Cloning and Expression of γ-Adaptin, a Component of Clathrin-coated Vesicles Associated with the Golgi Apparatus

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Abstract. Adaptins are the major components of adaptors, the protein complexes that link clathrin to transmembrane proteins (e.g., receptors) in coated pits and vesicles. The plasma membrane adaptor contains an α-adaptin subunit and a β-adaptin subunit, while the Golgi adaptor contains a γ-adaptin subunit and a β′-adaptin subunit. A partial cDNA clone encoding γ-adaptin was isolated from a bovine brain expression library by screening with antibodies, and was used to obtain a cDNA clone from a mouse brain library containing the full coding sequence. The identity of the clones was confirmed by protein sequencing. The deduced amino acid sequence of γ-adaptin was found to be homologous to that of α-adaptin, with several stretches of identical amino acids or conservative substitutions in the first ~70 kD, and 25% identity overall. Weaker homology was seen between γ- and β-adaptins. Like both α- and β-adaptins, γ-adaptin has a proline and glycine-rich hinge region, dividing it into NH_2- and COOH-terminal domains. A chimeric γ-adaptin was constructed from the mouse and bovine cDNAs and transfected into Rat 1 fibroblasts. Immunofluorescence microscopy was carried out using an mAb which recognizes an epitope present on the chimera but not found on the rodent protein. The construct was found to have a distribution typical of endogenous γ-adaptin. Using this transfection system, it should now be possible to exchange domains between α- and γ-adaptins, to try to find out how adaptors are targeted to the appropriate membrane compartment of the cell, and how they recruit the appropriate receptors into the coated vesicle.

CLATHRIN-coated vesicles associated with the Golgi apparatus were first observed over twenty years ago, and were most extensively studied at that time in the rat vas deferens (Friend and Farquhar, 1967). Since then, virtually all eukaryotic cells have been shown to contain Golgi-associated coated vesicles as well as endocytic coated vesicles associated with the plasma membrane. The region of the Golgi apparatus from which the clathrin-coated vesicles bud is the compartment now known as the *trans*-Golgi network (TGN)[1], thought to be a major sorting station in the cell (Griffiths and Simons, 1986). In mammalian cells, at least one function of the Golgi coated vesicles is probably to concentrate newly synthesized lysosomal enzymes bound to mannose-6-phosphate receptors, thus diverting the enzymes away from the constitutive or default pathway to the cell surface and targeting them to prelysosomes (Geuze et al., 1985; Lobel et al., 1989). In addition, nascent secretory granules in cells with a regulated secretory pathway are often partially coated with clathrin (Orci et al., 1984), suggesting that Golgi coated vesicles may also play a role in the formation of secretory granules in such cells (Tooze and Tooze, 1986).

Evidence from in vitro studies indicates that the sorting process that occurs when coated vesicles are formed is mediated by protein complexes called adaptors. The cytoplasmic domains of selected membrane proteins have been shown to bind to adaptors (Pearse, 1988; Glickman et al., 1989), which in turn bind to clathrin (Keen et al., 1979; Pearse and Robinson, 1984; Keen, 1987). These interactions are believed to cause such membrane proteins (and their ligands, since the proteins are usually receptors) to become packaged into a coated vesicle for transfer to another part of the cell. Different adaptors are associated with the plasma membrane and the Golgi apparatus (Robinson and Pearse, 1986; Robinson, 1987; Ahle et al., 1988), and are thought to account for the different specificities of the two types of coated vesicles (Glickman et al., 1989).

Both Golgi and plasma membrane-associated adaptors are protein complexes made up of four subunits: two proteins of ~100 kD, called adaptins, and two smaller proteins of ~50 and ~20 kD (Pearse and Robinson, 1984; Keen, 1987; Ahle et al., 1988). The adaptins have been divided into three classes: α, β, and γ. Both adaptors have a β-adaptin subunit, which appears to provide the clathrin binding site of the adaptor complex (Ahle et al., 1989). In addition, the Golgi adaptor has a γ-adaptin subunit, while the plasma membrane receptor has an α-adaptin subunit. In spite of the similarities between the sizes of the four subunits of the Golgi and plasma membrane adaptors, only the two β-adaptins have

1. *Abbreviations used in this paper*: CNBr, cyanogen bromide; TGN, *trans*-Golgi network.

been shown to be related by peptide mapping and antibody cross-reactivity (Ahle et al., 1988).

cDNA clones encoding both α- and β-adaptins have recently been isolated and sequenced (Robinson, 1989; Kirchhausen et al., 1989; Ponnambalam et al., 1990). Southern blotting has demonstrated that there are two α-adaptin genes (Robinson, 1989), one of which is primarily expressed in neurons (Robinson, 1987, 1989). Two distinct cDNAs have also been cloned for the β-adaptins (Kirchhausen et al., 1989), which may correspond to the two types of β-adaptin, β and β′, found in the plasma membrane and Golgi adaptors, respectively. No obvious sequence homology has been detected between the α- and β-adaptins. However, they both have a proline and glycine-rich region between amino acids 600 and 750 which has been shown to be extremely protease sensitive (Kirchhausen et al., 1989). Ultrastructural observations have revealed that the plasma membrane adaptor has an unusual structure, appearing as a brick-like "head" flanked by two smaller "ears", connected by flexible hinges (Heuser and Keen, 1988). The protease-sensitive stretches of the α- and β-adaptins correspond to the two hinges, with each adaptin contributing a ~30-kD COOH-terminal ear. Although comparable ultrastructural studies have not yet been carried out on the Golgi adaptor, the similar susceptibility of the γ- and β′-adaptins to proteolysis suggests that the two adaptors have the same general structure (Schroeder and Ungewickell, personal communication).

Since the β-adaptin subunits of the plasma membrane and Golgi adaptors are thought to attach the complexes to clathrin, it seems likely that the adaptor-specific adaptins, α and γ, will be found to have adaptor-specific functions, such as targeting to the appropriate membrane or binding to the right receptors. A comparison of the primary structures of the α- and γ-adaptins as deduced from their cDNAs, followed by in vitro mutagenesis and "cut and paste" experiments to construct chimeric adaptins, should lead to a better understanding of such functions. To this end, I have cloned and sequenced γ-adaptin cDNAs from bovine and mouse brain libraries. I have also devised a system for expressing an engineered version of the protein in tissue culture cells so that it can be detected with antibodies, without any background from the cells' endogenous γ-adaptin.

Materials and Methods

Isolation, Sequencing, and Characterization of Clones

Recombinant DNA techniques were generally those described by Sambrook et al. (1990). A λgt11 bovine brain cDNA library was purchased from Clonetech (Palo Alto, CA) and used for expression cloning (Huynh et al., 1983). Screening was carried out with a mixture of two monoclonal antibodies against γ-adaptin, generously provided by E. Ungewickell (Max Planck Institute, Martinsried, FRG) (Ahle et al., 1988), followed by ^{125}I-labeled affinity-purified goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO). Four independent clones were isolated from 5×10^5 p.f.u. and were shown by restriction mapping and sequencing to be products of the same gene. A ~1.3-kb fragment (corresponding to bases 871–2156 in the final mouse sequence; see Fig. 1) was labeled by nick translation and used to screen a λgt11 mouse brain cDNA library kindly provided by Y. Citri (Weizmann Institute of Science, Rehovot, Israel) (Martinez et al., 1987). Among the positive phages obtained in this second screen was one containing an insert that encoded the complete protein sequence.

Inserts were subcloned into Bluescript plasmid vectors (Stratagene Cloning Systems, San Diego, CA) for restriction mapping and for production of single-stranded DNA using helper phage. Sequencing was carried out by the dideoxy chain termination method (Sanger et al., 1977), using restriction fragments and Exo III–nested deletions. To complete the sequence of the entire coding region in both directions, synthetic oligonucleotides were used as primers to fill in any remaining gaps.

Protein sequencing was carried out on purified γ-adaptin obtained from bovine brain coated vesicles by Tris extraction, gel filtration, hydroxylapatite chromatography, and preparative SDS-PAGE (Pearse and Robinson, 1984; Ahle et al., 1988). Peptides were generated with cyanogen bromide (CNBr) and separated by HPLC as previously described (Robinson, 1989), and were sequenced by I. Fearnley (MRC Laboratory of Molecular Biology, Cambridge, England) (Walker et al., 1987). In addition, intact γ-adaptin as well as γ-adaptin digested with CNBr or V8 protease (Cleveland et al., 1977) were subjected to electrophoresis and transferred to polyvinylidene difluoride filters for microsequencing (Matsudaira, 1987).

Expression in Tissue Culture Cells

To construct a new full-length γ-adaptin for expression in rodent cells which would be recognized by mAbs against the bovine protein, a mouse–cow cDNA chimera was prepared, making use of a Pst I site shared by both cDNAs. A bovine Pst I/Eco RI fragment, corresponding to bases 1221–2156 in the mouse sequence, was subcloned into Bluescript SK(-). The mouse Pst I fragment immediately 5′ to this fragment was then inserted, followed by the 3′ Eco RI fragment from cow. In both cases, restriction mapping was carried out to select for clones with the correct orientation. The entire insert was then cut out with Bam HI and Cla I. The vector for transfection was constructed from two plasmids kindly donated by H. Pelham (MRC Laboratory of Molecular Biology, Cambridge, England): pHYK and pUSX1 (Munro and Pelham, 1987). pHYK, an expression vector encoding lysozyme, provided the framework for the new vector, but the existing adenovirus major late promoter was excised with Sac II and Hind III and replaced with a Sac II/Hind III fragment of pUSX1 containing the SV40 early promoter. A Hind III/Eco RI fragment, containing the coding sequence for lysozyme, was then replaced by a double-stranded oligonucleotide containing restriction sites for Bgl II, Not I, Kpn I, and Cla I. This modified vector was cut with Bgl II and Cla I and ligated to the Bam HI/Cla I γ-adaptin insert to produce plasmid pHYKS4.

Rat 1 cells were transfected with the above construct by the calcium phosphate coprecipitation method. After 1 d in culture, they were trypsinized and plated onto multiwell glass slides. The next day, the cells were fixed with methanol for 5 min at −20°C, followed by acetone for 30 s at −20°C, and then prepared for double labeling immunofluorescence, essentially as described (Robinson, 1987). The primary antibodies were mouse mAb 100/3 against bovine γ-adaptin (Ahle et al., 1988) and a rabbit polyclonal antiserum against the Golgi membrane protein TGN38, generously provided by P. Luzio (Department of Clinical Biochemistry, Cambridge University, England). The anti-TGN38 antiserum had been raised against a bacterially expressed fusion protein (Luzio et al., 1990). The second antibodies were fluorescein-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-rabbit IgG (Sigma Chemical Co.), which had been preabsorbed with rabbit IgG and mouse IgG, respectively, to remove cross-reacting antibodies.

Results

Isolation and Characterization of γ-Adaptin Clones

A λgt11 bovine brain cDNA expression library was screened with a mixture of two mAbs against γ-adaptin raised by Ungewickell and co-workers: mAb 100/3 (Ahle et al., 1988), and another mAb which may recognize the same epitope since it labels the same bands on one-dimensional peptide maps (data not shown). Five positive clones were obtained. Two of these clones had identical inserts; the other inserts, although different, appeared from restriction mapping and partial sequencing to have been derived from the same original mRNA.

Because none of the inserts were long enough to encode a full-length γ-adaptin, the cloned cDNA was used as a probe to screen a mouse brain cDNA library previously shown to contain full-length α-adaptin inserts. One of the

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-305                                                           CGCAGTCGCCTCCTCCTCGGCAAATAGTCCTCGGCGGGGGCCGAGTCCTCGGGTCATCCGGGGTG

-240  GGAGACGGCACGGGCTGCAGTCGGAGGGACGCGGGGCGGCCGGCGAGAGGGAGCCGGGGAGCGGCGTCTCCCGGGGAAGCGCGAGGCGTGAGGTAGAGGCGCCCTCCTCGCCCGGGATCC

-120  CTCCGCCTGCAGTCGCGGTGCTGAGGCCGAGGGAGCCGCCATTTTGGATGTTCGGTTCCTGCTGCCACTGCCGCCGCCGCCCCCGAAGCTGCTGGTTTCCTTCGAGGTTTCGGGCCGAGG

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   1   M  P  A  P  I  R  L  R  E  L  I  R  T  I  R  T  A  R  T  Q  A  E  E  R  E  M  I  Q  K  E  C  A  A  I  R  S  S  F  R  E
   1  ATGCCAGCCCCCATCAGATTGCGGGAGCTGATCCGGACCATCCGGACAGCCCGAACCCAAGCTGAGGAACGAGAAATGATCCAGAAAGAATGTGCTGCAATCCGGTCCTCATTTAGAGAA

  41   E  D  N  T  Y  R  C  R  N  V  A  K  L  L  Y  M  H  M  L  G  Y  P  A  H  F  G  Q  L  E  C  L  K  L  I  A  S  Q  K  F  T
 121  GAAGACAATACATACCGGTGTCGGAATGTGGCAAAGTTACTATATATGCACATGCTGGGCTACCCTGCTCACTTTGGACAGTTGGAGTGCCTCAAGCTAATCGCCTCACAAAAATTCACA

  81   D  K  R  I  G  Y  L  G  A  M  L  L  L  D  E  R  Q  D  V  H  L  L  M  T  N  C  I  K  N  D  L  N  H  S  T  Q  F  V  Q  G
 241  GACAAACGCATTGGCTATTTAGGAGCAATGCTATTATTAGATGAAAGACAAGATGTGCATCTTCTCATGACCAACTGTATCAAGAATGATCTTAATCATAGCACGCAGTTTGTGCAGGGG

 121   L  A  L  C  T  L  G  C  M  G  S  S  E  M  C  R  D  L  A  G  E  V  E  K  L  L  K  T  S  N  S  Y  L  R  K  K  A  A  L  C
 361  CTAGCACTTTGTACCCTCGGCTGCATGGGCTCCTCGGAGATGTGCAGAGATCTTGCGGGAGAGGTAGAAAAGCTTCTGAAAACCTCCAACTCTTACTTAAGAAAAAAGGCAGCACTGTGT

 161   A  V  H  V  I  R  K  V  P  E  L  M  E  M  F  L  P  A  T  K  N  L  L  N  E  K  N  H  G  V  L  H  T  S  V  V  L  L  T  E
 481  GCTGTTCACGTCATCAGGAAGGTTCCTGAACTTATGGAGATGTTTTTACCAGCAACAAAAAATTTATTGAATGAGAAGAACCATGGTGTCCTTCACACGTCTGTAGTCCTCCTCACAGAG

 201   M  C  E  R  S  P  D  M  L  A  H  F  R  K  L  V  P  Q  L  V  R  I  L  K  N  L  I  M  S  G  Y  S  P  E  H  D  V  S  G  I
 601  ATGTGTGAACGGAGCCCAGACATGCTCGCACATTTCAGAAAGCTTGTGCCCCAATTAGTTCGTATTCTAAAGAACCTCATCATGTCCGGATATTCCCCAGAACATGATGTCTCTGGTATC

 241   S  D  P  F  L  Q  V  R  I  L  R  L  L  R  I  L  G  R  N  D  D  D  S  S  E  A  M  N  D  I  L  A  Q  V  A  T  N  T  E  T
 721  AGTGACCCCTTTTTGCAGGTACGAATTTTGCGGTTATTAAGAATTTTAGGACGGAATGACGATGATTCAAGTGAAGCTATGAATGATATATTAGCACAGGTTGCCACTAATACAGAGACT

 281   S  K  N  V  G  N  A  I  L  Y  E  T  V  L  T  I  M  D  I  K  S  E  S  G  L  R  V  L  A  I  N  I  L  G  R  F  L  L  N  N
 841  AGTAAAAATGTAGGAAATGCTATTCTGTATGAGACGGTTTTGACTATCATGGATATTAAATCAGAGAGTGGATTGCGAGTTCTAGCCATAAATATTCTTGGTCGATTCTTATTGAACAAT

 321   D  K  N  I  R  Y  V  A  L  T  S  L  L  K  T  V  Q  T  D  H  N  A  V  Q  R  H  R  S  T  I  V  D  C  L  K  D  L  D  V  S
 961  GACAAAAATATTAGATATGTTGCTCTGACATCTCTGTTGAAGACTGTGCAGACAGATCATAACGCTGTACAGAGGCATAGAAGCACAATTGTGGACTGTCTGAAAGATCTGGATGTCTCC

 361   I  K  R  R  A  M  E  L  S  F  A  L  V  N  G  N  N  I  R  G  M  M  K  E  L  L  Y  F  L  D  S  C  E  P  E  F  K  A  D  C
1081  ATAAAAAGACGTGCAATGGAACTGAGTTTTGCCCTGGTGAATGGGAATAATATCCGAGGCATGATGAAAGAATTACTTTATTTCTGGATTCCTGTGAGCCAGAATTTAAAGCTGATTGT

 401   A  S  G  I  F  L  A  A  E  K  Y  A  P  S  K  R  W  H  I  D  T  I  M  R  V  L  T  T  A  G  S  Y  V  R  D  D  A  V  P  N
1201  GCATCTGGAATCTTCCTTGCTGCAGAAAAGTACGCACCTTCCAAACGGTGGCATATAGATACAATTATGCGTGTCTTGACAACGGCAGGAAGTTATGTTCGCGATGATGCAGTTCCTAAC

 441   L  I  Q  L  I  T  N  S  V  E  M  H  A  Y  T  V  Q  R  L  Y  K  A  I  L  G  D  Y  S  Q  Q  P  L  V  Q  V  A  A  W  C  I
1321  TTGATTCAGTTAATAACCAACAGTGTGGAGATGCACGCTTACACCGTCCAGCGCCTGTACAAGGCGATTCTCGGTGACTATTCTCAACAACCCTTGGTACAAGTGGCTGCGTGGTGTATA

 481   G  E  Y  G  D  L  L  V  S  G  Q  C  E  E  E  E  P  I  Q  V  T  E  D  E  V  L  D  I  L  E  S  V  L  I  S  N  M  S  T  S
1441  GGTGAATATGGCGATCTTCTTGTGTCTGGCCAGTGTGAAGAGGAAGAGCCTATTCAGGTGACAGAAGATGAAGTGCTGGATATTTTAGAAAGTGTCCTGATCTCTAATATGTCCACCTCT

 521   V  T  R  G  Y  A  L  T  A  I  M  K  L  S  T  R  F  T  C  T  V  N  R  I  K  K  V  V  S  I  Y  G  S  S  I  D  V  E  L  Q
1561  GTGACAAGAGGCTATGCTCTCACTGCCATTATGAAGCTGTCTACCCGATTCACCTGTACTGTAAACCGAATTAAGAAAGTGGTTTCCATCTATGGGAGCAGCATCGACGTAGAGCTCCAG

 561   Q  R  A  V  E  Y  N  A  L  F  K  K  Y  D  H  M  R  S  A  L  L  E  R  M  P  V  M  E  K  V  T  T  N  G  P  S  E  I  V  Q
1681  CAGAGGGCAGTAGAGTACAACGCACTTTTTAAGAAGTATGACCACATGAGGTCTGCCCTACTTGAAAGAATGCCTGTCATGGAAAAAGTGACCACAAATGGCCCTTCGGAGATCGTGCAG

 601   T  N  G  E  T  E  P  A  P  L  E  T  K  P  P  P  S  G  P  Q  P  T  S  Q  A  N  D  L  L  D  L  L  G  G  N  D  I  T  P  V
1801  ACAAATGGAGAGACAGAACCAGCGCCCCTAGAGACTAAACCACCACCCTCAGGGCCACAACCCACAAGCCAGGCCAATGATCTATTGGATTTGTTGGGAGGAAATGACATAACACCTGTT

 641   I  P  T  A  P  T  S  K  P  A  S  A  G  G  E  L  L  D  L  L  G  D  I  T  L  T  G  A  P  A  A  A  P  T  P  A  S  V  P  Q
1921  ATCCCAACTGCACCTACAAGCAAACCAGCATCAGCTGGTGGAGAACTTCTCGACTTGCTAGGAGACATCACCCTGACAGGTGCTCCAGCTGCTGCTCCTACCCCTGCCTCAGTGCCACAG

 681   I  S  Q  P  P  F  L  L  D  G  L  S  S  Q  P  L  F  N  D  I  A  P  G  I  P  S  I  T  A  Y  S  K  N  G  L  K  I  E  F  T
2041  ATATCCCAGCCCCCCTTCTTGTTGGATGGGCTTTCCTCACAGCCTCTCTTCAATGACATCGCTCCAGGCATCCCCTCCATCACAGCGTACAGTAAGAACGGCTTGAAGATAGAGTTCACC

 721   F  E  R  S  N  T  N  P  S  V  T  V  I  T  I  Q  A  S  N  S  T  E  L  D  M  T  D  F  V  F  Q  A  A  V  P  K  T  F  Q  L
2161  TTTGAACGGTCAAACACCAACCCCAGCGTAACAGTGATAACGATACAGGCCTCTAACAGCACAGAGCTAGACATGACGGACTTTGTTTTCCAGGCTGCAGTACCAAAGACATTCCAGCTG

 761   Q  L  L  S  P  S  S  S  V  V  P  A  F  N  T  G  T  I  T  Q  V  I  K  V  L  N  P  Q  K  Q  Q  L  R  M  R  I  K  L  T  Y
2281  CAGCTTCTGTCTCCTAGCAGCAGCGTTGTCCCAGCCTTTAATACAGGGACCATCACACAAGTCATTAAAGTTCTGAATCCACAGAAGCAACAGCTGCGAATGCGGATCAAGCTCACATAT

 801   N  H  K  G  S  A  M  Q  D  L  A  E  V  N  N  F  P  P  Q  S  W  Q
2401  AATCACAAGGGCTCGGCAATGCAAGATCTAGCAGAAGTGAACAATTTCCCCCCTCAGTCCTGGCAATGAGGATGCAGCACCATTCTCATTCTTATCCCACTCAATCAAAGGAATTCTGGG

2521  AAGGAGGTTGTGATTGCTGGCAAGTCCCCCCAACTGTACCATGGGCACGAGGAGCTGAGAGAACTGTTGAGGAGGGTTTG
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Figure 1. DNA sequence and deduced protein sequence of a mouse brain cDNA clone encoding γ-adaptin. The NH_2-terminal sequence that was obtained from purified bovine γ-adaptin is indicated. The initiator methionine is cleaved off in the mature protein, leaving an NH_2-terminal proline. These sequence data are available from EMBL/GenBank/DDBJ under accession number X54424.

clones obtained in this screen contained the complete coding sequence for γ-adaptin, as shown in Fig. 1. The identity of the clone was confirmed by protein sequencing. The NH_2 terminus of the intact protein was sequenced out to ten residues, and was subsequently isolated as a CNBr fragment which gave an unambiguous sequence of 24 amino acids identical to that deduced from the cDNA. Other peptides that were sequenced were less clean and thus more difficult to interpret, but most could still be found in the deduced protein sequence (data not shown).

The predicted molecular weight of the protein is 91,352. This is somewhat smaller than γ-adaptin's published molecular mass of 104 kD, based on its mobility on SDS polyacrylamide gels (Ahle et al., 1988). However, the relative mobil-

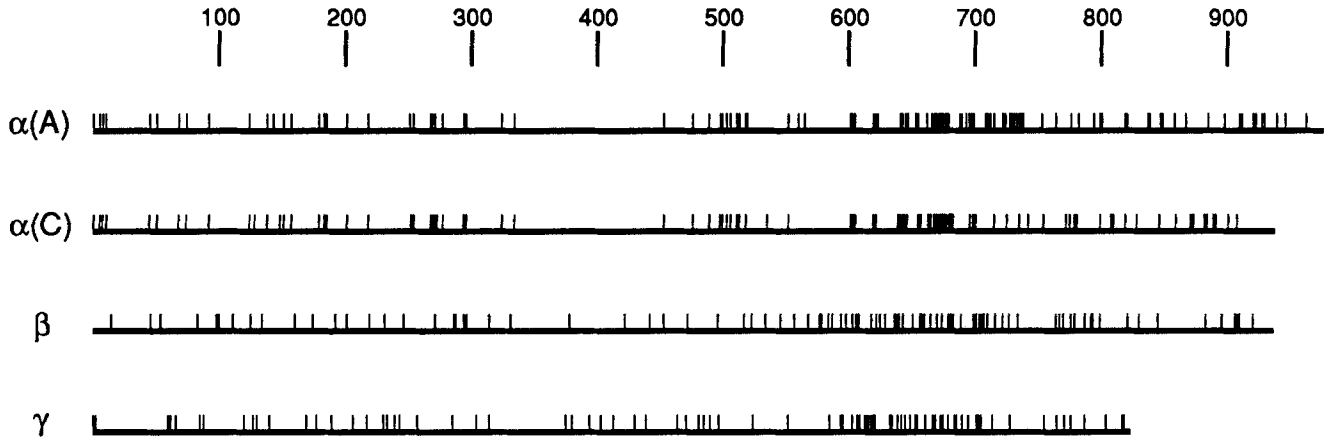

Figure 2. Diagrams of the sequences of the two α-adaptins, α(A) and α(C) (Robinson, 1989), β-adaptin (Ponnambalam et al., 1990), and γ-adaptin, with the positions of the prolines and glycines indicated. The scale at the top refers to the amino acid number.

ity of the protein seems to depend upon the gel system used (e.g., see Glickman et al., 1989). A comparison of the mouse and bovine cDNAs provided further evidence that the COOH-terminal coding sequence was correct: the deduced amino acid sequences of the two COOH termini are identical, although there are several differences at the nucleotide level; and there is complete divergence of the two DNA sequences immediately after the stop codons. Overall, the mouse and bovine protein sequences are highly homologous, with only 8 amino acid substitutions in the 553 residues that were compared.

Comparison of γ-Adaptin with α- and β-Adaptins

Both α- and β-adaptins have proline and glycine-rich stretches between amino acids 600 and 700–750, shown to correspond to the hinge regions of the two proteins (Robinson, 1989; Kirchhausen et al., 1989; Ponnambalam et al., 1990). Fig. 2 shows linear diagrams of all three types of adaptin, α, β, and γ, with the prolines and glycines indicated. It is clear that γ-adaptin has a similar stretch of sequence in the same position, while the COOH-terminal "ear" domain of γ-adaptin is smaller than that of α- or β-adaptin. The putative γ-adaptin hinge is very hydrophilic and particularly rich in acidic residues (11 acidic and only 2 basic residues between amino acids 600 and 700), and its contains several prolines next to alanines. These features also appear in the other adaptin hinges (Robinson, 1989; Kirchhausen et al., 1989; Ponnambalam et al., 1990).

Although the α- and β-adaptins have a similar amino acid content at their hinge regions, they do not show any apparent sequence homology when compared on Diagon plots. However, it seemed likely that α and γ-adaptins might be more closely related, since they probably occupy similar positions in the two adaptor complexes and carry out analogous functions, even though their peptide maps do not show any apparent similarities (Ahle et al., 1988). The Diagon plots in Fig. 3 reveal that γ-adaptin does indeed show sequence homology with α-adaptin, but no obvious homology with β-adaptin. A comparison of the γ- and α-adaptin sequences is shown in Fig. 4. The longest stretch of identical amino acids is the sequence DVELQQRAVEY (amino acids 556–566 in γ-adaptin, 572–582 in α-adaptin), although there are many other homologous patches containing identical amino acids or conservative substitutions, giving an overall identity of 25.0%. Interestingly, the homology is restricted to the NH_2 termini of the two proteins, up to and including their hinge regions; the COOH-terminal ears do not appear to be homologous.

When protein databases were searched for any other proteins homologous to γ-adaptin, no significant matches appeared. Similarly, no other proteins have been found that are homologous to the α- and β-adaptins (Robinson, 1989; Kirchhausen et al., 1989). However, because many sequences (including those for α- and β-adaptins) are only accessible as nucleic acid sequences, a search was also carried out using the TFASTA program (Pearson and Lipman, 1988)

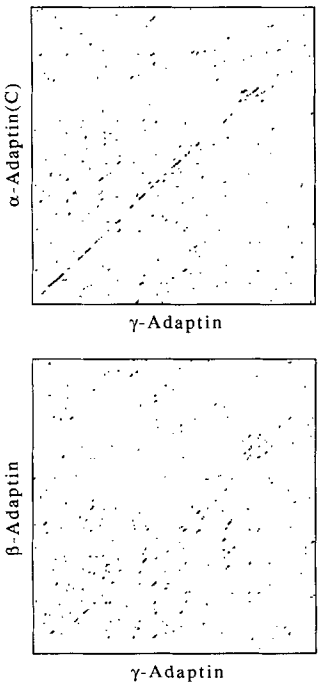

Figure 3. Diagon plots of γ-adaptin compared with α-adaptin(C) (*top*) and β-adaptin (*bottom*). The plots were made with the Staden V15.0 program using the parameters at their default settings (span length = 11, proportional score = 132, identities score = 8) (Staden, 1982).

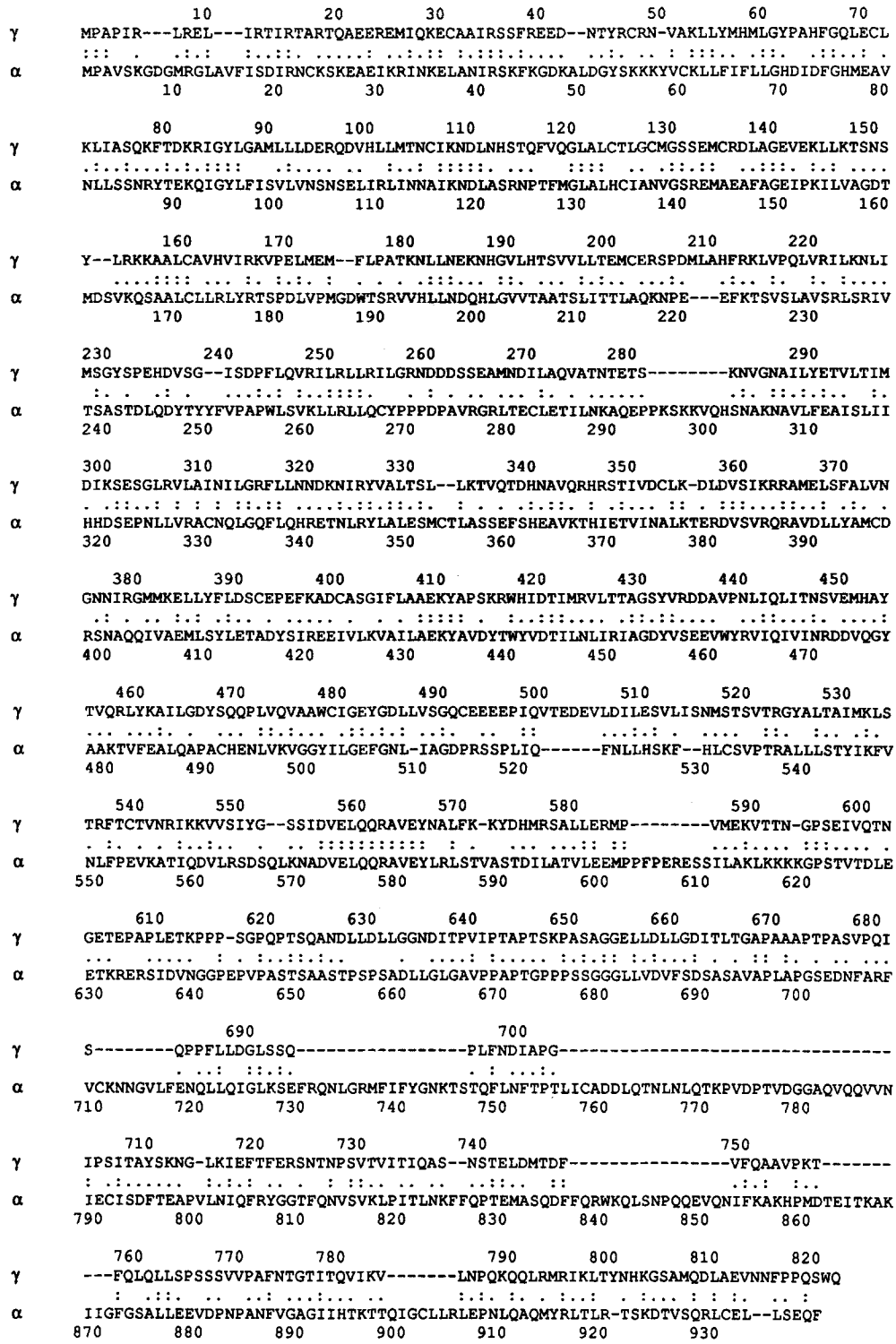

Figure 4. Comparison of the sequences of γ-adaptin (*upper sequence*) and α-adaptin(C) (*lower sequence*). The sequences were aligned by the FASTA computer program (Pearson and Lipman, 1988). Identical amino acids are indicated with two dots and conservative substitutions with one dot. These sequence data are available from EMBL/GenBank/DDBJ under accession number X54424.

to search DNA databases with the γ-adaptin protein sequence. Not surprisingly, the two closest homologues were found to be the two α-adaptins, with optimized scores of 774 for α(C) and 516 for α(A). Interestingly, the next closest homologue was β-adaptin, with an optimized score of 286. None of the other matches in either the DNA or the protein databases (>40,000 sequences) had scores of over 100. Unlike α- and γ-adaptins, however, γ- and β-adaptins and α- and β-adaptins do not share any long stretches of identical amino acids. Nevertheless, the FASTA program (Pearson and Lipman, 1988) reveals that γ and β have an overall amino acid identity of 15.7%, while α and β have an overall identity of 17.8%.

Expression of a γ-Adaptin Chimera in Fibroblasts

A major reason for cloning the adaptin cDNAs was to ad-

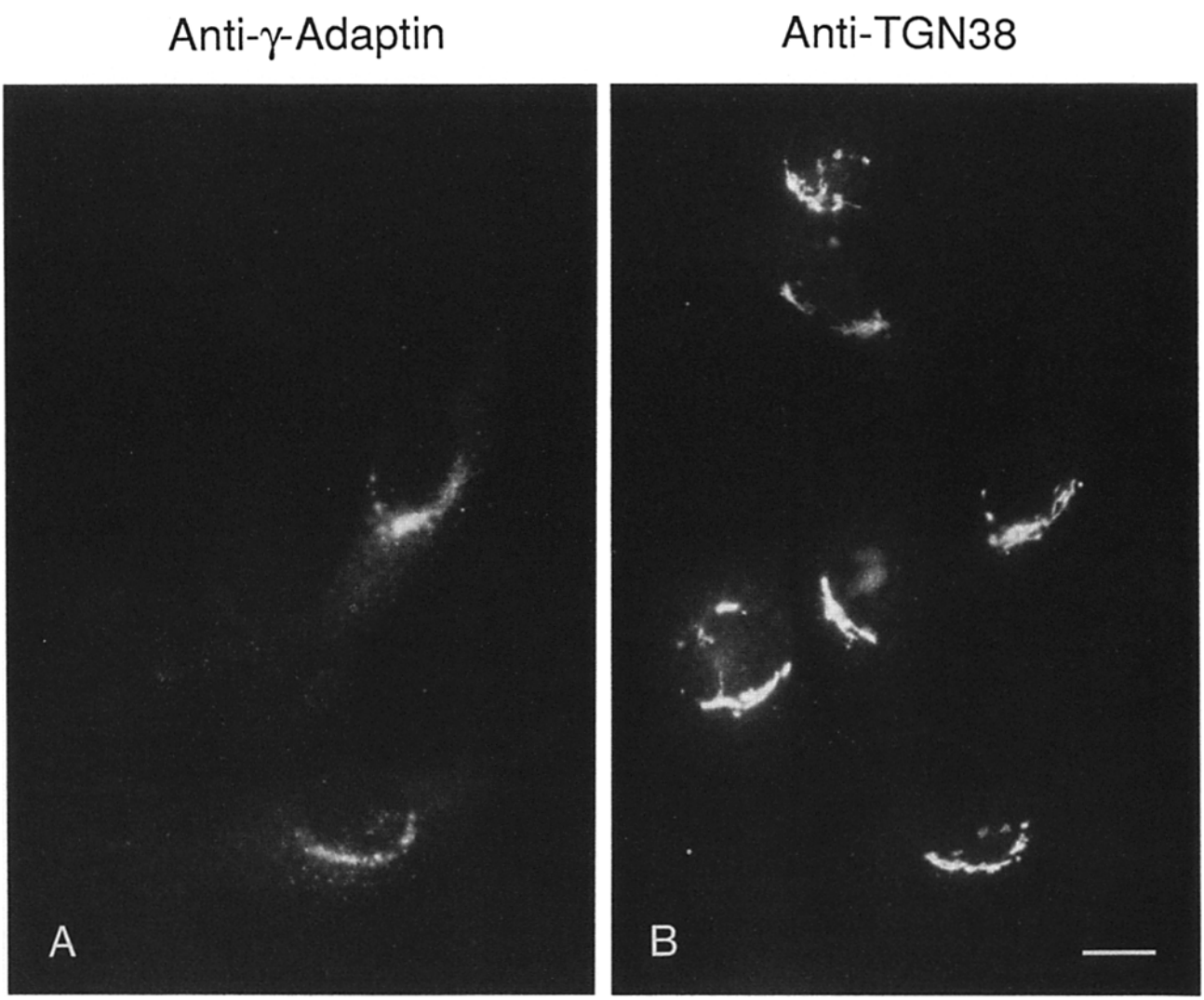

Figure 5. Immunofluorescence micrographs of Rat 1 cells transfected with a γ-adaptin chimera, constructed from the mouse and bovine cDNAs. The monoclonal anti-γ-adaptin antibody (*A*) does not recognize the protein from rodents and thus only stains the transfected cells. Cells expressing the antigen often come in pairs, probably the result of cell division after transfection. Double labeling with an antiserum against TGN38 (*B*) shows the position of the *trans*-Golgi network in the cells. Bar, 10 μm.

dress questions about adaptor function, using in vitro mutagenesis followed by expression in tissue cultures cells. However, before beginning such experiments, it is necessary to develop a system in which the proteins encoded by the cDNAs can be distinguished from the cell's endogenous adaptins. In the case of γ-adaptin, a means of specifically labeling the transfected protein is suggested by the observation that the epitope(s) recognized by the antibodies used for library screening occur in γ-adaptin from most mammals, but not from rodents. Although the full-length γ-adaptin cDNA was obtained from mouse, the epitope must occur in the bovine cDNA clones that were detected with the antibodies in the initial screen. Moreover, the epitope must be contained between amino acids 495 and 677, since that is the region common to all of the clones. From the major NH_2-terminal sequence of a CNBr fragment reacting with mAb 100/3, one can tentatively map the epitope to the hinge region, between amino acids 588 and 677.

To construct a full-length γ-adaptin that would react with the antibodies, a chimeric cDNA was made, containing the mouse sequence from amino acids 1 to 408, followed by the bovine sequence, taking advantage of a Pst I site shared by both cDNAs. This construct was inserted into a mammalian expression vector downstream from the SV40 early promoter and used to transfect Rat 1 fibroblasts. To maximize transient expression, the cells were then kept in culture for 2 d before they were assayed with mAb 100/3.

Because the transfection efficiency was only ~1–5%, the amounts of antigenic γ-adaptin that were being made were too low to be detected by Western blotting. However, by immunofluorescence microscopy, the few cells that were expressing the γ-adaptin chimera could be easily identified. The amount of expression was variable, probably because the cells took up variable amounts of DNA. Fig. 5 *A* shows two transfected cells with moderate to high expression, together with several nontransfected cells. In the transfected cells, the γ-adaptin chimera has a punctate distribution mainly in the perinuclear region, similar to the distribution

of endogenous γ-adaptin in cells that react with the antibody (Ahle et al., 1988). Fig. 5 *B* shows the same cells double labeled with an antiserum against an integral membrane protein of the TGN, TGN38, raised by Luzio and co-workers (Luzio et al., 1990). The perinuclear labeling is very similar with the two antibodies, consistent with ultrastructural evidence that the Golgi-associated coated vesicles bud from the TGN. However, there are also scattered dots labeled with the anti-γ-adaptin antibody that are closer to the cell periphery. Similar observations were made when Madin-Darby bovine kidney (MDBK) cells were labeled to reveal endogenous γ-adaptin and galactosyl transferase, a marker for *trans* Golgi cisternae (Ahle et al., 1988), although one could not rule out the possibility that the TGN might have a more widespread distribution than the Golgi stack. The present results indicate that the peripheral γ-adaptin labeling is not due to coated vesicles associated with the TGN, suggesting either that they were originally derived from the TGN but have now moved some distance away, or that they are associated with some other compartment such as endosomes.

Discussion

Previous reports describing the cloning and sequencing of α- and β-adaptins (Robinson, 1989; Kirchhausen et al., 1989; Ponnambalam et al., 1990) have provided information both about the structure of adaptins and about their heterogeneity. The cloning of γ-adaptin permits a similar analysis of the third class of adaptin, with the added advantage that γ-adaptin can be compared with both of the other adaptin classes which have already been cloned.

Like α- and β-adaptins, γ-adaptin has a two-domain structure separated by a proline and glycine-rich hinge, as predicted by proteolysis studies. The COOH-terminal ear domain is somewhat smaller than those of α- and β-adaptins. However, the distance from the end of the proline and glycine-rich stretch to the COOH terminus of the protein is only 117 amino acids instead of 200–250 amino acids. The anti-γ-adaptin antibody mAb 100/3, raised by Ahle and coworkers, apparently binds to the hinge region of the protein, providing further evidence, in addition to the proteolysis data, that the hinge is exposed in the native adaptor complex, since the antibody has been successfully used for immunoprecipitation of whole adaptors (Ahle et al., 1988).

Both α- and β-adaptins are heterogeneous: there are two α-adaptin genes and at least two β-adaptin genes, as well as tissue-specific splicing of β-adaptin (Robinson, 1989; Kirchhausen et al., 1989; Ponnambalam et al., 1990). Although there is no evidence for more than one form of γ-adaptin, either from the protein chemistry of Ahle et al. (1988) or from the data reported here, preliminary Southern blotting suggests that there might be more than one γ-adaptin gene (unpublished observations), although alternative explanations have not been ruled out. This will be an important point to establish, since clathrin-coated vesicles associated with the TGN have been implicated in two distinct post-Golgi pathways in regulated secretory cells: targeting of proteins to lysosomes and targeting of proteins to (or removal of proteins from) secretory granules (Griffiths and Simons, 1986; Tooze and Tooze, 1986). Thus, it is possible that such cells might use different forms of γ-adaptin to bud coated vesicles with different specificities from the same compartment.

A major finding from the sequence analysis was that γ-adaptin shows marked homology with α-adaptin, and weaker homology with β-adaptin. The regions that are conserved between α- and γ-adaptins could be important for binding to the other components of the adaptor complex, in particular to the β-adaptin subunit, which is very similar in the plasma membrane and Golgi adaptors (Ahle et al., 1988). Consistent with this possibility, the greatest homology is seen in the NH_2-terminal ~60 kD, which is the part of the protein thought to interact with the other adaptor components.

The homology between α- and γ-adaptins strongly suggests that they arose from a common ancestral gene, while their weaker homology with β-adaptins suggests that even earlier in evolution, there may have been a single progenitor adaptin gene. The division of clathrin-coated vesicles into two populations, one associated with the plasma membrane and one associated with the Golgi apparatus, is found in virtually all eukaryotes, including plants (Hillmer et al., 1988): in fact, the plant equivalent of the TGN is called the partially coated reticulum. It is tempting to speculate that the duplication of the ancestral α/γ gene may have preceded the divergence of coated vesicles into these two populations. If so, the homology between α- and γ-adaptins should facilitate the preparation of probes to look for adaptins in lower eukaryotes, such as yeast. In addition, if there are previously uncharacterized adaptins in mammalian cells, such widely crossreacting probes may be useful for identifying them.

The relative lack of homology between the COOH-terminal ear domains of α- and γ-adaptins suggests that these domains may be involved in an adaptor-specific function. Two such functions are targeting the adaptor complex to the right membrane compartment of the cell, and binding to the cytoplasmic tails of the appropriate transmembrane receptors. It seems likely that these are two separate functions, and that adaptors are not targeted solely by receptor binding, since most of these receptors occur in other membrane compartments in addition to those where adaptors are found. With the cloning of both α- and γ-adaptins, it may now be possible to dissect these two functions with a mutational analysis of the proteins.

The transfection system reported here provides a means of testing such mutations in vivo. The immunofluorescence localization of the γ-adaptin chimera encoded by the cDNA suggests that the protein is equilibrating with endogenous γ-adaptin and is being used to make Golgi adaptors and coated vesicles. One experiment that may be particularly informative, and which is made feasible by the two-domain structure of the adaptins, will be to swap the NH_2 and COOH termini of α and γ, and then to use immunofluorescence to find out whether such constructs are targeted to the plasma membrane or to the Golgi region. In addition, by overproducing genetically engineered adaptins, it may be possible to compete with the cell's endogenous adaptins for the other subunits of the adaptor complex. In this way, one might be able to create a dominant mutation (Herskowitz, 1987), and then to study the effect of such a phenotype on the sorting of other proteins.

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