shown recently, phosphorylation of Arp3 by the *Legionella pneumophila* kinase LegK2 inhibits actin polymerization at the surface of bacterial-containing phagosomes [59].

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 has not been investigated in detail. While the major subunit Arp3 is detected in all tissues, a gene encoding the

isoform ARP3β has been detected predominantly in brain neuronal cells and was proposed to play a role in the development and/or maintenance of nerve cells [60]. 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 [61]. ARPC5 was also found to display a second isoform, named ARPC5B, expressed constitutively in many tissues but with the highest levels in the brain, while the original ARPC5A was found highly enriched in the spleen and thymus [62].

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 detected Arp2/3 complexes consisting of a core composed of Arp2, Arp3 and ARPC2, together with α-actinin and vinculin, or Arp2, Arp3, ARPC2, ARPC3 and vinculin, that is, 'hybrid complexes' [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, which can mediate the recruitment of the complex to focal adhesions and compete with ARPC1B in HeLa cells. Accordingly, knock-down of ARPC1B had a positive effect on focal adhesion and stress fiber formation, in line with an equilibrium shifted towards formation of Arp2/3-vinculin hybrid complexes [8].

Listeria monocytogenes Infection

L. monocytogenes, as mentioned above, uses the Arp2/3 complex for entry into cells and for actin-based motility. 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]. Knockdown of ARPC1B but not of ARPC1A significantly diminished bacterial entry, while inactivation of ARPC1A had a more profound impact on actin tail formation than inactivation of ARPC1B. The use of small interfering RNAs targeting the subunits ARPC5A (the product of the *ARPC5* gene) and ARPC5B (the product of the *ARPC5L* gene) did not alter bacterial entry nor actin tail formation, suggesting that the ARPC5 subunits are dispensable for both cellular processes. ArpC4 was also shown to be dispensable for *L. monocytogenes* early invasion of host cells [9]. At this stage, taking into account the central place of the ARPC4 subunit in the Arp2/3 complex function according to previous functional and structural results [53,54], it is not possible to exclude that residual ARPC4 levels may explain the absence of phenotype upon anti-ARPC4 RNAi treatment during bacterial entry.

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 are significantly more efficient at promoting actin assembly than those containing ARPC1A and ARPC5A. Interestingly, 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 confirm that the subunits Arp2 and Arp3 play a critical role as an actin nucleation core module, whereas the role of the other subunits is regulatory, determining the efficiency of actin nucleation as well as localization of the complex [8,53,54]. In the case of the *L. monocytogenes* model, it is interesting to mention that vinculin inactivation by siRNA did not perturb bacterial cellular invasion nor actin-based motility [9], raising the possibility that other cellular molecule(s) not yet identified participate in 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 coexist in cells (see Outstanding Questions; Figure 1, Key Figure, presents a summary of currently described complexes and their mode of regulation). Differential expression of the Arp2/3 complex subunits in various cells may drive this diversity.

In the case of the *L. monocytogenes* system, in wild-type cells the subunit ARPC5 can be detected at both bacterial entry sites and actin comet tails by fluorescence microscopy, indicating that although ARPC5 is dispensable, Arp2/3 complexes containing this subunit may still be functional 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 cases. This hypothesis is supported by the observation that ARPC4 can be found predominantly early during bacterial actin comet tail formation and that knockdown 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]. It is highly anticipated that other examples of diverse Arp2/3 complexes in other systems will soon be reported.

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Box 1

Activation of the Arp2/3 Complex by NPFs

The Arp2/3 complex is by itself an inefficient actin nucleator, and requires the binding of nucleation promoting factors (NPFs) to stimulate its nucleation activity. While preformed actin filaments are also Arp2/3 complex activators [20], the most efficient NPFs include the bacterial surface proteins ActA and RickA from *L. monocytogenes* and *R. conorii* respectively [15,33,61] as well as the eukaryotic WASP (Wiskott–Aldrich syndrome protein) [62], SCAR/WAVE (suppressor of cyclic AMP repressor/WASp-family verprolin-homologous protein) [20], WASH (WASP and Scar homologue) [63], WHAMM (WASP homolog associated with actin, membranes and microtubules) [64], and JMY (junction mediating and regulatory protein, p53 cofactor) [65]. These NPFs characteristically display a WCA domain (W: WASP-homology 2 domain or WH2, C: Connector, A: Acidic; this domain is also known as VCA domain: Verprolin-Connector and Acidic-rich) that increases the affinity of Arp2/3 complex for the mother filament, activating the complex [4,66]. NPFs like ActA and WASP dimerize to deliver actin monomers to the Arp2/3 complex [67]. NPFs dissociate from the Arp2/3 complex and may participate in multiple rounds of activation [68].

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Box 2

Actin Branching by the Arp2/3 Complex

The Arp2/3 complex generates new actin filaments that branch-off from the side of pre-existing filaments at a 70° angle to form a Y-branched network [23]. The use of electron tomography to reconstruct the branch junction suggested that the Arp2 and Arp3 subunits reorganize into a dimer, providing a template for elongation of the daughter filament [52]. The remaining subunits, in particular ARPC2 and ARPC4, were reported to make substantial contacts with the mother filament and allow the anchoring of Arp3 as the first subunit of the daugther filament [52]. Subsequent mutagenesis studies have confirmed a role for ARPC2 and ARPC4 in providing an actin-filament-binding interface which is critical for nucleation and branch stability [69] and suggest for the yeast Arc40 (ARPC1 in mammalian cells, see nomenclature in Table 1) multiple essential roles, including suppression of spontaneous nucleation by the Arp2/3 complex and propagation of NPF activation signals [70].

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Trends

Mass spectrometry analysis of proteins extracted from focal adhesions identified two Arp2/3 hybrid complexes: the first is composed of Arp2, Arp3, ARPC2 and ARPC3 together with vinculin; the second is composed of Arp2, Arp3, ARPC2 and vinculin together with α-actinin.

Functional studies of Arp2/3 complex subunits during *Listeria monocytogenes* cell invasion and actin-based motility suggest that diverse complexes participate at each infection stage. The subunit ARPC1B is predominantly required for cellular entry while the subunit ARPC1A is predominantly required for intracellular actin polymerization. Moreover, the subunit ARPC5 is dispensable for both processes.

Arp2/3 complexes containing the subunits ARPC1B and ARPC5B are more efficient at promoting actin assembly by vaccinia virus than complexes containing ARPC1A and ARPC5A subunits.

Post-translational modifications of different subunits affect the efficiency of Arp2/3 complex activity. Phosphorylation of Arp2 and ARPC1B enhances actin nucleation, while phosphorylation of Arp3 attenuates it.

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Glossary

Focal adhesions: large multiprotein complexes that act as transmembrane links between the extracellular matrix and the actin cytoskeleton. The protein network associated to integrin receptor that bridges between the extracellular matrix and the termini of actin stress fibers is called ‘the adhesome’.

Listeria monocytogenes: a Gram-positive bacterium responsible for a food-borne disease named listeriosis, which can lead to meningitis in newborns and abortions in pregnant women. *L. monocytogenes* is the prototype intracellular pathogen, inducing its internalization within nonphagocytic cells, lysing its phagosome and using an actin-based motility system to promote cell-to-cell spread.

Nucleation promoting factors (NPFs): factors that activate the Arp2/3 complex to polymerize actin above the basal nucleation rate threshold displayed by the native complex. The activities of the NPFs are regulated by signal transduction pathways that coordinate actin cytoskeleton polymerization in time and space.

Vaccinia virus: a prototypic poxvirus closely related to variola virus, the causative agent of smallpox. Poxviruses are enveloped DNA viruses that have the particular ability to exist in two infectious forms: mature virions are viral particles contained within a single membrane, while extracellular virions are contained within two concentric membranes. Vaccinia virus particles are propelled on the tip of actin tails at the surface of infected host cells.

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Outstanding Questions

The regulation of the Arp2/3 complex diversity in cells is unclear at present. Available subunits could determine the composition of the complexes in some cellular contexts. No evidence exists for subunit exchange in already formed Arp2/3 complexes, but this possibility can not be formally excluded.

Which other molecules may be associated with canonical subunits of the Arp2/3 complex? A future challenge will be to isolate and characterize the various Arp2/3 complexes, and assess their activity *in vitro*.

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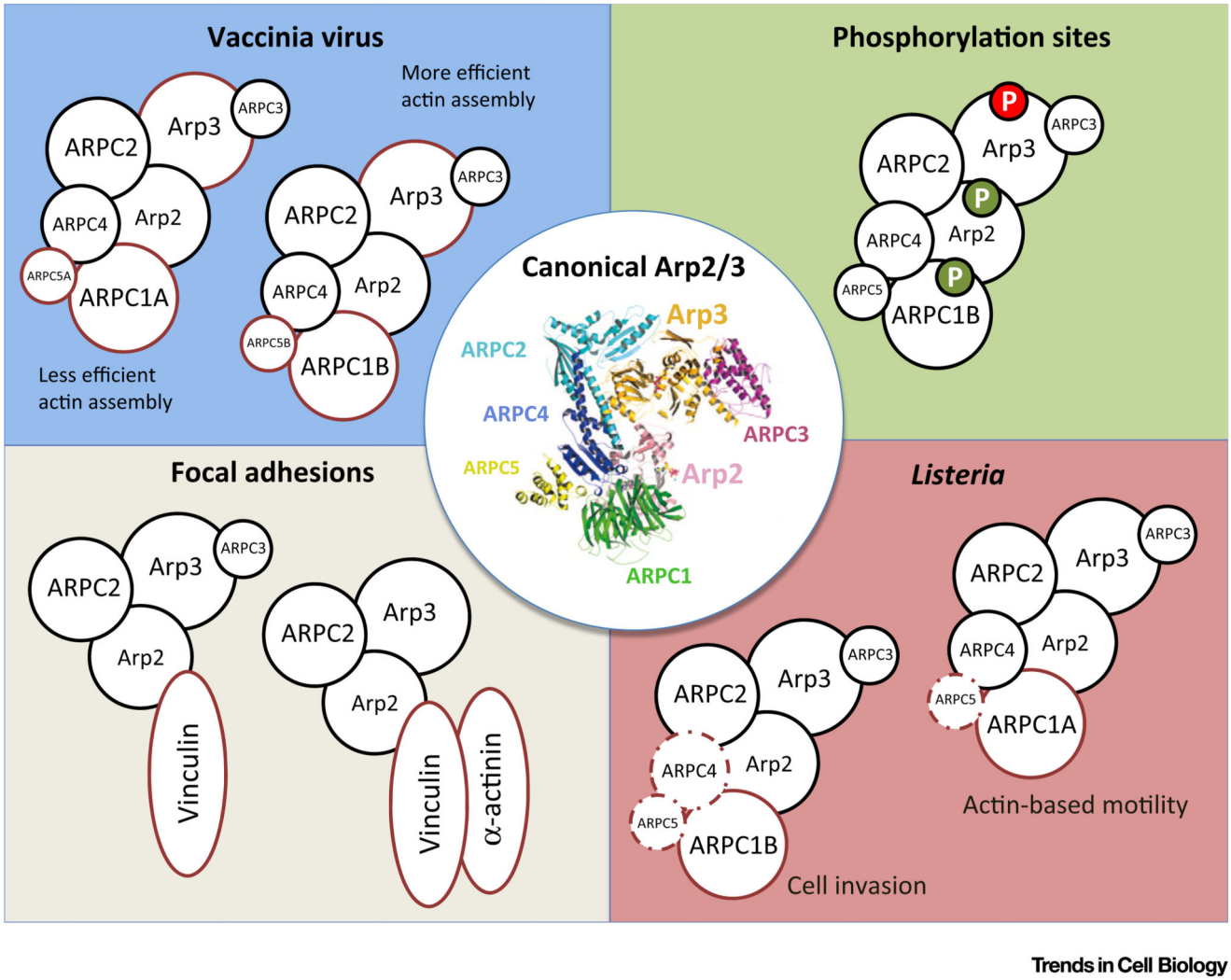

Figure 1. Key Figure: Diversity of Arp2/3 Complexes

Central circle (white): ribbon diagram of the canonical 7-subunit Arp2/3 complex [63]; copyright (2004) National Academy of Sciences, U.S.A. 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).

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Table 1

Arp2/3 Complex Nomenclature

Common Vertebrate Name	***Homo sapiens***		***Mus musculus***		***Danio rerio***		***Drosophila melanogaster***		***Caenorhabditis elegans***	***Leishmania major***	***Saccharomyces cerevisiae***	***Arabid thalian***
Arp2	Arp2		Arp2		Arp2	Arp2A Arp2B	Arp2		Arp2	Arp2	Arp2	Arp2
Arp3	Arp3	Arp3A Arp3B	Arp3	Arp3A Arp3B	Arp3	Arp3A Arp3B	Arp3		Arp3	Arp3	Arp3	Arp3
p41	ARPC1	ARPC1A ARPC1B	ARPC1	ARPC1A ARPC1B	ARPC1	ARPC1A ARPC1B	ARPC1		ARPC1	ARPC1	Arc40/Sop2	ARPC1
p34	ARPC2		ARPC2		ARPC2		ARPC2		ARPC2	ARPC2	ARC35	ARPC2
p21	ARPC3		ARPC3		ARPC3		ARPC3	ARPC3A ARPC3B	ARPC3		ARC18	ARPC3
p20	ARPC4		ARPC4		ARPC4		ARPC4		ARPC4	ARPC4	ARC19	ARPC4
p16	ARPC5	ARPC5A ARPC5B	ARPC5		ARPC5	ARPC5A ARPC5B	ARPC5		ARPC5		ARC15	ARPC5