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Adaptor protein complexes and disease at a glance

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ABSTRACT

Adaptor protein (AP) complexes are heterotetramers that select cargo for inclusion into transport vesicles. Five AP complexes (AP-1 to AP-5) have been described, each with a distinct localisation and function. Furthermore, patients with a range of disorders, particularly involving the nervous system, have now been identified with mutations in each of the AP complexes. In many cases this has been correlated with aberrantly localised membrane proteins. In this Cell Science at a Glance article and the accompanying poster, we summarize what is known about the five AP complexes and discuss how this helps to explain the clinical features of the different genetic disorders.

KEY WORDS: Adaptor protein complex, Clathrin, Membrane traffic, Endocytosis, Golgi, Hereditary spastic paraplegia

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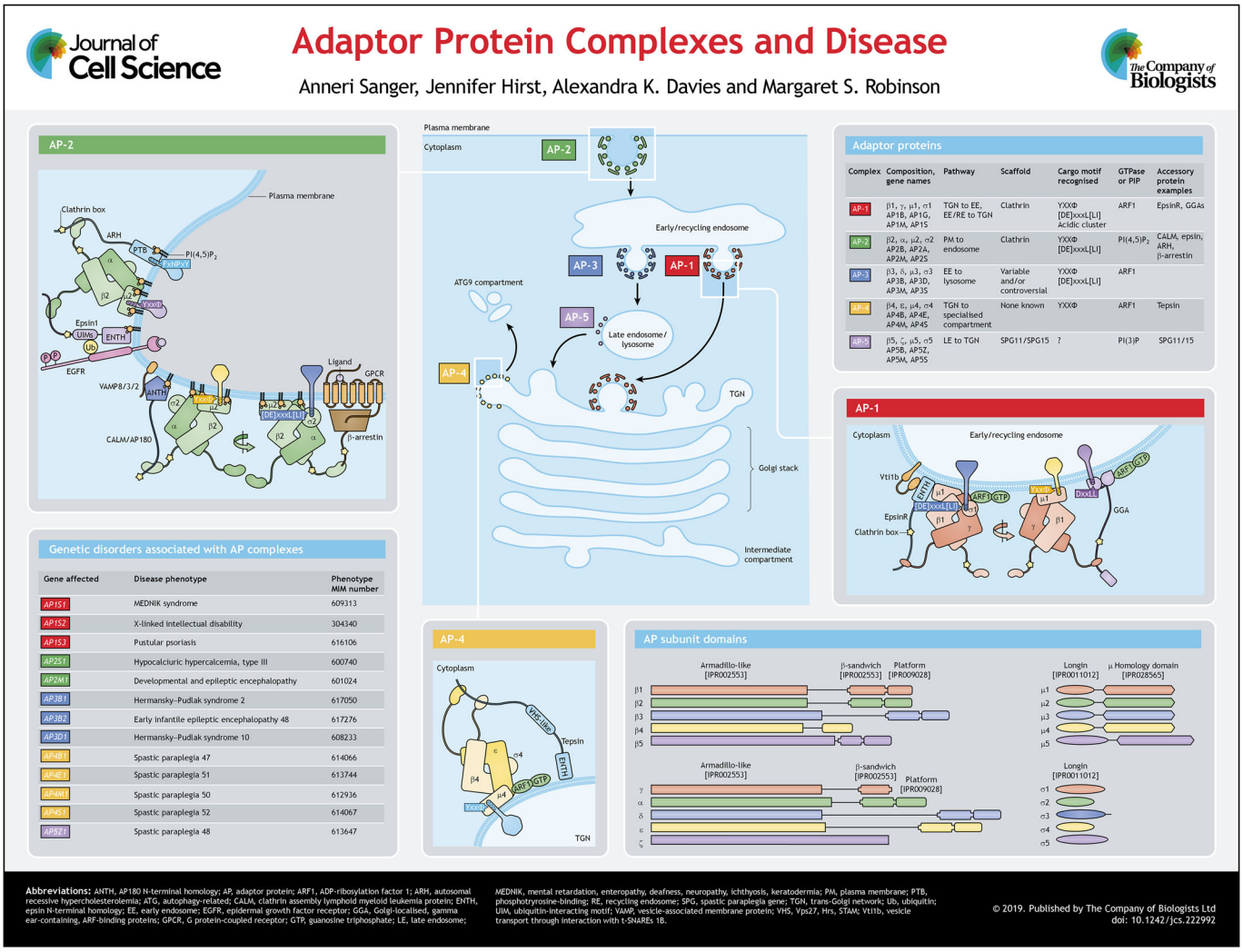
Introduction

Nearly a third of the proteins encoded by the human genome are predicted to be integral membrane proteins, all of which have to get to the right part of the cell. There are several ways of moving membrane proteins from one subcellular compartment to another, but perhaps the most common way is to package them into coated vesicles. The coat is made up of protein complexes, which are recruited onto a particular membrane compartment where they shape the membrane into a vesicle and collect the vesicle cargo. After the vesicle buds, it sheds its coat and goes on to fuse with a different



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membrane compartment. COPI- and COPII-coated vesicles facilitate trafficking between the ER and the Golgi, while many of the post-Golgi trafficking pathways make use of adaptor protein (AP) complexes. The AP complexes are evolutionarily related to part of the COPI coat, and their function is to select cargo for inclusion into vesicles (Traub and Bonifacino, 2013; Robinson, 2015). Several genetic disorders have now been identified where the causative mutation is in an AP subunit. This results in membrane proteins failing to get packaged correctly into vesicles, so that they end up in the wrong part of the cell. In this article and the accompanying poster, we provide a brief overview of the AP complexes and how they are thought to work, and we discuss the disease-causing mutations that have been found in each of the complexes.

Structure and localisation of AP complexes

Five AP complexes have been identified. The first to be discovered were AP-1 and AP-2, which are abundant components of clathrin-coated vesicles (CCVs) (Zaremba and Keen, 1983; Pearse and Robinson, 1984; Keen, 1987). The two APs coordinate CCV formation by providing a link between the membrane and the clathrin scaffold. They also recruit accessory proteins to the CCV, some of which act as cargo-specific adaptors (Traub, 2009). AP-1 is associated with the trans-Golgi network (TGN) and early and/or recycling endosomes, whereas AP-2 is located at the plasma membrane (Robinson, 2015) (see poster). Both AP-1 and AP-2 are heterotetramers, consisting of two large (∼100 kDa) subunits, γ and β1 in the AP-1 complex, and α and β2 in the AP-2 complex; a medium-sized (∼50 kDa) μ subunit; and a small (∼20 kDa) σ subunit (see poster). The N-terminal domains of the large subunits, together with the medium and small subunits, form a compact core, while the C-terminal domains of the large subunits form appendages that connect to the core by flexible linkers.

Two additional adaptor protein complexes, AP-3 and AP-4, were found in the 1990s on the basis of their homology to AP-1 and AP-2 (Robinson and Bonifacino, 2001). AP-3 is made up of δ, β3, μ3 and σ3 subunits, and AP-4 of ε, β4, μ4 and σ4 subunits. AP-3 is present on the same endosomes as AP-1, whereas AP-4 localises to the TGN (see poster). In 2011, a fifth AP complex was discovered, which localises to late endosomes. AP-5 had previously escaped detection because its subunits have little sequence similarity to those of the other APs (Hirst et al., 2011). In 2014, a sixth complex, TSET, was discovered (Hirst et al., 2014). TSET is associated with the plasma membrane, although it is slightly more closely related to the early-Golgi COPI complex than to the post-Golgi AP complexes. All seven of these complexes (the five APs, TSET and COPI) are ancient and present in most eukaryotes, although there are examples of secondary loss: for instance, TSET has been lost from animals and fungi.

Getting to the correct membrane

In order for the AP complexes to function, they need to be recruited onto the correct membrane. Three of the complexes, AP-1, AP-3 and AP-4, make use of the small GTPase ARF1, which also facilitates the recruitment of COPI. ARF1 is recruited onto the membrane first, coordinated by the exchange of GDP for GTP. The active GTP-bound ARF1 then recruits coat proteins and other effectors (Donaldson and Jackson, 2011). Structures have been solved for both COPI and AP-1 bound to activated ARF1, and the binding mechanisms are very similar, involving the two large subunits (Ren et al., 2013; Dodonova et al., 2015). However, binding to activated ARF1 is clearly not the only interaction that contributes to the recruitment of these coats, because COPI, AP-1, AP-3 and AP-4 all have distinct subcellular localisations. The current view is that recruitment is by coincidence detection, with multiple medium-to-low affinity interactions ensuring that the coat is recruited onto the correct membrane (Kirchhausen et al., 2014).

AP-2 and AP-5 don't appear to use ARF1, but they both have a strong phosphoinositide requirement, making use of phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3-phosphate (PI3P), respectively (see poster). PIP2 is found mainly on the plasma membrane, where AP-2 is recruited, and three of the AP-2 subunits have PIP2-binding sites (Collins et al., 2002). AP-5 is closely associated with two other proteins, SPG11 (spatacsin) and SPG15 (spastizin, also known as ZFYVE26). SPG15 has a PI3P-binding FYVE domain, and treatment of cells with the PI3-kinase inhibitor wortmannin causes the AP-5–SPG11–SPG15 complex to become cytosolic rather than associated with late endosomes (Hirst et al., 2013). However, much of the PI3P in the cell is on early endosomes, providing further evidence for the coincidence detection hypothesis.

In addition to facilitating recruitment, the binding of AP-2 to PIP2 and of AP-1 to ARF1 induce a conformational change, from a 'closed' to an 'open' conformation (Jackson et al., 2010; Ren et al., 2013; Kelly et al., 2014). Opening up the complex exposes binding sites for cargo and clathrin. These interactions further stabilise the open conformation, and help to nucleate the assembly of the coat (Mettlen and Danuser, 2014).

Selecting the correct cargo

Most cargo proteins interact with coats through short linear motifs in their cytoplasmic tails. There are two well-characterised cargo-binding sites on the APs, one for the tyrosine-based motif YxxΦ (where Φ is a bulky hydrophobic residue), and one for the dileucine motif, [D/E]xxxL[L/I] (see poster). Yeast two-hybrid experiments, which can be used to investigate protein–protein interactions, were the first to show that the YxxΦ motif binds to the AP medium subunits, specifically to the C-terminal 'μ homology domain' (MHD) (Ohno et al., 1995). Subsequent X-ray crystallography studies on AP-2 showed how the tyrosine and bulky hydrophobic residues fit into compatible pockets, which are inaccessible when the complex is in its closed conformation (Owen and Evans, 1998; Jackson et al., 2010). Membrane proteins that have YxxΦ motifs in their cytosolic tails include intracellular sorting receptors for lysosomal hydrolases (e.g. cation-dependent and cation-independent mannose-6-phosphate receptors), endocytic receptors (e.g. the transferrin receptor), lysosomal membrane proteins (e.g. LAMP1 and LAMP2), and TGN-resident proteins [e.g. TGN38 (also known as TGOLN2) and furin] (Bonifacino and Traub, 2003).

The molecular basis for dileucine sorting was also first uncovered in yeast, in this case by using the three-hybrid system, which is a way of investigating three-way protein interactions. Two of the AP subunits act in concert to bind the motif: the small subunit, and one of the large subunits (Janvier et al., 2003; Doray et al., 2007). Structural studies on AP-2 showed that the two leucines are accommodated by hydrophobic pockets in the σ2 subunit, while the acidic residue (D or E) sits on a positively charged patch provided by the α and σ2 subunits (Kelly et al., 2008). Like the YxxΦ motif, the dileucine motif is found in many different proteins, and can act as both an internalisation signal and an intracellular sorting signal (Bonifacino and Traub, 2003).

Making a vesicle

Clathrin-coated vesicle formation at the plasma membrane has been extensively studied, using methods ranging from live cell imaging to

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in vitro reconstitution. It seems clear that the ability of clathrin to self-assemble into spherical cages is essential for shaping the membrane (Hinrichsen et al., 2006; Dannhauser and Ungewickell, 2012). The best-characterised clathrin-binding motif, found on AP-1 and AP-2, as well as on other clathrin-associated proteins, is the clathrin box, LΦ[D/E/N]Φ[D/E], where Φ is a bulky hydrophobic residue (Dell'Angelica et al., 1998). This motif is found in the β subunits of both AP-1 and AP-2, and interestingly also in the β subunit of AP-3, even though AP-3 is not enriched in CCVs (Dell'Angelica et al., 1998). The current view is that AP-3 is able to interact with clathrin (Peden et al., 2004), but that this interaction may not be essential for AP-3 function (Peden et al., 2002).

However, there is no evidence for any sort of interaction between clathrin and either AP-4 or AP-5, raising the question of how (or even if) they make vesicles. Studies on COPI and COPII, as well as on several clathrin-associated proteins, show that there are several ways to bend a membrane into a vesicle. Part of the COPII coat forms an outer cage that is superficially similar to the clathrin cage (Stagg et al., 2006), while COPI, together with bound ARF1, assembles on membranes into an inherently curved structure (Dodonova et al., 2015). Some of the proteins associated with CCVs have large hydrodynamic radii and are thought to bend membranes by molecular crowding (Stachowiak et al., 2012), while others may generate curvature by driving a wedge into a membrane (Ford et al., 2002). A key experiment will be to try to make AP-4- or AP-5-coated vesicles *in vitro*, using minimal components: e.g., AP-4, its accessory protein tepsin, and ARF1, or AP-5 together with SPG11 and SPG15. This should reveal whether and/or how AP-4 and AP-5 shape membranes.

Genetic disorders of AP subunits

If a mutation in a gene is non-lethal, then there is a good chance that it will turn up in the human population. Disease-causing mutations have now been found in all of the AP complexes, even in AP-1 and AP-2, which are essential for life in animals. The lack of any one subunit renders the entire complex non-functional (Robinson, 2015), so loss-of-function mutations in any subunit of a particular complex should have the same phenotype.

AP-1

Mice without any AP-1 die as embryos (Zizioli et al., 1999; Meyer et al., 2000), but mammals have three genes encoding the AP-1 small subunit, and loss of any one of them is non-lethal. Indeed, individuals with genetic disease have been identified with mutations in each of the three (see poster). The first of these mutations was discovered in a large-scale study on X-linked intellectual disability, where three families were found with truncating mutations in the *AP1S2* gene, encoding σ1B (Tarpey et al., 2006). Subsequent studies identified autosomal recessive mutations in both of the other σ1 genes. Mutations in *AP1S1* (σ1A) affect many cell types and cause a unique syndrome called MEDNIK (an acronym for mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis and keratodermia) (Montpetit et al., 2008). Mutations in *AP1S3* (σ1C) mainly affect the skin, causing an autoinflammatory condition called pustular psoriasis (Setta-Kaffetzi et al., 2014). There is also a mouse model for σ1B deficiency (Glyvuk et al., 2010) (Box 1).

Why do mutations in the three genes cause such different phenotypes? Part of the explanation may be that they are expressed at different levels in different cell types. There is limited information about σ1 protein expression, but in HeLa cells, σ1A is the most abundant of the three, followed by σ1C and σ1B (Itzhak et al., 2016). In contrast, in mouse neurons, σ1A is about twice as abundant as σ1B, while σ1C is undetectable (Itzhak et al., 2017). Moreover, northern blotting and RT-PCR on mouse tissues indicate that σ1A and σ1B are more strongly expressed in brain than in other tissues, while expression of σ1C is relatively weak in brain (Glyvuk et al., 2010). This may help to explain why mutations in σ1A (*AP1S1*) or σ1B (*AP1S2*) affect the nervous system, while mutations in σ1C (*AP1S3*) do not.

Another possibility is that the different σ1 isoforms may have different cargo preferences. Several membrane proteins have been found to exhibit aberrant localisations or expression levels in cells that are deficient in one of the σ1 genes, including Toll-like receptor 3 (TLR-3) in *AP1S3*-knockdown macrophages (Setta-Kaffetzi et al., 2014), sortilin in adipocytes from *AP1S2*-knockout mice (Baltes et al., 2014), and ATP7A in fibroblasts from a patient homozygous for a null mutation in *AP1S1* (Martinelli et al., 2013).

The ATP7A phenotype is probably the best understood. ATP7A and ATP7B are copper-transporting ATPases that maintain copper homeostasis in mammalian cells, with ATP7A expressed ubiquitously and ATP7B expressed mainly in hepatocytes. Both proteins are localised at the TGN under control conditions, but are translocated to the plasma membrane in response to elevated copper levels (La Fontaine and Mercer, 2007). ATP7A and ATP7B have dileucine motifs and are packaged into CCVs in an AP-1-dependent manner (Hirst et al., 2012; Jain et al., 2015; Yi and Kaler, 2015), which helps to maintain their normal steady state localisation. Lack of ATP7A or ATP7B cause Menkes disease and Wilson disease,

Box 1. Mouse models.

A number of the AP subunit genes that are mutated in individuals with AP complex-associated genetic disease have been knocked out in mice, and in most cases the phenotypes are very similar (e.g. Glyvuk et al., 2010; Khundadze et al., 2019). There are also mouse knockouts of genes that have not (yet) been implicated in disease.

The AP-1 medium subunit is encoded by two genes in mammals: *AP1M1*, which is expressed ubiquitously and is essential for embryonic development (Meyer et al., 2000), and *AP1M2*, which is expressed only in epithelial cells, where it contributes to basolateral protein targeting (Fölsch et al., 1999; Guo et al., 2013). Mouse *AP1M2* knockouts are viable, although there is increased mortality in the first 8 weeks. In addition, the mice develop spontaneous chronic colitis and intestinal epithelial cell hyperplasia (Takahashi et al., 2011). This has been correlated with mistrafficking of certain cytokine receptors and E-cadherin, both of which are normally restricted to the basolateral cell surface (Hase et al., 2013).

Complete loss of AP-2 is early embryonic lethal (Mitsunari et al., 2005), but mice lacking the single-copy AP-2 β subunit gene *AP2B1* survive until birth but then die shortly afterwards, the only obvious abnormality being that they have a cleft palate (Li et al., 2010). The reason for their viability is that the β subunit of AP-1 is able to substitute. Many organisms share a common β1/β2 subunit, and the duplication that gave rise to *AP1B1* and *AP2B1* occurred relatively recently (Dacks and Robinson, 2017); therefore, β1 and β2 are sufficiently similar that they can interact with each other's partners. Why loss of β2 results in a cleft palate is less clear, but it could be related to effects on TGF-β receptor endocytosis (Li et al., 2010).

AP-3 has ubiquitously expressed and neuronal-specific genes not only for its β subunits, but also for its μ subunits. In mouse, knocking out the neuronal-specific medium subunit gene *AP3M2* produces a similar phenotype to knocking out *AP3B2*. In both cases, the mice have spontaneous epileptic seizures, correlated with a reduction in GABA transporters in synaptic vesicles (Nakatsu et al., 2004; Seong et al., 2005).

All three of these examples provide insights into human pathophysiology, and point towards candidate genes to be investigated in patients with similar clinical features.

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respectively, both of which share some clinical features with MEDNIK syndrome (Martinelli et al., 2013). Thus, mislocalisation of copper-transporting ATPases can account for at least some of the symptoms of σ1A deficiency. What is less clear is whether σ1A has the highest affinity of all the σ1 gene products for ATP7A and ATP7B, or whether it is simply more abundant than the other two in those tissues that are most affected by the disease.

AP-2

Two human genetic disorders involving AP-2 subunits have been described, both of which are caused by autosomal dominant missense mutations. The first is familial hypocalciuric hypercalcemia type 3 (FHH3), caused by a mutation in the single-copy small subunit gene, *AP2S1* (see poster). Multiple families have now been identified where a highly conserved arginine residue, R15, is mutated to a cysteine, a histidine or a leucine (Nesbit et al., 2013b). There are two other types of familial hypocalciuric hypercalcemia: FHH1, caused by mutations in the calcium-sensing G-protein-coupled receptor (GPCR) CaSR, and FHH2, caused by mutations in the α subunit of the G-protein that interacts with CaSR (Nesbit et al., 2013a). Thus, it seems likely that the missense mutations in *AP2S1* affect the trafficking of CaSR, a possibility supported by the finding that there is increased surface expression of the receptor in cells expressing mutated σ2 (Nesbit et al., 2013b).

Interestingly, the crystal structure of AP-2 with bound peptides shows that the conserved R15 residue in σ2 makes contact with the [D/E]xxxL[L/I] motif, specifically with the first residue, which is normally acidic (Kelly et al., 2008). Moreover, the cytosolic tail of CaSR contains two leucines in a row, leading to the suggestion that normally CaSR is internalised in a dileucine- and AP-2-dependent manner, but that this does not occur when R15 is mutated (Nesbit et al., 2013b). However, there are a few inconsistencies with this hypothesis. First, it has been shown that mutating R15 does not affect dileucine binding *in vitro* (Kelly et al., 2008), and second, the sequence in the CaSR cytoplasmic tail (RHQPLL) does not conform to a canonical dileucine motif, and there is no evidence that it contributes to the localisation of the protein. GPCRs are normally internalized via the cargo-selective adaptor β-arrestin, which binds to the β2 appendage (Goodman et al., 1996; Keyel et al., 2008), so it is not clear what a mutation in the AP-2 small subunit would do. Moreover, internalization of GPCRs is usually a mechanism for inactivating them, so impaired internalization would be expected to activate rather than inactivate the receptor (Goodman et al., 1996). There is also the question of why this mutation appears to affect only a single cargo protein.

A more recent study helps to explain at least some of these discrepancies (Gorvin et al., 2018). The authors showed that sustained signalling by CaSR occurs from endosomes rather than from the plasma membrane, and they also reported that the mutation in σ2 led to increased colocalisation between CaSR and clathrin in static structures (Gorvin et al., 2018). This suggests that in the cells of individuals with these particular mutations in σ2, the receptor may be getting stuck in coated pits rather than failing to access them. Clearly, there is a lot more to unravel before we really understand the molecular basis for FHH3.

The second autosomal dominant missense mutation is in the AP-2μ subunit (see poster) (Helbig et al., 2019), recently identified in a large-scale study on epilepsy, in which four out of 2624 affected individuals were found to have the same *de novo* variant. A single base C-to-T substitution causes a highly conserved arginine, R170, to be replaced by a tryptophan. This residue is just upstream from the MHD, and *in silico* modelling suggests that the R17W mutant may be impaired in its ability to undergo the conformational change that is essential for cargo recognition, a possibility supported by the finding that the mutant only partially rescues transferrin uptake in μ2-deficient cells. Precisely why the mutation causes epilepsy is less clear, but clathrin-mediated endocytosis plays a key role in synaptic vesicle retrieval in neurons. Moreover, *de novo* missense mutations in dynamin (DNM1), which facilitates the scission of CCVs, also cause epilepsy, albeit with a different clinical phenotype (EuroEPINOMICS-RES Consortium et al., 2014).

In addition to these relatively rare mutations in AP-2 subunits, there are much more common mutations that have been identified in cargo and machinery for the AP-2 pathway (see Box 2).

AP-3

Unlike AP-1 and AP-2, AP-3 is non-essential in animals. Recessive disease-causing mutations have been identified in three of the AP-3 subunits: the single-copy δ gene, *AP3D1*, and both of the β3 genes, *AP3B1*, which is expressed ubiquitously, and *AP3B2*, which is neuronal-specific (Dell'Angelica et al., 1997; Simpson et al., 1997). Mutations in *AP3B1* cause Hermansky–Pudlak syndrome type 2, a multisystem disorder characterised by albinism, prolonged bleeding, immunodeficiency and pulmonary fibrosis (see poster) (Dell'Angelica et al., 1999). All of these abnormalities can be correlated with aberrant trafficking to lysosomes and lysosome-related organelles, such as melanosomes, platelet dense bodies and dendritic cell phagosomes (Dell'Angelica et al., 1999; Mantegazza et al., 2012). Mutations in

Box 2. Genetic disorders of cargo and accessory proteins.

In addition to mutations in adaptor proteins that affect cargo localisation, there are several examples of mutations in the cargo proteins themselves that cause them to be mislocalised. Probably the most famous of these is a mutation in the low-density lipoprotein (LDL) receptor, discovered by the Brown and Goldstein labs, that causes the receptor to remain on the cell surface instead of being internalised by clathrin-mediated endocytosis (Anderson et al., 1977). This results in almost as severe a case of familial hypercholesterolaemia as that found in patients with no LDL receptors on the cell surface at all. The Brown and Goldstein labs went on to show (not trivial in the mid-1980s) that the causative mutation was a tyrosine-to-cysteine substitution, leading to the discovery of the first internalization signal, FxNPxY (Davis et al., 1986; Chen et al., 1990). Ironically, this is not a sorting signal for AP complexes, but for cargo-selective adaptors with PTB domains, such as autosomal recessive hypercholesterolemia (ARH, also known as LDLRAP1), the clathrin adaptor disabled homolog 2 (DAB2), and the endocytic adaptor protein NUMB (Mishra et al., 2002a,b). Most of the LDL clearance occurs in the liver, where ARH is highly expressed, and mutations in ARH also cause hypercholesterolaemia (Garcia et al., 2001), as indicated by the protein name.

Another example of a mutation in a cargo protein that prevents it from interacting with adaptors is the premature truncation of the anion exchanger AE1 (also known as SLC4A1), which leads to autosomal-dominant distal renal tubular acidosis. The truncation removes a YxxΦ motif, which is essential for its basolateral localisation, most likely by interacting with AP-1 (Devonald et al., 2003). However, disease-causing mutations in membrane proteins don't necessarily have to remove sorting signals; they could also introduce new sorting signals. A recent study identified a new type of genetic disorder: the 'dileucineopathies'. Single-base substitutions were found to create dileucine motifs and cause membrane proteins that normally reside mainly on the plasma membrane to redistribute to endosomes (Meyer et al., 2018). In the case of the glucose transporter GLUT1 (also known as SLC2A1), this was shown to cause impaired glucose transport across the blood–brain barrier, resulting in intellectual disability and seizures (Meyer et al., 2018).

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AP3D1 cause a similar phenotype, but with neurological problems as well, including neurodevelopmental delay, impaired hearing and severe seizures (see poster) (Ammann et al., 2016). There are also individuals with genetic disorders associated with mutations in *AP3B2*, who have the neurological symptoms only (see poster) (Assoum et al., 2016). These observations are consistent with the expression patterns of the three genes.

Cell biological studies have shown that lack of AP-3 causes increased surface expression of several lysosomal membrane proteins (Dell'Angelica et al., 1999), as well as missorting of the melanosomal proteins tyrosinase and OCA2 (Huizing et al., 2001; Theos et al., 2005; Sitaram et al., 2012). However, none of these proteins are completely mislocalised, seemingly because other AP complexes contribute to their steady-state distribution. The lysosomal membrane proteins all have YxxΦ and/or dileucine motifs and are efficiently retrieved by AP-2 and clathrin when they appear on the cell surface (Janvier and Bonifacino, 2005). Similarly, the melanosomal protein tyrosinase, which has both a YxxΦ and a dileucine motif, shows colocalisation with both AP-1 and AP-3, and can be sorted by either one (Theos et al., 2005). The cytomegalovirus glycoprotein gp48, which has a dileucine motif, is another example of a cargo protein that can be sorted by either AP-1 or AP-3 (Reusch et al., 2001). These findings may help to explain why AP-3 deficiency in humans is non-lethal.

AP-4

Patients with autosomal recessive mutations in any of the four AP-4 subunits present with severe intellectual disability and developmental delay, as well as progressive weakness and spasticity of the legs, i.e., spastic paraplegia (see poster) (Verkerk et al., 2009; Abou Jamra et al., 2011). Some of these patients had originally been misdiagnosed with cerebral palsy, suggesting that mutations in AP-4 may be more common than previously thought (Ebrahimi-Fakhari et al., 2018). There are also AP-4 knockout mice, which have a much milder phenotype than human patients (Matsuda et al., 2008), possibly because of the progressive nature of the illness. However, abnormalities were observed in the neurons of these mice, with AMPA receptor-containing autophagosomes accumulating in axons (Matsuda et al., 2008). This is consistent with the patient studies, because spastic paraplegias are caused by defects in the long axons of motor neurons (Blackstone et al., 2011).

Three independent studies have recently demonstrated that loss of AP-4 impairs the transport of the core autophagy protein ATG9A from the TGN to peripheral sites, which leads to dysregulation of autophagy (Mattera et al., 2017; Davies et al., 2018; De Pace et al., 2018; Ivankovic et al., 2019). This occurs in diverse cell types, including patient-derived fibroblasts and primary neurons from AP-4 knockout mice, suggesting that ATG9A trafficking is a ubiquitous function of AP-4. Defective autophagy is implicated in the aetiology of many neurodegenerative diseases (Menzies et al., 2017) and neuronal-specific loss of Atg9a in mice causes severe neurological phenotypes (Yamaguchi et al., 2018). Thus, it seems likely that missorting of ATG9A contributes, at least in part, to the neuropathology observed in AP-4-deficient patients.

Two proteins of unknown function, SERINC1 and SERINC3, colocalise with ATG9A in AP-4-dependent vesicles and are also mislocalised in AP-4-deficient cells (Davies et al., 2018). Other proteins reported to be sorted by AP-4 include amyloid precursor protein (APP) (Burgos et al., 2010) and transmembrane AMPA receptor regulatory proteins, or TARPs (Matsuda et al., 2008). Both APP and ATG9A have a tyrosine-based sorting signal, YxxΦE, which preferentially binds to subunit μ4 rather than to μ1, μ2 or μ3 (Burgos et al., 2010; Mattera et al., 2017). However, the other proteins that are thought to be sorted by AP-4 do not have a YxxΦE motif. Further mechanistic studies are required to understand cargo recognition by AP-4, and to assess the possible contribution of proposed AP-4 cargo proteins to disease caused by AP-4 deficiency.

AP-5

Disease-causing mutations in humans have so far only been identified for the AP-5 ζ subunit, encoded by *AP5Z1* (Słabicki et al., 2010; Hirst et al., 2016). Patients with homozygous or compound heterozygous mutations in this gene present with spastic paraplegia and other neurological abnormalities (see poster). Mutations in the AP-5-associated proteins SPG11 and SPG15 are more common, and the phenotype is similar for all three disorders, with spastic paraplegia accompanied by cognitive impairment and thin corpus callosum (viewed by MRI imaging of the brain) (Słabicki et al., 2010; Hirst et al., 2016; Pensato et al., 2014).

Fibroblasts from patients with mutations in AP5Z1, SPG11 or SPG15 accumulate aberrant endolysosomes (Hirst et al., 2015), similar to the fibroblasts of patients with lysosomal storage diseases, and accumulations of these organelles in axons may be the reason behind the neurological phenotype. Our recent search for AP-5 cargo proteins, carried out by identifying membrane proteins that are mislocalised in *AP5Z1*-knockout HeLa cells, suggests that the function of AP-5 is to retrieve a number of proteins from late endosomes and recycle them back to the Golgi complex (Hirst et al., 2018). These proteins may interact indirectly with AP-5 by binding to the transmembrane sorting receptor sortilin (Hirst et al., 2018). But whatever the mechanism for cargo recognition by AP-5, it seems unlikely that it involves canonical YxxΦ or dileucine motifs, because the sequences of the AP-5 subunits are so divergent from those of the other APs.

Perspectives

The first description of an AP complex was forty years ago, when CCV components of ∼100 kD and ∼50 kD were shown to promote clathrin assembly *in vitro*, hence the name AP, which was originally an acronym for assembly polypeptides (Keen et al., 1979). Since then, there have been many studies on the AP complexes, identifying all the different family members and finding out what they do. Because there has been so much basic cell biology on the five APs, in some cases, we have at least a partial understanding of why a particular mutation leads to a particular disease. However, in other cases, the mechanistic basis for the pathology is unclear. A more thorough understanding of the workings of all of the AP complexes will help us to understand the aetiology of the various diseases, and could eventually lead to the development of new treatments. Conversely, by finding out more about the consequences of AP deficiency in individuals with AP complex-associated genetic disease, we will be able to learn more about the cellular functions of the five AP complexes in all eukaryotes.

Acknowledgements

We thank both present and past members of the Robinson lab for their input.

Competing interests

The authors declare no competing or financial interests.

Funding

Work in the Robinson lab is funded by the Wellcome Trust (086598 and 214272).

Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.222992.supplemental.

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