Recent studies have described a widely expressed adaptor-like complex, named AP-3, which is likely involved in protein sorting in exocytic/endocytic pathways. The AP-3 complex is composed of four distinct subunits. Here, we report the identification of one of the subunits of this complex, which we call β3A-adaptin.

The AP-3 complex is functionally related to AP-1 and AP-2, and that it is likewise involved in the regulation of intracellular protein trafficking. In this paper we report the cloning of a novel cDNA encoding a 140-kDa subunit of the ubiquitous AP-3 complex. This protein, referred to as β3A-adaptin, is located on the Golgi network and the plasma membrane, and with coated vesicles that originate from these organelles. In addition to clathrin, these coats contain specific protein complexes known as adaptors or APs. One of these adaptors, AP-1, is a component of the trans-Golgi network clathrin coats and consists of four subunits: γ- and β1-adaptins (~100 kDa), μ1 (~47 kDa), and σ1 (~19 kDa). Another adaptor, AP-2, is associated with plasma membrane clathrin coats and is composed of four subunits: α- and β2-adaptins (~100 kDa), μ2 (~50 kDa), and σ2 (~17 kDa). The analogous subunits of AP-1 and AP-2 display significant homology to each other; in addition, the adaptor complexes themselves exhibit a similar overall structure of a “head” with two protruding “ears,” each separated from the head by a flexible “hinge” (3–5, 46).

A major function of AP-1 and AP-2 is to link clathrin lattices to the corresponding membranes. This role is fulfilled by the γ- and β1-adaptin subunits of AP-1 and the α- and β2-adaptin subunits of AP-2 (6–9). The adaptors are also responsible for the recognition of sorting signals present in the cytosolic domains of integral membrane proteins (10–21), an event that leads to the concentration of these proteins within clathrin-coated areas of the trans-Golgi network and the plasma membrane. Recent evidence suggests that the μ1 subunit of AP-1 and the μ2 subunit of AP-2 are directly involved in signal recognition (16, 18, 22).

In the past few years, it has become clear that the structure and function of clathrin coats serve as paradigms for other protein coats. Indeed, some subunits of the non-clathrin coat, COP, are structurally related to subunits of AP-1 and AP-2 (23–26). In addition, recent studies have identified other mammalian proteins that display significant homology to AP-1 and AP-2 subunits and are components of previously unknown coats. Pevsner et al. (27) isolated cDNAs encoding two proteins, named p47A and p47B, that exhibit ~80% identity to each other and ~30% identity to μ1 and μ2. Newman et al. (28) described another protein, named β-NAP, that is ~30% identical to β1- and β2-adaptins. Finally, Watanabe et al. (29) and Dell’Angelica et al. (30) reported the identification of two proteins named α3A and ε3B; these proteins are 84% identical to each other and ~30% identical to σ1 and σ2. Some of these subunits are expressed in a wide variety of tissues and cell lines (p47A, σ3A, and ε3B), while others are only expressed in brain, spinal cord, and neuronal cell lines (p47B and β-NAP).

Not surprisingly, these homologs of AP-1 and AP-2 subunits were found to exist as heterotetrameric assemblies resembling adaptor complexes (30, 31). One of these complexes, expressed in neuronal cells, contains β-NAP and either p47A or p47B (31).

A similar complex, which was named AP-3, is expressed in all cells examined to date and contains subunits of ~160, ~140, ~47, and ~22 kDa (30). The ~47-kDa protein corresponds to p47A while the ~22-kDa protein is either α3A or ε3B. The ~140-kDa subunit of this complex is immunologically related to the neuronal protein β-NAP but distinct from it based on its expression in various non-neuronal cell lines (30).

In this paper we report the cloning of a novel cDNA encoding the ~140-kDa subunit of the ubiquitous AP-3 complex. This protein, referred to as β3A-adaptin, is closely related to β-NAP and displays significant homology to β1- and β2-adaptins. Consistent with it being a subunit of the ubiquitous AP-3 complex, β3A-adaptin is expressed in a wide variety of tissues and cell lines. Biochemical analyses show that β3A-adaptin is phosphorylated on serine residues in vivo and is absent from clathrin-coated vesicles. The similarity of β3A-adaptin to β1- and β2-adaptins lends support to the idea that AP-3 is structurally and functionally related to AP-1 and AP-2, and that it is likewise involved in the regulation of intracellular protein trafficking.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) U81504.

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β3A-adaptin, a Subunit of the Adaptor-like Complex AP-3*

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Characterization of β3A-adaptin

EXPERIMENTAL PROCEDURES

Cloning of β3A-adaptin cDNA—Two EST clones (GenBank accession codes R02669 and T96538) from human fetal liver/spleen (Washington University-Merck EST Project) were found to encode portions of a novel protein with significant homology to β-NAP. Based on these partial sequences, a full-length cDNA encoding this protein, named β3A-adaptin, was isolated from a Marathon-Ready cDNA library (CLONTECH, Palo Alto, CA) by a combination of 5′-RACE PCR, using the Advantage® cDNA PCR kit (CLONTECH). The specific primers used in a first and second (nested) 5′-RACE reactions were complementary to nucleotides 3181–3205 and 2955–2981 of the β3A-adaptin cDNA, respectively. The first and second (nested) primers used for 3′-RACE PCR corresponded to nucleotides 1773–1800 and 1898–1922 of the full-length cDNA, respectively. Both 5′ and 3′ nested PCR products were cloned into the pNoTA/T7 shuttle vector (5 Prime → 3 Prime, Inc., Boulder, CO). Several independent clones were isolated and sequenced to guard against errors introduced by the DNA polymerase during PCR amplification. Both strands were sequenced by the dideoxy method.

Cells—The sources and culture conditions for all the human cell lines used in this study are described elsewhere (29).

Northern Blot and RT-PCR Analyses of mRNA Expression—Northern blot analysis was carried out as described before (30). The β3A-adaptin probe was obtained by PCR using a 5′ primer corresponding to nucleotides 1989–1992 and a 3′ primer complementary to nucleotides 2955–2981 of the full-length cDNA, respectively. The probe used for detection of the β-NAP mRNA consisted of a 450-base pair fragment, which was PCR-amplified from the EST clone 165789 (Washington University-Merck EST Project) using 5′ and 3′ primers corresponding to nucleotides 2000–2028 and 2424–2449 of the full-length β-NAP cDNA (GenBank accession number: U37673), respectively. The above sets of primers were also used for RT-PCR analysis of the expression of β3A-adaptin and β-NAP mRNAs in cell lines; the analysis was performed by using the Gene Amp® XL RNA PCR kit (Perkin-Elmer, Branchburg, NJ) according to the manufacturer’s instructions.

Production of GST Fusion Proteins—To prepare a series of GST proteins bearing different segments of β3A-adaptin, the corresponding cDNA fragments were engineered by PCR to be cloned in-frame into the pGEX-5X-1 vector (Pharmacia Biotech, Uppsala, Sweden). The β3A-adaptin cDNA segments corresponding to amino acids 1–287, 1–642, and 810–1094 were cloned into the EcoRI-NotI sites of the vector, while the segment encoding amino acids 643–809 was cloned into the BamHI-NotI sites of the vector. The DNA sequence of all the constructs was confirmed by manual sequencing. Fusion proteins were expressed in Escherichia coli cells and then affinity-purified using glutathione-Sepharose 4B beads (Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer’s instructions. Antibodies—Monoclonal anti-α-adaptin antibody 100/2 was obtained from Sigma. The preparation and purification of polyclonal rabbit antibodies to p47 (A and B) and α3 (A and B) were described previously (29). The preparation of an antiserum against a GST fusion protein that bears the hinge region of β-NAP (GST-β-NAP31-275) has also been described previously (29). Here, this antiserum was passed through a column containing GST coupled to Affi-Gel 15 (Bio-Rad), to remove anti-GST antibodies, and then affinity-purified (32) using as a ligand a peptide comprising residues 647–796 of β-NAP. The purified antibody, herein referred to as β3H1, recognizes the hinge region of both β-NAP and β3A-adaptin, as inferred from immunoprecipitation experiments with the corresponding fragments translated in vitro. The β3H7 antibody was raised in rabbits by immunization with the GST-β3A-adaptin fusion protein. The antibody was affinity-purified using as a ligand the same fusion protein coupled to Affi-Gel 15 beads, and was subsequently immunoadsorbed with GST, followed by GST-β-NAP647–796 to obtain a monospecific antibody to the hinge region of β3A-adaptin. The lack of cross-reactivity between the β3H7 antibody and β-NAP was corroborated by immunoprecipitation experiments and by immunoblotting. The β3A and β3C antibodies were obtained by immunizing rabbits with GST-β3A1–642 and GST-β3A610–1094 fusion proteins, followed by affinity purification on immobilized GST-β3A1–287 and GST-β3A810–1094, respectively. Both antibodies were absorbed with GST. Polyclonal rabbit antibodies to BSA (Cappel, Cochranville, PA) or to the FLAG epitope (Santa Cruz Biotechnology, Santa Cruz, CA) were used as irrelevant antibody controls. The preparation of an antibody to human δ-adaptin is described elsewhere.2

Immunoprecipitation and Immunoblotting—Metabolic labeling of M1 cells with [35S]methionine, immunoprecipitation-recapture experiments, and immunoblot analysis were performed as described previously (30).

Alkaline Phosphatase Treatment—The subunits of the AP-3 complex were isolated from [35S]methionine-labeled M1 cells by immunoprecipitation-recapture (30). The immunoprecipitates were resuspended in 50 mM Tris-HCl (pH 8.5), 1 mM EDTA and divided into two aliquots. One of the aliquots was treated with 1 milliunit of calf intestinal alkaline phosphatase (Boehringer Mannheim) for 1 h at 37 °C; the other one was mock-treated. Samples were analyzed by SDS-PAGE (33) followed by fluorography.

Metabolic Labeling with [32P]Orthophosphate and Phosphoamino Acid Analysis—M1 cells were grown to almost confluence in Dulbecco’s modified Eagle’s medium supplemented with 9% (v/v) fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamicin. Cells were detached from the plates by treatment with 0.5 mg/ml trypsin, 10 mM EDTA in phosphate-free Dulbecco’s modified Eagle’s medium, 25 mM HEPES, collected by low-speed centrifugation, and then washed twice with phosphate-free Dulbecco’s modified Eagle’s medium, 25 mM HEPES, 0.1% BSA. Subsequently, cells were suspended in phosphate-free Dulbecco’s modified Eagle’s medium, 25 mM HEPES, 0.1% BSA containing 2 μCi/ml [32P]Orthophosphate and incubated at 37 °C for 3 h. After the incubation, cells were washed three times with ice-cold phosphate-buffered saline containing 5 mM EDTA, 1 mM orthovanadate, and 1 mM sodium fluoride, and then lysed by incubation for 15 min on ice with 1% (w/v) Triton X-100, 0.3 mM NaCl, 50 mM Tris-HCl (pH 7.4), 10 mM iodoacetamide, 5 mM EDTA, 1 mM orthovanadate, 1 mM sodium fluoride, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 2 μg/ml leupeptin, 0.1% (w/v) BSA. Immunoprecipitation of the AP-3 complex with an anti-α3 antibody, dissociation of the complex, and subsequent immunoprecipitation of the subunits with specific antibodies were carried out as described previously (30). Phosphoamino acid analysis of metabolically [32P]-labeled β3A-adaptin was performed by two-dimensional thin layer electrophoresis (34).

Other Materials and Methods—The preparation of cytosol from M1 cells and its fractionation by gel filtration were performed as described previously (30). For isolation of the complex by affinity chromatography, the cytosol was passed through a Protein A-Sepharose column (1.6 ml) and then loaded onto a 0.4 ml column containing 0.7 mg of purified anti-α3 antibody which had been covalently coupled to Protein A-Sepharose (32). Bound proteins were eluted with 0.1 μM glycine (pH 2.5). A crude membrane fraction and purified clathrin-coated vesicles from bovine brain were the kind gift of Lois Greene and Evan Eisenberg (National Heart, Lung, and Blood Institute, National Institutes of Health).

RESULTS

Molecular Cloning and Sequence Analyses of a cDNA Encoding β3A-adaptin—Our previous work demonstrated the existence in non-neuronal cells of a protein immunologically related to the neuronal β-NAP (30). To identify this protein, we searched EST data bases for β-NAP homologs. Two EST clones from human fetal liver/spleen were found to encode a novel protein with significant homology to β-NAP. The complete cDNA was obtained using 5′- and 3′-RACE procedures based on partial sequences of the EST clones. Analysis of the sequence of this 3950-base pair cDNA revealed a long open reading frame encoding a protein of 1094 amino acids with a predicted molecular mass of 121,350 Da. The protein displayed significant homology not only to β-NAP (Fig. 1), but also to the Saccharomyces cerevisiae chromosome VII open reading frame YGR261c and to mammalian β1- and β2-adaptins (Fig. 2). The new human protein was named β3A-adaptin.

Based on a hydropathy plot, β3A-adaptin can be divided into at least three distinct regions: an amino-terminal (A) region spanning residues 1–642, a strongly hydrophilic segment (H) comprising residues 643–809, and a carboxyl-terminal (C) region.

1 The abbreviations used are: RACE, rapid amplification of cDNA ends; BSA, bovine serum albumin; EST, expressed sequence tag; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; RT, reverse transcription; kb, kilobase pair(s).

2 C. E. Oui, J. E. Moreira, E. C. DellAngelica, G. Poy, D. Wassarman, and J. S. Bonifacino, submitted for publication.
region spanning residues 810–1094 (Fig. 2, A and B). This structure is reminiscent of the domain organization of other adaptins (3–5).

The homology of β3A-adaptin to β-NAP extends over the entire length of their polypeptide chains (Fig. 1), although the degree of sequence similarity varies in the three regions (Fig. 2B). The percentage of conserved amino acid residues shared by the two proteins is highest in the A region; this region also exhibits the highest degree of similarity to the S. cerevisiae YGR261c protein and to the mammalian β-adaptins (Fig. 2B).

In the β1- and β2-adaptins, the amino-terminal segment corresponds to a “head” or “core” domain of the proteins where interactions with the other adaptor subunits are thought to take place. The head domains of β1-adaptin, β2-adaptin, and β-NAP have been shown to contain up to 14 Arm repeats (35, 36), a degenerate motif that probably functions as a protein-protein interaction element. Analysis of the β3A-adaptin sequence reveals that this protein also contains 12 or 13 Arm repeats in the A region (not shown).

The H region of β3A-adaptin is strongly hydrophilic and rich in acidic residues (31%) and serine residues (26%) (Fig. 1). This region contains many potential sites for phosphorylation by the serine/threonine kinases casein kinase I and casein kinase II (21 and 25 sites, respectively; Refs. 37 and 38). The H region of β3A-adaptin is 50% identical to the analogous segment in β1- and β2-adaptins and is absent from YGR261c (Fig. 2B). In the β-adaptins, this segment corresponds to the “ear” or “appendage” domain.

A phylogenetic tree constructed with the above sequences and that of the more distantly related COPI subunit, β-COP, (Fig. 2C) shows that β3A-adaptin, β-NAP, and YGR261c all belong to a group that may have diverged from the others early in the evolution of the family. The mammalian β1 and β2-adaptins, a D. melanogaster β-adaptin and two other S. cerevisiae adaptins (Sc ADB1 and Sc ADB2) cluster together in a separate branch. The β-COPs from rat, fly, and yeast are distantly related to the members of the other two groups. Thus, these sequence analyses define a group of proteins (β3A-adaptin, β-NAP, and YGR261c) that are more closely related to clathrin-associated β-adaptins than to COPI components.

**Tissue and Cell Expression of the β3A-adaptin mRNA**–The pattern of expression of the β3A-adaptin mRNA in various human tissues and cell lines was examined by Northern blot analysis (Fig. 3). A single ~4.2-kb β3A-adaptin message was
Characterization of β3A-adaptin

Fig. 2. Comparison of the sequence of β3A-adaptin with those of other coat proteins. A, Kyte-Doolittle hydropathy plot of human β3A-adaptin. The sequence of β3A-adaptin was divided into three regions based on the hydropathy profile and the degree of identity to analogous regions in other adaptins. The three regions were designated A (amino-terminal), H (bingle), and C (carboxyl-terminal). B, schematic representation of the structure of human β3A-adaptin, β-NAP, the S. cerevisiae (Sc) YGR261c gene product, and rat β1-adaptin, showing regions of sequence homology (corresponding domains are indicated by identical shading patterns). C, a phylogenetic tree comparing the sequence of human β3A-adaptin with those of other coat proteins from different species was constructed using the Darwin program (52). The length of each branch represents the phosphogenetic distance based on the hydropathy profile and the degree of identity to analogous regions in other adaptins. The three regions were designated A (amino-terminal), H (bingle), and C (carboxyl-terminal). B, schematic representation of the structure of human β3A-adaptin, β-NAP, the S. cerevisiae (Sc) YGR261c gene product, and rat β1-adaptin, showing regions of sequence homology (corresponding domains are indicated by identical shading patterns). Amino acid numbers demarcating the different regions are shown on top of each scheme. C, a phylogenetic tree comparing the sequence of human β3A-adaptin with those of other coat proteins from different species was constructed using the Darwin program (52). The length of each branch represents the phosphogenetic distance based on the hydropathy profile and the degree of identity to analogous regions in other adaptins.

Fig. 3. Analysis of β3A-adaptin mRNA expression in different tissues and cell lines. A, Northern blot analysis of the human fibroblast cell line M1, which expresses the β3A-adaptin mRNA but not the β-NAP mRNA (Fig. 3). Antibodies to both the H and C regions of β3A-adaptin (β3H7 and β3C1, respectively) were found to recognize a protein that migrated as a 140-kDa polypeptide on SDS-PAGE and that co-eluted on a gel filtration column with the 140-kDa protein α3 (Fig. 4A), a known component of the AP-3 complex (30). The peak elution of β3A-adaptin and α3 corresponded to a complex with Stokes radius of ~85 Å, as previously reported (30).

To address directly the question of whether β3A-adaptin is a component of AP-3, this complex was affinity-purified from M1 cells on an anti-α3 column and the eluate was analyzed by Western blotting with anti-α3 and anti-β3A-adaptin antibodies (Fig. 4B). The anti-α3 antibody recognized minor and major species of 20–22 kDa, as previously shown (30), whereas the different antibodies to β3A-adaptin recognized a ~140-kDa species (Fig. 4B). Neither band was observed when the same procedure was performed on a control column without anti-α3 antibody (data not shown). Thus, β3A-adaptin co-purifies with α3 on affinity chromatography.

The association of β3A-adaptin with α3 was also analyzed using an immunoprecipitation-recapture technique (30). This technique consisted of immunoprecipitating the AP-3 complex from [35S]methionine-labeled M1 cells using an antibody to α3 and, after dissociation in the presence of SDS, isolating the individual subunits of the complex by re-precipitation with specific antibodies. Re-precipitation with the β3H1 antibody (Fig. 5A) and with the antibodies β3H7, β3A1, and β3C1 (not shown) further confirmed that β3A-adaptin is indeed a component of the AP-3 complex. Fig. 5A also illustrates that, in
### Characterization of β3A-adaptin

#### FIG. 4. The β3A-adaptin protein is a component of the AP-3 complex.

A. cytosol from M1 cells was fractionated on a Superose 6 gel filtration column and fractions 28–36 were subjected to Western blot analysis with antibodies to σ3 and to the H (β3H7) and C (β3C1) regions of β3A-adaptin. Both β3A-adaptin and σ3 co-eluted in this column, peaking at a fraction corresponding to a Stokes radius of ~85 Å (30). B, the AP-3 complex was purified by affinity chromatography on an anti-σ3 antibody column. Western blots of the purified complex were probed with an irrelevant antibody (anti-FLAG), with an antibody to σ3, or with the anti-β3A-adaptin antibodies β3A1, β3H7, and β3C1. The positions of molecular markers (in kDa) are indicated at the left.

addition to σ3 (A or B) and β3A-adaptin, the other components of AP-3 are p47 (27) and another protein known as δ-adaptin.2

**β3A-adaptin Is a Phosphoprotein**—Because of the many potential phosphorylation sites predicted from the sequence (see above), we were interested in determining whether the protein was phosphorylated in vivo. To this end, we treated [35S]methionine-labeled β3A-adaptin isolated from the cytosol of M1 cells with alkaline phosphatase and determined the migration of the untreated and treated samples on SDS-PAGE. The alkaline phosphatase treatment resulted in decreased migration of the entire population of labeled β3A-adaptin molecules, as can be seen in Fig. 5A (lanes 5 and 6), and with better resolution in Fig. 5B. This experiment suggested that β3A-adaptin is a phosphorylated protein at steady state. In contrast to β3A-adaptin, none of the other subunits of AP-3 changed their phosphorylation state (Fig. 5A).

The fact that the AP-3 complex exists both in soluble and membrane-bound pools (30) led us to investigate whether phosphorylation of β3A-adaptin correlates with association to membranes. We found that both the cytosolic and membrane-bound forms of β3A-adaptin were equally sensitive to alkaline phosphatase (Fig. 5A). Thus, these experiments suggest that the AP-3 complex is not highly enriched in the clathrin-coated vesicle preparation relative to the crude membrane fraction (Fig. 7). This was in contrast to β3A-adaptin which was not detected in the clathrin-coated vesicles, although it was present in high amounts in the crude membrane fraction (Fig. 7). Similarly, p47 and σ3 were not detected in the clathrin-coated vesicle fraction (Fig. 7). Thus, these experiments suggest that the AP-3 complex is not associated with clathrin-coated vesicles.

#### FIG. 5. Alkaline phosphatase treatment affects the electrophoretic mobility of β3A-adaptin.

A, the AP-3 complex was isolated from a cytosolic extract of [35S]methionine-labeled M1 cells by immunoprecipitation with an antibody to σ3. The complex was then dissociated in the presence of SDS and subjected to re-precipitation with antibodies to BSA (nonspecific control), δ-adaptin, β3A-adaptin, p47, and σ3, as indicated in the figure. The immunoprecipitates were treated for 1 h at 37 °C in the absence (−) or presence (+) of alkaline phosphatase and then resolved by SDS-PAGE on a 4–20% polyacrylamide gel. Notice the slight decrease in the electrophoretic mobility of β3A-adaptin upon treatment with alkaline phosphatase. Other subunits of AP-3 were not noticeably affected. B, analysis of the effect of alkaline phosphatase on the migration of [35S]methionine-labeled β3A-adaptin isolated from either cytosol or membranes. Samples were resolved by SDS-PAGE on a 8% acrylamide gel; this gel system allowed a better resolution of phosphorylated and dephosphorylated forms of β3A-adaptin. The positions of molecular markers (in kDa) are indicated on the left.

bodies to two other components of the AP-3 complex, p47 (A and B) and σ3 (A and B), and to a component of AP-2, α-adaptin, as a control. Two forms of α-adaptin (αa and αc; Ref. 39) were highly enriched in the clathrin-coated vesicle preparation relative to the crude membrane fraction (Fig. 7). This was in contrast to β3A-adaptin which was not detected in the clathrin-coated vesicles, although it was present in high amounts in the crude membrane fraction (Fig. 7). Similarly, p47 and σ3 were not detected in the clathrin-coated vesicle fraction (Fig. 7). Thus, these experiments suggest that the AP-3 complex is not associated with clathrin-coated vesicles.

**DISCUSSION**

In this study we describe a novel human protein named β3A-adaptin. The β3A-adaptin mRNA is expressed in a wide variety of tissues and cell lines and the protein itself has been shown to exist in various cell lines (this study; Ref. 30). Biochemical analyses demonstrate that β3A-adaptin is a subunit
Characterization of β3A-adaptin

of the ubiquitous adaptor-like protein complex AP-3, which has been previously shown to exist in association with trans-Golgi network and/or endosomal compartments (30). The other subunits of the AP-3 complex are δ-adaptin,2 p47A (Ref. 27; now called μ3A), and δ3A or δ3B (29, 30) (Fig. 5). The AP-3 complex is not associated with clathrin-coated vesicles, suggesting that it may be a component of a different coat. In this regard, the ubiquitous AP-3 complex resembles the complex containing the brain-specific β-NAP, which is also absent from clathrin-coated vesicles (28, 31).

The existence of a closely-related homolog of the brain-specific β-NAP was first evidenced by the immunoprecipitation of a ~140-kDa protein from non-neuronal cells using an antibody to β-NAP (30). Since β-NAP is not expressed in non-neuronal cells (Refs. 28 and 31, this study), this protein had to be an immunologically cross-reactive homolog. Sequence analyses now show that β3A-adaptin shares 61% overall identity and 75% overall similarity with β-NAP. β-NAP itself is part of a complex with either the brain-specific p47B (now called μ3B) or the ubiquitous p47A (μ3A) subunits, and with two other proteins that are also similar to subunits of AP-3 (31). This suggests that both the ubiquitous β3A-adaptin-containing complex and the brain-specific β-NAP-containing complex have a similar structure and may even share some common subunits. Moreover, in cells that express both the ubiquitous and brain-specific subunits (i.e. neurons), the subunits could combine to generate several different complexes.

In addition to β-NAP, other coat proteins display a lower but significant degree of homology to β3A-adaptin. This includes the S. cerevisiae gene product, YGR261c, also known as APL6/YKS5. This protein is likely to be the yeast counterpart of β3A-adaptin and/or β-NAP and, in fact, has been shown to be part of a complex with three other proteins (APL5/YKS4, APM3/YKS6, and APS3/YKS7) which are closely related to AP-3 subunits.3 β3A-adaptin also has a significant degree of homology to the clathrin-associated adaptor subunits β1- and β2-adaptins and to the COPI subunit β-COP. The fact that β3A-adaptin is related to all of these organellar coat proteins and the ability of p47A (μ3A) to bind tyrosine-based sorting signals (30) strongly suggest that the AP-3 complex may be similarly involved in the regulation of intracellular protein trafficking.

An examination of the β3A-adaptin sequence reveals at least three distinct regions, named A (amino-terminal), H (hinge), and C (carboxyl-terminal) (Fig. 2B). The three regions are likely analogous to structural and functional domains that have been well defined in β1- and β2-adaptins (40, 41). The A region is homologous to the head or core domains of β1- and β2-adaptins, which are involved in interactions with the other subunits of AP-1 and AP-2 (9, 40, 42). The H region is analogous to the hinge or “stalk” domains of β1- and β2-adaptins, which mediate interactions with clathrin (7, 43). Finally, the C region of β3A-adaptin might be analogous to the carboxy appendage domains of β1- and β2-adaptins. The function of the β1- and β2-adaptin carboxy domains is still unclear, although the analogous domain of α-adaptin has been shown to bind regulatory molecules such as dynamin (44) and Eps15 (45). The domain organization of δ-adaptin is also thought to resemble those of α- and γ-adaptin.2 These similarities suggest that AP-3 may have an overall structure analogous to AP-1 and AP-2.

A salient feature of β3A-adaptin is that it is phosphorylated on serine residues (Figs. 5 and 6). Moreover, β3A-adaptin is the only subunit of AP-3 that is detectably phosphorylated under the conditions of our experiments. Although the sites of phosphorylation within β3A-adaptin have not been located, the presence of a large number of consensus sequences for phosphorylation by casein kinase I and II in the H region suggests that this region might be highly phosphorylated. While the degree of identity of β3A-adaptin to β-NAP and S. cerevisiae YGR261c is lower in the H region as compared with the A region, it is noteworthy that the overall characteristics of the segment are conserved among these proteins, including the presence of many potential sites of phosphorylation by casein

3 S. Lemmon and L. Robinson, personal communication.
kinase I and II. Indeed, β-NAP is also heavily phosphorylated both in vivo and in vitro (28) and YGR261c displays a genetic interaction with casein kinase I in yeast cells. Components of the clathrin-associated adaptors (47–50) and of COPI (51) have also been shown to be phosphorylated, suggesting that phosphorylation might be a common mechanism for regulating coat function within cells.

The findings presented here add to the growing evidence that AP-3 is a structural and functional homolog of AP-1 and AP-2. Indeed, all three complexes are capable of binding reversibly to membranes and have a similar heterotetrameric structure. Moreover, the analogous subunits of the three complexes are structurally related to each other and may even exhibit a similar domain organization. Finally, the analogous subunits might play similar roles. For instance, the p47A (μ3A) subunit of AP-3 is capable of binding tyrosine-based sorting signals (22, 30), like its relatives of AP-3 is capable of binding tyrosine-based sorting signals (22, 30), like its relatives 3A-adaptin and Kirchhausen, T. (1996) *EMBO J.* 15, 5789–5795

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