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Neuronal Functions of Adaptor Complexes Involved in Protein Sorting

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Abstract

Selective transport of transmembrane proteins to different intracellular compartments often involves the recognition of sorting signals in the cytosolic domains of the proteins by components of membrane coats. Some of these coats have as their key components a family of heterotetrameric adaptor protein (AP) complexes named AP-1 through AP-5. AP complexes play important roles in all cells, but their functions are most critical in neurons because of the extreme compartmental complexity of these cells. Accordingly, various diseases caused by mutations in AP subunit genes exhibit a range of neurological abnormalities as their most salient features. In this article, we discuss the properties of the different AP complexes, with a focus on their roles in neuronal physiology and pathology.

Introduction

Neurons are the most extremely asymmetric and polarized cells in metazoans. They are organized into a complex array of domains (soma, dendrites and axon) and subdomains (e.g., dendritic spines, axon initial segment, pre- and postsynaptic terminals) (Fig. 1A). Each of these domains and subdomains has specialized functions that are performed by distinct complements of subcellular organelles and proteins [1,2]. The establishment and maintenance of this heterogeneous distribution of intracellular components rely on a variety of molecular mechanisms that deliver them to their corresponding neuronal locations. For organelles, selective transport depends on adaptor proteins that couple them to cytoskeletal motors, a subject that has been covered in previous reviews [1,2,3]. On the other hand, the sorting of specific proteins to different locations is controlled by a different set of adaptors that mediate incorporation of the proteins into specific populations of transport carriers [1,4]. In this minireview, we focus on the neuronal functions of a family of these latter adaptors known as adaptor protein (AP) complexes.

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Characteristics of AP complexes

The AP complex family comprises five members named AP-1, AP-2, AP-3, AP-4 and AP-5 (Fig. 2A, Table 1), which mediate sorting of transmembrane cargos in post-Golgi compartments of the biosynthetic, secretory, endocytic and lysosomal-targeting pathways [5]. These complexes are present in most eukaryotic species and cells, although some were lost in certain lineages during evolution [5]. AP complexes are composed of four subunits that are homologous among the different AP complexes (Fig. 2A). The four AP subunits are also homologous to subunits of the Golgi-associated COPI-F subcomplex [5]. In addition, the AP μ 1-5 subunits are homologous to proteins of the muniscin family, an evolutionary remnant of the ancient multiprotein complex TSET [5]. Because the relationship of COPI and TSET to AP-1-5 is more distant, they will not be covered in this article.

AP complexes associate with the cytosolic face of membranes through interactions with docking factors (e.g., Arf-family GTPases and/or phosphoinositides), and subsequently recruit scaffolding proteins (e.g., clathrin, SPG11-SPG15), leading to the formation of supramolecular assemblies known as protein coats (Fig. 2B,C, Table 1). Once in place, AP complexes recognize sorting signals in the cytosolic tails of transmembrane cargos, resulting in the capture of these cargos within coated areas of the membrane (Fig. 2B, Table 1). This is followed by budding of coated transport carriers that deliver the cargo proteins to different cellular compartments. Each AP complex has a characteristic intracellular distribution, signal-recognition specificity, and, ultimately, function (Table 1). Several of the AP complex subunits occur as two or three isoforms encoded by different genes (Table 1), further diversifying the properties of the complexes. Of particular interest, some of the subunit isoforms are specifically expressed in the brain, as is the case for the AP-3 subunits β 3B and μ 3B. Additional diversity arises from alternative splicing of subunit mRNAs, as is the case for brain-specific variants of the AP-2 α C and β 2 subunits [6,7]. Mutations in genes encoding AP subunits and other coat proteins are causes of human diseases that we refer to as “coatopathies” (Table 2). Because of the ubiquity of AP complexes, AP coatopathies often affect multiple organ systems. However, many of these diseases exhibit a prevalence of neurological symptoms, in a few cases due to the mutation of a brain-specific isoform (e.g., β 3B), but in most cases because the unique architecture of neurons makes these cells especially vulnerable to defects in protein sorting.

AP-1: somatodendritic sorting

AP-1 is a clathrin-associated complex that, in most cell types, mediates sorting between endosomes and the *trans*-Golgi network (TGN) (Table 1). In neurons, however, AP-1 has been implicated in the polarized sorting of a subset of transmembrane receptors and transporters to the somatodendritic domain [8,9,10,11•]. This function involves packaging of the somatodendritic proteins into a specific population of transport carriers budding from the TGN or endosomes within the soma (Fig. 1B), or retrieval of somatodendritic proteins from the axon by means of retrograde transport carriers (Fig. 1D). The importance of AP-1 for both generic and neuron-specific processes is underscored by the existence of human coatopathies caused by mutations in the σ 1A, σ 1B or σ 1C subunit isoforms (Table 2). Mutations in σ 1A are the cause of the autosomal recessive MEDNIK syndrome, an acronym

for mental retardation, enteropathy, deafness, neuropathy, ichthyosis and keratoderma [12]. Mutations in $\sigma 1B$ cause the X-linked mental retardation disorder known as Fried or Pettigrew syndrome [13,14]. Finally, mutations in $\sigma 1C$ are associated with susceptibility to pustular psoriasis, an inflammatory skin disorder [15]. The neurological features of MEDNIK and Fried/Pettigrew syndromes probably derive from mislocalization of neuronal AP-1 cargos that specifically require sorting by $\sigma 1A$ and $\sigma 1B$, respectively. This distinctive requirement could stem from a different signal-recognition specificity of the $\sigma 1$ isoforms, or their expression in different neuronal populations. The missorted cargos that account for the neurological dysfunction in these diseases, however, remain to be determined.

AP-2: pre- and post-synaptic endocytosis

AP-2 is the prototypical adaptor for clathrin-mediated endocytosis in all cells (Table 1). In neurons, AP-2 participates in specialized endocytic processes at both pre- and post-synaptic terminals (Fig. 1C,D). The release of neurotransmitters by fusion of synaptic vesicles (SV) with the pre-synaptic plasma membrane is quickly followed by endocytic retrieval of SV proteins for reuse in new SV formation. Early electron microscopy studies revealed the presence of numerous clathrin-coated pits and vesicles in the vicinity of SV exocytic sites [16,17] (Fig. 2C). It was therefore natural to assume that clathrin and its endocytic partner AP-2 were responsible for SV protein retrieval (Fig. 1D). In support of this role, perturbation of the expression or activity of AP-2 subunits was shown to impair SV protein retrieval and SV reformation [18,19,20,21,22]. However, other clathrin adaptors, such as AP-1 [23] and the monomeric protein stonin 2 [24,25,26], were also found to contribute to the recycling of specific SV components. Recent studies have challenged the importance of the clathrin/AP-2-dependent mechanism of SV recycling, and instead proposed that SV proteins are retrieved by an ultrafast process involving large, uncoated invaginations of the pre-synaptic plasma membrane [27,28]. In this alternative scenario, clathrin/AP-2 could still be involved in sweeping up the leftovers from ultrafast endocytosis, or reforming SV from endosomes [29] (Fig. 1D).

In contrast to the controversy concerning the involvement of AP-2 in SV protein retrieval at pre-synaptic terminals, there is consensus that AP-2 plays an important role in endocytosis of neurotransmitter receptors at post-synaptic sites (Fig. 1C). For example, AP-2 participates in the endocytosis of both NMDA- and AMPA-type glutamate receptors from the post-synaptic plasma membrane, a key process for the regulation of synaptic strength and plasticity [30,31,32,33•].

In addition to the canonical roles of AP-2 in endocytosis, a recent study demonstrated a function of AP-2 as a linker for TrkB-containing autophagosomes to the p150^{Glued} subunit of dynactin, enabling their dynein-mediated retrograde transport from the distal axon towards the soma [34] (Fig. 1E). Thus, AP-2 appears to function as an adaptor for both coat-dependent protein sorting and microtubule-dependent organelle transport.

AP-3: sorting to synaptic vesicles, dense core vesicles and the axon

AP-3 is mainly associated with coated profiles on endosomal tubules, where it partly co-localizes with clathrin [35,36]. Although AP-3 directly interacts with clathrin [35], the functional relevance of this interaction has been disputed [36]. Instead, the protein VPS41 has been proposed as an alternative AP-3 scaffold [37] (Table 1). In most cells, AP-3 functions to decrease the recycling of lysosomal membrane proteins from endosomes to the plasma membrane [36,38] (Table 1). In specialized cell types, however, AP-3 is critical for the biogenesis of lysosome-related organelles such as melanosomes and platelet dense bodies [39]. Accordingly, mutations in the ubiquitous β 3A subunit of AP-3 cause the pigmentation and bleeding disorder known as Hermansky-Pudlak syndrome (HPS) type 2 (HPS2) [38] (Table 2). There is also extensive evidence that mammalian AP-3 has specialized functions in neurons, including the sorting of transmembrane proteins to specific SV populations [40,41] (Fig. 1D), AMPA receptors from early to late endosomes [32], and neuropeptides to dense core vesicles [42,43], in the latter case in cooperation with VPS41. Moreover, recent studies in *C. elegans* have shown that AP-3 is required for the axonal targeting of various receptors and transporters [11•] (Fig. 1B). Not surprisingly, then, mice and humans with mutations in the ubiquitous δ subunit [39,44•] or the brain-specific μ 3B [41] or β 3B subunits [45•] of AP-3 exhibit severe neurological abnormalities, including neurodevelopmental delay and seizures (Table 2).

AP-4: somatodendritic sorting and autophagy

AP-4 is part of a non-clathrin coat that mediates export of a subset of cargos from the TGN to more distal compartments of the endomembrane system [46,47,48,49] (Table 1). Although AP-4 subunits are ubiquitously expressed in mammals, the consequences of AP-4 deficiency are most prominent in neurons. Indeed, ablation of the AP-4 β 4 gene in mouse resulted in motor abnormalities and mislocalization of somatodendritic AMPA receptors to axonal autophagosomes [48]. On the basis of this latter phenotype, AP-4 was proposed to function in polarized sorting to the somatodendritic domain [48] by a pathway that might be alternative to that mediated by AP-1 [8,9,10,11•]. Mutations in the four AP-4 subunits were also identified as causes of hereditary spastic paraplegia (SPG) with intellectual disability and thin corpus callosum [50,51] (Table 2). Neurological dysfunction in AP-4-deficient mice and humans could result from the aforementioned missorting of AMPA receptors to the axon. However, a recent study showed that AP-4 is also involved in export of the transmembrane autophagy protein ATG9A from the TGN to endosomes and/or pre-autophagosomal structures [52•]. Autophagy defects could thus also contribute to the neuropathogenesis of AP-4 deficiency. Heterozygous variants in the gene encoding the ϵ subunit of AP-4 were also found to segregate with familial stuttering, a disorder of volitional speech control [53]. The neuropathological basis for this disorder, however, is currently unknown.

AP-5: lysosomal degradation

AP-5 is the most recently described member of the heterotetrameric AP complex family in mammals [54,55] (Fig. 2, Table 1). This complex is also part of a non-clathrin coat, which

instead comprises the products of two SPG genes, SPG11 (spatacsin) and SPG15 (spastizin), as scaffolds [54,55,56]. Mutations in the ζ subunit of AP-5, like mutations in SPG11, SPG15 and AP-4 subunits, cause a form of complicated hereditary spastic paraplegia with intellectual disability and thin corpus callosum [54] (Table 2). However, unlike AP-4, AP-5 is associated with late endosomes and lysosomes [56] and appears not to participate in ATG9A export from the TGN [52•]. Instead, AP-5 mediates retrieval of cargos such as the cation-independent mannose 6-phosphate receptor (CI-MPR) and the Golgi proteins GOLIM4 and GOLM1 from late endosomes to the Golgi complex [57•], thus contributing to endo-lysosomal homeostasis [58,59,60•].

Concluding remarks

From the above discussion it is clear that, while AP complexes play important functions in most cells, they are particularly essential for proper neuronal function. This special requirement stems from their canonical roles in cargo sorting and transport carrier formation at different stages of the neuronal endomembrane system, including various processes that are specific to neurons. However, protein coats in general and AP complexes in particular also appear to function in non-canonical ways. For instance, to the already described function of AP-2 in coupling dynein-dynactin to autophagosomes for retrograde transport along the axon [34], we can add the interaction of AP-1 with kinesins for anterograde transport of TGN- or endosome-derived carriers [61,62]. This function would be akin to that of other adaptors that link organelles to microtubule motors [3]. Fully elucidating both the canonical and non-canonical functions of AP complexes in neurons will be essential to explain the pathogenesis of AP coatopathies that affect the central nervous system. Particularly important will be to identify the complete sets of cargos sorted by different AP complexes. For example, copper metabolism defects in MEDNIK syndrome due to mutations in AP-1 σ 1A are likely due to missorting of the copper transporters ATP7A and/or ATP7B [63]. This finding prompted the use of zinc acetate, a standard treatment for ATP7B-deficient Wilson's disease patients, as a therapy for MEDNIK syndrome, resulting in amelioration of liver disease and behavioral disturbances [63]. Knowledge of key cargos that are missorted in other AP coatopathies could similarly inform therapeutic interventions to mitigate symptoms of these diseases.

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Highlights

- AP complexes mediate intracellular sorting of transmembrane proteins
- Although present in all cells, AP complexes have specialized functions in neurons
- Mutations in AP subunit genes cause various neurological disorders
- Studies of AP complexes could help the development of therapies for these disorders

Figure 1.

Neuronal processes mediated by AP complexes. **A**. Schematic representation of a neuron with its domains and subdomains. AIS: axon initial segment. **B**. AP complexes involved in polarized sorting from the soma. AP-1 and AP-4 are shown to sort proteins from the TGN and/or endosomes to the somatodendritic (SD) domain [8,9,10,11•]. In contrast, AP-3 sorts proteins to the axon [11•]. **C**. AP-2 mediates endocytosis of receptors at the post-synaptic terminal [30,31,32,33•]. RE: recycling endosome. **D**. AP complexes involved in sorting at the pre-synaptic terminal. AP-1 mediates retrieval of escaped somatodendritic proteins to the soma [9]. AP-2 is shown to participate in endocytosis of synaptic vesicle (SV) proteins from the plasma membrane and biogenesis of SV from endosomes [18,19,20,21,64,29]. AP-3 contributes to SV biogenesis from synaptic vesicle precursors (SVP) [40,41]. **E**. Non-canonical function of AP-2 in coupling axonal autophagosomes containing BDNF-activated TrkB receptors to dynein-dynactin for retrograde transport towards the soma [34].

Figure 2.

Properties of AP complexes. **A.** Schematic representation of AP complexes indicating their subunit composition and domain organization. **B.** Architecture of a generic coat. Docking factors recruit AP complexes to the membrane, after which the AP complexes recruit scaffolding proteins and concentrate transmembrane cargos by virtue of interactions with sorting signals in the cytosolic domains of the cargos. **C.** Clathrin-coated pits and vesicles (arrows) at a pre-synaptic terminal [16] ©1973 Heuser, John et al. *Journal of Cell Biology*. 57(2):315-344. doi:10.1083/jcb.57.2.315.

Table 1

Properties of AP complexes^a

Adaptors	AP-1	AP-2	AP-3	AP-4	AP-5
Subunits/genes ^b	$\gamma 1/AP1G1$ $\gamma 2/AP1G2$ $\beta 1/AP1B1$ $\mu 1A/AP1M1$ $\mu 1B/AP1M2$ $\sigma 1A/AP1S1$ $\sigma 1B/AP1S2$ $\sigma 1C/AP1S3$	$\alpha A/AP2A1$ $\alpha C/AP2A2$ $\beta 2/AP2B1$ $\mu 2/AP2M1$ $\sigma 2/AP2S1$	$\delta/AP3D1$ $\beta 3A/AP3B1$ $\beta 3B/AP3B2^c$ $\mu 3A/AP3M1$ $\mu 3B/AP3M2^c$ $\sigma 3A/AP3S1$ $\sigma 3B/AP3S2$	$\epsilon/AP4E1$ $\beta 4/AP4B1$ $\mu 4/AP4M1$ $\sigma 4/AP4S1$	$\zeta/AP5Z1$ $\beta 5/AP5B1$ $\mu 5/AP5M1$ $\sigma 5/AP5S1$
Scaffolds	Clathrin	Clathrin	Clathrin, VPS41	Unknown	SPG11 (spatacsin)-SPG15 (spastizin)
Docking factors	Arf, PtdIns(4) <i>P</i>	PtdIns(4,5) <i>P</i>	Arf, PtdIns(3) <i>P</i>	Arf	PtdIns(3) <i>P</i>
Sorting signals ^d	YXXØ [DE]XXXL[L] <i>I</i> Non-canonical	YXXXØ [DE]XXXXL[L] <i>I</i>	YXXØ [DE]XXXXL[L] <i>I</i>	YXXXØE Non-canonical	Unknown
Localization ^e	TGN/TEN	PM	TEN	TGN	LE/lysosomes
General functions	Sorting between TGN and endosomes; polarized sorting	Endocytosis	Sorting to lysosome-related organelles (LRO); contribution to sorting of lysosomal membrane proteins	Export from the TGN to endosomes and autophagosomal structures	Retrograde transport from endosomes to the TGN
Neuronal functions	Somatodendritic sorting; spine morphogenesis; Notch trafficking	Synaptic vesicle recycling; post-synaptic receptor endocytosis; axonal retrograde transport of autophagosomes	Synaptic vesicle biogenesis, particularly those containing GABA and choline transporters; dense core vesicle biogenesis; axonal sorting	Somatodendritic sorting of AMPA and $\delta 2$ glutamate receptors,	Lysosomal and autophagic degradation; maintenance of neuronal health

^aReferences to some of the information in this table are cited in the text.^bAP complexes are heterotetramers composed of four subunits, some of which exist as two or three isoforms.^cBrain-specific subunits.^dAmino acids in the signals are represented in the single letter code. X represents any amino acid and Ø a bulky hydrophobic amino acid.^eTGN: trans-Golgi network, TEN: tubular endosomal network, PM: plasma membrane, LE: late endosome.

Table 2Diseases caused by defective AP complexes^a

Adaptors	Subunits/Genes	Diseases	Neuropathological manifestations ^b
AP-1	$\sigma 1A/AP1S1$	MEDNIK syndrome	Psychomotor retardation, microcephaly, sensorineural deafness, peripheral neuropathy
	$\sigma 1B/AP1S2$	Fried/Pettigrew syndrome	Severe mental retardation, early hypotonia, progressing to spasticity and contractures, choreoathetosis, hydrocephalus, fourth ventricle enlargement with cerebellar hypoplasia, seizures, iron accumulation in the basal ganglia, neuroaxonal dystrophy
	$\sigma 1C/AP1S3$	Susceptibility to pustular psoriasis type 15 (PSORS15)	No neurological features reported
AP-2	$\sigma 2/AP2S1$	Familial hypocalciuric hypercalcemia type III (HHC3)	Headaches
AP-3	$\delta/AP3D1$	Hermansky-Pudlak syndrome type 10 (HPS10)	Severe neurodevelopmental delay, microcephaly, seizures, hearing impairment, atrophy of the telencephalon, enlarged cerebrospinal fluid spaces
	$\beta 3A/AP3B1$	Hermansky-Pudlak syndrome type 2 (HPS2)	Mild neurodevelopmental delay or no neurological symptoms
	$\beta 3B/AP3B2$	Early-onset epileptic encephalopathy (EOEE) type 48	Severe developmental delay, intellectual disability, absent speech, poor motor development, seizures, optic atrophy, postnatal microcephaly
AP-4	$\epsilon/AP4E1$	Hereditary spastic paraplegia type 51 (SPG51)	Neonatal hypotonia that progresses to hypertonia and spasticity, severe intellectual disability with poor or absent speech, seizures, microcephaly, enlarged ventricles, cortical and cerebellar atrophy, diffuse white matter loss, thin corpus callosum, hippocampal globoid formation, thin hippocampus Heterozygous variants of AP4E1 linked to familial stuttering
	$\beta 4/AP4B1$	Hereditary spastic paraplegia type 47 (SPG47)	
	$\mu 4/AP4M1$	Hereditary spastic paraplegia type 50 (SPG50)	
	$\sigma 4/AP4S1$	Hereditary spastic paraplegia type 52 (SPG52)	
AP-5	$\zeta/AP5Z1$	Hereditary spastic paraplegia type 48 (SPG48)	Spastic paraplegia, neuropathy, parkinsonism, cognitive impairment, white matter lesions, thin corpus callosum

^aReferences to some of the information in this table are cited in the text.^bMany of these diseases have non-neurological features that are not listed here.