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Cloning, expression, and localization of a novel γ-adaptin-like molecule

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Abstract We describe the cloning, expression, and localization of γ_2-adaptin, a novel isoform of γ-adaptin. The predicted human and mouse γ_2-adaptin proteins are ~90 kDa and 64.4% and 61.7% identical to γ-adaptin, respectively. γ_2-Adaptin was localized to the Golgi, its localization distinct from γ-adaptin. The membrane association of γ- and γ_2-adaptin could further be distinguished by differential sensitivity to the fungal metabolite brefeldin A, γ_2-adaptin binding being insensitive to drug treatment. Together, these results suggest that γ_2-adaptin plays a role in membrane transport distinct from that played by γ-adaptin.

Key words: Adaptin; Adaptor; Golgi; Human; Mouse

1. Introduction

Intracellular membrane traffic in eukaryotic cells is typified by the budding of transport vesicles from progenitor membranes and the subsequent fusion of vesicles with target membranes. The mechanism of transport vesicle budding must confer the vesicle's identity as defined by its cargo and the components required for vesicle targeting and fusion upon reaching its destination. Vesicle budding typically requires recruitment of cytosolic coat proteins, such as clathrin, COPI, and COPII. These coat proteins interact directly and indirectly with integral membrane proteins at the bud site, and are likely to participate in the selection of cargo (reviewed in [1–3]).

Clathrin is the best characterized coat protein involved in the formation of transport vesicles. The clathrin-mediated formation of transport vesicles from the plasma membrane and *trans*-Golgi network requires the activity of multi-component adaptor protein (AP) complexes to recruit clathrin to the cytosolic face of these membranes. The AP-1 complex recruits clathrin to the TGN and AP-2 recruits clathrin to the plasma membrane (reviewed in [4]). A third adaptor complex, termed AP-3, is also found on the TGN, as well as on peripheral cytoplasmic foci, that may correspond to endosomal structures [5,6]. This adaptor complex may be a component of a non-clathrin coat [6], although a recent study has shown that it can associate with clathrin [7].

Adaptors are heterotetrameric structures consisting of two heavy chains termed 'adaptins' and one each of a medium (μ) and small (σ) chain. The AP-1 adaptor complex associated with clathrin coats on the TGN consists of two adaptins (γ- and β1-adaptin) of ~100 kDa, μ1 (~47 kDa), and σ1 (~19 kDa). AP-2, the plasma membrane adaptor, is also comprised of two adaptins (α- and β2-adaptin, each ~100 kDa), μ2 (~50 kDa), and σ2 (~17 kDa). The AP-3 heterotetramer has two somewhat larger adaptin subunits, δ- and β3-adaptin (~140 kDa and ~160 kDa), μ3 (~47 kDa), and σ3 (~22 kDa). There are neuron specific isoforms of the AP-2 and AP-3 adaptor complexes containing neuronal-specific adaptins, μ chains, and σ chains. All adaptor complexes have a similar overall structure consisting of an N-terminal, block-like, 'head' domain, a hydrophilic, usually proline and glycine-rich, 'hinge' domain, followed by a C-terminal 'ear' domain [4–6,8].

AP-1 and AP-2 recruit clathrin and participate in molecular sorting [3]. AP-1 and AP-3 are thought to mediate transport from the TGN to the endocytic pathway; the corresponding activity for AP-2 is from the plasma membrane to the endosome. Clathrin on early endosomes is also likely to participate in molecular sorting at this organelle. However, until recently [7] none of the known adaptor complexes were found associated with clathrin on endosomes [3,6,9,10]. The role of μ and σ chains in adaptor complexes has been discussed extensively elsewhere [4].

There are at least twelve well characterized sorting events in intracellular membrane trafficking described in eukaryotic cells [4]. Notably, there are more pathways than coat proteins to account for them. This observation, and the assumption that molecular sorting must require a protein coat [1,2,4,11], prompted us to search for novel adaptin molecules. Here we report the cDNA cloning and initial characterization of a novel, ubiquitously expressed, γ-adaptin-like molecule, we term 'γ_2-adaptin'.

2. Materials and methods

2.1. Cell lines, antibodies, and reagents

HeLa cells were grown on plastic tissue culture plates in MEM, heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 10 μg/ml streptomycin, 2 mM L-glutamine, at 37°C in 5% CO_2. Fluorescent secondary antibodies were from Jackson ImmunoResearch Laboratories. Partial human cDNAs were from the American Type Culture Collection (Rockville, MD, USA). An oligo(dT) primed mouse embryonic brain λZAPII cDNA library was generously provided by M. Tiemeyer (Yale University School of Medicine, New Haven, CT, USA). All molecular biology reagents were purchased from New England Biolabs (Beverly, MA, USA), unless otherwise noted. Brefeldin A was from Epicenter Technologies (Madison, WI, USA). Mouse monoclonal anti-γ-adaptin (100/3) and FITC-labeled *Helix pomatia* lectin were purchased from Sigma (St. Louis, MO, USA). Monoclonal anti-β-COP was generously provided by T. Kreis, University of Geneva, Geneva, Switzerland. Chemical fixatives for electron microscopy

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were obtained from Electron Microscope Sciences (Ft. Washington, PA, USA), protein A-gold conjugates were purchased from J.W. Slot, The University of Utrecht (Cell Biology, Utrecht, The Netherlands). All other chemicals for electron microscopy were obtained from Sigma, St. Louis, MO, USA.

2.2. Database searches, sequencing, and sequence analysis

An NCBI BLAST search of the EST databases using the published mouse γ-adaptin cDNA as the query sequence (GenBank accession number X54424) was performed. The mouse γ_2-adaptin was cloned using array cloning [12]. Primers were derived from a mouse EST (GenBank accession number AA185250) corresponding to the 3′ end of mouse γ_2-adaptin. The T49401 partial human cDNA of human γ_2-adaptin was used as a probe for Southern analysis for array cloning. A 78-bp gap corresponding to amino acids 189–215 in the open reading frame of the mouse γ_2-adaptin was cloned by RT-PCR of mouse first strand cDNA from thymus. PCR was performed using PLATINUM *Taq* DNA polymerase as per the manufacturer's instructions (Life Technologies, Gaithersburg, MD, USA). Confirmation of the amino acid sequence of mouse γ_2-adaptin was generously provided by H. Cen and R. Williams (Chiron, Emeryville, CA, USA). The full-length cDNAs for both the mouse and the human γ_2-adaptins were toxic to bacteria and could not be maintained as complete cDNAs. Sequence analysis and compilation of sequencing information was performed using LaserGene Navigator (DNAStar, Madison, WI, USA). Sequencing of both strands of cDNAs was done by the W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University, New Haven, CT, USA. Oligos were obtained from the Program for Critical Technologies in Molecular Medicine, Yale University, New Haven, CT, USA.

2.3. Northern analysis

Northern analysis of a multiple human mRNA (Clontech, Palo Alto, CA, USA) was performed as per the manufacturer's instructions using [α-^{32}P]dCTP-labeled (Amersham, Arlington Heights, IL, USA), random primed, γ_2-adaptin cDNAs (ESTs T49401 and H41406), or control β-actin, as probes prepared according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA). Blots were stripped and reprobed as recommended by the manufacturer. Images were acquired using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA) or Hyperfilm MP (Amersham, Arlington Heights, IL, USA).

2.4. Peptides and antibody production

Peptides for antibody production, affinity purification, and peptide competition experiments were: N-LEKVLQSHMSLPA-C (γ_2-adaptin) and N-LESVLISNMSTSV-C (mouse γ-adaptin), see Fig. 1b. Peptides were obtained from the W.M. Keck Foundation Biotechnology Resource Laboratory, HHMI, Yale University, New Haven, CT, USA. The γ_2-adaptin peptide was glutaraldehyde conjugated to KLH (Pierce, Rockford, IL, USA). The KLH-γ_2-adaptin peptide conjugate was used to immunize rabbits (Pocono Rabbit Farm, Canadensis, PA, USA). Where required, polyclonal anti-peptide antibodies were affinity purified from crude serum using γ_2-adaptin peptide conjugated to tresyl chloride-activated agarose (Sigma, St. Louis, MO, USA). Crude anti-γ_2-adaptin peptide antiserum was used for all experiments, unless otherwise stated.

2.5. Immunofluorescence microscopy and peptide competition

HeLa cells were cultured on glass coverslips for 24–48 h as described [13]. Peptide competition of the binding of affinity purified anti-γ_2-adaptin peptide antibody was performed by pre-incubating ~50 μg/ml primary antibodies in the presence of 20 μg/ml γ_2-adaptin peptide, or the corresponding peptide from mouse γ-adaptin peptide, on ice for 45 min prior to addition to the coverslip. Cells were visualized using a Zeiss Axiophot fluorescence microscope with a rhodamine and fluorescein filter set. Photographic images were scanned from 35-mm black and white negatives using a Polaroid SprintScan35 scanner (Cambridge, MA, USA), then processed in parallel using Adobe Photoshop 4.0 (Mountain View, CA, USA).

2.6. Electron microscopy

HeLa cells were grown for 72 h on plastic substrate and fixed 1 h in 4% buffered formaldehyde containing 0.2% glutaraldehyde (pH 7.4). Double strength fixative was added to cells and medium at 37°C, left for 5 min and then replaced with fresh single strength fixative and left for 1 h on ice. The fixative was replaced with PBS/10% FCS, the cells scraped and pelleted. Cell pellets were embedded in 10% gelatin, infused in 2.3 M sucrose, frozen in liquid nitrogen, and sectioned in an Ultracut S ultramicrotome (Leica, Deerfield, IL, USA) at −110°C to −130°C using a diamond knife (DiatomeU.S., Ft. Washington, PA, USA). Sections were handled and immunolabeled using established methods [14,15].

3. Results

3.1. Molecular cloning and sequence analysis of human and mouse cDNAs encoding γ_2-adaptin

Using mouse γ-adaptin (X54424) [16] as our probe in a TBLASTN search of dbest [17], we identified four overlapping human ESTs (T49401, T80403, H41406, and AA0051855) that together comprised a 3.312-kb nucleotide sequence consisting of 5′-UTR, 2.256 kb of open reading frame, and 3′-UTR (GenBank accession AF068706). A TBLASTN 2.0 search of the mouse dbest [18] using the translated open reading frame of this human γ-adaptin homolog recovered a single significant EST (AA185250) coding for the 29 C-terminal amino acids and 0.25 kb of 3′-UTR for the corresponding mouse γ-adaptin homolog. The full length cDNA (GenBank accession AF068706) was cloned using an array of a mouse embryonic brain cDNA library.

The novel human and mouse sequences clearly fall within the γ-adaptin sub-family of the adaptin family of proteins (Fig. 1a). Indeed, their high degree of homology to γ-adaptin led us to designate them 'γ_2-adaptin'. The human and mouse γ_2-adaptin cDNAs encode proteins of 786 amino acids (~87 kDa) and 791 amino acids (~88 kDa), respectively, that are 86.5% identical and 88.2% similar. Mouse γ_2-adaptin is 61.7% identical and 70% similar to mouse γ-adaptin, and human γ_2-adaptin is 64.4% identical and 73.1% similar to mouse γ-adaptin. The highest degree of homology is in the head domains of γ- and γ_2-adaptin, and like most of the members of the adaptin family, the hinge domain of γ_2-adaptin is proline- and glycine-rich relative to other regions of the molecule (Fig. 1b). Though both γ- and γ_2-adaptin have an overall similarity of sequence and structure, γ_2-adaptin has a truncated 'ear' domain (Fig. 1c), which is the shortest of all the mammalian adaptins identified (data not shown). The reduction of the ear domain in γ_2-adaptin may have functional significance in that the ear domain of α-adaptin has been shown to interact with Eps15, a molecule that is found on the periphery of coated pits on the plasma membrane (see Section 4) [19–23].

3.2. Tissue distribution of γ_2-adaptin in mouse and human tissues

Human tissues were examined by Northern analysis to de-

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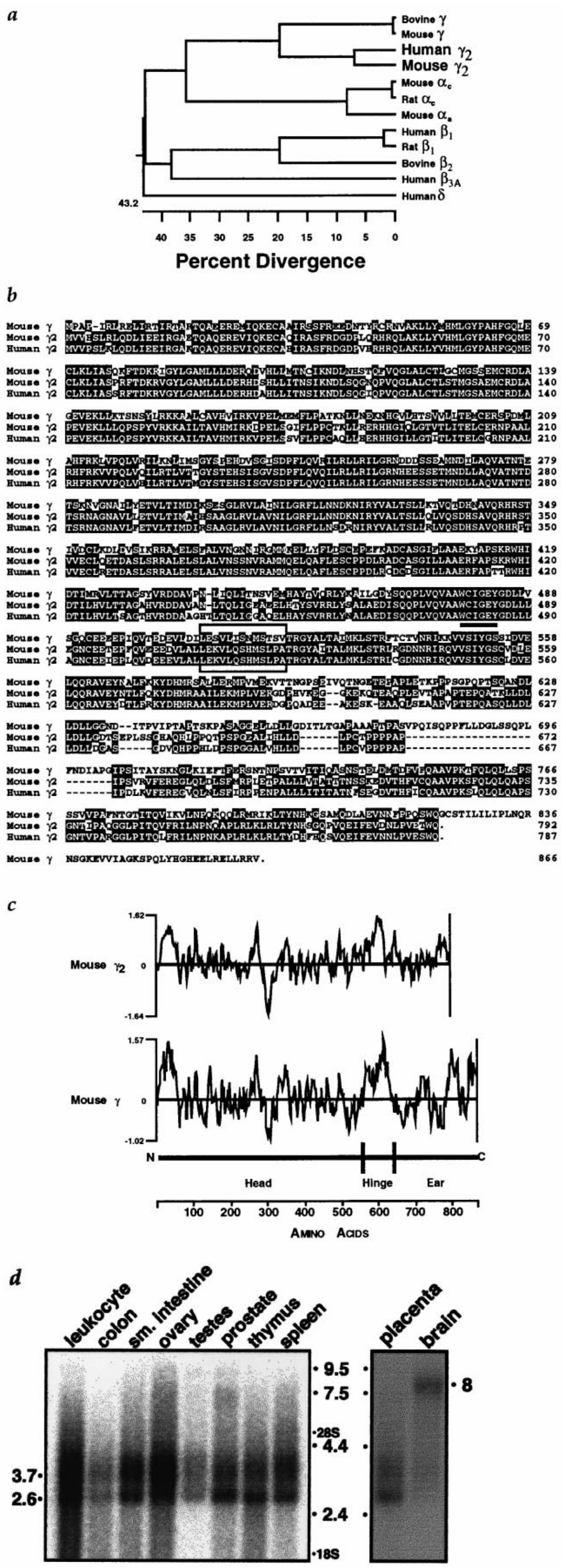
Fig. 1. Analysis of predicted amino acid sequences of γ_2-adaptins and tissue distribution. a: A dendrogram showing the relationship between representative known adaptins (see [4] for accession numbers). b: Alignment of γ_2-adaptins, and human γ_2-adaptin. Identical residues are highlighted. Boxed peptides for anti-γ_2-adaptin antiserum and peptide competition experiments. The underlined sequence is the 'WIIDGY' domain found in adaptin molecules [6]. c: Kyte-Doolittle hydropathy plots of γ-adaptins divided into three domains: Head, Hinge, and Ear. d: Northern analysis showing ubiquitous expression of γ_2-adaptin. The central column of numbers are the molecular weight markers and 28S and 18S ribosomal subunits. Molecular weights of messages revealed using the γ_2-adaptin probe on outer sides of the blots.



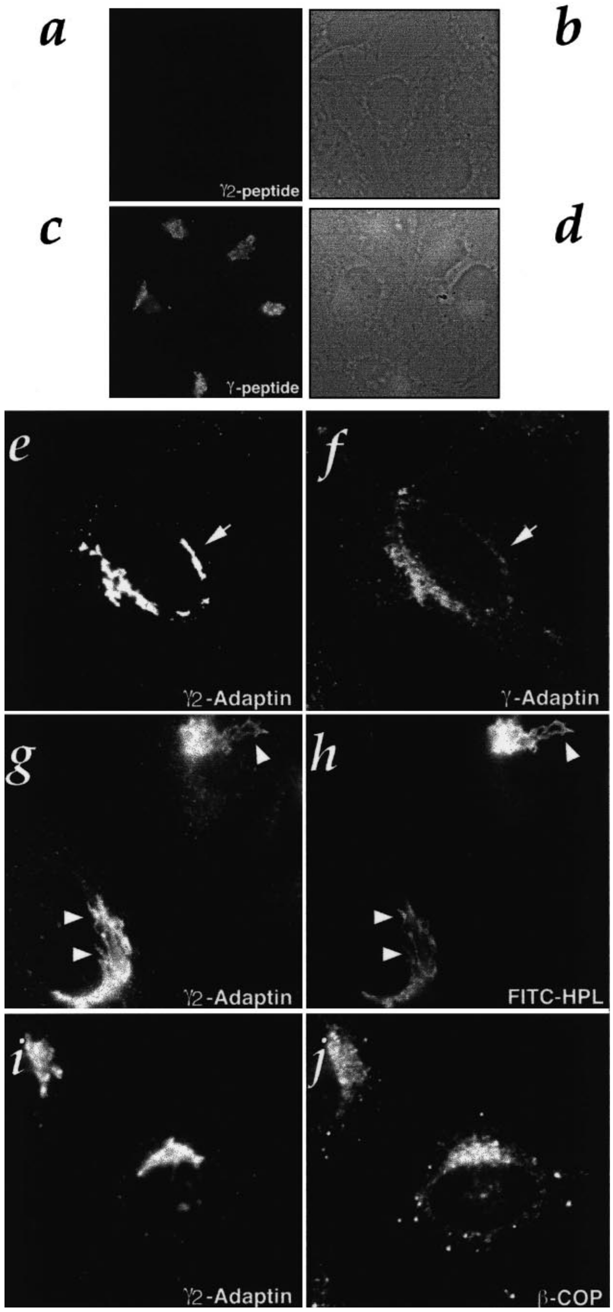


Fig. 2. Peptide competition and colocalization of γ_2-adaptin with Golgi markers. Indirect immunofluorescence of HeLa cells using affinity purified γ_2-adaptin peptide antibodies molecule reveals a Golgi-like perinuclear staining pattern (a, b phase) that can be competed for with the γ_2-adaptin peptide (c, d phase). Colocalization of γ_2-adaptin (e, g, i) compared to γ-adaptin (f), FITC-labeled *Helix pomatia* lectin (h), and β-COP (j). Arrows indicate perinuclear region of cells where there is little colocalization between γ_2- and γ-adaptin. Arrowheads indicate areas of colocalization between γ_2-adaptin and FITC-HPL.

termine the expression pattern of γ_2-adaptin (Fig. 1d). Northern analysis revealed equally abundant 2.6- and 3.7-kb messages; the 2.6-kb message may represent an abundant splicing intermediate. Interestingly, Northern analysis of message from brain revealed the primary message for γ_2-adaptin to be ∼8 kb, suggesting a brain-specific isoform of γ_2-adaptin. Northern analysis (Fig. 1d) and RT-PCR (data not shown) demonstrated that the γ_2-adaptin message was ubiquitously expressed.

3.3. Cellular localization, differential brefeldin A sensitivity, and relative distribution of γ_2-adaptin vs. γ-adaptin

Several peptides derived from the predicted amino acid sequences of human and mouse γ_2-adaptin were used to generate antisera. One peptide (N-LEKVLQSHMSLPA-C, see box Fig. 1b) generated an antiserum suitable for immunofluorescence and immunoelectron microscopy in HeLa cells, as well as all other mammalian cell lines examined (mouse, rat, hamster, and canine – data not shown). Unfortunately, this antiserum proved to be unsuitable for Western analysis and immunoprecipitation of endogenous proteins from cell lysates.

Both crude antiserum and affinity purified antibodies to the γ_2-adaptin peptide reveal a strong perinuclear, Golgi-like, staining pattern (Fig. 2). To be certain that this pattern was not a cross-reaction, we performed a peptide competition assay with the immunizing peptide and the corresponding peptide from γ-adaptin (Fig. 1b). The γ_2-adaptin peptide completely inhibited antibody recognition of γ_2-adaptin in HeLa cells (Fig. 2a) while the corresponding γ-adaptin peptide had no effect on the staining of this Golgi-like structure (Fig. 2b). A comparable result was observed in a mouse cell line (data not shown).

Association of γ-adaptin to Golgi membranes is sensitive to the fungal metabolite brefeldin A (BFA) [24,25]. When BFA is added to cells in vivo the majority of γ-adaptin rapidly becomes cytosolic (∼2 min). β-COP, a component of 'coatomer', a Golgi-associated coat protein complex involved in vesicle budding [1,2], has the same reversible BFA sensitivity as γ-adaptin. However, BFA has little effect upon the distribution of α-adaptin [24]. To test whether γ_2-adaptin's behavior was more like that of β-COP and γ-adaptin, or that of α-adaptin, BFA was added to HeLa cells grown on coverslips. The localization of γ_2-adaptin was compared to that of γ-adaptin after 15 min in 10 μM BFA-supplemented growth media. γ_2-Adaptin remains membrane bound in the presence of BFA (Fig. 3a) while γ-adaptin (Fig. 3b) and β-COP (data not shown) dissociate from membranes. These data provide additional evidence in support of γ_2-adaptin being a unique entity distinct from γ-adaptin.

We next examined the intracellular distribution of γ_2-adaptin relative to a number of markers of the secretory pathway including γ-adaptin (Fig. 2e,f). Though there was partial co-

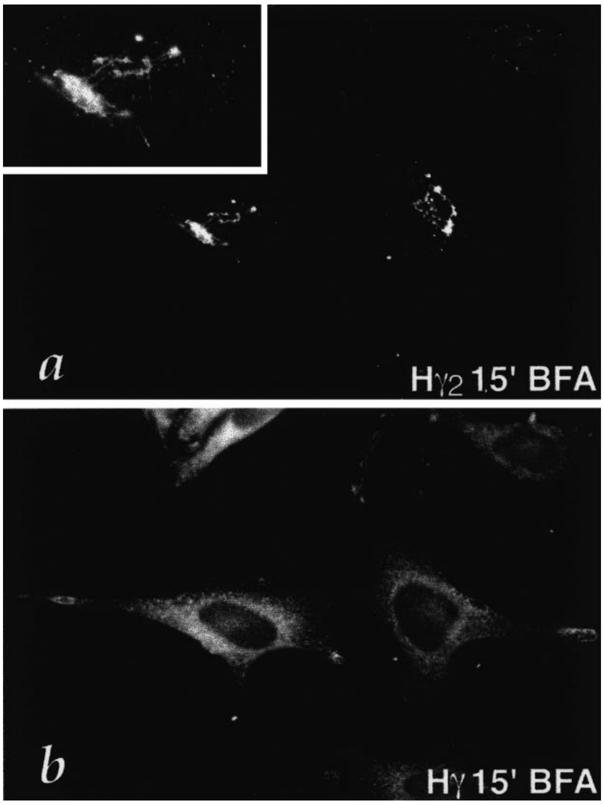


Fig. 3. γ_2-Adaptin in HeLa cells has a differential sensitivity to brefeldin A compared to γ-adaptin. HeLa cells were double labeled for γ_2-adaptin and γ-adaptin. See Fig. 2 for colocalization of γ_2-adaptin and γ-adaptin. a, b: After 15 min of exposure to BFA, γ_2-adaptin remains associated with membranes while γ-adaptin becomes cytosolic. Inset: Note association of γ_2-adaptin staining with probable Golgi-membrane tubules.

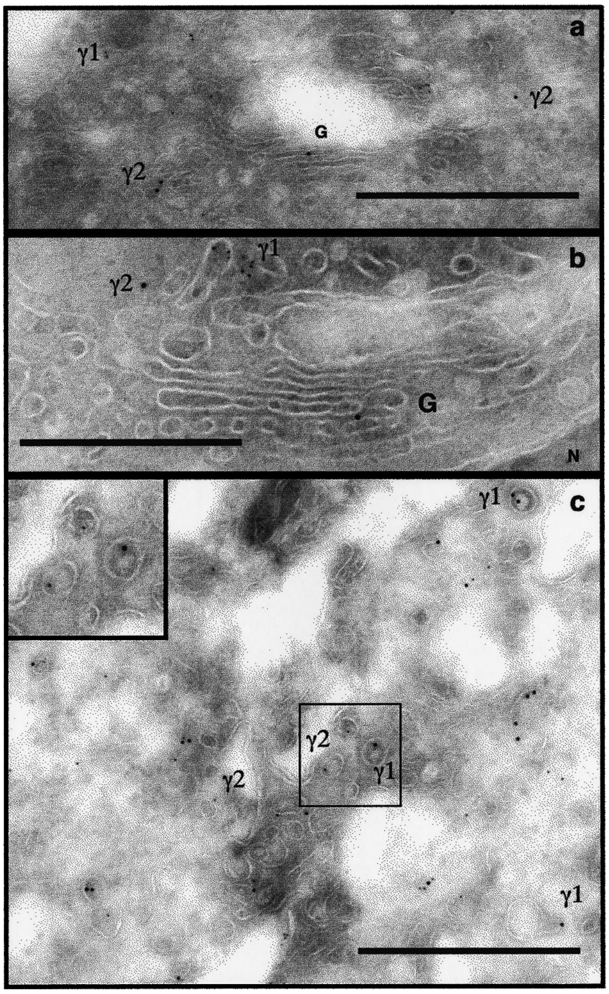


Fig. 4. Immunogold electron microscopic localization of γ_2- and γ-adaptin. Frozen thin sections were prepared from HeLa cells and labeled with antibodies against γ_2-adaptin and γ-adaptin. a: Golgi stacks (G) label with γ_2-adaptin (10 nm gold) and with γ-adaptin (5 nm gold). Bar = 0.5 μm. b: Higher magnification of Golgi stacks in a. Bar = 0.25 μm. c: Vesicular structures with electron-dense coat-like material are positive for γ_2-adaptin (5 nm gold) and γ-adaptin (10 nm gold). Bar = 0.5 μm. Inset in c is higher magnification view of boxed region.

localization with a number of markers, including γ-adaptin, the best colocalization with γ_2-adaptin was observed for a *cis*-Golgi/intermediate compartment marker, FITC-labeled *Helix pomatia* lectin (FITC-HPL) [26] (Fig. 2g,h). In addition to the colocalization with FITC-HPL, γ_2-adaptin also colocalized with β-COP (Fig. 2i,j), a marker of the ER and Golgi apparatus [1,2,24]. The peripheral punctate staining pattern observed in cells stained for γ_2-adaptin did not correspond to endocytic structures as determined by immunofluorescent comparison with early endocytic and recycling compartments (FITC-labeled human transferrin) [13], late endosomes (mannose 6-phosphate receptor), lysosomes (lysosomal glycoprotein, lgp95) [27] (data not shown).

Immunoelectron microscopy of HeLa cells confirmed the distinct localizations of γ- and γ_2-adaptin in the Golgi apparatus. γ_2-Adaptin labeling was found to be associated with vesicles of the Golgi complex, and occasionally with Golgi cisternae (Fig. 4a,b). Although antibody to γ-adaptin labeled membrane structures in close proximity to those positive for γ_2-adaptin, where membranes were clearly discernible, labeling was exclusive for one or the other adaptin. Interestingly, an electron-dense coat structure appeared to surround vesicles that were positive for either γ- or γ_2-adaptin (Fig. 4c). At least in the case of γ-adaptin, this coat is likely to reflect the presence of clathrin [28].

4. Discussion

In this study we describe a novel member of the adaptin family, γ_2-adaptin, identified based upon its similarity to the AP-1 adaptor protein, γ-adaptin. This adaptin is widely expressed in both human and murine tissues, as well as in a number of cell lines from other species. In addition, it is possible that there is a brain-specific isoform of γ_2-adaptin. γ_2-Adaptin is found on the Golgi apparatus, both on Golgi stacks as well as on vesicular structures in close proximity to vesicles labeling for γ-adaptin in the TGN (Fig. 4).

There is a high degree of sequence similarity between γ- and γ_2-adaptin ($>60\%$). However, γ_2-adaptin has a truncated ear domain compared to γ-adaptin, although the amino acid similarity between γ_2-adaptin and γ-adaptin remains high in the regions where the ear domains of the two adaptins align. This may be significant because the ear domain of α-adaptin interacts with the epidermal growth factor receptor tyrosine kinase substrate Eps15 which, given its localization in nascent clathrin-coated pits at the plasma membrane and its primary structure, is suggestive of a role in the regulation of the formation of clathrin-coated vesicles [19–23]. It is possible that the ear domains of other adaptins also interact with Eps15-like molecules. Therefore, the structural differences between the ear domains of γ- and γ_2-adaptin may reflect functional differences between these molecules.

The differential sensitivity of γ-adaptin and γ_2-adaptin to the fungal metabolite brefeldin A suggests different mechanisms for regulation of the recruitment of these two adaptins to membranes. Recruitment of γ-adaptin as a component of the AP-1 adaptor, and the subsequent recruitment of clathrin, to the TGN depends upon the activity of the small GTP-binding protein, ARF1. ARF proteins are recruited to membranes in their GTP-bound conformation. BFA inhibits the exchange of GDP for GTP on ARFs, thus maintaining them in their inactive, cytosolic, conformation [29,30]. In the presence of BFA coat protein components, both γ-adaptin as part of the AP-1/clathrin coat and the β-COP-containing coatomer complex, rapidly become cytosolic [2,24]. In contrast, γ_2-adaptin remains associated with Golgi membranes in the presence of BFA (Fig. 3), even after extended periods (2 h) in the presence of the drug (data not shown). This result suggests that the γ_2-adaptin membrane association may be regulated by a different ARF GTPase than γ-adaptin, or by an altogether different regulatory mechanism [24].

There is a high degree of colocalization between γ_2-adaptin and the Golgi markers β-COP and *Helix pomatia* lectin (Fig. 2). This and the presence of γ_2-adaptin on the stacks of the Golgi as well as on vesicles closely associated with vesicles positive for γ-adaptin (Fig. 4), suggests that this adaptin may play a more general role in the trafficking of molecules through the secretory pathway. γ-Adaptin in the TGN is involved in the sorting of integral membrane proteins from the TGN to the late endosome and its presence on this structure is indicative of the binding of clathrin [25,27,28,31–33]. γ_2-Adaptin has a wider distribution than γ-adaptin at the electron microscopic level.

The localization of the novel γ_2-adaptin to a distinct population of membranes with electron-dense material present around vesicles, distinct from those membranes populated by the clathrin-recruiting γ-adaptin, suggests that there may be a novel coat protein associated with γ_2-adaptin-containing membranes. The AP-3 adaptor complex found on the TGN associated with vesicular structures in close proximity to clathrin-coated vesicles does not colocalize with clathrin in the Golgi apparatus [6,34], though AP-3 on endocytic structures appears to do so [7]. This observation suggests that there are more classes of vesicles whose contents are determined by the activity of adaptors in the Golgi apparatus and associated structures. It is possible that both γ-adaptin and γ_2-adaptin are capable of recruiting clathrin to membranes and that the differences in localization between these molecules reflect a difference in vesicle cargo. Further investigation will be required to understand the significance of the structural differences between γ_2- and γ-adaptin, potential differences between the components of the AP-1 adaptor and the putative γ_2-adaptin-associated adaptor, and the additional heterogeneity of vesicles associated with the Golgi apparatus.

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References

[1] Rothman, J.E. and Wieland, F.T. (1996) Science 272, 227–234.
[2] Schekman, R. and Orci, L. (1996) Science 271, 1526–1533.
[3] Mellman, I. (1996) Annu. Rev. Cell Dev. Biol. 12, 575–625.
[4] Lewin, D.A. and Mellman, I. (1998) Biochim. Biophys. Acta 1401, 129–145.
[5] Dell'Angelica, E.C., Ohno, H., Ooi, C.E., Rabinovich, E., Roche, K.W. and Bonifacino, J.S. (1997) EMBO J. 16, 917–928.
[6] Simpson, F., Pedren, A.A., Christopoulou, L. and Robinson, M.S. (1997) J. Cell Biol. 137, 835–845.
[7] Dell'Angelica, E.C., Klumperman, J., Stoorvogel, W. and Bonifacino, J.S. (1998) Science 280, 431–434.
[8] Heuser, J.E.A.K. (1988) J. Cell Biol. 107, 877–886.
[9] Stoorvogel, W., Oorschot, V. and Geuze, H.J. (1996) J. Cell Biol. 132, 21–33.
[10] Cowles, C.R., Odorizzi, G., Payne, G.S. and Emr, S.D. (1997) Cell 91, 109–118.

[11] Pearse, B.M. and Robinson, M.S. (1990) Annu. Rev. Cell Biol. 6, 151–171.
[12] Israel, D.I. (1993) Nucleic Acids Res. 21, 2627–2631.
[13] Daro, E., van der Sluijs, P., Galli, T. and Mellman, I. (1996) Proc. Natl. Acad. Sci. USA 93, 9559–9564.
[14] Webster, P., IJodo, J.W., Chicoine, L.M. and Fikrig, E. (1998) J. Clin. Invest. 101, in press.
[15] Liou, W., Geuze, H.J. and Slot, J.W. (1996) Histochem. Cell Biol. 106, 41–58.
[16] Robinson, M.S. (1990) J. Cell Biol.
[17] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[18] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Nucleic Acids Res. 25, 3389–3402.
[19] Benmerah, A., Gagnon, J., Begue, B., Megarbane, B., Dautry, V.A. and Cerf, B.N. (1995) J. Cell Biol.
[20] Wong, W.T., Schumacher, C., Salcini, A.E., Romano, A., Castagnino, P., Pelicci, P.G. and Di, F.P. (1995) Proc. Natl. Acad. Sci. USA 92, 9530–9534.
[21] Benmerah, A., Begue, B., Dautry, V.A. and Cerf, B.N. (1996) J. Biol. Chem. 271, 12111–12116.
[22] Tebar, F., Sorkina, T., Sorkin, A., Ericsson, M. and Kirchhausen, T. (1996) J. Biol. Chem. 27, 28727–28730.
[23] Wendland, B., Mccaffery, J.M., Xiao, Q. and Emr, S.D. (1996) J. Cell Biol. 135, 1485–1500.
[24] Robinson, M.S. and Kreis, T.E. (1992) Cell 69, 129–138.
[25] Traub, L.M., Ostrom, J.A. and Kornfeld, S. (1993) J. Cell Biol. 123, 561–573.
[26] Krijnse-Locker, J., Ericsson, M., Rottier, P.J. and Griffiths, G. (1994) J. Cell Biol. 124, 55–70.
[27] Kornfeld, S. and Mellman, I. (1989) Annu. Rev. Cell Biol. 5, 483–525.
[28] Schmid, S.L. (1997) Annu. Rev. Biochem. 66, 511–548.
[29] Donaldson, J.G., Finazzi, D. and Klausner, R.D. (1992) Nature 360, 350–352.
[30] Donaldson, J.G. and Klausner, R.D. (1994) Curr. Opin. Cell Biol. 6, 527–532.
[31] Le Borgne, R., Schmidt, A., Mauxion, F., Griffiths, G. and Hoflack, B. (1993) J. Biol. Chem. 268, 22552–22556.
[32] Le Borgne, R., Griffiths, G. and Hoflack, B. (1996) J. Biol. Chem. 271, 2162–2170.
[33] Le Borgne, R. and Hoflack, B. (1997) J. Cell Biol. 137, 335–345.
[34] Simpson, F., Bright, N.A., West, M.A., Newman, L.S., Darnell, R.B. and Robinson, M.S. (1996) J. Cell Biol. 133, 749–760.