The EMBO Journal vol.12 no.7 pp.2847–2853, 1993

A 102 kDa subunit of a Golgi-associated particle has homology to β subunits of trimeric G proteins

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Communicated by R.A.Weiss

We have identified a 102 kDa protein, p102, which is found on the cytoplasmic face of Golgi membranes, exocytic transport vesicles and in the cytosol. A monoclonal antibody that cross-reacts with p102 is able to immunoprecipitate a 500–600 kDa protein complex containing p102 and additional subunits. The composition of this p102-containing protein complex resembles that of the Golgi coatomer complex, which constitutes the coat of non-clathrin coated vesicles. One of the subunits of the p102 complex reacts with a monoclonal antibody that detects β-COP, a subunit of the Golgi coatomer complex. Like β-COP, p102 exists in a brefeldin A-sensitive association with Golgi membranes. The sequence of p102 contains an N-terminal domain composed of six repeats which are similar to those found in the β subunit of trimeric G proteins and other regulatory proteins. We suggest that p102 may be involved in regulating membrane traffic in the constitutive exocytic pathway.

Key words: brefeldin A/coatomer/Golgi/G-proteins/TCP-1

Introduction

The biochemical analysis of vesicular traffic in the exocytic pathway has identified certain polypeptides that may be specifically involved in membrane recognition and translocation events (Rothman and Orci, 1992). For example, a protein complex of M_r 500–700 kDa (Duden *et al.*, 1991; Waters *et al.*, 1991) has been isolated from the cytosol and from the surface of the 75 nm (intercisternal) Golgi transport vesicles (Malhotra *et al.*, 1989; Serafini *et al.*, 1991). This protein complex, or coatomer complex, is believed to constitute the coat of the vesicles of the constitutive exocytic pathway. The coatomer consists of α-, β-, γ- and δ-coatomer proteins (COPS) and additional proteins (Waters *et al.*, 1991), with the β-COP subunit (Duden *et al.*, 1991) displaying sequence homology to the α-, β- and γ-adaptin proteins which interact with clathrin at the surfaces of clathrin coated vesicles (Robinson, 1992). Recently the bovine γ-COP sequence was shown to be highly related to the sec21p gene of *Saccharomyces cerevisiae* (Stenbeck *et al.*, 1992), an essential gene required for vesicle budding in ER to Golgi transport whose product is a subunit of a 700 kDa coatomer-like particle (Hosobuchi *et al.*, 1992). Characterization of the other coatomer subunits may help define the mechanisms involved in regulating membrane target specificity.

Using a fortuitous cross-reactivity with two of seven monoclonal antibodies generated against the C-terminal half of mouse TCP-1, we have identified two large protein particles. These particles are called the p102 and p115 particles because of the estimated molecular weights of the anti-TCP-1 cross-reactive polypeptides in the respective particles. Characterization of the non-membrane bound form of the p102 particle revealed strong similarities to the coatomer of Golgi transport vesicles including similar molecular weight, number and M_r of subunits, membrane-bound to free cytosolic ratio and brefeldin A sensitivity. Furthermore, immunoprecipitated p102 particle contains a polypeptide recognized by monoclonal antibody M3A5 (Allan and Kreis, 1986), which recognizes β-COP (Duden *et al.*, 1991). Based upon indirect immunofluorescence experiments, the Golgi distributions of β-COP and p102 overlap substantially.

The cloning and sequencing of the gene encoding p102 revealed a 30 kDa N-terminal domain with homology to the G_β subunit of trimeric G protein complexes. Based on this homology we suggest that p102 may, like G_β subunits, mediate interaction with membranes.

Results

Anti-TCP-1 monoclonal antibodies are cross-reactive by Western blot analysis

Seven monoclonal antibodies were previously raised against the C-terminal half of mouse TCP-1 expressed in *Escherichia coli* (Willison *et al.*, 1989). When the post-nuclear supernatants (PNS) from different species and cell types were Western blotted with two of the seven antibodies (23C and 72A), three predominant cross-reacting polypeptides were detected in mammalian cell extracts with approximate molecular weights of 115, 102 and 63 kDa (Figure 1). Significantly, monoclonal antibodies 23C and 72A did not react with primate TCP-1 (Figure 1, lanes 16, 17 and 18) and this result suggested that TCP-1 might not be responsible for the Golgi localized immunofluorescence signal seen previously with this pair of antibodies (Willison *et al.*, 1989). We showed recently that the Golgi localization was not due to TCP-1 since monospecific monoclonal anti-TCP-1 antibodies stain the cytoplasm and do not stain membrane structures (Lewis *et al.*, 1992). Since monoclonal antibodies 23C and 72A stain the Golgi in a wide variety of cell types derived from different species (Willison *et al.*, 1989), it seemed likely that either the 115 kDa (p115) or the 102 kDa (p102) polypeptides were responsible for the Golgi signal, as they are evolutionarily highly conserved as judged by Western blotting (Figure 1). It also seemed likely that p102, rather than p115, is Golgi localized because monkey cells (Figure 1, lower panel, lane 16) and some mouse tissues contain little or no p115 as measured by Western blotting with 72A, yet all these cells show strong Golgi localized immunofluorescence signals.

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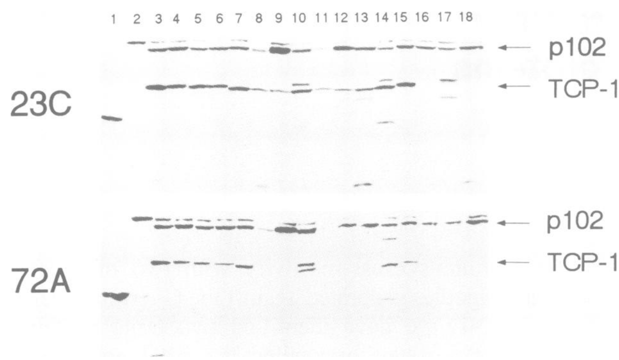

Fig. 1. Western blotting of post-nuclear supernatant (PNS) lysates with anti-mouse TCP-1 monoclonal antibodies 23C (top panel) and 72A (bottom panel). PNS homogenates are from the following cell types: (1) *Schizosaccharomyces pombe*; (2) a *Xenopus laevis* kidney-derived tissue culture line; (3) nontransformed chicken embryo fibroblasts, CEF-38; (4) normal rat kidney fibroblasts; (5) baby hamster kidney cells, BHK-21; (6) Chinese hamster ovary, wild type background, CHO-WTB; (7−13) mouse tissues: testis germ cells, brain, liver, spleen, heart, kidney and ovary; (14) BALB/c AnN mouse B-cell tumour line, LK-35.2; (15) Madin−Darby canine kidney cells; (16) African green monkey, COS; (17) human thymoma, CEM; (18) human epidermoid carcinoma, H.Ep2. A representative 10 min ECL exposure is shown.

p102 is a component of a 500−600 kDa particle

Biochemical fractionation of PNS of [^{35}S]methionine-labelled cells was performed to determine whether p102 co-fractionated with membrane components. Figure 2a shows the distribution of ^{35}S-labelled polypeptides upon sucrose gradient fractionation of PNS from human cells, H.Ep2. Figure 2b shows the distribution of intact Golgi and lysosomal membranes in the gradient. Figure 2c shows a Western blot of the gradient fractions probed with monoclonal antibody 23C to detect p102 and p115. p115 sedimented at around 20% sucrose density, similar to the TCP-1 particle (Lewis *et al.*, 1992), but showed no co-sedimentation with membranes. P115 must be a component of an independent protein particle unrelated either to the TCP-1 chaperonin or the p102 particle because it does not co-precipitate or co-purify with either of these protein particles (data not shown).

In contrast to p115, p102 displayed two peaks of distribution across the sucrose gradient fractions (Figure 2c). Under normal growth conditions, 14% of the total p102 signal co-sedimented with membranes (fractions 2−6, 28−38% sucrose), while 86% appeared to be soluble (fractions 9−12, peak 17.5% sucrose). The non-membrane-associated p102 sedimented with an approximate molecular weight of 500−600 kDa rather than its monomeric M_r of 102 kDa, suggesting that it might be a component of a large protein complex. Immunoprecipitation of p102 from the soluble peak sucrose gradient fractions with 23C identified a number of polypeptides co-precipitating with p102 (Figure 3, lane 1). This polypeptide profile looked very similar to that of the β-COP complex biochemically purified by Waters *et al.* (1991), with one protein of M_r ~150 kDa, four in the 100 kDa range, one of 60 kDa and one of 32 kDa. These polypeptides all differed in mobility from the subunits of the TCP-1 complex precipitated from the same sucrose gradient fractions with monospecific anti-TCP-1 monoclonal antibody (Figure 3, lane 2), and were not immunoprecipitated by a negative control (anti-VSV-G)

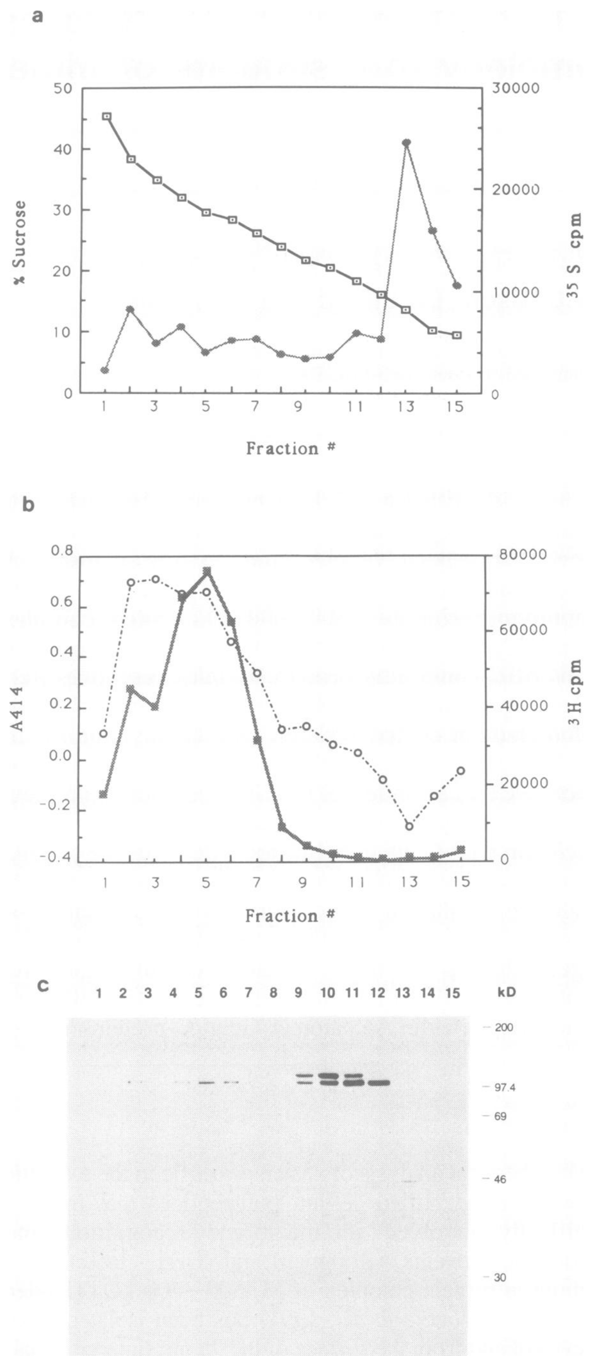

Fig. 2. Sucrose density gradient fractionation of [^{35}S]methionine-labelled H.Ep2 cell PNS homogenates. Fifteen fractions were collected across a 10.2−40% linear sucrose gradient from the bottom (heavy, fraction 1) to the top (light, fraction 15). The percentage sucrose per fraction is shown in panel **a** (open squares), with the distribution of total protein across the gradient, as incorporated [^{35}S]methionine c.p.m. per fraction in hatched ovals. The distribution of intact Golgi membranes was determined by incorporated [^{3}H]UDP-galactose (panel **b**, open circles) in a galactosyl transferase assay, and of intact lysosomal membranes by absorption at 414 nm using the substrate *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide in a β-hexosaminidase assay (panel b, hatched squares). The sedimentation of p102 and p115 within the gradient was determined by Western blot analysis of individual gradient fractions probed with the anti-mouse TCP-1 monoclonal antibody 23C (panel c). The apparent molecular mass of the p102 complex was determined from sedimentation of known native proteins [thyroglobulin (669 kDa), jack bean urease (545 kDa), apoferritin (443 kDa), β-amylase (200 kDa), bovine serum albumin (69 kDa) and ovalbumin (45 kDa)] run on a parallel gradient.

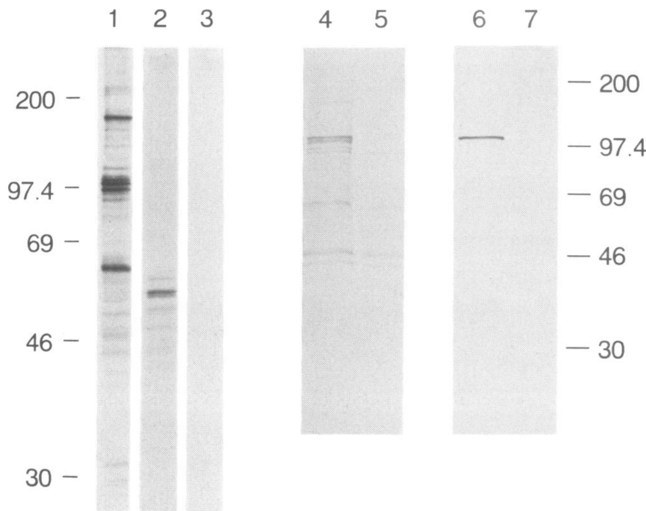

Fig. 3. Immunoprecipitation of p102 and associated polypeptides. Lanes 1–3 show immunoprecipitates of the p102 complex sucrose peak fraction from [^{35}S]methionine-labelled H.Ep2 cells. Monoclonal antibodies 23C and 72A were used to precipitate the p102 complex (lane 1); 91A and 84A, to precipitate the TCP-1 complex (lane 2); or mouse anti-VSV-G monoclonal antibody, P5D4, as a negative control (lane 3). The p102 complex contains polypeptides which are of similar size to α-COP (150 kDa), β-COP (110 kDa), γ-COP (98 kDa) and δ-COP (61 kDa), and two additional species of ~100 kDa and a smaller species of 32 kDa. Lanes 4 and 5 are immunoprecipitations of the peak p102 pool of sucrose gradient fractions of ^{35}S-labelled H.Ep2 cell lysate precipitated with 23C/72A (lane 4) or monoclonal anti-VSV-G antibody P5D4 (to serve as a negative control, lane 5), fractionated by SDS–PAGE, transferred to a nitrocellulose filter and autoradiographed for ^{35}S. Lanes 6 and 7 show the same filter after probing with mouse monoclonal antibody M3A5 followed by alkaline phosphatase-conjugated goat anti-mouse second antibody.

antibody (Figure 3, lane 3). To determine whether β-COP can co-precipitate with p102 (Figure 3, lanes 4–7), radiolabelled p102 complex was immunoprecipitated from the soluble sucrose peak fractions, electrophoresed and transferred to a nitrocellulose membrane. The central panel in Figure 3 (lanes 4 and 5) is an autoradiographic exposure of the membrane after transfer showing the immunoprecipitated p102 complex (lane 4) and a negative control immunoprecipitation from the same sucrose fraction with an anti-VSV-G antibody (lane 5). The right-hand panel in Figure 3 (lanes 6 and 7) shows the same membrane, which has been probed with the anti-β-COP monoclonal antibody M3A5 (Allan and Kreis, 1986) (kindly given by Thomas Kreis) and developed using an alkaline phosphatase colour detection assay. Although M3A5 was originally raised against components of microtubules (Allan and Kreis, 1986), it reacts strongly with β-COP, which has an estimated M_r of 110 kDa and an M_r of 107.5 kDa calculated from the longest open reading frame of the cDNA (Duden *et al.*, 1991). A single band of similar mobility to β-COP was detected in the lane containing immunoprecipitated p102 complex (Figure 3, lane 6) but not in the negative control lane (Figure 3, lane 7), demonstrating that β-COP and p102 can be co-precipitated. The M3A5-detected band and p102 often co-migrate in our gels, and this may be one reason why p102 has not been visualized before. Under some electrophoresis conditions, p102 and β-COP do resolve in SDS–PAGE gels, and both exist in apparently stoichiometric amounts, with p102 migrating as the lower of the two bands. Two-dimensional gel Western blotting analysis of the immunoprecipitated complex showed that p102 focused poorly and was retained at the basic end of the gel, whereas β-COP had an estimated pI of 6.3–6.5 (data not shown). The apparent equimolarity of the two bands in immunoprecipitates, along with our observation that the immunoprecipitation conditions used quantitatively recover p102, suggest that the majority or all of p102 in the soluble peak is associated with β-COP. The extent of the association of p102 and β-COP was further assessed by Western blot analysis of sucrose gradient-fractionated PNS from different cell types using 23C and M3A5, which supported the idea that p102 and β-COP co-fractionate (data not shown). All of the above biochemical data are consistent with p102 being a subunit of coatomer.

Localization of p102 is brefeldin A-sensitive

Brefeldin A is a fungal metabolite which disrupts various steps in the exocytic pathway (Misumi *et al.*, 1986). In *Saccharomyces cerevisiae* brefeldin A blocks early transport steps in the secretory pathway (Graham *et al.*, 1993), and in some mammalian cell types export from distal Golgi compartments to the cell surface is inhibited (Miller *et al.*, 1992). Brefeldin A causes very rapid dissociation of β-COP from Golgi membranes into the cytosol (Donaldson *et al.*, 1990; Lippincott-Schwartz, 1993). Using indirect immunofluorescence co-staining in HeLa cells we directly compared the subcellular localization of p102 and β-COP in the presence and absence of 1 μg/ml brefeldin A. Cells were co-stained for p102, with mAb 23C, and for β-COP with anti-EAGE antibody (Duden *et al.*, 1991) (kindly given by Thomas Kreis). The staining patterns p102 and β-COP virtually overlapped in untreated cells (Figure 4A). Upon treatment with brefeldin A the time courses of dispersal and the distributions of the p102 and β-COP signals were again very similar with dispersal becoming apparent by 1 min (Figure 4B) and almost complete by 2 min (Figure 4D). The dispersal of signal was reversible by treatment with forskolin (data not shown). These results show that p102 responds to brefeldin A in a similar manner to β-COP and support the idea that these two molecules are involved together in membrane interaction events.

Isolation of p102 cDNA clones

Two human cDNA expression libraries were screened with monoclonal antibody 23C and several clones of different lengths were isolated. Figure 5 shows the translation of the longest open reading frame. The sequence encodes a 906 residue polypeptide with a molecular weight of 102.5 kDa and a C-terminus LDD. These three C-terminal residues are the same in mouse TCP-1, where the epitope recognized by 23C and 72A is known to reside, and account for the cross-reaction of the two monoclonal antibodies. A glutathione S-transferase fusion protein containing the 10 C-terminal residues of p102 reacted with both antibodies on Western blots (data not shown). To confirm that the p102 cDNA encodes the Golgi-associated polypeptide, a polyclonal rat antiserum was raised against a bacterially expressed 302 amino acid fragment of the protein (residues 307–609). On Western blots of a pool of sucrose gradient fractions of mouse testis germ cell PNS, enriched for the p102 particle, the antiserum recognized a protein (Figure 6B) that co-migrated with the 102 kDa polypeptide recognized by monoclonal antibodies 23C and 72A (Figure 6A).

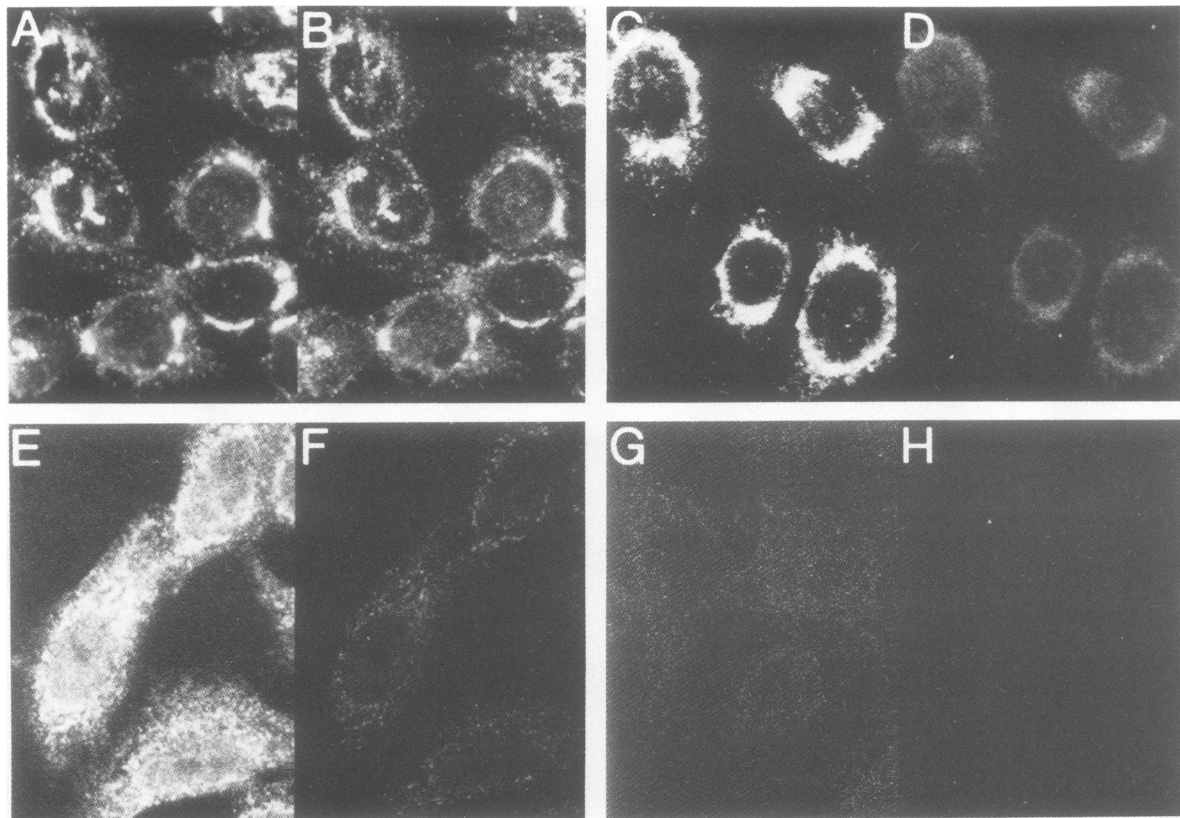

Fig. 4. Effect of brefeldin A on the subcellular distribution of p102 and β-COP. Panels A–H show the disruption of p102 (**A**, **C**, **E** and **G**) and β-COP (**B**, **D**, **F** and **H**) distribution in HeLa cells by brefeldin A (1 μg/ml) at 0 s (A and B), 60 s (C and D), 90 s (E and F) and 120 s (G and H). Cells were co-stained for p102 and β-COP by sequential incubation with 23C, anti-rat-lissamine-rhodamine, anti-EAGE and anti-rabbit-FITC.

Monoclonal antibody M3A5 recognized a protein of similar mobility to β-COP in the same sucrose fractions (Figure 6C). Indirect immunofluorescence co-staining with the anti-p102 polyclonal antiserum and 23C showed completely overlapping signals, suggesting the same protein was being detected (Figure 6D and E).

p102 contains multiple β-transducin-like repeats

Analysis of the sequence of p102 is consistent with the cytosolic localization of the protein. Searches of various databases (Swissprot release 22; Edinburgh) showed that the p102 sequence is novel. There are some short stretches of homology, whose significance is unclear, to *Drosophila* cGMP-dependent protein kinases (p102 residues 411–422), a yeast ATP-dependent RNA helicase (residues 730–789) and human β-*N*-acetyl-hexosaminidase (residues 417–468). At the N-terminus of p102 are six copies of a well-described repeated protein motif; the WD-40 repeat (van der Voorn and Ploegh, 1992). This repeat is found in the β subunit of guanine regulatory proteins, some cell cycle regulatory genes and transcription factors, as well as other proteins which differ in function and subcellular localization. In these proteins each full repeat unit is ~40 residues in length, and 2–7 repeats are found in tandem linked by short spacers of variable lengths. Figure 7 shows an alignment of the p102 WD-40 repeats with those from β-transducin and yeast CDC4. The lengths of the spacer regions between the p102 repeat units most closely resemble those found in β-transducin, although the repeats themselves are not strikingly similar, apart from the conserved residues, to those found

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MPLRIDIKRK LTARSDRVKS VDLHPTEPWM LASLYNGSVC   40
VWNHETQTLV KTFEVCDLPV RAAKFVARKN WVVTGADDMQ   80
IRVFNYNTLE RVHMFEAHSD YIRCIAVHPT QPFILTSSDD  120
MLIKLWDWDK KWSCSQVFEG HTHYVMQIVI NPKDNNQFAS  160
ASLDRTIKVW QLGSSSPNFT LEGHEKGVNC IDYYSGGDKP  200
YLISGADDRL VKIWDYQNKT CVQTLEGHAQ NVSCASFHPE  240
LPIIITGSED GTVRIWHSST YRLESTLNYG MERVWCVASL  280
RGSNNVALGY DEGSIIVKLG REEPAMSMDA NGKIIWAKHS  320
EVQQANLKAM GDAEIKDGER LPLAVKDMGS CEIYPQTIQH  360
NPNGRFVVVC GDGEYIIYTA MALRNKSFGS AQEFAWAHDS  400
SEYAIRESNS IVKIFKNFKE KKSFKPDFGA ESIYGGFLLG  440
VRSVNGLAFY DWDNTELIRR IEIQPKHIFW SDSGELVCIA  480
TEESFFILKY LSEKVLAAQE THEGVTEDGI EDAFEVLGEI  520
QEIVKTGLWV GDCFIYTSSV NRLNYYVGGE IVTIAHLDRT  560
MYLLGYIPKD NRLYLGDKEL NIISYSLLVS VLEYQTAVMR  600
RDFSMADKVL PTIPKEQRTR VAHFLEKQGF KQQALTVSTD  640
PEHRFELALQ LGELKIAYQL AVEAESEQKW KQLAELAISK  680
CQFGLAQECL HHAQDYGGLL LLATASGNAN MVNKLAEGAE  720
RDGKNNVAFM SYFLQGKVDA CLELLIRTGR LPEAAFLART  760
YLPSQVSRVV KLWRENLSKV NQKAAESLAD PTEYENLFPG  800
LKEAFVVEEW VKETHADLWP AKQYPLVTPN EERNVMEEGK  840
DFQPSRSTAQ QELDGKPASP TPVIVASHTA NKEEKSLLEL  880
EVDLDNLELE DIDTTDINLD EDILDD                 906
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Fig. 5. Deduced amino acid sequence of p102 open reading frame.

in any particular WD-40 repeat-containing protein. p102 is similar to CDC4 in that it contains six full repeats, although the repeats are found at the N-terminus of p102 rather than the C-terminus, as in CDC4.

Discussion

Mouse TCP-1 shares a C-terminal sequence with p102

Through the cloning of the p102 gene we have discovered why two anti-mouse TCP-1 monoclonal antibodies, 23C and

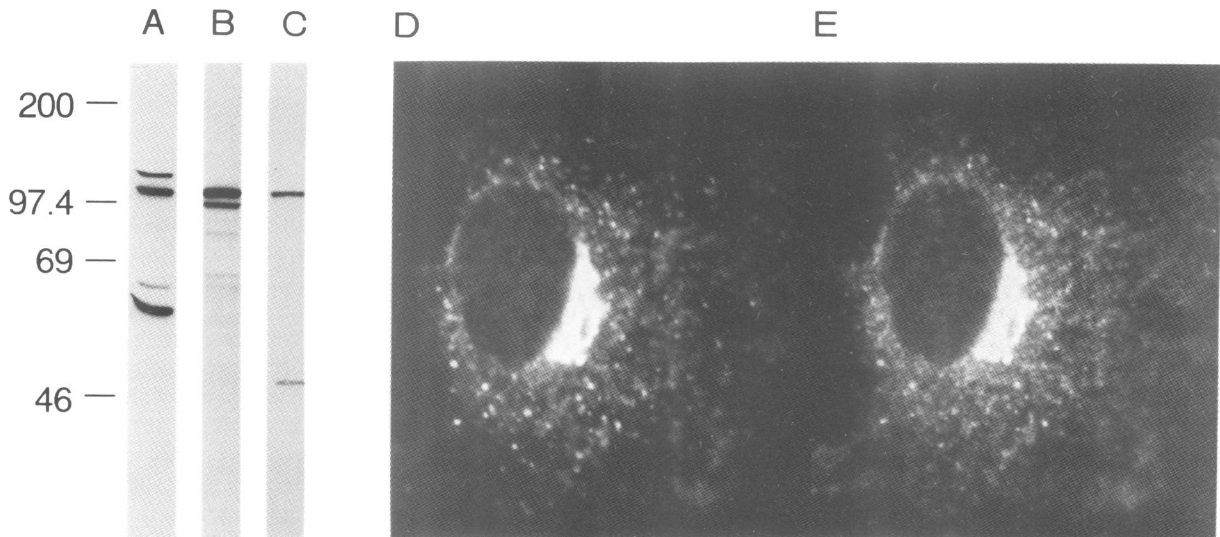

Fig. 6. Western blot of 8% SDS−PAGE gel of a pool of sucrose gradient fractions of mouse testis germ cell PNS (see Figure 2 legend), enriched for p102 complex, probed with monoclonal antibody 23C (panel **A**); affinity-purified anti-p102 polyclonal antibodies (panel **B**); monoclonal antibody M3A5 (panel **C**). The lower band in lane B may represent a proteolytic fragment of p102, lacking the C-terminus since it does not cross-react with 23C, or it may be a different protein. Its distribution in sucrose gradients is the same as that of p102. Panels **D** and **E**: co-staining of HeLa cells by indirect immunofluorescence using 23C (D) and anti-p102 polyclonal antibodies (E).

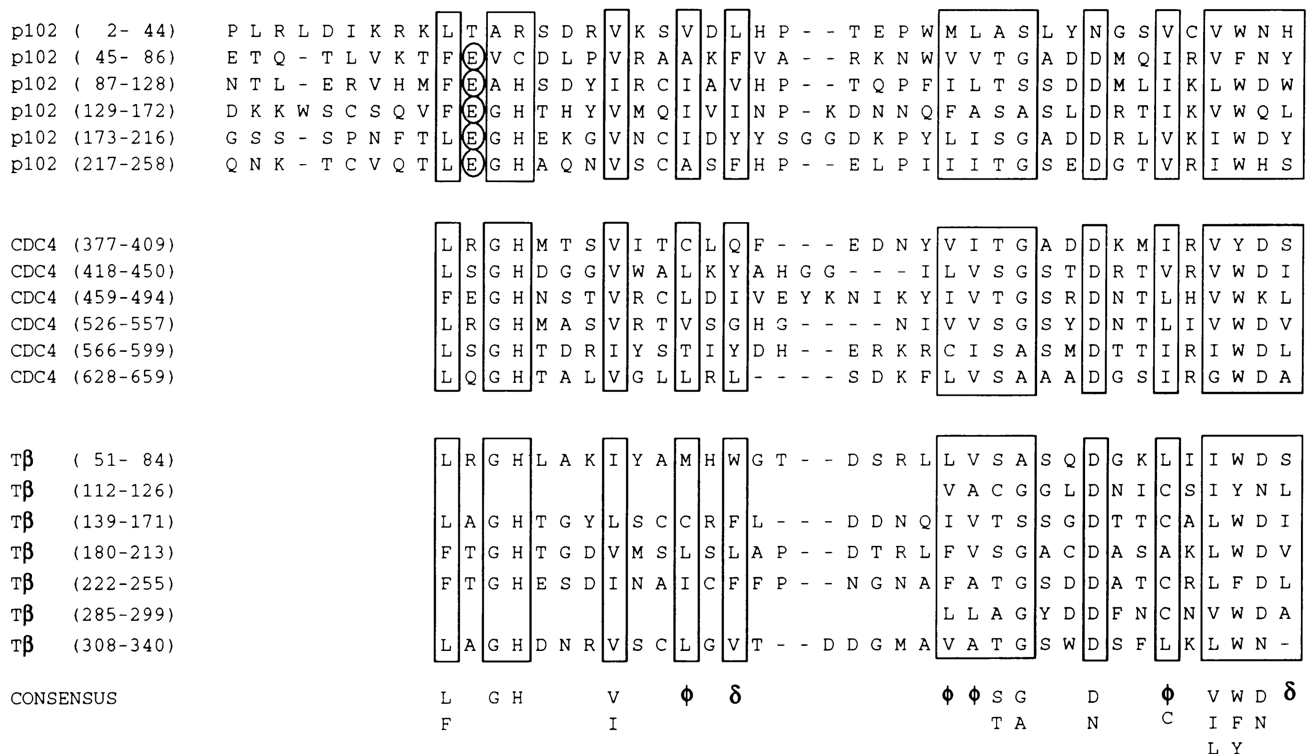

Fig. 7. Alignments of WD-40 repeat regions from p102 compared with consensus sequences of conserved regions of WD-40 repeats from the human β-transducin and *S.cerevisiae* CDC4 proteins (van der Voorn and Ploegh, 1992). Highly conserved residues between the proteins are boxed, and highly conserved residues within a single protein are circled. The consensus sequence is shown below the aligned repeats; ϕ = hydrophobic residues preferred, δ = non-charged.

72A, produced strong Golgi localized immunofluorescence signals. The last three C-terminal residues of mouse TCP-1 and p102 are LDD and these residues comprise part of the epitope recognized by these two antibodies. Apart from the C-termini, no other obvious sequence homology between p102 and TCP-1 was found. The cross-reactivity may be entirely fortuitous; however, it may reflect a common structural feature of the C-termini of p102 and TCP-1. It is notable that both of these proteins are components of large heteromeric complexes which can be immunoprecipitated under native conditions with monoclonal antibodies 23C and 72A, indicating that the C-termini of both p102 and TCP-1 are exposed on the outside of their respective complexes. It is also worth noting that the anti-β-COP monoclonal

antibody, M3A5, cross-reacts with a microtubule associated protein (Allan and Kreis, 1986) and that p102 shares an epitope with TCP-1, which has been demonstrated to fold tubulin (Yaffe *et al.*, 1992); perhaps β-COP and p102 can interact with microtubules in some manner?

Subcellular localization of p102

The precise distribution of coatomer throughout the exocytic pathway is not completely defined. Genetic experiments in yeast suggest involvement of coatomer in the ER to Golgi pathway (Hosobuchi *et al.*, 1992). Subcellular localization of coatomer in mammalian cells with anti-β-COP antibodies find it in the intermediate compartment between the ER and the Golgi apparatus (Lippincott-Schwartz, 1993) as defined by the p53 marker (Schweizer *et al.*, 1990) and by co-localization with the G protein of VSV ts045 at 15°C (Duden *et al.*, 1991). An important question is whether coatomer has a constant subunit composition when bound to the membranes of different Golgi compartments and in the cytosol. The presence of substoichiometric amounts of other proteins in preparations of coatomer (Rothman and Orci, 1992) suggests that the association of some subunits may vary depending upon the position of the coatomer in the transport pathway and/or its dynamic state. The production of antibodies to the other components of coatomer should allow the question of variation in subunit composition to be addressed. In indirect immunofluorescence co-staining experiments p102 and β-COP show overlapping signals, and the cytosolic form of p102 co-immunoprecipitates with β-COP. Both these polypeptides behave similarly in biochemical fractionation experiments and the composition of the p102 particle resembles that of coatomer. These data suggest that p102 is an integral subunit of coatomer.

Previous studies showed that mAbs 23C and 72A stained peripheral, punctate structures as well as perinuclear structures, implying localization of p102 to Golgi membranes and to vesicles being transported away from the Golgi apparatus (Willison *et al.*, 1989). The localization of p102 in the trans Golgi network was determined using the ts045 mutant of VSV at 19°C and the overlap between 23C and anti-VSV-G staining was complete (Willison *et al.*, 1989). Anti-β-COP antibodies showed similar co-staining with VSV-G in ts045 infected cells at 20°C except that not all peripherally located vesicles co-stained with both antibodies (Duden *et al.*, 1991). Electron microscopy localization with mAb 23C showed that labelling was heaviest in regions around the Golgi but largely absent from the cisternae themselves (Willison *et al.*, 1989). mAb 23C was able to bind to the Golgi apparatus after microinjection into the cytoplasm of living cells, indicating that membrane localization was to the cytosolic face of Golgi membranes rather than in the lumen (Willison *et al.*, 1989). Further experiments are required to determine the precise localization of p102 on the membranes of different Golgi compartments.

Homology of p102 to β subunits of trimeric G proteins

What is the significance of the presence of WD-40 repeats in p102? p102 may be solely a structural component of the protein complex or it may also be involved in the association of the complex with membranes. In the latter case, the G_β-like domain of p102 might interact directly with a membrane-localized G_α protein in the target membranes of the Golgi. There is increasing evidence that GTP-binding proteins play an important regulatory role in transport processes (Hall, 1990). ADP-ribosylation factor (ARF) is required for binding of the coatomer to Golgi membranes (Donaldson *et al.*, 1992a), and an ARF exchange factor may be a target for brefeldin A (Donaldson *et al.*, 1992b; Helms and Rothman, 1992). Trimeric G proteins are implicated through the use of GTP-γ-S, which inhibits Golgi transport assays, or $[AlF_4]^-$ which activates G_α subunits (Burgoyne, 1992), and it has been shown that $G_{\alpha i3}$ is concentrated on Golgi membranes (Ercolani *et al.*, 1990). It is possible that p102 is involved in trimeric G protein interactions.

Another way of considering the function of p102 is to suppose that the WD-40 repeats interact with tetratricopeptide (TPR) repeat-containing proteins in the particle or on membranes. TPR repeat proteins contain multiple copies of a conserved 34 amino acid sequence and, like WD-40 repeat-containing proteins, have different functions and subcellular localizations (Goebl and Yanagida, 1991). One of them, MAS70, is known to be associated with mitochondrial membranes and plays a role in mitochondrial protein import (Woolford, 1989). There is genetic evidence that TPR proteins can interact with WD-40 proteins (Goebl and Yanagida, 1991). We examined the sequence of β-COP and found a single TPR-like repeat between residues 80 and 114. Possibly β-COP and p102 interact directly through their respective repeats. It will be interesting to search for other TPR repeat-containing proteins on the cytosolic face of Golgi membranes and in coatomer complexes.

Recently a WD-40 repeat-containing protein has been implicated in membrane transport in yeast. The *SEC13* gene of *S.cerevisiae* is required for vesicle formation from the ER and it encodes a 33 kDa polypeptide, Sec13p, which is composed of six copies of the WD-40 repeat (Pryer *et al.*, 1993). Interestingly, like p102, Sec13p exists in a large particle (700 kDa) in the cytosol and is peripherally associated with membranes.

Materials and methods

Cell fractionation

For the Western blotting experiment in Figure 1, PNS were prepared by lysis in 1% Triton X-100 in PBS containing protease inhibitors (PMSF 1 mM, chymostatin 5 μg/ml, antipain 5 μg/ml, pepstatin A 5 μg/ml, leupeptin 10 μg/ml and aprotinin 0.3 TIU/ml) and phosphatase inhibitors (Na_2VO_4 2 mM and NaF 50 mM). Protein from $2-6 \times 10^5$ cells for cell lines and tissues or 8×10^5 *Schizosaccharomyces pombe* cells was loaded per lane. Protein loadings were adjusted, not more than 3-fold, to produce equivalent Coomassie stained profiles in each lane. For the sucrose gradient fractionation experiment in Figure 2, PNS and sucrose gradients were the very same as those used previously (Lewis *et al.*, 1992). SDS–PAGE, Western blotting and ECL detection (Amersham) were as previously described (Lewis *et al.*, 1992).

Anti-TCP-1 monoclonal antibodies

Monoclonal anti-mouse TCP-1 antibodies have been described previously (Willison *et al.*, 1989). The epitopes on mouse TCP-1 which are recognized by monoclonal antibodies 23C and 72A were mapped using oligopeptides in a binding assay. The epitopes for both 23C and 72A reside at the C-terminus of TCP-1. The critical residues for binding are the final three residues, LDD (residues 554–556), and changes to or additions to the C-terminal amino acid, D_{556}, abrogate binding. Human TCP-1 is not recognized by 23C or 72A because residue 555 is N rather than D as in mouse TCP-1 (Willison *et al.*, 1986; Kirchhoff and Willison, 1990).

Immunoprecipitation

Pooled 15.4–21.5% sucrose fractions containing both p102 peak and TCP-1 peak were used for immunoprecipitation to show that each complex can

be specifically precipitated from a mixture containing both. The fractions were diluted with an equal volume of 50 mM HEPES pH 7.2, 90 mM KCl and adjusted to 0.5% Triton X-100. Monoclonal antibodies 23C and 72A (3 μg of each) were added to 1 ml of diluted sucrose gradient fraction and incubated for 30 min on ice. Rabbit anti-rat immunoglobulins (12 μg) were then added and incubated for 30 min on ice, followed by addition of protein A−Sepharose beads (Pharmacia) and incubation overnight at 4°C. The beads were washed three times in 50 mM HEPES pH 7.2, 90 mM KCl, 0.5% Triton X-100. Immunoglobulins, eluted by boiling in 2 × Laemmli loading buffer, were subjected to SDS−PAGE. Western blots of the immunoprecipitates with mouse monoclonal antibody, M3A5, were developed with alkaline phosphatase-conjugated goat anti-mouse second antibody (Pierce).

Antiserum preparation

A GST fusion protein containing amino acid residues 307−609 of p102 was expressed in *E.coli* using bacterial expression vector pGEX-2T (Smith and Johnson, 1988) and purified essentially as described. The thrombin-cleaved p102 moiety was purified by electroelution from 10% SDS−PAGE gels overnight at 100 mA in SDS−PAGE running buffer. The protein was concentrated and used to immunize a rat. Antiserum was affinity purified by binding to Reacti-Gel^{Tm} HW-65 beads (Pierce) coupled to purified thrombin-cleaved fusion protein, and eluting with 50 mM glycine, pH 2.0 in 2−5 ml fractions, which were immediately adjusted to pH 7.3 with 1 M Tris pH 8.0.

Indirect immunofluorescence

HeLa cells were plated onto 13 mm glass coverslips 24−72 h before use. After treatment with brefeldin A (1 μg/ml; Sigma), cells were fixed and permeabilized by incubation in methanol:acetone (1:1) at −20°C for 5 min. Coverslips were pre-blocked by incubation in PBS containing 0.5 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and 0.2% BSA (PBS/BSA) for 15 min. Indirect immunofluorescence with rat anti-p102 monoclonal antibody (23C) and affinity purified rabbit anti-β-COP antibody anti-EAGE (Duden *et al.*, 1991) (kindly given by Thomas Kreis) was detected with donkey anti-rat conjugated lissamine−rhodamine and donkey anti-rabbit conjugated FITC, respectively. Both incubations with primary and secondary antibodies were carried out in PBS/BSA for 1 h at room temperature. Samples were visualized on a Nikon Optiphot fluorescence microscope equipped with a Bio-Rad MRC600 confocal imaging system. For co-staining with 23C and anti-p102 polyclonal antibodies, HeLa cells were fixed and permeabilized by incubation in 100% methanol at −20°C for 8 min. Coverslips were pre-blocked by incubation in PBS containing 0.5 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and 1% BSA (fraction V, Sigma) for 15−20 min. Co-staining was carried out by indirect immunofluorescence with sequential incubation in affinity purified anti-p102 polyclonal antiserum, followed by anti-rat TRITC (Sigma), biotinylated monoclonal antibody 23C and avidin-conjugated FITC (Vector Labs, Burlingame, CA). All incubations were carried out in the PBS/BSA solution for 1−1.5 h at room temperature.

cDNA cloning and sequencing

Two human cDNA expression libraries were screened with monoclonal antibody 23C essentially according to the protocol supplied with the Stratagene picoBlue ImmunoDetection Kit, except that the 37°C IPTG induction step was left overnight, and the filters were blocked in 1 M glycine, 5% powdered milk, 1% (w/v) egg albumin and 5% fetal calf serum. After a second antibody incubation with goat anti-rat calf alkaline phosphatase (Pierce), filters were developed in BCIP/NBT solution. Several overlapping clones were obtained by screening ~300 000−400 000 plaques from a λgt11, oligo(dT)-primed, human liver cDNA library (kindly provided by Alan Ashworth), and an equivalent number of plaques from a λZAPII random primed HT1080 cell line library (kindly given by Philip Mitchell). The sequence was obtained by [^{35}S]dATP dideoxy chain sequencing of both strands of PCR products and Bluescript subclones, using oligonucleotide primers. The sequence of most of the open reading frame was contained in a single cDNA clone, HT14.2. The most 5′ 70 bp of coding sequence were obtained by PCR from HT1080 library pools. The EMBL accession number is X70476.

Acknowledgements

We thank Hugh Paterson for help with confocal microscopy, Thomas Kreis for the M3A5 and EAGE antibodies, Philip Mitchell for the HT1080 cDNA library, Alan Ashworth for the human liver cDNA library and helpful discussions, and Ken Merrifield for help with computer analysis. This work is funded by a grant to the Institute of Cancer Research from the Cancer Research Campaign and the Medical Research Council.

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Received on January 28, 1993; revised on April 20, 1993

Note added in proof

Stenbeck *et al.* have independently identified the bovine cDNA of p102 (see accompanying paper).