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Essay

Adaptins[D]
The Final Recount

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This essay constitutes the second article in the "Genome Annotation Series" initiated by Thomas D. Pollard, Essay Editor. The first essay, by J.S. Berg, B.C. Powell, and R.E. Cheney, appeared in the April 2001 issue of *Molecular Biology of the Cell* (Vol. 12, Issue 4, pp 780–794).

Adaptins are subunits of adaptor protein (AP) complexes involved in the formation of intracellular transport vesicles and in the selection of cargo for incorporation into the vesicles. In this article, we report the results of a survey for adaptins from sequenced genomes including those of man, mouse, the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the plant *Arabidopsis thaliana*, and the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. We find that humans, mice, and *Arabidopsis thaliana* have four AP complexes (AP-1, AP-2, AP-3, and AP-4), whereas *D. melanogaster*, *C. elegans*, *S. cerevisiae,* and *S. pombe* have only three (AP-1, AP-2, and AP-3). Additional diversification of AP complexes arises from the existence of adaptin isoforms encoded by distinct genes or resulting from alternative splicing of mRNAs. We complete the assignment of adaptins to AP complexes and provide information on the chromosomal localization, exon-intron structure, and pseudogenes for the different adaptins. In addition, we discuss the structural and evolutionary relationships of the adaptins and the genetic analyses of their function. Finally, we extend our survey to adaptin-related proteins such as the GGAs and stonins, which contain domains homologous to the adaptins.

OVERVIEW OF THE ADAPTIN FAMILY

The term "adaptin" was coined by Barbara Pearse (1975) to designate a group of ~100 kDa proteins that copurified with clathrin upon isolation of clathrin-coated vesicles. The ~100 kDa-proteins were later found to be subunits of heterotetrameric adaptor protein (AP) complexes, and the term "adaptin" was extended to all subunits of these complexes. Four basic AP complexes have been described: AP-1, AP-2, AP-3, and AP-4. Each of these complexes is composed of two large adaptins (one each of $\gamma/\alpha/\delta/\epsilon$ and β1–4, respectively, 90–130 kDa), one medium adaptin (μ1–4, ~50 kDa), and one small adaptin (σ1–4, ~20 kDa) (Figure 1A) (reviewed by Kirchhausen, 1999; Lewin and Mellman, 1998; Robinson and Bonifacino, 2001). The analogous adaptins of the four AP complexes are homologous to one another (21–83% identity at the amino acid level). In general, the subunits of different AP complexes are not interchangeable, with the exception of some nonmammalian β1/2 hybrid proteins (see below), and possibly mammalian β1 and β2, which can be components of both AP-1 and AP-2. Some of the adaptins occur as two or more closely-related isoforms encoded by different genes. Additional diversity arises from alternative splicing of adaptin mRNAs. Thus, cells that express several of these adaptin variants have the potential to assemble a diverse array of AP complexes. AP-1, AP-2, and AP-3 are expressed in all eukaryotic cells examined to date. AP-4, on the other hand, is ubiquitously expressed in man (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), and the plant *Arabidopsis thaliana*, but not in the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, and the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

AP complexes are components of protein coats that associate with the cytoplasmic face of organelles of the secretory and endocytic pathways. The complexes participate in the formation of coated vesicular carriers, as well as in the selection of cargo molecules for incorporation into the carriers. AP-2 mediates rapid endocytosis from the plasma membrane, while AP-1, AP-3, and AP-4 mediate sorting events at the *trans*-Golgi network (TGN) and/or endosomes (Figure 1B). AP-1 and AP-2 function in conjunction with clathrin, whereas AP-4 is most likely part of a nonclathrin coat. Mammalian (but not yeast) AP-3 has been shown to interact with clathrin, but the functional significance of this

[D]Online version of this essay contains supplemental tabular material. Online version is available at www.molcellbiol.org.

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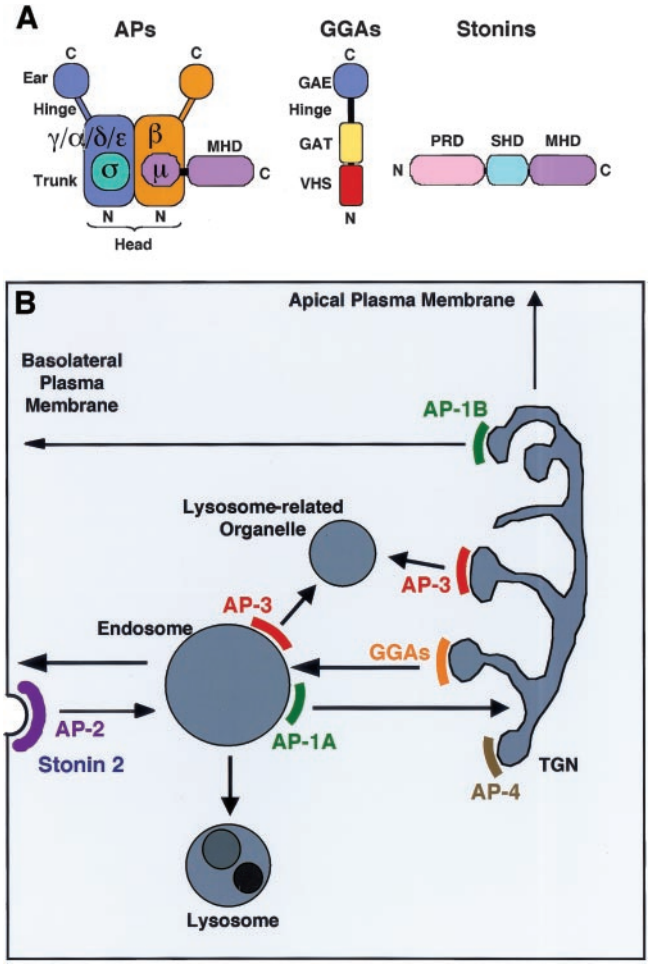

Figure 1. Schematic representation of adaptins and related proteins (A) and of the trafficking pathways in which they might be involved (B). The scheme in A depicts the structure of a generic AP complex, a GGA, and a stonin. N and C indicate the amino- and carboxy-termini of the proteins, respectively. GAE, γ-adaptin ear-homology domain; GAT, GGA, and TOM1-homology domain; MHD, μ-homology domain; PRD, proline-rich domain; SHD, stonin-homology domain; VHS, Vps27p/HRS/STAM-homology domain. B is a scheme of postGolgi trafficking pathways, showing the putative localization and role AP complexes, GGAs, and stonins within the cell. Of these, only the localization and role of AP-2 have been established with certainty. All the others should be considered tentative.

interaction is still unclear. The AP complexes have the overall shape of a "head" with two protruding "ears" connected to the head by flexible "hinge" domains (Figure 1A).

Recent studies have identified two additional families of proteins, the GGAs (Golgi-localizing, γ-adaptin ear homology, ARF-binding proteins) (Boman *et al.*, 2000; Dell'Angelica *et al.*, 2000b; Hirst *et al.*, 2000; Poussu *et al.*, 2000; Takatsu *et al.*, 2000), and the stonins (Andrews *et al.*, 1996; Martina *et al.*, 2001; Walther *et al.*, 2001), which share partial homology with the adaptins but are not components of AP complexes (Figure 1A). The GGAs contain a carboxy-terminal domain homologous (28–30% identity at the amino acid level) to the ear domain of the γ-adaptin subunit of AP-1. They function as monomeric adaptors for ARF (ADP-ribosylation factor)-dependent recruitment of clathrin to the TGN, and they mediate sorting of mannose 6-phosphate receptors and sortilin from the TGN to endosomes (Nielsen *et al.*, 2001; Puertollano *et al.*, 2001a; Puertollano *et al.*, 2001b; Takatsu *et al.*, 2000; Zhdankina *et al.*, 2001; Zhu *et al.*, 2001). The stonins are related to the *D. melanogaster* stoned B protein and exhibit homology (22–25% identity at the amino acid level) to the carboxy-terminal domain of the μ adaptins (Andrews *et al.*, 1996; Martina *et al.*, 2001; Walther *et al.*, 2001). The available evidence points to a role for at least some of the stonins in endocytosis (Fergestad and Broadie, 2001; Fergestad *et al.*, 1999; Martina *et al.*, 2001; Stimson *et al.*, 2001).

The adaptins are also distantly related (16–21% identity at the amino acid level) to subunits of the heteroheptameric COPI (coat protein I) or coatomer complex, a protein coat that functions in ER-Golgi and endosomal transport pathways. The large AP subunits are related to the β-COP and γ-COP subunits of COPI, while the medium and small AP subunits are related to the δ-COP and ζ-COP subunits of COPI, respectively. Together, β-, γ-, δ- and ζ-COP constitute the heterotetrameric F-COPI subcomplex (Fiedler *et al.*, 1996). COPI comprises three additional subunits named α-COP, β′-COP, and ε-COP that are not related to the adaptins. These subunits constitute the B-COPI subcomplex (Fiedler *et al.*, 1996), which is thought to subserve a function similar to that of clathrin.

Because of the critical roles of adaptins and related proteins in intracellular protein trafficking, it is of utmost importance to identify the complete repertoire of these proteins in eukaryotes. This goal is now achievable thanks to the recent completion of the sequencing of the genomes of humans and model organisms such as *M. musculus*, *D. melanogaster*, *C. elegans*, *A. thaliana*, *S. cerevisiae*, and *S. pombe* (Adams *et al.*, 2000; The *C. elegans* Sequencing Consortium, 1998; Goffeau *et al.*, 1996; The *Arabidopsis* Genome Initiative, 2000; Lander *et al.*, 2001; Venter *et al.*, 2001). The following sections describe the findings of a genome-wide survey for adaptins, GGAs, and stonins in these organisms. COPI subunits are beyond the scope of this essay and are only discussed in relation to the adaptins.

METHODS OF ANALYSIS AND SUMMARY OF TABLES

An inventory of adaptins was compiled from information published in the literature or obtained from the following internet resources: the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov), The Genome Database (http://www.gdb.org), the Mouse Genome Informatics at The Jackson Laboratory (http://www.informatics.jax.org/), the *D. melanogaster* genome at NCBI (http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/7227.html), the Sanger Center *C. elegans* Genome Project (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml), and The Stanford University *Saccharomyces* Genome Database search page (http://genome-www.stanford.edu/Saccharomyces/). To search for novel human adaptins, we used the TBLASTN algorithm (http://www.ncbi.nlm.nih.gov/genome/seq/page.cgi?F=HsBlast.html&&ORG=Hs) at the NCBI human genome BLAST web page. Adaptins in organisms other than human

Table 1. Adaptins and related proteins identified in various eukaryotes

Adaptins	*H. sapiens*	*M. musculus*	*A. thaliana*	*D. melanogaster*	*C. elegans*	*S. cerevisiae*	*S. pombe*
AP-1							
Large	γ1 γ2	γ1 γ2	γ-I γ-II γ-III	γ	γ	γ	γ
	β1	β1	β1/2-I β1/2-II	β1/2[a]	β1/2[a]	β1	β1
			β1/2-III[b]				
Medium	μ1A μ1B	μ1A μ1B	μ1	μ1	μ1-I μ1-II	μ1-I μ1-II	μ1
Small	σ1A σ1B	σ1A σ1B	σ1-I σ1-II	σ1	σ1	σ1	σ1
	σ1C	σ1C					
AP-2							
Large	α1 α2	α1 α2	α	α	α	α	α
	β2	β2	β1/2-I β1/2-II	β1/2[a]	β1/2[a]	β2	β2
			β1/2-III[b]				
Medium	μ2	μ2	μ2	μ2	μ2	μ2	μ2
Small	σ2	σ2	σ2	σ2	σ2	σ2	σ2
AP-3							
Large	δ	δ	δ	δ	δ	δ	δ
	β3A β3B	β3A β3B	β3	β3	β3	β3	β3
Medium	μ3A μ3B	μ3A μ3B	μ3	μ3	μ3	μ3	μ3
Small	σ3A σ3B	σ3A σ3B	σ3	σ3	σ3	σ3	σ3
AP-4							
Large	ε	ε	ε	n.f.[c]	n.f.[c]	n.f.[c]	n.f.[c]
	β4	β4	β4				
Medium	μ4	μ4	μ4				
Small	σ4	σ4	σ4				
GGAs	GGA1	GGA1	n.f.	GGA	GGA	Gga1p	Gga1p
	GGA2	GGA2				Gga2p	Gga2p
	GGA3	GGA3					
Stonins	Stonin 1	Stonin 1	n.f.[c]	Stoned B	Stonin	n.f.[c]	n.f.[c]
	Stonin 2/ Stoned B	Stonin 2/ Stoned B					

See Supplemental Tables 1–9 (online) for more details

[a] The β1/2 adaptins of *C. elegans* and *D. melanogaster* are most likely components of AP-1 and AP-2.

[b] The assignment of β1/2-I to β1/2-III adaptins of *A. thaliana* both to AP complexes is tentative.

[c] n.f., these adaptins were not found in these organisms.

were found using the BLASTP and TBLASTN algorithms at the NCBI web page. Homologous hit sequences were reanalyzed using the same algorithm to check for the closest human relative and assigned a name accordingly. Consensus secondary structure predictions and sequence alignments were performed using the Multialign and ClustalW programs available at the Pôle Bio-informatique Lyonnais (http://npsa-pbil.ibcp.fr/). Protein family (Pfam) domains are listed in the Pfam Homepage (http://pfam.wustl.edu/) at Washington University, St. Louis, MO.

Table 1 lists all the adaptins and related proteins found in mammals and other eukaryotes with sequenced genomes. Table 2 summarizes the phenotypes resulting from disruption, RNA interference, or naturally-occurring mutations of genes encoding adaptins and adaptin-related proteins in organisms from yeast to humans. Supplemental Tables 1 and 2 (all supplemental Tables are found online) contain an inventory of the names, chromosomal location, number of exons and size of the genes, and the accession codes for all human and mouse adaptins, respectively. Supplemental Table 3 lists potential human pseudogenes. Supplemental Tables 4–9 summarize information on adaptins in *D. melanogaster*, *A. thaliana*, *C. elegans*, *S. cerevisiae*, *S. pombe*, and other organisms, in that order.

MAMMALIAN ADAPTINS AND RELATED PROTEINS: ISOFORMS AND GENETIC ANALYSES OF THEIR FUNCTION

AP-1 Adaptins

Both humans and mice express two γ (γ1 and γ2), one β (β1), two μ (μ1A and μ1B) and three σ (σ1A, σ1B, and σ1C) adaptin(s) (Table 1, Supplemental Tables 1 and 2). Although there is only one gene encoding β1, two isoforms can be generated by alternative splicing of exon 15 (Peyrard *et al.*, 1994). σ1C is a novel isoform of σ1 encoded on chromosome 2 that was identified in our analyses. The predicted amino acid sequence for σ1C adaptin is equally homologous to the σ1A and σ1B adaptins (Supplemental Figure 1A). All of these proteins are known or predicted to assemble into various forms of the heterotetrameric AP-1 complex.

The AP-1 subunits are expressed in all mammalian tissues and cells examined except for μ1B, which is exclusively expressed in polarized epithelial cells (Ohno *et al.*, 1999). For σ1C, human ESTs can be found from a variety of sources, such as kidney (accession number BG166479), colon (BG386072), brain (BF697657), and B-cells (BG340480), sug-

Table 2. Genetic analyses of the function of adaptins and related proteins

Complex	Organism	Methodology	Phenotype
AP-1			
γ1	*M. musculus*	Targeted disruption	Embryonic lethal at day 4.5 pc
μ1A	*M. musculus*	Targeted disruption	Embryonic lethal at day 13.5 pc, accumulation of mannose-6-phosphate receptors in endosomes
γ	*C. elegans*	dsRNAi	Embryonic lethal
β1/2	*C. elegans*	dsRNAi	Embryonic lethal, inhibition of yolk endocytosis
μ1-I	*C. elegans*	Naturally-occurring mutation (*unc-101*)	50% embryonic lethal, uncoordinated movement
μ1-II	*C. elegans*	dsRNAi	Arrested development at larval L1 stage
σ1	*C. elegans*	dsRNAi	Embryonic lethal
γ	*S. cerevisiae*	Targeted disruption	Synthetic lethal with clathrin temperature sensitive mutation, mild defect in α-factor processing
β1	*S. cerevisiae*	Targeted disruption	Synthetic lethal with clathrin temperature sensitive mutation, mild defect in α-factor processing
μ1	*S. cerevisiae*	Targeted disruption	Synthetic lethal with clathrin temperature sensitive mutation, mild defect in α-factor processing
σ1	*S. cerevisiae*	Targeted disruption	Synthetic lethal with clathrin temperature sensitive mutation, mild defect in α-factor processing
AP-2			
α	*D. melanogaster*	P-element enhancer trap insertion	Several alleles - most severe are embryonic lethal, synapses devoid of vesicles
α	*C. elegans*	dsRNAi	Embryonic lethal, inhibition of yolk endocytosis
μ2	*C. elegans*	dsRNAi	50% embryonic lethal, dumpy phenotype
σ2	*C. elegans*	dsRNAi	50% embryonic lethal, dumpy phenotype
α	*S. cerevisiae*	Targeted disruption	None observed
β2	*S. cerevisiae*	Targeted disruption	None observed
μ2	*S. cerevisiae*	Targeted disruption	None observed
σ2	*S. cerevisiae*	Targeted disruption	None observed
AP-3			
β3A	*H. sapiens*	Naturally-occurring mutation (HPS type 2)	Pigmentation defect, bleeding disorder, misrouting of lysosomal membrane proteins to the plasma membrane
δ	*M. musculus*	Naturally-occurring mutation (mocha)	Pigmentation defect, bleeding disorder, lysosomal abnormalities, inner ear degeneration, neurological defects
β3A	*M. musculus*	Naturally-occurring mutation (pearl)	Pigmentation and bleeding defects
δ	*D. melanogaster*	Naturally-occurring mutation (*garnet*)	Pigmentation defect
β3	*D. melanogaster*	Naturally-occurring mutation (*ruby*)	Like *garnet*
μ3	*D. melanogaster*	Naturally-occurring mutation (*carmine*)	Like *garnet*
σ3	*D. melanogaster*	Naturally-occurring mutation (*orange*)	Like *garnet*
δ	*S. cerevisiae*	Targeted disruption	Missorting of alkaline phosphatase and Vam3p
β3	*S. cerevisiae*	Targeted disruption	Missorting of alkaline phosphatase and Vam3p
μ3	*S. cerevisiae*	Targeted disruption	Missorting of alkaline phosphatase and Vam3p
σ3	*S. cerevisiae*	Targeted disruption	Missorting of alkaline phosphatase and Vam3p
Related proteins			
Gga1p	*S. cerevisiae*	Targeted disruption	Vacuolar protein sorting and α-factor maturation defects
Gga2p stoned B	*D. melanogaster*	Naturally-occurring mutation (*stoned*)	Paralysis at the nonpermissive temperature

gesting that it may be ubiquitously expressed. Homozygous disruptions of the genes encoding γ1 or μ1A cause embryonic lethality in mice, indicating that the AP-1 complex is essential for viability (Zizioli *et al.*, 1999; Meyer *et al.*, 2000) (Table 2). An embryonal fibroblast cell line deficient in μ1A adaptin exhibited accumulation of mannose 6-phosphate receptors in endosomes, suggesting a role for the AP-1 complex containing μ1A (i.e., AP-1A) in sorting from endosomes to the TGN (Meyer *et al.*, 2000). The absence of μ1B expression in the polarized epithelial cell line LLC-PK1 (Ohno *et al.*, 1999), on the other hand, was linked to impaired sorting of LDL receptor and other transmembrane proteins to the basolateral plasma membrane domain (Fölsch *et al.*, 1999) (Table 2). Thus, the form of AP-1 containing μ1B (i.e., AP-1B) appears to be involved in basolateral targeting. The functional importance of other mammalian AP-1 subunit isoforms (e.g., γ2, σ1A, σ1B, σ1C) is unknown.

BLASTP searches of GenBank revealed an additional human cDNA termed FLJ10813 encoding a protein that is distantly related to the medium adaptins, with human and mouse μ1B being the most homologous (24% identity and 37% similarity at the amino acid level, but with 17% sequence gaps). The FLJ10813 protein is predicted to be truncated at the carboxy terminus relative to the μ chains, sug-

gesting that it may not function as a μ adaptin. Interestingly, while no homologues can be found in *D. melanogaster*, *C. elegans*, or *S. cerevisiae*, a homologous protein (accession number AC006234) exists in *A. thaliana*.

AP-2 Adaptins

Genes encoding two α (α1 and α2), one β (β2), one μ (μ2) and one σ (σ2) adaptin(s) have been found in both humans and mice (Table 1, Supplemental Tables 1 and 2). The human and mouse α2 adaptin sequences have been known for some time (Robinson, 1989; Faber *et al.*, 1998), whereas the sequence of human α1 adaptin is annotated as a hypothetical protein in GenBank (accession number CAB66859). Human α1 and α2 adaptin share 81% identity and 88% similarity at the amino acid level. Although there are fewer isoforms of AP-2 subunits as compared with AP-1 subunits, additional diversity arises from alternative splicing of some mRNAs. In mammals such as mouse and pig, the α1 adaptin mRNA is alternatively spliced in brain and skeletal muscle to generate a protein with 21 additional amino acids in the hinge region (Ball *et al.*, 1995). Alternative splicing of exon 5 of the mouse μ2 gene leads to the presence or absence of His142 and Gln143 in the protein. Although the longer isoform is more abundant, both forms are fully capable of interacting with tyrosine-based sorting signals (Ohno *et al.*, 1998). Finally, a splice variant of σ2 adaptin termed σ2Δ has been identified in human leukocytes (Holzmann *et al.*, 1998). All AP-2 subunits are ubiquitously expressed in mammals. No genetic analyses of AP-2 function in mammals have been reported to date.

AP-3 Adaptins

Both humans and mice contain genes encoding one δ, two β3 (β3A and β3B), two μ3 (μ3A and μ3B), and two σ3 (σ3A and σ3B) adaptin(s) (Table 1, Supplemental Tables 1 and 2). δ, β3A, μ3A, σ3A, and σ3B are expressed ubiquitously, while β3B and μ3B are specifically expressed in neurons and neuroendocrine cells (Pevsner *et al.*, 1994; Newman *et al.*, 1995). Several putative splice variants of δ adaptin lacking codons 170–260, 117–285, and 746–877 have been identified through searches of EST databases and PCR amplification (Ooi *et al.*, 1997). In humans, mutations in the gene encoding β3A adaptin cause Hermansky-Pudlak syndrome type 2 (HPS-2), a genetic disorder characterized by defective melanosomes and platelet dense granules (Dell'Angelica *et al.*, 1999b) (Table 2). A similar disorder has been described in mice bearing mutations in the genes encoding δ adaptin (mocha, Kantheti *et al.*, 1998) and β3A adaptin (pearl, Feng *et al.*, 2000; Feng *et al.*, 1999; Yang *et al.*, 2000) (Table 2). In addition, δ adaptin-deficient mice exhibit neurological defects that are not observed in β3A adaptin-deficient mice or HPS-2 patients (Kantheti *et al.*, 1998). Fibroblasts from AP-3-deficient humans and mice exhibit increased trafficking of lysosomal membrane proteins such as CD63, lamp-1, and lamp-2 via the plasma membrane (Dell'Angelica *et al.*, 2000a; Dell'Angelica *et al.*, 1999b; Yang *et al.*, 2000). A similar missorting of melanosomal and platelet dense granule proteins could underlie the organellar defects in the AP-3 mutants.

AP-4 Adaptins

Both humans and mice have only one gene encoding each of the ε, β4, μ4, and σ4 adaptin subunits of AP-4 (Table 1, Supplemental Tables 1 and 2) (Dell'Angelica *et al.*, 1999a; Hirst *et al.*, 1999). While no isoforms or splice variants of the AP-4 subunits ε, β4, or μ4 have been reported to date, the human σ4 mRNA appears to be subject to alternative splicing (accession numbers NP_009008 and AAH01259). Both splice isoforms share the first 102 residues, but they contain unrelated carboxy-terminal sequences of 42 (one exon) and 57 amino acids (two exons), respectively. Genomic sequences can be found for both splice variants, but no data are available on their tissue expression or differential incorporation of the proteins into AP-4. There are also no data on the disruption of AP-4 subunit gene expression in mammals, although indirect evidence has suggested a possible involvement of this complex in protein sorting to lysosomes (Aguilar *et al.*, 2001).

GGAs and Stonins

Unlike the conventional adaptins, GGAs and stonins are monomeric proteins. Genes encoding three GGAs (GGA1, GGA2 and GGA3) and two stonins (stonin 1 and stonin 2) have been described in both humans and mice (Table 1, Supplemental Tables 1 and 2) (Boman *et al.*, 2000; Dell'Angelica *et al.*, 2000b; Hirst *et al.*, 2000; Martina *et al.*, 2001; Poussu *et al.*, 2000; Takatsu *et al.*, 2000). Alternative splicing has been reported for the GGA2 and GGA3 mRNAs. For GGA2, this results in a truncated form of the protein (accession number AAK38634) comprising residues 1–194 of the full length GGA2, plus an additional ~30 residues at the carboxy terminus (Nielsen *et al.*, 2001). This short GGA2 form, therefore, has a complete VHS domain but no GAT, hinge or GAE domains. There is also a short form of GGA3 (accession number AAF42849) that lacks 33 residues from the VHS domain as compared with the full-length GGA3 (Dell'Angelica *et al.*, 2000b), and that appears not to interact with the acidic di-leucine motif in the cytoplasmic tails of the mannose 6-phosphate receptors (Takatsu *et al.*, 2001). No genetic disruptions of the expression of GGAs and stonins in mammals have been reported.

STRUCTURAL FEATURES OF MAMMALIAN ADAPTINS AND RELATED PROTEINS

The availability of a complete catalogue of mammalian adaptins should now allow detailed analyses of the structural features that account for both the conservation and specialization of their functions. A schematic representation of all human adaptins and adaptin-related proteins is shown in Figure 2.

Large Adaptins

A region at the amino terminus of the large adaptins, comprising ~600 amino acid residues in γ1, γ2, α1, α2, ε, β3A, and β3B, ~530 residues in β1, β2, and β4, and ~490 residues in δ, corresponds to the so-called "trunk" or "Adaptin_N" homology domain (pfam 01602) (Figure 2). This domain is predicted to be rich in α-helical secondary structure. In the β adaptins, it has been proposed to comprise 13–14 armadillo

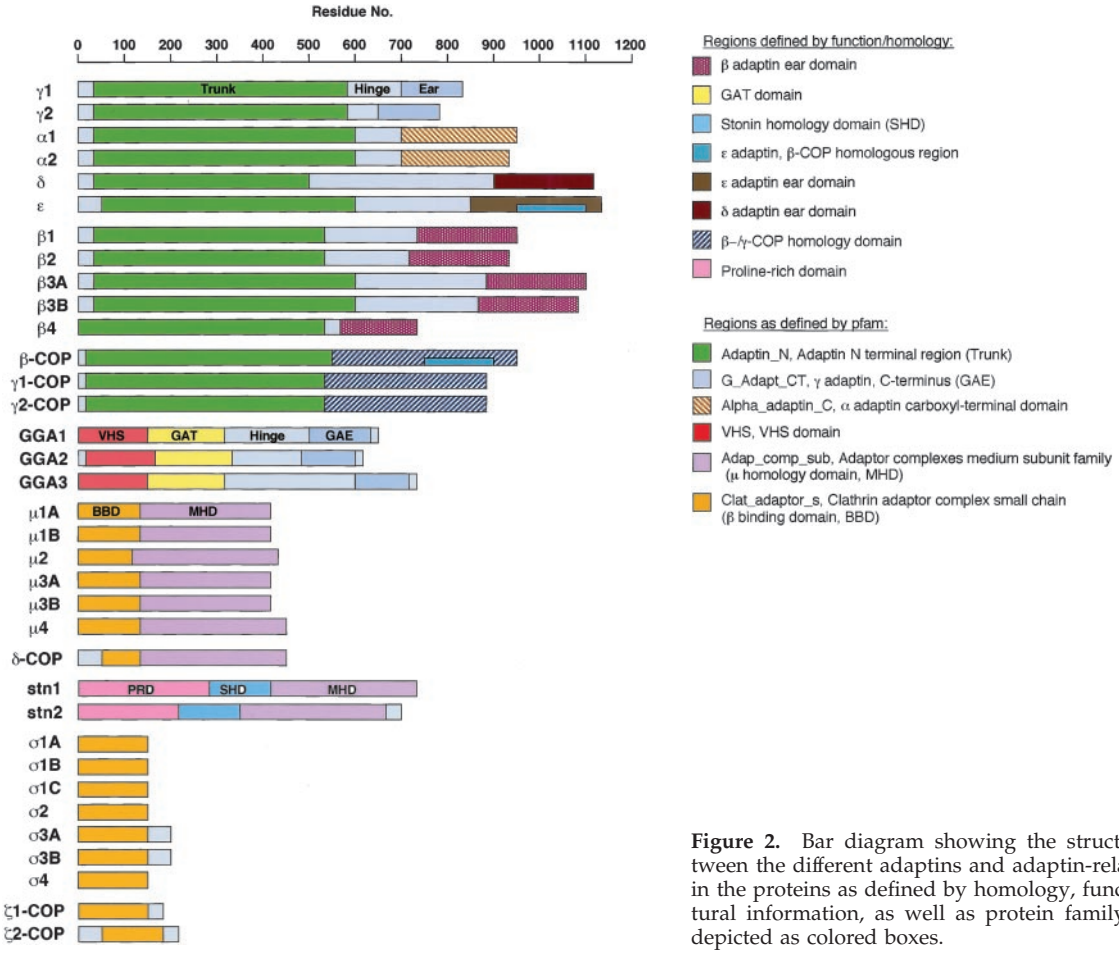

Figure 2. Bar diagram showing the structural relationships between the different adaptins and adaptin-related proteins. Regions in the proteins as defined by homology, function, or tertiary structural information, as well as protein family (Pfam) domains are depicted as colored boxes.

(arm) repeats, ~40-residue sequences that fold into two short α-helices linked by joining loops (Groves and Barford, 1999). Arm repeats have not been recognized in the amino-terminal region of the γ/α/δ/ϵ adaptins. However, their homology to the β adaptins suggests that they could be composed of more divergent double helix-loop repeats. An "Adaptin_N" homology domain is also present within the amino-terminal ~500 residues of the β-COP, γ1-COP, and γ2-COP subunits of COPI.

The "Adaptin_N" homology domain is involved in inter-subunit interactions between the large adaptins and with the medium and small adaptins. It is also responsible for targeting of AP-1 and AP-2 to the TGN and plasma membrane, respectively. Page and Robinson (1995) have demonstrated that the trunk domains of γ1 and α2 interact with σ1A and σ2, respectively, while the β adaptins are more promiscuous, as both β1 and β2 can interact with either μ1A and μ2 in yeast two-hybrid assays. The ability of the γ/α large chains to bind specifically to the small chains has been shown to correlate with their in vivo targeting to the TGN or the plasma membrane. The trunk domains of β1 and β2 are also the site of interaction with dileucine-based signals (Rapoport *et al.*, 1998; Greenberg *et al.*, 1998).

The "hinge" domains of the large chains are of variable length, ranging from 46 residues in β4 adaptin to 410 residues in δ adaptin, and exhibit little if any sequence homology (Figure 2). With the exception of γ2 and δ adaptin, the hinge regions of the adaptins are enriched in serine residues, many of which are potential targets for phosphorylation (Newman *et al.*, 1995; Wilde and Brodsky, 1996; Dell'Angelica *et al.*, 1997; Faundez and Kelly, 2000). The hinge domains of β1, β2, β3A, and β3B adaptins contain clathrin-binding motifs conforming to the consensus, L(L,I)(D,E,N)(L,F)(D,E) (Dell'Angelica *et al.*, 1998; Kirchhausen, 2000).

Homology in the carboxy-terminal "ear" domains of the large adaptins is lower than that in the trunk domains, especially among the γ/α/δ/ϵ adaptins (Figure 2). This probably reflects the functional diversity of the various AP complexes. The carboxy-terminal ~300 amino acids of α1 and α2 adaptin contain what is known as the "Alpha _adaptin_C" domain (pfam 02296) (Figure 2). The ear domain of the α2 adaptin has been shown to interact with many regulators of coat assembly and/or vesicle formation that contain DPF/W motifs, such as epsin, eps15, and amphiphysin (reviewed by Slepnev and De Camilli, 2000). The three-dimensional structure of the ear domain of mouse α2 adaptin (residues 695–938) was solved with the use of x-ray crystallography (Owen *et al.*, 1999; Traub *et al.*, 1999). This

domain consists of two structurally unrelated subdomains. The amino-terminal subdomain forms a nine-stranded β-sandwich that has similarity to the immunoglobulin fold and possibly acts as a structural anchor or spacer for the carboxy-terminal subdomain. This latter domain harbors the binding site for the DPF/W containing proteins, which is centered around residue W840. This domain contains a five-stranded β-sheet that is flanked by three α-helices and bears no resemblance to other known domain structures.

The carboxy-terminal ~120 residues of the γ1 and γ2 adaptins share the so-called "G_Adapt_CT" domain (pfam 02139) with the carboxy-terminal ~120 residues of the three GGAs (Figure 2). Functionally, this homology is mirrored in the ability of the γ1 ear and the GGA GAE domains to interact with γ-synergin and rabaptin-5 (Hirst *et al.*, 2000; Page *et al.*, 1999; Takatsu *et al.*, 2000). Although the alignment of δ and ε adaptin with the γ and α adaptin sequences shows little conservation outside the trunk domain, a comparison of the predicted secondary structures allows the tentative assignment of ear domains for δ and ε. In δ adaptin, the ear domain starts around residue 900 while in ε adaptin, it starts around residue 840. For both δ and ε adaptins, this domain is predicted to comprise a part rich in β-sheet followed by an α-helical segment of ~100 residues, similarly to the α adaptins. The carboxy-terminal domains of γ1-COP, γ2-COP, and β-COP are largely dissimilar from those of the AP large subunits. However, there is a small stretch of homology (22% amino acid identity, 41% similarity) between residues 946 and 1094 of ε adaptin and residues 745 and 895 of β-COP. This homology is exclusive for β-COP and ε adaptin since it is not observed in γ1-COP or γ2-COP, or in the γ, α, and δ adaptins.

Although the ear domains of the γ/α/δ/ε adaptins do not share sequence homology with the β ear domains, the tertiary structures of the ear domains of α2 adaptin (Owen *et al.*, 1999; Traub *et al.*, 1999) and β2 adaptin (Owen *et al.*, 2000) are remarkably similar. The ear domains of the other β adaptins exhibit significant sequence homology to β2, suggesting that they may also display a similar three-dimensional structure.

Medium and Small Adaptins

The amino-terminal domain of the μ adaptins, consisting of 120–140 residues, interacts with the β adaptins of the corresponding complexes, and is hence referred to as "β-binding domain" (BBD, Aguilar *et al.*, 1997) or "Clat_adaptor_s" region (pfam 01217) (Figure 2). This region exhibits homology to a 90-residue sequence from the amino-terminal domain of δ-COP (20–27% amino acid identity, 44–51% similarity), as well as to the entire or almost entire length of the small adaptins (~20% amino acid identity, ~40% similarity). The small adaptins are in turn homologous to ζ1-COP and ζ2-COP (17–23% amino acid identity, 43–49% similarity). In addition, σ3A, σ3B, and ζ1-COP have short extensions at their carboxy-termini and ζ2-COP at both its amino- and carboxy-termini. By analogy with the amino-terminal domain of the μ adaptins, it is tempting to speculate that the entire length of the σ adaptins may be engaged in interactions with the γ/α/δ/ε adaptins, perhaps having the sole purpose of stabilizing the AP complex. The crystal structure of this domain has not been solved, although theoretical analyses predict a high content of α-helices.

The 290–320-residue carboxy-terminal domain of the μ adaptins (Figure 2) is known as the "YXXØ-signal-binding domain" (Aguilar *et al.*, 1997) or "Adap_comp_sub" domain (pfam 00928) and binds YXXØ-motifs (Y is tyrosine, X is any amino acid and Ø is leucine, isoleucine, phenylalanine, methionine or valine) present in the cytosolic domains of some transmembrane proteins. This domain has an all β-sheet, "banana-shaped" tertiary structure including two hydrophobic pockets that accommodate the tyrosine and bulky hydrophobic residues of YXXØ-type signals (Owen and Evans, 1998). This domain of μ2 has also been shown to interact with synaptotagmins (Haucke *et al.*, 2000). Interestingly, this domain is shared with δ-COP (Serafini *et al.*, 1991; Waters *et al.*, 1991; Radice *et al.*, 1995; Tunnacliffe *et al.*, 1996) and the stonins (Andrews *et al.*, 1996; Martina *et al.*, 2001). Although neither of these proteins bind YXXØ signals, the ability to interact with synaptotagmins appears to be conserved in the stonins (Martina *et al.*, 2001; Walther *et al., 2001).*

ADAPTIN PSEUDOGENES IN THE HUMAN GENOME

In addition to genes encoding the AP subunits described above, TBLASTN searches at the NCBI human genome BLAST web page using the known adaptin protein sequences as queries revealed additional DNA sequences homologous to adaptin genes (Supplemental Table 3). However, these sequences encoded only fragments of the predicted adaptins, had deletions, insertions or frame shifts that resulted in premature termination codons, or had no introns, all characteristics of pseudogenes (Supplemental Table 3). Of these, only an intronless gene on chromosome 17 could potentially give rise to a protein identical to σ1B residues 1-142 (amino acid identity). Although the absence of introns is a salient feature of pseudogenes, transcribed intronless paralogs have nonetheless been described (Venter *et al.*, 2001), making it possible that this sequence encodes an additional σ1 adaptin.

ADAPTINS AND RELATED PROTEINS IN OTHER ORGANISMS

Adaptins in other organisms (Table 1, Supplemental Tables 4–9) have been tentatively identified based on the homology to their mammalian counterparts, with the exception of the *S. cerevisiae* adaptins that have also been assigned by Yeung *et al.* (1999) based on biochemical and genetic evidence. Like mammals, *A. thaliana* contains genes encoding subunits of the four AP complexes. In contrast, *D. melanogaster*, *C. elegans*, *S. cerevisiae*, and *S. pombe* possess genes encoding subunits of AP-1, AP-2, and AP-3, but not AP-4. The domain organization and other structural features of adaptins in these organisms are predicted to be very similar to those of mammalian adaptins.

AP-1 Adaptins

As in mammals, AP-1 is the complex that exhibits the greatest subunit diversification in other organisms (Table 1, Supplemental Tables 4–9). The *A. thaliana* genome contains genes encoding three γ (γ-I, γ-II and γ-III), three β1/2 (β1/

2-I, β1/2-II and β1/2-III), one μ1, and two σ1 (σ1-I and σ1-II) adaptin(s) (Supplemental Table 4). γ-I had been previously given the names γ1 and γ2 by Schledzewski *et al.* who isolated two sequences with 98% identity at the amino acid level [accession number AAC28338 (γ1) and CAB39730 (γ2)]. In a TBLASTN search, however, gene T23E23.7 was most closely related to both γ1 and γ2 (94% identity and similarity at the amino acid level), making it likely that both proteins originate from the same gene. The difference in amino acid composition between the γ1 and γ2 forms of γ-I could be the result of alternative splicing. γ-II shares 70% amino acid sequence identity with γ-I. γ-III has several stretches of homology to both γ-I and γ-II. However, it shows several features that would make it an unusual γ adaptin. The γ-III "Adaptin_N" region of homology (pfam 01602) is truncated and shifted carboxy-terminally relative to the other *A. thaliana* γ adaptins. Moreover, γ-III lacks the γ adaptin hinge and ear domains. Since there is no corroborative evidence for the existence of a transcript encoding γ-III, this DNA could be an artifact or a pseudogene. Full sequences are available for genes encoding *A. thaliana* β1/2-I and β1/2-II, whereas that encoding β1/2-III has been only partially sequenced. The β1/2 adaptins share 93–98% identity at the amino acid level and are thus more closely related to each other than to either human β1 or β2 adaptin. This suggests that they are products of relatively recent gene duplications. As is the case for mammalian β1 and β2 adaptins, the *A. thaliana* β1/2 adaptins could be subunits of both AP-1 and AP-2 complexes. Two σ1 adaptins that are homologous to mammalian σ1 adaptins have been identified in *A. thaliana*. These are constitutively expressed in all tissues of the plant, with higher levels being found in reproductive tissues (Maldonado-Mendoza and Nessler, 1997). Only one gene encoding a μ1 homolog could be identified in *A. thaliana*.

D. melanogaster contains only one gene encoding each of the subunits of AP-1 (γ, β1/2, μ1 and σ1 adaptins) (Supplemental Table 5), while *C. elegans* contains genes encoding one γ, one β1/2, two μ1 (μ1-I and μ1-II) and one σ1 adaptin(s) (Supplemental Table 6). Both *D. melanogaster* and *C. elegans* have a single β1/2 adaptin, which is probably a subunit of both AP-1 and AP-2. While *D. melanogaster* has a single μ1 adaptin, *C. elegans* has two, μ1-I (Unc-101) and μ1-II (Apm-1). The 56% identity and 70% similarity at the amino acid level of these two proteins points to a gene duplication within the nematodes group. Both proteins are transcribed in all cells and at all stages of development. Null mutations of *unc-101* are lethal in 50% of the animals (Lee *et al.*, 1994), and a dsRNAi against *apm-1* results in larval lethality (Shim *et al.*, 2000). Simultaneous interference with both μ1 adaptins by dsRNAi is embryonic lethal in 100% of the animals, as is dsRNAi against γ, β1 or σ1 (Shim *et al.*, 2000) (Table 2).

S. cerevisiae has one γ (Apl4p), one β1 (Apl2p), two μ1 (Apm1p and Apm2p) and one σ1 (Aps1p) adaptin (Supplemental Table 7). This allows for the assembly of two AP-1 complexes that differ in their μ1 subunit (Yeung *et al.*, 1999). While Apm1p is a classical μ chain, Apm2p is unusually large and cannot complement for Apm1p in *apm1* null mutants (Stepp *et al.*, 1995). Disruption of genes encoding AP-1 subunits is not detrimental to *S. cerevisiae* cells (Nakai *et al.*, 1993; Phan *et al.*, 1994; Rad *et al.*, 1995; Stepp *et al.*, 1995) (Table 2). However, they exhibit synthetic lethality with the temperature-sensitive clathrin heavy chain allele *chc1*^{ts} at the nonpermissive temperature (Phan *et al.*, 1994). Even at the permissive temperature, there is a defect in α-factor secretion in the double mutants, indicating a role for yeast AP-1 in sorting at the TGN (Phan *et al.*, 1994; Stepp *et al.*, 1995).

AP-2 Adaptins

A. thaliana, *D. melanogaster*, *C. elegans*, *S. cerevisiae*, and *S. pombe* have single genes encoding the α, μ2 and σ2 subunits of AP-2 (Table 1, Supplemental Tables 4–8). As discussed above, *A. thaliana* possesses three genes encoding hybrid β1/2 adaptins (β1/2-I, -II, and -III) that could be components of AP-2 as well as AP-1. Similarly, *D. melanogaster* and *C. elegans* have single genes encoding a hybrid β1/2 adaptin that is also probably shared by AP-1 and AP-2. In *D. melanogaster*, α and μ2 are most highly expressed in the central nervous system. Disruption of *D. melanogaster* α adaptin expression resulted in alleles with various degrees of severity ranging from embryonic lethality to adult flies that could neither walk nor fly (González-Gaitán and Jäckle, 1997) (Table 2). dsRNAi of α or β1/2 adaptin in *C. elegans* resulted in the inhibition of yolk endocytosis and embryos proved to be inviable (Grant and Hirsh, 1999). Embryos obtained from μ2(RNAi) or σ2(RNAi) mothers exhibited developmental phenotypes (Levy *et al.*, 1993; Shim and Lee, 2000) (Table 2). *S. cerevisiae* has homologues of the mammalian AP-2 adaptor complex subunits [Apl3p (α), Apl1p (β2), Apm4p (μ2), and Aps2p (σ2)], but their deletion has no apparent effect on endocytosis or any other protein sorting step yet analyzed (Munn, 2001) (Table 2).

AP-3 Adaptins

AP-3 subunits are encoded by single genes in *A. thaliana*, *D. melanogaster*, *C. elegans*, *S. cerevisiae*, and *S. pombe* (Table 1, Supplemental Tables 4–7). We found the *C. elegans* σ3 adaptin sequence by using the TBLASTN algorithm at the Sanger Center on a search for homologues of *D. melanogaster* σ3 (Supplemental Figure 1B). In agreement with studies of AP-3 function in mammals described above, *D. melanogaster* AP-3 appears to be involved in the biogenesis of pigment granules (Kretzschmar *et al.*, 2000; Lloyd *et al.*, 1999; Mullins *et al.*, 2000; Mullins *et al.*, 1999; Ooi *et al.*, 1997; Simpson *et al.*, 1997) (Table 2). Mutations of the genes encoding the *S. cerevisiae* δ (Apl5p), β3 (Apl6p), μ3 (Apm3p) or σ3 (Aps3p) impaired the sorting of alkaline phosphatase (ALP) and the t-SNARE Vam3p to the vacuole (Cowles *et al.*, 1997), an organelle that is the yeast counterpart of mammalian lysosomes (Table 2).

AP-4 Adaptins

In addition to mammals, a complete set of AP-4 subunits has only been found in *A. thaliana* (Table 1, Supplemental Table 3). Homologues of μ4 have been identified in *G. gallus* (Wang and Kilimann, 1997) and *D. discoideum* (de Chassey *et al.*, 2001), the genomes of which have not yet been completely sequenced. However, homologues of ε, β4 and σ4 subunits have yet to be identified in these organisms. There-

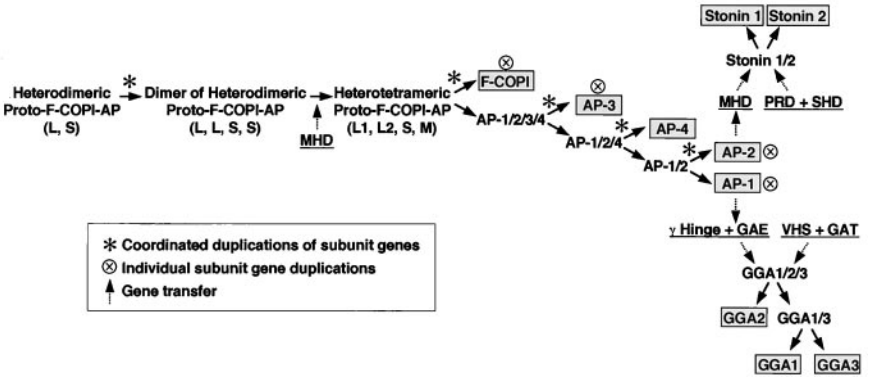

Figure 3. Possible evolution of the COPI-AP coat components. The order of the appearance of distinct complexes was deduced from the phylogenetic analysis of all adaptins present in *A. thaliana*, *C. elegans*, *D. melanogaster*, *H. sapiens*, *M. musculus*, and *S. cerevisiae*. The GGAs and stonins are hypothesized to have evolved after the γ and μ2 adaptins were differentiated, respectively, based on their higher degree of homology to domains from these adaptins. Asterisks indicate coordinated rounds of gene duplication, while dashed arrows show gene transfer. See text for more details.

fore, the existence of an AP-4 complex in these organisms remains to be formally established.

GGAs and Stonins

Sequences homologous to the GGA proteins could be identified in *C. elegans* and *D. melanogaster* (Table 1, Supplemental Tables 4–6), but no data are available on their expression pattern, localization or function. *S. cerevisiae* and *S. pombe* each contain two GGA proteins that are more closely related to each other than to the mammalian proteins. Single deletions of genes encoding these proteins in *S. cerevisiae* resulted in no obvious phenotype, but the *gga1*Δ *gga2*Δ double deletion strain displays defects in CPY and proteinase A sorting to the vacuole, Pep12p sorting from the Golgi to a prevacuolar compartment, α-factor maturation, and vacuolar morphology (Black and Pelham, 2000; Costaguta *et al.*, 2001; Dell'Angelica *et al.*, 2000b; Hirst *et al.*, 2000; Mullins and Bonifacino, 2001; Zhdankina *et al.*, 2001) (Table 2).

A BLASTP search for homologues of the mammalian stonins in other organisms also identified homologues in *D. melanogaster* and *C. elegans*, but not in *A. thaliana*, *S. cerevisiae* and *S. pombe*, suggesting that these proteins may be specific to the animal lineage (Table 1, Supplemental Tables 4–9). The only member of this family for which a genetic analysis of its function has been performed is the *D. melanogaster* stoned B protein. This protein is one of two polypeptides produced from a dicistronic message that is transcribed from the *stoned* gene (Andrews *et al.*, 1996). The other polypeptide translated from this message, stoned A, is structurally unrelated to the adaptins. Temperature-sensitive *stoned* mutants display uncoordinated leg and wing movements characteristic of neurological dysfunction at the nonpermissive temperature (Phillips *et al.*, 2000) (Table 2). The mutants also exhibit decreased uptake of FM1–43 in nerve terminals, suggesting that the neurological defects are due to impaired synaptic vesicle recycling (Phillips *et al.*, 2000).

EVOLUTION OF ADAPTINS AND RELATED PROTEINS

COPI and AP Complexes

All eukaryotes for which sequence information is available have one COPI and at least three of the four AP complexes (AP-1, AP-2 and AP-3), suggesting that the diversification of heterotetrameric coat complexes occurred before the branching of the major eukaryotic kingdoms [see Doolittle (1999) for a review of recent phylogenetic classifications]. The existence of AP-4 in *A. thaliana* and some vertebrates (*G. gallus*, *M. musculus*, and *H. sapiens*) suggests that this complex evolved before the separation of the plant and animal ancestors. The homologies between the two sets of large subunits of the AP complexes (γ/α/δ/ε and β1–4) and the F-COPI subcomplex (γ- and β-COP) indicate that they all derived from a single ancestral large chain (denoted as L in Figure 3). Similarly, the μ and σ subunits of AP complexes as well as the δ and ζ subunits of the F-COPI subcomplex likely derived from a common ancestral small chain (denoted as S in Figure 3). These two proteins must have come together to form a proto-F-COPI-AP hemicomplex (Schledzewski *et al.*, 1999) (L, S in Figure 3). This complex could have been a heterodimer or a heterotetramer composed of two identical heterodimers (L, L, S, S in Figure 3). Genes encoding the subunits of this ancestral complex must have undergone successive rounds of coordinated gene duplication (indicated by asterisks in Figure 3) to give rise to the F-COPI and AP complexes. A first round of gene duplication and accumulation of mutations resulted in the emergence of two distinct pairs of large and small subunits (L1, L2, S1, S2). Later, one of the small subunits acquired a precursor of the μ subunit signal-binding domain (MHD in Figure 3), leading to the emergence of an ancestral μ subunit (M) (Schledzewski *et al.*, 1999). The two large subunits, together with the medium and small subunits, constituted the proto-F-COPI-AP heterotetrameric complex (L1, L2, M, S in Figure 3). Another round of gene duplication involving all four subunits of this complex followed by evolutionary divergence led to the appearance of distinct F-COPI and proto-AP (AP-1/2/3/4) complexes (Figure 3). From this point on, the F-COPI and proto-AP complexes followed different evolutionary paths. F-COPI acquired the ability to bind to the three proteins that conform the B-COPI subcomplex to form a heteroheptameric COPI complex (Fiedler *et al.*, 1996). Isoforms of the γ-COP and ζ-COP subunits arose by separate gene duplications (indicated by ⊗ in Figure 3) in plants (ζ-COP) and vertebrates (γ-COP and ζ-COP), but no new sets of four subunits were derived from the F-COPI subunits. In contrast, the genes encoding the four subunits of the proto-AP complex underwent at least three more rounds of gene duplication (Figure 3).

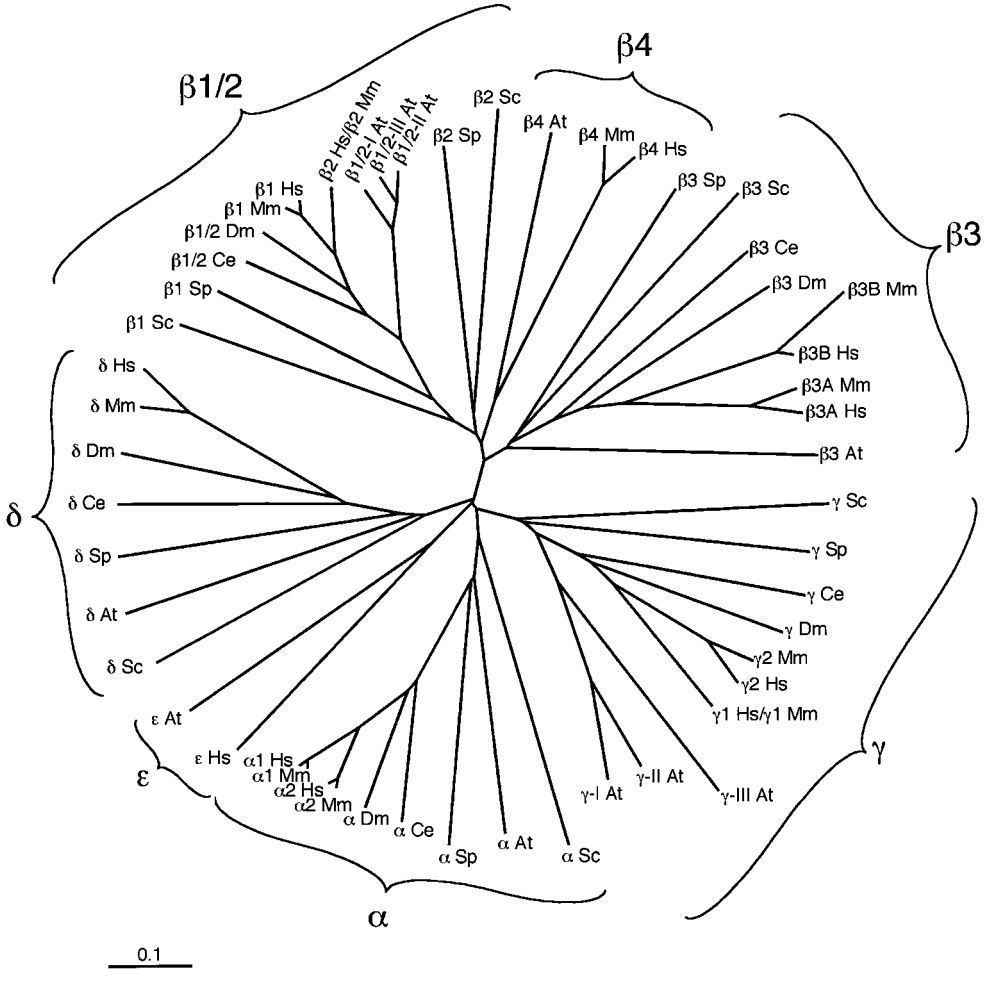

Figure 4. An unrooted phylogenetic tree displaying the evolutionary relationship between the large adaptins from *A. thaliana* (*At*), *C. elegans* (*Ce*), *D. melanogaster* (*Dm*), *H. sapiens* (*Hs*), *M. musculus* (*Mm*), *S. cerevisiae* (*Sc*), and *S. pombe* (*Sp*). The $\gamma/\alpha/\delta/\epsilon$ and $\beta 1-4$ subunits from these organisms were aligned, and a phylogenetic tree was calculated using the EMBL European Bioinformatics Institute ClustalW algorithm (*http://www2.ebi.ac.uk/clustalw*) and displayed using the TreeviewPPC program. The evolutionary distance is indicated by substitutions per site.

The probable sequence in which the four AP complexes evolved was inferred from phylogenetic analyses using the EMBL European Bioinformatics Institute ClustalW algorithm (http://www2.ebi.ac.uk/clustalw) and the Treeview-PPC program (Figures 4 and 5). Although differences in the evolutionary rates of different proteins can lead to erroneous phylogenetic reconstructions (Brocchieri, 2001), the heterotetrameric structure of the four AP complex allows combined measures of evolutionary distance to be derived. These analyses indicate that the precursor of modern-day AP-3 branched out first from the proto-AP complex. The AP-4 complex appears to have evolved next. Some animals such as *C. elegans* and *D. melanogaster* do not have an AP-4, while *A. thaliana* and mammals do. This indicates that, although AP-4 is ancient, some organisms may have lost the genes for this complex. Distinct AP-1 and AP-2 complexes were the last ones to evolve, initially sharing a single β subunit. The sharing of a β subunit has persisted to date in organisms such as *C. elegans* and *D. melanogaster*, which have only one $\beta 1/2$ subunit common to both AP-1 and AP-2. Duplications of genes encoding $\beta 1/2$ precursors may have occurred relatively recently in some lineages giving rise to two or three genes encoding closely related paralogs that may be subunits of both AP-1 and AP-2.

AP-1 and AP-2 subunits in the yeasts *S. cerevisiae* and *S. pombe* evolved somewhat differently, since the duplication of the $\beta 1/2$ subunit gene appears to have occurred very early in evolution after the separation from the common yeast/nematode ancestor. Thus, the *S. cerevisiae* AP-1 β adaptin, Apl2p, is most homologous to the mammalian $\beta 1$ and $\beta 2$ proteins while the *S. cerevisiae* AP-2 β adaptin, Apl4p, is quite different from the $\beta 2$ subunit of mammalian AP-2. Similarly, the *S. cerevisiae* $\mu 2$ adaptin, Apm4p, is equally homologous to the mammalian $\mu 1$ and $\mu 2$ proteins, which suggests an early duplication of genes encoding the $\beta 1/2$-$\mu 1/2$ hemicomplex in *S. cerevisiae*. An early duplication of the gene encoding $\mu 1$ may also be responsible for the appearance of a second, distinct $\mu 1$ in *S. cerevisiae*, Apm2p, which remains a relative outlier in the phylogenetic tree.

The transition between flies and vertebrates is characterized by the duplication of genes encoding individual subunits of the AP complexes (indicated by the $\otimes$ in Figure 3). These resulted in the emergence of two or more isoforms of certain subunits, including γ ($\gamma 1$ and $\gamma 2$), $\mu 1$ ($\mu 1$A and $\mu 1$B), $\sigma 1$ ($\sigma 1$A, $\sigma 1$B and $\sigma 1$C), α ($\alpha 1$ and $\alpha 2$), $\beta 3$ ($\beta 3$A and $\beta 3$B), $\mu 3$ ($\mu 3$A and $\mu 3$B), and $\sigma 3$ ($\sigma 3$A and $\sigma 3$B) in humans. Some of the isoforms continued to be expressed in all cells, while others became specific to some cells or tissues. For example,

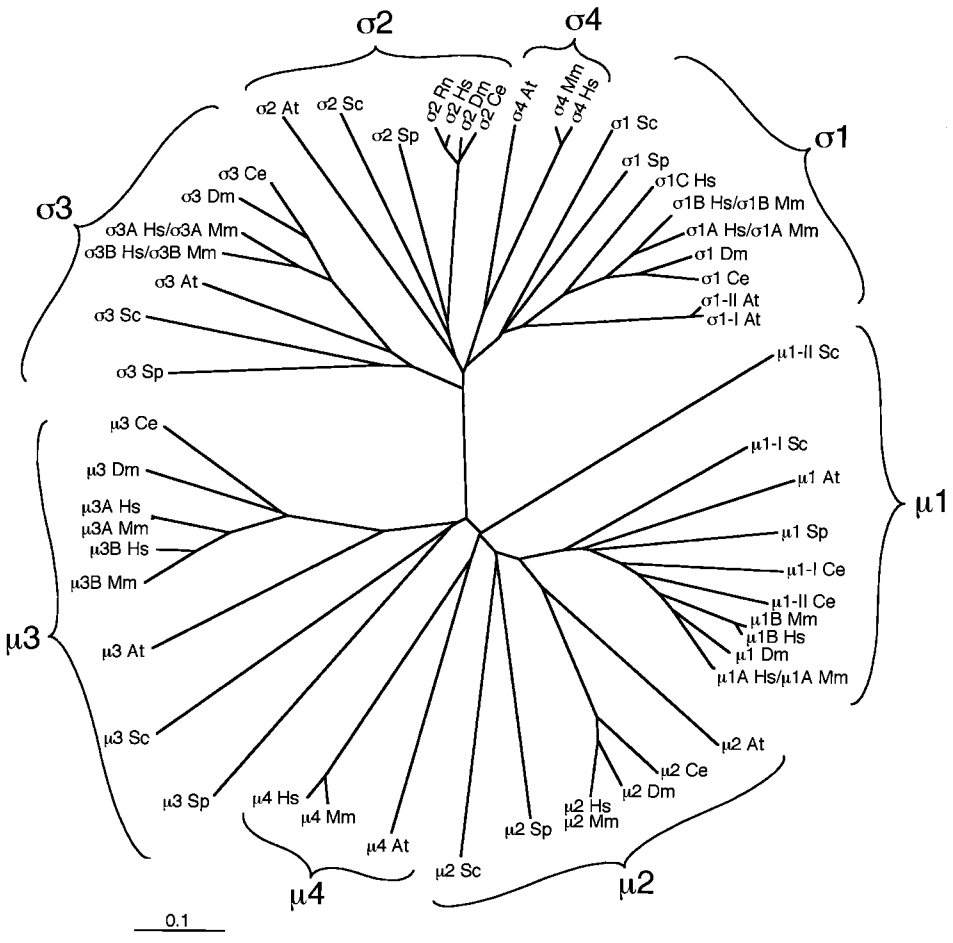

Figure 5. Analysis of the evolutionary relationship between the small and medium adaptins calculated as described for Figure 4. Note that for σ2, the *R. norvegicus* (*Rn*) sequence was used, as the *M. musculus* σ2 sequence is not yet available.

β3A and μ3A are ubiquitous while β3B and μ3B are exclusively found in brain or other neural tissues. Similarly, μ1A is ubiquitous while μ1B is expressed only in epithelial cells. It thus appears that more complex organisms built specificity on top of their already established AP complexes by selectively replacing some of their preexisting subunits with new isoforms.

GGAs and Stonins

All three mammalian GGA proteins contain a domain that is homologous to the ear domain of the γ adaptins, but are otherwise structurally distinct from the γ adaptins. A duplication and transfer of the γ ear domain probably took place after the emergence of AP-1 but before the separation of the major eukaryotic kingdoms (Figure 3). The complete GGA gene was duplicated in *S. cerevisiae* and *S. pombe*, giving rise to two GGA isoforms. *C. elegans* and *D. melanogaster* have only one GGA gene but mice and humans have three. GGA2 was likely the first one to branch out, while GGA1 and GGA3 separated later (Figure 3).

The stonins have a carboxy-terminal domain homologous to the signal-binding domain of the μ adaptins, more specifically to mammalian μ2. This suggests that genomic sequences encoding the μ2 signal-binding domain were duplicated and translocated next to sequences encoding the proline-rich (PRD in Figure 3) and stonin homology domains (SHD in Figure 3) thus creating the stonin ancestor (stonin 1/2 in Figure 3). Since stonin orthologues have been identified in *C. elegans*, *D. melanogaster*, mice and humans, but not yeast or *A. thaliana*, it appears that they evolved in the animal lineage. Similarly to adaptins and GGAs, however, the stonin gene was duplicated in vertebrates, leading to the human and mouse stonin 1 and stonin 2 genes (Figure 3).

CONCLUSIONS AND PROSPECTS

In this essay we have assembled a comprehensive inventory of adaptins and related proteins encoded in the genomes of various eukaryotic organisms. We have tentatively assigned them to complexes according to the mammalian AP nomenclature and described the structural and evolutionary relationships between these proteins. Our analyses resulted in the identification of several new gene products that had not yet been annotated as adaptins or related proteins. With a systematic classification of the adaptins in hand, we can now attempt to explain the full range of protein sorting events mediated by the adaptins. Many aspects of adaptin function

remain to be elucidated, including: (1) the exact intracellular localization and distribution of AP complexes and related proteins; (2) the signal-binding specificity of all the adaptins and related proteins; (3) the nature of the cellular processes in which AP complexes and related proteins are involved; (4) the function of ubiquitous and tissue-specific adaptin isoforms; (5) the regulation of expression of adaptins during development. We expect rapid advances in the elucidation of these issues through refinement of established morphological, biochemical, and genetic methodologies, as well as the adoption of recent advances in genetic manipulation such as RNA interference in mammalian cells.

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