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The p24 family of transmembrane proteins at the interface between endoplasmic reticulum and Golgi apparatus

G. Emery, J. Gruenberg, and **M. Rojo***

Département de Biochimie, Université de Genève, Genève

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Summary. The p24 family of small transmembrane proteins was discovered recently in yeast and mammalian cells, and some of its members have been implicated in biosynthetic protein transport. The p24 proteins are proposed to act on transport vesicles as receptors for coat and/or cargo, but their precise function(s) remain controversial. Here, we describe this protein family, and we review the available experimental data concerning their localization and function. Finally, we hypothesize about a possible role of p24 proteins in organelle morphogenesis.

Keywords: p24 family; Membrane traffic; Transmembrane protein; Protein sorting; Protein transport; Coat protein.

Abbreviations: CGN cis-Golgi network; COP coat protein; ER endoplasmic reticulum; VSV-G vesicular stomatitis virus glycoprotein G.

Introduction

The strict compartmentalization of cellular metabolism relies on the specific properties of each organelle, and thus on the specific localization of organellar proteins to their target compartment. The proteins destined to the distinct compartments of the vacuolar apparatus (endoplasmic reticulum, Golgi apparatus, plasma membrane, endosomes, lysosomes) are all first imported into the endoplasmic reticulum (ER), and then sorted to their final destination (Palade 1975). Research in the field of membrane traffic is devoted to unravel the mechanisms and to identify the factors that ensure precise sorting and transport of proteins and membranes. These studies have led to the identification of numerous proteins: most of them are soluble, and reversibly attach to membranes while exerting their function (Rothman 1994, Gruenberg and Maxfield 1995). Integral membrane proteins, however, are supposed to confer membrane identity: they may function as receptors for soluble factors, enable mutual recognition of membranes, and even catalyze budding and fusion processes. The recent discovery of a new family of transmembrane proteins involved in biosynthetic protein transport and conserved from yeast to mammals (the p24 family; Schimmöller et al. 1995, Stamnes et al. 1995) was very challenging, as they may account for some of the proposed activities.

Several experimental approaches implicate p24 proteins in protein secretion, but their precise function is controversial. In yeast, it was found that null mutants of *EMP24* and *ERV25* secrete a subset of proteins with delayed kinetics when compared to wild-type cells (Schimmöller et al. 1995, Belden and Barlowe 1996). In addition, yeast cells lacking emp24p are deficient in the retention of ER-resident proteins (Elrod-Erickson and Kaiser 1996). In mammalian cells, microinjected antibodies against the cytoplasmic tail of p23 inhibit transport of a transmembrane cargo molecule (Rojo et al. 1997), and of a bacterial toxin that is transported retrogradely through the biosynthetic pathway (Majoul et al. 1998). Accordingly, it was established (i) that the expression of Xenopus p23 is coordinately increased (together with constituents of the ER translocation machinery) when secretion of a prohormone is stimulated (Holthuis et al. 1995), and (ii) that mammalian p23 and p24 are enriched in rat pan-

* Correspondence and reprints: Institut de Myologie, INSERM U523, Groupe Hospitalier Pitié-Salpêtrière, 47 boulevard de l'Hôpital, F-75651 Paris Cedex 13, France.

creas when compared to rat tissues with a lower secretory activity (Blum et al. 1996).

The p24 family of small transmembrane proteins

Most p24 proteins were originally identified by microsequencing in membrane preparations from vertebrate and yeast cells, and they were named according to their apparent molecular mass in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (gp25L: Wada et al. 1991; emp24: Singer-Krüger et al. 1993; p24: Stamnes et al. 1995; erv25: Belden and Barlowe 1996; Tmp21: Blum et al. 1996; p23: Sohn et al. 1996, Rojo et al. 1997). New mammalian homologues were then named, by analogy, p26 and gp27, although their deduced molecular mass was also 22–24 kDa (Dominguez et al. 1998). Here, we will use this nomenclature for all metazoan p24 proteins (Fig. 1). A further mammalian p24 protein (Gayle et al. 1996; a putative ligand for the T1/ST2 receptor) will be named Tp24. Yeast homologues other than emp24p and erv25p will be identified as proposed (Fig. 1), yp24b–f (Fiedler et al. 1996).

Homology searches in DNA sequence databases confirmed that this protein family occurs in animals, fungi, and plants (Fig. 1). The dendogram of Fig. 1 is derived from the alignment of the 60 to 70 C-terminal amino acids of each homologue: this region of the sequence is well conserved between homologues (Fig. 2), and is also available in those homologues where only partial sequences (expressed sequence tag) are known. The tree of p24 proteins (Fig. 1) depicts 4 main branches with sequences of different species (p23, p24, p25, p26) and two branches that only contain yeast sequences (yp24b/yp24e, yp24f). The degree of similarity within one branch is always above 40%, but can reach significantly higher values (e.g., 95–100% between all vertebrate and above 67% between all metazoan p23 and p24 sequences). The similar organization of the p24 family, and the high degree of similarity within a subfamily, strongly suggests that metazoan homologues within a subfamily represent species orthologs of the same protein (e.g., human and Caenorhabditis p23, p24, p25, p26, Tp24). The significant sequence similarity between human p23/p24 and yeast erv25/emp24 (ca. 30% over the complete sequence and up to 50% in the C-terminal domain) indicates that they may also represent species orthologs. The complete genomes of *Caenorhabditis elegans* and of *Saccharomyces cerevisiae* are known

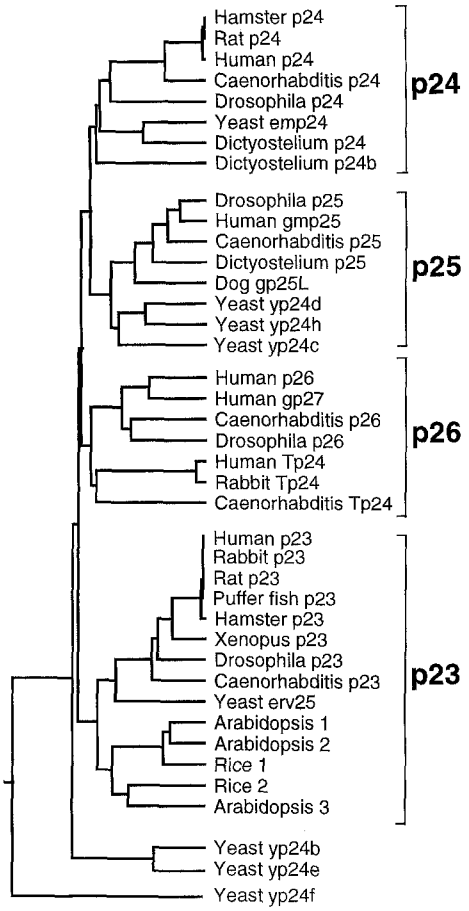


Fig. 1. Dendogram of p24 proteins. The p24 family has 4 subfamilies with sequences of different species (p23, p24, p25, p26) and two subfamilies with only yeast sequences (yp24b/yp24e, yp24f). The length of the branches is proportional to the degree of sequence divergence. The degree of similarity within one subfamily is always above 40%, but can reach significantly higher values. New p24 homologues were identified in Genbank/EMBL/DDBJ databases using BLAST (Altschul et al. 1990). The dendogram was performed with the 60–70 C-terminal amino acids by the clustal algorithm with default parameters (megalign program, DNAstar for MacIntosh). The accession numbers (Genbank/EMBL/DDBJ or SWISS-PROT) of the sequences are (from top to bottom): U26264, X92097, X92098, AF014940, AC002446, X67317, C84126, C92061, AA141200, X90872, AL031011, C92861, X53592, Q05359, Z72524, U00059 (the sequences of human p26 and gp27 are not available on databases, their peptide sequences are from Dominguez et al. [1998]), AF039713, AI124275, U41804, U41805, Z68316, X97442, X98303, X97443, U40761, AJ001513, X90517, AA699272, Z74035, Z49810, AC005313, T46519, C98563, C74602, AC003970, P39704, Z74924, Z74066

(The C. elegans sequencing consortium 1998): the different organization of the p24 family in yeast and in *C. elegans* (Fig. 1) precludes the existence of a unique yeast ortholog to each metazoan protein (and vice versa). The p24 family also exists in plants (Fig. 1), but

the low number of available sequences precludes any thorough analysis at this stage.

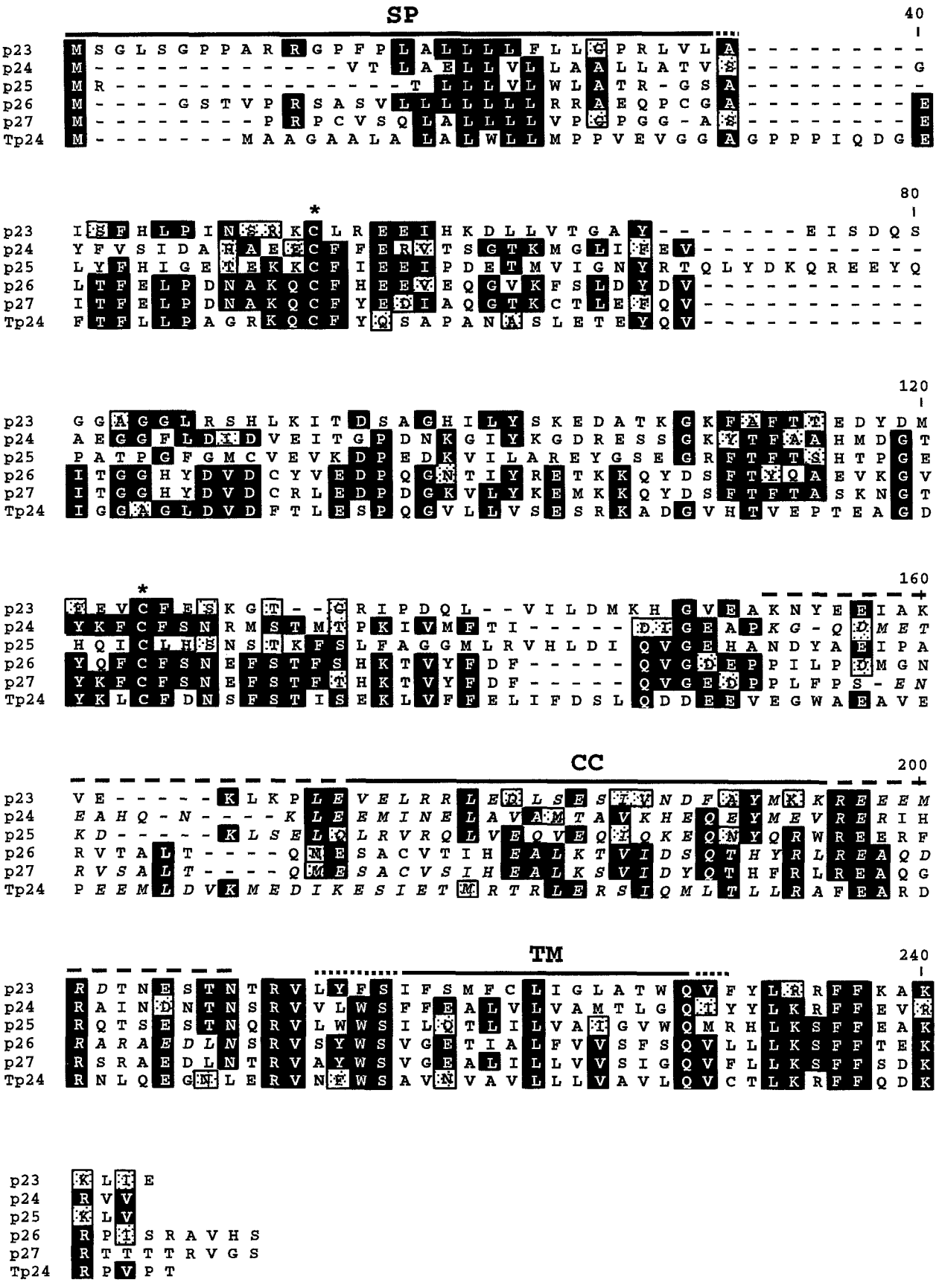
The proteins of the p24 family all share the same topology (Fig. 2): an N-terminal signal peptide, a domain with heptad repeats of hydrophobic amino acids suited for coiled-coil interactions, a transmembrane domain, and a short cytoplasmic tail. This type I transmembrane topology has been confirmed by protease protection experiments (Blum et al. 1996, Sohn et al. 1996, Rojo et al. 1997, Dominguez et al. 1998). Two proteins, p25 and p27, have been shown to be N-glycosylated (gmp25, gp27; Dominguez et al. 1998). The remarkable degree of conservation of certain amino acid motifs (Fig. 2) indicates that they are highly relevant for function. The two cysteine residues (Fig. 2) that are conserved throughout the family suggest the existence of a disulfide bridge in the luminal domain. The putative coiled-coil domains enable intermolecular interactions, and the fact that these domains are at similar positions relative to the transmembrane domains (Fig. 2), suggests that interactions may take place not only between copies of the same protein, but also between different p24 proteins. The transmembrane domains contain polar residues and a conserved glutamine at the membrane–cytosol interface. The cytoplasmic tail is also highly conserved: all human homologues contain a double phenylalanin (FF) and a dibasic (KK, RR, or RK) motif. The C-terminal amino acids are hydrophobic in all yeast (Nakamura et al. 1998) and in two mammalian homologues (Fig. 2). Extensive sequence analysis does not reveal any motifs indicative of an enzymatic activity.

Localization

Yeast erv25p was localized to the ER by immunofluorescence microscopy (Belden and Barlowe 1996). Mammalian p23 was localized to the cis-Golgi network (CGN) and to the intermediate compartment between ER and Golgi apparatus by fluorescence microscopy (Sohn et al. 1996, Rojo et al. 1997) (Fig. 3) and by immunogold labelling of cell cryosections (Rojo et al. 1997). Mammalian gmp25 was localized by electron microscopy both to the CGN and to membranes of the ER (Dominguez et al. 1998). The known p24 proteins appear to continuously cycle between ER and Golgi (Rojo et al. 1997, Nickel et al. 1997, Dominguez et al. 1998). In addition, it was also found that yeast emp24 and erv25 form a protein complex (Belden and Barlowe 1996), and that several mammalian p24 proteins distribute to fractions of intermediate density between (heavy) ER and (light) Golgi membranes (Dominguez et al. 1998). The latter study also proposed that p24 proteins might depend on each other for localization. Altogether, these biochemical observations indirectly suggest that other p24 proteins may also localize to the interface between ER and Golgi. The dynamic localization of p24 proteins to the ER–Golgi interface is in agreement with a function in secretion, as numerous sorting and transport events occur at this location: (i) at the ER, newly synthesized proteins are sorted from ER-resident proteins and transported to the Golgi apparatus, (ii) in the Golgi, anterograde cargo molecules (that are further transported to endosomes, lysosomes, or plasma membrane) are sorted both from Golgi-resident proteins and from "escaped" ER proteins that are retrieved to the ER (Gruenberg and Maxfield 1995, Teasdale and Jackson 1996, Kaiser and Ferro-Novick 1998).

The localization and trafficking of many type I transmembrane proteins depends on amino acid motifs (determinants) present on their cytoplasmic and transmembrane domains (Kirchhausen et al. 1997, Letourneur and Cosson 1998, and references therein). The p24 proteins display different amino acid motifs in the latter domains (diphenylalanine, dilysine, leucine-valine; Fig. 2) that determine and/or modulate localization and transport of chimeric reporter molecules (Fiedler et al. 1996, Fiedler and Rothman 1997, Nickel et al. 1997, Nakamura et al. 1998) and of p24 proteins (Dominguez et al. 1998). The presence of motifs for anterograde and/or retrograde transport is in agreement with the cycling of p24 proteins between ER and Golgi. However, it is important to note that

Fig. 2. Alignment of human p24 proteins. Residues in black are conserved in 3 of 6 homologues. The positions of the conserved cysteine residues are indicated with asterisks. The positions of the signal peptide (*SP*), the coiled-coil (*CC*) and the transmembrane domains (*TM*) are indicated with a line (solid, present in all; dashed, present in 5 homologues). The p24 proteins all share the same topology. Numerous amino acid motifs are highly conserved throughout their sequences. The transmembrane and the coiled-coil domains were determined with the protean program (DNAstar), using the Goldman–Engelman–Steitz algorithm (Engelman et al. 1986) with default parameters and the coiled-coil algorithm with thresholds 1.3 (p23, p24, p25, Tp24) or 1.125 (p26, p27), respectively. The length of the signal peptide was predicted as described (Nielsen et al. 1997). For more details on methods, see legend to Fig. 1


SP
CC
TM
p23
p24
p25
p26
p27
Tp24

the proper transport and localization of p24 proteins depends on the luminal domain (Nickel et al. 1997) and on other p24 proteins (Dominguez et al. 1998).

Function

Traffic between ER and Golgi is accomplished by two complexes of coat proteins (COPI and COPII) that form coated transport vesicles: COPII vesicles bud from the ER and transport anterograde cargo (for a review, see Kuehn and Schekman 1997), COPI vesicles can bud from different membranes along the biosynthetic pathway and have been implicated in both retrograde and anterograde transport (for a review, see Cosson and Letourneur 1997). It has been shown that peptides equivalent to the cytoplasmic tails of some p24 proteins are able to interact with COPI and COPII proteins in vitro (Fiedler et al. 1996, Sohn et al. 1996, Dominguez et al. 1998). Mutations that affect the COP-binding capacity of such peptides affect the localization of p24 proteins (Dominguez et al. 1998) and of reporter molecules appended with p24 cytoplasmic tails (Fiedler et al. 1996, Sohn et al. 1996, Nickel et al. 1997). Therefore, it has been proposed that such interactions mediate loading into COPII and/or COPI vesicles in vivo.

The budding of COPI and COPII vesicles has been reconstituted in vitro with purified components (for reviews, see Kuehn and Scheckman 1997, Cosson and Letourneur 1997), and it has been shown that some p24 proteins partition into COPI- (mammals) and COPII-coated vesicles (yeast) generated in vitro (Schimmöller et al. 1995, Belden and Barlowe 1996, Stamnes et al. 1995). In addition, p23 was found to be strongly enriched in a fraction of purified COPI-coated vesicles generated in vitro (Sohn et al. 1996). These findings, together with the ability of peptides derived from p24 cytoplasmic tails to bind COPs in vitro, led to the proposal that p24 proteins are essential for vesicle biogenesis and/or COP recruitment (p24: Stamnes et al. 1995; p23: Sohn et al. 1996). In contrast, we found that the p23 protein was present, but not enriched, in dense gradient fractions enriched in COPI-coated vesicles (Rojo et al. 1997). At present we have no explanations for this discrepancy, except for slight differences in the experimental protocols. However, other findings also indicate that the function of p24 proteins is not restricted to the interaction with COPs and to the biogenesis of COP-coated vesicles: (i) COPII-coated vesicles can be generated in vitro from yeast ER membranes that are devoid of emp24p, erv25, or both (Belden and Barlowe 1996), and (ii) the membrane association of COPI does not vary with the amount of p23 on membranes (Rojo et al. 1997). In addition, antibodies that bind to the cytoplasmic tail of p23 (in vivo and in vitro) inhibit anterograde transport of transmembrane cargo (in vivo), but do not affect membrane association of COPI (in vitro). Finally, visualization of anterograde transport (Rojo et al. 1997) revealed that p23 colocalizes with anterograde cargo (vesicular stomatitis virus glycoprotein G, VSV-G) and COPI proteins when transport is inhibited at 15 °C (Fig. 3D), but segregates from cargo (VSV-G) and COPI when transport resumes (Fig. 3E).

Yeast emp24p and erv25p were the first proteins reported to function in cargo selection: null mutants of *EMP24* and *ERV25* (i) secret a subset of proteins with delayed kinetics, when compared to wild-type yeast cells, and (ii) are deficient in the retention of ER-resident proteins (Schimmöller et al. 1995, Belden and Barlowe 1996, Elrod-Erickson and Kaiser 1996). The presence of yeast p24 proteins on COPII-coated vesicles led to the proposal that they may act as cargo adaptors/receptors on transport vesicles, where they would specifically interact with cargo molecules. Until now, it has not been possible to detect a specific association between a p24 protein and a cargo molecule. Given the abundance, variety, and dynamics of p24 proteins (see above), it is possible that interactions of cargo molecules with p24 proteins are weak and/or transient, and cannot be revealed by classical biochemical approaches. Alternatively, it is possible that p24 proteins do not directly interact with cargo molecules, and that they mediate cargo selection by contributing to organelle structure (see below).

Several p24 proteins are relatively abundant, and can be visualized by protein stain in gels of subcellular membrane fractions (e.g., Singer-Krüger et al. 1993, Stamnes et al. 1995, Belden and Barlowe 1996, Rojo et al. 1997). In addition, we found by electron microscopical analysis of purified membranes that p23 is a major component of the CGN, where it may make up to 30% of all integral membrane proteins (Rojo et al. 1997). Therefore, we proposed that p23 contributes to CGN structure, and that this contribution is necessary for efficient sorting and transport (Rojo et al. 1997). This hypothesis, which has also been formulated for yeast p24 proteins, is compatible with a role in cargo selection (Elrod-Erickson and Kaiser 1996).

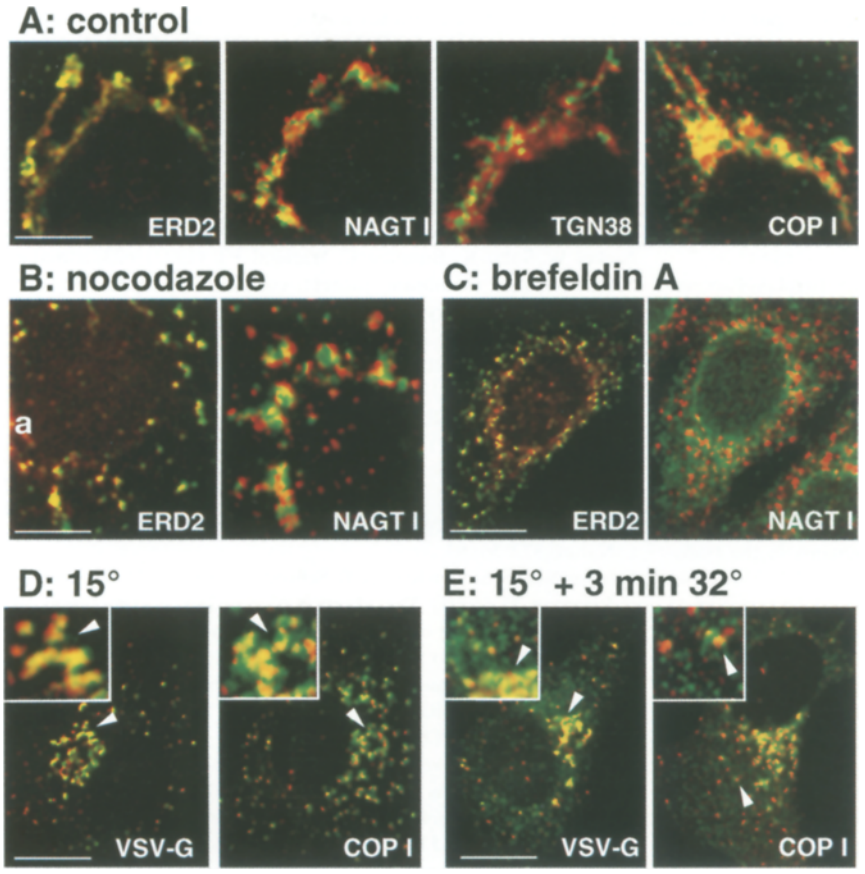


Fig. 3. Localization of p23 to the CGN (**A–D**) and visualization of transient transport intermediates that are positive for cargo (VSV-G) and coat (COPI), but negative for p23 (**E**). Shown is the merge between the fluorescence signal of p23 (red) and that of the indicated markers (green) in confocal microscopy; overlapping signals appear in yellow. **A** p23 localizes to the perinuclear ribbon of Golgi membranes, where it colocalizes substantially (yellow signal) with markers of the CGN (ERD2) and partially (separate green and red signals) with markers of medial Golgi (NAGTI), of the trans-Golgi network (TGN38), or with COPI. **B** The disruption of the Golgi ribbon by depolymerization of the microtubule network with nocodazole allows to better visualize the colocalization of p23 with proteins of the CGN (ERD2) and the segregation from medial Golgi (NAGTI). **C** The treatment with brefeldin A redistributes Golgi markers (NAGTI) to the endoplasmic reticulum and CGN-proteins (p23, ERD2) to brefeldin A remnants. **D** The arrest of membrane traffic at 15 °C leads to colocalization of p23 with cargo molecules (VSV-G) and coat proteins (COPI) in the intermediate compartment between ER and Golgi. **E** After release of the temperature block, transport resumes: p23 is absent from transport intermediates that contain cargo (VSV-G) or COPI. Bars: 5 μm. See Rojo et al. (1997), for details

At this stage, it is not known whether different p24 homologues (i) fulfill similar functions at different locations, (ii) display different functions at the same location, or (iii) act synergistically at the same compartment(s), but it is possible that other p24 proteins are also morphogenic. The presence of putative coiled-coil domains in p24 proteins at similar positions relative to the transmembrane domain enables heterotypic interactions between different p24 proteins. Therefore, p24 proteins may modulate organelle morphology by extensive heterotypic interactions and/or oligomerizations. Indeed, direct and indirect experimental evidences indicate that p24 proteins interact with each other in vivo, and that these interactions are necessary for protein stability and localization (Belden and Barlowe 1996, Dominguez et al. 1998). This working model is also speculative, and more work will be necessary to reveal the true function of p24 proteins in secretion.

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