The polarized epithelia-specific μ 1B-adaptin complements μ 1A-deficiency in fibroblasts

Eeva-Liisa Eskelinen, Christoph Meyer¹, Hiroshi Ohno², Kurt von Figura¹ & Peter Schu^{1,+}

School of Life Sciences, Center for High Resolution Imaging and Processing, University of Dundee, Dundee DD1 5EH, UK, ¹Zentrum für Biochemie und Molekulare Zellbiologie, Department Biochemie II, Universität Göttingen, Heinrich-Düker-Weg 12, D-37073 Göttingen, Germany and ²Division of Molecular Membrane Biology, Cancer Research Institute, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-0934, Japan

Received December 6, 2001; revised February 6, 2002; accepted March 4, 2002

The heterotetrameric AP-1A adaptor complex of clathrin-coated vesicles is ubiquitously expressed. The μ 1-adaptin subunit of the complex exists as the ubiquitous μ 1A and the polarized epithelia-specific μ 1B, which are 80% identical. In polarized epithelia, μ 1B is incorporated into the AP-1B complex, which is required for basolateral plasma membrane sorting of the low-density lipoprotein receptor. Binding of AP-1B to subdomains of the *trans*-Golgi network (TGN) appears to be part of the mechanism by which protein sorting is mediated. We expressed μ 1B in μ 1A-deficient fibroblasts to test for μ 1B function in non-polarized cells. AP-1B complexes were formed and bound to the TGN and to endosomes. Moreover, AP-1B restored the AP-1A-dependent sorting of mannose 6-phosphate receptors between endosomes and the TGN. This demonstrates that μ 1A and μ 1B do have overlapping sorting functions and indicates that AP-1A and AP-1B mediate protein sorting along parallel pathways between the TGN and endosomes in polarized epithelia.

INTRODUCTION

The clathrin adaptor protein complex AP-1A has been localized to the perinuclear *trans*-Golgi network (TGN). Microscopic and biochemical analysis indicates that it mediates vesicular protein transport from the TGN to endosomes. Mouse 'knock-outs' of AP-1A subunits revealed that AP-1A is indispensable for embryonic development and that it is also required for endosome-to-TGN transport (Zizioli *et al.*, 1999; Meyer *et al.*, 2000). The AP-1A complex consists of four adaptin subunits, which are ubiquitously expressed: the two ≥ 100 kDa adaptins γ 1 and β 1, the 47 kDa μ 1A-adaptin and the 19 kDa σ 1-adaptin (Hirst and Robinson, 1998; Kirchhausen, 1999). A polarized epithelia-specific μ 1B isoform exists that is 80% identical to μ 1A (Ohno

et al., 1999). μ 1 and β 1 bind tyrosine and leucine containing sorting signal sequences within the cytoplasmic tails of vesicle cargo proteins (Rapoport *et al.*, 1998; Rodionov and Bakke, 1998; Heilker *et al.*, 1999). Binding of the YxxQ type protein sorting motif to μ 2 of the homologous plasma membrane AP-2 complex has been analysed by co-crystallization and X-ray analysis (Owen and Evans, 1998). The region in μ 2 binding the YxxQ motif is conserved in μ -adaptins and is identical in μ 1A and μ 1B. The μ 1B-containing complex is named AP-1B and appears to mediate basolateral TGN to plasma membrane sorting of the low-density lipoprotein receptor (LDL-R) (Fölsch *et al.*, 1999). Basolateral sorting of LDL-R is due to AP-1B binding to its non-canonical Y-based sorting signal motifs (Fölsch *et al.*, 2001).

It is not known by which mechanisms AP-1B mediates basolateral sorting. It appears that AP-1A and AP-1B bind to specific regions of the TGN, and it could be that this subdomain specificity is part of the mechanism by which polarized sorting is mediated (Fölsch *et al.*, 1999). We wanted to test whether μ 1B function is restricted to polarized epithelia as indicated by its expression pattern and transformed μ 1B cDNA into μ 1A-deficient fibroblasts.

In the μ 1A^{-/-} fibroblasts, sorting of mannose 6-phosphate receptors MPR46 and MPR300 was analysed. They recycle between the TGN and endosomes mediating intracellular sorting of lysosomal enzymes. MPR300 also mediates endocytosis of ligands such as IGF-II. In various cell types, ~50% of receptors are concentrated in the perinuclear TGN (Klumperman *et al.*, 1993). In μ 1A^{-/-} cells, they are redistributed from the TGN into early endosomes at the expense of the TGN. They recycle between these endosomes and the plasma membrane, but transport to the TGN is blocked, indicating that AP-1A also mediates endosome-to-TGN transport (Meyer *et al.*,

*Corresponding author. Tel: +49 551 395949; Fax: +49 551 395979; E-mail: pschu@gwdg.de

E.-L. Eskelinen *et al.*

2000, 2001). MPRs contain multiple AP-1A binding motifs, whose precise functions in sorting are not known (Höning *et al.*, 1997; Heilker *et al.*, 1999). Sorting between the TGN and endosomes involves a dileucine motif present at the C-termini of MPR300 and MPR46 (Johnson and Kornfeld, 1992; Tikkanen *et al.*, 2000). The dileucine motif binds to $\beta 1$, but binding to μ -adaptins has also been demonstrated (Rapoport *et al.*, 1998; Storch and Braulke, 2001).

RESULTS

$\mu 1B$ expression restores membrane binding of $\gamma 1$ and clathrin

Murine $\mu 1B$ cDNA was stably expressed in $\mu 1A^{-/-}$ cells. The anti- $\mu 1A$ peptide serum does not recognize $\mu 1B$, and thus $\mu 1B$ mRNA levels were determined. $\mu 1B$ mRNA was expressed in clone 3 to levels ~2-fold higher than those of $\mu 1A$ (data not shown). Clone 3 was used for all further experiments. Protein expression was determined indirectly by analysis of AP-1 complex assembly. In $\mu 1A$ -deficient fibroblasts, $\gamma 1$, $\beta 1$ and $\sigma 1$ form a trimeric complex, which, in gel filtration experiments, elutes two fractions later than AP-1A (Meyer *et al.*, 2000). AP-1B complex assembly was analysed by gel filtration of cytosolic proteins, and the distribution of $\gamma 1$ in the fractions was analysed by western blotting. Fractionation of AP-2 served as a control and was followed by anti- α -adaptin western blotting. In $\mu 1B$ -expressing cells, $\gamma 1$ fractionated as in AP-1A-expressing control cells, demonstrating the formation of the AP-1B complex (Figure 1). Adaptins not incorporated into a complex are degraded (Zizioli *et al.*, 1999).

In control cells, $\gamma 1$ and clathrin are concentrated at the perinuclear TGN and clathrin is also found on vesicles throughout the cell, presumably AP-2 endocytic vesicles. The trimeric $\gamma 1$ - $\beta 1$ - $\sigma 1$ complex of $\mu 1A^{-/-}$ cells does not bind to membranes. Membrane staining by $\gamma 1$ in confocal immunofluorescence microscopy is lost, as is the perinuclear concentration of clathrin, whereas clathrin labelling of vesicles throughout the cytoplasm is not affected (Meyer *et al.*, 2000). $\mu 1B$ expression in the $\mu 1A^{-/-}$ cells restored the perinuclear concentration of $\gamma 1$ and of clathrin, indicating the formation of functional AP-1B complexes (Figure 2).

AP-1B relocates MPRs to the TGN

In control cells, the majority of MPR46 and MPR300 is concentrated in the perinuclear TGN, and only 5–10% of receptors are found in early endosomes (Klumpperman *et al.*, 1993; Meyer *et al.*, 2001). In $\mu 1A^{-/-}$ cells, MPR46 and MPR300 are redistributed at the expense of the TGN into endosomes, which are labelled for the early endosomal antigen EEA1. MPR46 and MPR300 recycle between the endosomes and the plasma membrane but are not transported out of endosomes back to the TGN (Meyer *et al.*, 2000, 2001). Formation of AP-1B in the $\mu 1A^{-/-}$ cells caused the redistribution of the MPR46 from EEA1-positive endosomes back to the TGN, indicating restoration of endosome-to-TGN transport. Colocalization of MPR46 with EEA1 in the AP-1B-expressing fibroblasts is comparable with control cells (Figure 2).

Fig. 1. AP-1B complex formation in $\mu 1A^{-/-}$ fibroblasts. AP-1 and AP-2 cytoplasmic adaptor complexes were separated by gel filtration and detected by western blotting using anti- $\gamma 1$ or anti- α antibodies.

To further compare AP-1A and AP-1B distribution on the TGN, we determined colocalization of $\gamma 1$ in AP-1A- and AP-1B-expressing cells with MPR300 by double-immunofluorescence microscopy. AP-1A and AP-1B colocalized to a comparable extent with MPR300 (Figure 3).

Association of AP-1A and AP-1B to endosomes

AP-1A function is required for endosome-to-TGN transport of MPRs (Meyer *et al.*, 2000, 2001). Therefore, we asked whether AP-1A and AP-1B (labelled by $\gamma 1$ antibodies) can be detected on early endosomal compartments by immunogold electron microscopy. Endosomes were labelled by horseradish peroxidase (HRP) fluid phase endocytosis over 20 min. HRP was visualized by diaminobenzidine (DAB). To identify late endosomal compartments, the thin cryosections were also labelled for the lysosomal membrane protein LAMP-1. DAB-positive endosomes, containing no or only low amounts of LAMP-1, are likely to be early endosomes or endosomal carrier vesicles (Griffiths *et al.*, 1989), whereas strongly LAMP-1-positive vesicles (such as the structure in the lower left corner of Figure 4B) are late endosomes or lysosomes. AP-1A and AP-1B complexes were detected by anti- $\gamma 1$ antibodies and 10 nm gold. $\gamma 1$ was detected on the limiting membrane of endosomes in AP-1A-expressing wild-type cells (data not shown), in agreement with earlier reports in other cell types (Futter *et al.*, 1998;

Fig. 2. Distribution of γ 1 and clathrin heavy-chain (chc) in control (ct), μ 1A^{-/-} and μ 1A^{-/-}:: μ 1B fibroblasts. μ 1A^{-/-} cells do not show γ 1 and clathrin-heavy-chain binding to the TGN, whereas membrane binding of these proteins is observed in μ 1A^{-/-}:: μ 1B cells as in control cells. MPR46 subcellular distribution in these cell lines was detected by MPR46 and EEA1 confocal double-immunofluorescence microscopy. Control cells show perinuclear TGN concentration of MPR46 (red) and colocalization with EEA1 (green) is limited (four vesicles), whereas extensive colocalization of MPR46 and EEA1 is observed in μ 1A^{-/-} cells. In μ 1A^{-/-}:: μ 1B cells, perinuclear TGN localization of MPR46 is restored and colocalization with EEA1 is lost.

Mallard *et al.*, 1998). γ 1 was also detected on limiting membranes of early endosomes in AP-1B-expressing cells (Figure 4A–C). Occasionally, γ 1 was seen associated with a coated bud (Figure 4B). Quantitation of γ 1 labelling showed that 3.45 ± 0.18 and $5.57 \pm 1.31\%$ of total labelling was associated with limiting membranes of DAB-positive endosomes in wild-type and AP-1B-expressing cells, respectively. γ 1 was also seen associated with small vesicles located next to early endosomes in wild-type cells (data not shown) and AP-1B-expressing cells (Figure 4C and D). In addition, γ 1 was located to the TGN in both cell lines (shown for AP-1B, Figure 4E). AP-1B has also been detected at the TGN in polarized cells (Fölsch *et al.*, 2001).

AP-1B restores MPR-mediated intracellular protein sorting

The microscopic analysis indicated that AP-1B mediates MPR sorting between endosomes and TGN. This was analysed by determining intracellular lysosomal protein sorting. Receptor–ligand complexes are transported from the TGN to endosomes, where they dissociate. MPRs recycle back to the TGN, and their ligands are transported to lysosomes. Control cells secrete only ~10% of the Golgi precursor cathepsin D (Meyer *et al.*, 2000). This fraction does not increase by the addition of 5 mM mannose 6-phosphate (Man6P) to the medium (Figure 5A, p). Thus, uptake of cathepsin D does not contribute to its lysosomal sorting. μ 1A^{-/-} cells secrete 33% of cathepsin D into the culture medium.

Fig. 3. Colocalization of MPR300, AP-1A and AP-1B at the TGN analysed by anti- γ 1 and anti-MPR300 double-immunofluorescence confocal microscopy. Both cell lines show colocalization of γ 1 (green) and MPR300 (red).

This fraction increases to 60% in the presence of Man6P (Meyer *et al.*, 2000). Sorting of cathepsin D was analysed by metabolic labelling with [35 S]methionine for 1 h followed by a 4 h chase in the presence of Man6P. Expression of μ 1A and μ 1B restored intracellular sorting of cathepsin D. μ 1A: μ 1A cDNA cells secreted 17% of cathepsin D and μ 1A: μ 1B cDNA cells secreted 26% in the presence of Man6P (Figure 5A).

We further determined the secretion of the lysosomal enzyme β -hexosaminidase. Cells were washed with phosphate-buffered saline, and medium was added containing heat-inactivated and dialysed fetal calf serum (FCS). After 24 h, the medium was collected and cells were harvested. Enzymatic activity was determined in cell extracts and in the media (Figure 5B) (von Figura, 1978). Cultures of control cells contained 25% of the total β -hexosaminidase activity in the medium, and those of μ 1A $^{-/-}$ cells contained 45%. Expression of μ 1A or μ 1B in the latter reduced β -hexosaminidase activity in the medium to 30 and 33%, respectively ($n = 2$).

μ 1A $^{-/-}$ cells have an enhanced capacity to endocytose lysosomal proteins due to increased MPR300 recycling between the

Fig. 4. γ 1 localization to early endosomes and TGN in μ 1A $^{-/-}$: μ 1B fibroblasts. The cells were fed with HRP for 20 min and fixed, and HRP was then visualized by DAB reaction. Thin cryosections were labelled with antibodies against γ 1 (10 nm gold) and LAMP-1 (5 nm gold). Arrowheads indicate all gold particles localizing γ 1, whereas arrows indicate some of the LAMP-1-associated gold particles. Asterisks indicate the DAB reaction product. (A–D) Endosomes, (E) TGN. Plasma membrane (pm) is indicated in (A). The γ 1-positive coated bud in (B) is 64 nm in diameter. TGN was identified by the proximity of a Golgi stack (G) in (E). Scale bars = 200 nm.

plasma membrane and endosomes. This is determined by measuring the endocytosis of metabolically 35 S-labelled arylsulfatase A (ASA) added to the cell culture medium (Meyer *et al.*, 2000, 2001). Over 4 h, μ 1A $^{-/-}$ cells endocytosed four times more ASA than control cells (Figure 5C). Expression of μ 1A or μ 1B in the latter cells reduced endocytosis of ASA to 160 and 140%, respectively, of control cells ($n = 2$).

DISCUSSION

Expression of μ 1B is restricted to certain polarized epithelia, indicating the presence of a unique basolateral sorting machinery in these cell types (Ohno *et al.*, 1999; Meyer *et al.*, 2000). Expression of μ 1B in μ 1A $^{-/-}$ fibroblasts, however, led to the formation of the polarized epithelia-specific AP-1B complex. AP-1B binds to the perinuclear TGN and endosomes of

Fig. 5. Cathepsin D and β-hexosaminidase intracellular sorting in control (ct), μ1A^{-/-}, μ1A^{-/-}::μ1A and μ1A^{-/-}::μ1B cells. (A) Man6P was added to the medium to prevent endocytosis of secreted cathepsin D. Metabolically labelled cathepsin D was immunoprecipitated from cell extracts (C) and from the medium (M). The Golgi precursor form (p) and the endosomal intermediate processed form (i) are indicated. (B) Secretion of β-hexosaminidase to the medium. Shown here is the distribution of the enzymatic activity between the cells and the medium as a percentage of the total (*n* = 2). (C) Endocytosis of arylsulfatase A (ASA) by control (ct), μ1A^{-/-}, μ1A^{-/-}::μ1A and μ1A^{-/-}::μ1B cells (*n* = 2). Arbitrary units correspond to pixel numbers on phosphorimager screens (Fuji BAS1000).

fibroblasts as AP-1A. Moreover, AP-1B expression restored perinuclear TGN concentration of MPRs and their intracellular sorting between the TGN and endosomes demonstrating complementation of AP-1A function.

However, AP-1B does not restore all AP-1A functions. Prohormone convertase furin recycling between the TGN and endosomes also depends on AP-1A (Molloy *et al.*, 1999). Perinuclear concentration of furin is not restored by AP-1B expression (Fölsch *et al.*, 2001). Furin is mislocalized to endosomes not labelled by EEA1 (Meyer *et al.*, 2001). AP-1B and furin do not colocalize at the TGN, whereas AP-1A colocalizes with furin (Fölsch *et al.*, 2001). It appears that AP-1A and AP-1B bind to different TGN domains and endosomes. It is not clear whether cargo proteins, like the MPRs, are required for high-affinity binding of AP-1A to the TGN (Le Borgne and Hoflack, 1997; Zhu *et al.*, 1999). It is more likely that compartment-specific adaptor-receptor structures exist, which contribute to the compartment-specific binding of adaptors (Mallet and Brodsky, 1996; Seaman *et al.*, 1996). In addition, adaptors do have overlapping sorting signal recognition specificities, and thus binding to the cargo proteins cannot be the mechanism mediating compartment-specific binding (Heilker *et al.*, 1999). However, compartment receptor structures required for AP-1B-mediated sorting are not exclusively expressed in polarized epithelial cells in contrast to μ1B itself. Thus, only expression of μ1B in polarized epithelia would be required for basolateral sorting of certain cargo proteins such as the LDL-R (Meyer *et al.*, 2000; Fölsch *et al.*, 2001).

Why did the new μ1B develop and why is the basolateral sorting of the LDL-R not mediated by μ1A? It could be that proteins like the endocytic LDL-R follow additional trafficking pathways, which are incompatible with binding to the ubiquitous AP-1A. The normally basolateral sorted LDL-R also mediates apical-to-basolateral transcytosis of LDL at the blood-brain barrier. The mechanism of this pathway is not known (Dehouck *et al.*, 1997). In contrast to LDL-R, the MPRs have only been detected in basolateral endosomes and plasma membranes (Bresciani *et al.*, 1997). Evolution of polarized epithelia and of specific proteins such as the LDL-R might have required the evolution of new sorting signal sequence motifs and new sorting adaptins such as the μ1B.

LDL-R with a point mutation in the cytoplasmic domain fails to be basolaterally sorted in hepatocytes and in polarized MDCK cells (Koivisto *et al.*, 2001). μ1B is not expressed in hepatocytes but in MDCK cells (Fölsch *et al.*, 1999; Ohno *et al.*, 1999; Meyer *et al.*, 2000). Thus, there must be additional adaptins to μ1B required for basolateral sorting of LDL-R. The AP-1 homologous and ubiquitously expressed AP-4 complex is also required for basolateral sorting in polarized cell lines such as MDCK cells. MDCK cells expressing μ4 antisense-RNA mis-sort LDL-R and MPR46 to the apical site, whereas μ1B-deficient cells sort MPR46 basolaterally (Simmen *et al.*, 2002). Thus, polarized sorting appears to be mediated by AP-1B and AP-4 complexes, indicating that polarized sorting decisions are made at two different compartments: the TGN and endosomes. Thus, AP-1A and AP-1B appear to mediate protein sorting along parallel pathways between the TGN and endosomes and that additional sorting decisions are made in endosomes.

METHODS

Cellular and molecular biology. Mouse embryonic fibroblasts were cultivated in Dulbecco's modified Eagle's medium and 10% FCS. Mouse μ 1B and μ 1A cDNAs were cloned into the murine expression vector pMPSV. μ 1B and μ 1A cDNAs were stably transfected into μ 1A^{-/-} mouse embryonic fibroblasts by the effectene transfection kit (Qiagen). Clones were selected by hygromycin (500 μ g/ml) (Meyer *et al.*, 2000). Expression of μ -adaptins was analysed by northern blotting of 5 μ g total RNA and AP-1 complex formation (see below). Signals were quantified by phosphorimager analysis (Fuji BAS1000).

Adaptor assembly and protein sorting. Analysis of AP-1 complex formation was performed as described previously (Meyer *et al.*, 2000). Freshly prepared cytosolic adaptors were separated by gel chromatography on a Sephadex 200 column (SMART, Pharmacia). Column fractions were analysed by western blotting. Sorting of cathepsin D, β -hexosaminidase and [³⁵S]ASA endocytosis were performed as described previously (von Figura, 1978; Meyer *et al.*, 2000).

Microscopy and antisera. Cells were fixed with *p*-formaldehyde and permeabilized with saponin following standard protocols. Adaptins and clathrin were detected with anti- γ 1, anti- α and anti-clathrin heavy-chain mouse monoclonal antibodies (Transduction Laboratories). Affinity-purified anti- σ 1 serum was a generous gift from M. Robinson (Cambridge, UK). Anti-LAMP-1 rabbit serum was a gift from Masaru Himeno (Kyushu University, Japan). Anti- μ 1A peptide rabbit antiserum was described previously (Meyer *et al.*, 2000). The serum does not recognize μ 1B. Anti-cathepsin D, anti-MPR46 and anti-MPR300 sera were generously provided by R. Pohlmann and A. Hille-Rehfeld from the Institut. Anti-EEA1 was a generous gift from H. Stenmark (Oslo, Norway). Fluorophore-coupled secondary antibodies were from Jackson Immunoresearch. Confocal microscopy was performed using a Zeiss LSM 200 Series equipped with a Zeiss Plan Apochromat 63 $\times$ /1.40 and Carl Zeiss LSM version 3.95 software.

For electron microscopy, cells were fed with HRP for 20 min, fixed in 4% PFA, 0.1 M HEPES pH 7.4 for 2 h at room temperature, followed by 2% PFA at 4°C overnight. The DAB reaction was performed according to Griffiths *et al.* (1989). Cells were embedded in gelatine, infiltrated in 20% polyvinylpyrrolidone, 1.8 M sucrose, and frozen in liquid nitrogen. Thawed sections were labelled with rabbit anti-rat LAMP-1 and mouse monoclonal anti- γ 1. Secondary antibodies were goat Fab₂ coupled to 5 or 10 nm gold (British BioCell, Cardiff, UK). For quantitation of γ 1 labelling, sections were systematically screened at a magnification of 30 000 $\times$, using 10 $\times$ magnifying binoculars. Gold particles within a distance of 30 nm outside or 20 nm inside of endosomal limiting membrane were scored as endosome associated (arrowheads in Figure 4A and B, and the upper of the two 10 nm gold particles in Figure 4C). A minimum of 600 gold particles, from two or three separate grids, were counted for each cell line. Images were processed for presentation using Adobe Photoshop Version 5 and Deneba Canvas 5.0.

ACKNOWLEDGEMENTS

We thank M. Robinson, M. Himeno and H. Stenmark for antibodies. We also thank C. Hinners for excellent technical

assistance. This work is supported by the Deutsche Forschungsgemeinschaft SFB 523/A6.

REFERENCES

- Bresciani, R., Denzer, K., Pohlmann, R. and von Figura, K. (1997) The 46 kDa mannose-6-phosphate receptor contains a signal for basolateral sorting within the 19 juxtamembrane cytosolic residues. *Biochem. J.*, **327**, 811–818.
- Dehouck, B., Fenart, L., Dehouck, M.P., Pierce, A., Torpier, G. and Cecchelli, R. (1997) A new function for the LDL receptor: transcytosis of LDL across the blood–brain barrier. *J. Cell Biol.*, **138**, 877–889.
- Fölsch, H., Ohno, H., Bonifacino, J.S. and Mellman, I. (1999) A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. *Cell*, **99**, 189–198.
- Fölsch, H., Pypaert, M., Schu, P. and Mellman, I. (2001) Distribution and function of AP-1 clathrin adaptor complexes in polarized epithelial cells. *J. Cell Biol.*, **152**, 595–606.
- Futter, C.E., Gibson, A., Allchin, E.H., Maxwell, S., Ruddock, L.J., Odorizzi, G., Domingo, D., Trowbridge, I.S. and Hopkins, C.R. (1998) In polarized MDCK cells basolateral vesicles arise from clathrin- γ -adaptin-coated domains on endosomal tubules. *J. Cell Biol.*, **141**, 611–623.
- Griffiths, G., Back, R. and Marsh, M. (1989) A quantitative analysis of the endocytic pathway in baby hamster kidney cells. *J. Cell Biol.*, **109**, 2703–2720.
- Heilker, R., Spiess, M. and Crottet, P. (1999) Recognition of sorting signals by clathrin adaptors. *BioEssays*, **21**, 558–567.
- Hirst, J. and Robinson, M. (1998) Clathrin and adaptors. *Biochim. Biophys. Acta*, **1404**, 173–193.
- Höning, S., Sosa, M., Hille-Rehfeld, A. and von Figura, K. (1997) The 46 kDa mannose-6-phosphate receptor contains multiple binding sites for clathrin adaptors. *J. Biol. Chem.*, **272**, 19884–19890.
- Johnson, K.F. and Kornfeld, S. (1992) The cytoplasmic tail of the mannose 6-phosphate/insulin-like growth factor-II receptor has two signals for lysosomal enzyme sorting in the Golgi. *J. Cell Biol.*, **119**, 249–257.
- Kirchhausen, T. (1999) Adaptors for clathrin-mediated traffic. *Annu. Rev. Cell. Dev. Biol.*, **15**, 705–732.
- Klumperman, J., Hille, A., Veenendaal, T., Oorschot, V., Stoorvogel, W., von Figura, K. and Geuze, H.J. (1993) Differences in the endosomal distributions of the two mannose-6-phosphate receptors. *J. Cell Biol.*, **121**, 997–1010.
- Koivisto, U.M., Hubbard, A.L. and Mellman, I. (2001) A novel cellular phenotype for familial hypercholesterolemia due to a defect in polarized targeting of LDL receptor. *Cell*, **105**, 575–585.
- Le Borgne, R. and Hoflack, B. (1997) Mannose 6-phosphate receptors regulate the formation of clathrin-coated vesicles in the TGN. *J. Cell Biol.*, **137**, 335–345.
- Mallard, F., Antony, C., Tenza, D., Salamero, J., Goud, B. and Johannes, L. (1998) Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of Shiga toxin B-fragment transport. *J. Cell Biol.*, **143**, 973–990.
- Mallet, W.G. and Brodsky, F.M. (1996) A membrane-associated protein complex with selective binding to the clathrin coat adaptor AP1. *J. Cell Sci.*, **109**, 3059–3068.
- Meyer, C., Zizioli, D., Lausmann, S., Eskelinen, E.L., Hamann, J., Saftig, P., von Figura, K. and Schu, P. (2000) μ 1A adaptin-deficient mice: lethality, loss of AP-1 binding and rerouting of mannose 6-phosphate receptors. *EMBO J.*, **19**, 2193–2203.
- Meyer, C., Eskelinen, E.L., Guruprasad, R.M., von Figura, K. and Schu, P. (2001) μ 1A-deficiency induces a profound increase of MPR300/IGF-II receptor internalization rate. *J. Cell Sci.*, **114**, 4469–4476.
- Molloy, S.S., Anderson, E.D., Jean, F. and Thomas, G. (1999) Bi-cycling the furin pathway: from TGN localization to pathogen activation and embryogenesis. *Trends Cell Biol.*, **9**, 28–35.
- Ohno, H. *et al.* (1999) μ 1B, a novel adaptor medium chain expressed in polarized epithelial cells. *FEBS Lett.*, **449**, 215–220.

- Owen, D.J. and Evans, P.R. (1998) A structural explanation for the recognition of tyrosine-based endocytotic signals. *Science*, **282**, 1327–1332.
- Rapoport, I., Chen, C.Y., Cupers, P., Shoelson, S.E. and Kirchhausen, T. (1998) Dileucine-based sorting signals bind to the β chain of AP-1 at a site distinct and regulated differently from the tyrosine-based motif-binding site. *EMBO J.*, **17**, 2148–2155.
- Rodionov, D.G. and Bakke, O. (1998) Medium chains of adaptor complexes AP-1 and AP-2 recognize leucine-based sorting signals from the invariant chain. *J. Biol. Chem.*, **273**, 6005–6008.
- Seaman, M.N.J., Sowerby, P.J. and Robinson, M.S. (1996) Cytosolic and membrane-associated proteins involved in the recruitment of AP-1 adaptors onto the *trans*-Golgi network. *J. Biol. Chem.*, **271**, 25446–25451.
- Simmen, T., Höning, S., Icking, A., Tikkanen, R. and Hunziker, W. (2002) AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells. *Nature Cell Biol.*, **4**, 154–159.
- Storch, S. and Braulke, T. (2001) Multiple C-terminal motifs of the 46-kDa mannose 6-phosphate receptor tail contribute to efficient binding of medium chains of AP-2 and AP-3. *J. Biol. Chem.*, **276**, 4298–4303.
- Tikkanen, R., Obermüller, S., Denzer, K., Pungitore, R., Geuze, H.J., von Figura, K. and Höning, S. (2000) The dileucine motif within the tail of MPR46 is required for sorting of the receptor in endosomes. *Traffic*, **1**, 631–640.
- von Figura, K. (1978) Secretion of β-hexosaminidase by cultured human skin fibroblasts. *Exp. Cell Res.*, **111**, 15–21.
- Zhu, Y., Traub, L.M. and Kornfeld, S. (1999) High-affinity binding of the AP-1 adaptor complex to *trans*-Golgi network membranes devoid of mannose 6-phosphate receptors. *Mol. Biol. Cell*, **10**, 537–549.
- Zizioli, D., Meyer, C., Guhde, G., Saftig, P., von Figura, K. and Schu, P. (1999) Early embryonic death of mice deficient in γ-adaptin. *J. Biol. Chem.*, **274**, 5385–5390.

DOI: 10.1093/embo-reports/kvf092