An escort for GPCRs: implications for regulation of receptor density at the cell surface.

Lamia Achour, Catherine Labbé-Jullié, Mark Scott, Stefano Marullo

An escort for GPCRs: implications for regulation of receptor density at the cell surface.

Lamia Achour[1,2], Catherine Labbé-Jullié[1,2], Mark GH Scott[1,2] and Stefano Marullo[1,2]

1. Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), 75014 Paris, France
2. INSERM, U567, 75014 Paris, France.

G-protein-coupled receptors (GPCRs) are dynamically regulated by various mechanisms, which tune their response to external stimuli. Modulation of their plasma membrane density, via trafficking between sub-cellular compartments, constitutes an important process in this context. Substantial information has been accumulated on cellular pathways, which remove GPCRs from the cell surface for subsequent degradation or recycling. In comparison, much less is known about mechanisms controlling trafficking of neo-synthesised GPCRs from intracellular compartments to the cell surface. Although GPCR export to the plasma membrane is commonly considered to mostly implicate the default, unregulated secretory pathway, an increasing number of observations indicate that trafficking to the plasma membrane from the endoplasmic reticulum may be tightly regulated and involve specific protein partners. Moreover, a new paradigm is emerging in some cellular contexts, in which stocks of functional receptors retained within intracellular compartments may be rapidly mobilized to the plasma membrane to maintain sustained physiological responsiveness.

Established models of GPCR maturation

Introduction

Polytopic proteins (spanning membranes several times) are synthesized by ribosomes attached at the cytosolic face of the endoplasmic reticulum (ER) and enter cotranslationally in the ER lumen via a translocation complex (the translocon), to which they are targeted by hydrophobic signal sequences (Figure 1). Membrane insertion of transmembrane domains is driven by the translocon and orientation signals contained in the polypeptidic chain itself and it is assisted by molecular chaperones and folding factors[1, 2]. Most polytopic proteins fold properly with the aid of the general chaperone system, which comprises classical and lectin chaperones, in addition to enzymes that catalyze disulfide bond formation or peptidyl-prolyl cis-trans isomerization[3,4]. Once polytopic proteins have

achieved their native conformation, they leave the ER and are transported through the secretory pathway to their destination. This complex ER machinery constitutes the major quality-control system for proof-reading newly synthesized proteins: folding-defective polypeptides are exported across the ER membrane into the cytosol and destroyed by the ER-associated degradation pathway (ERAD)[5].

Several studies have investigated the implication of the general chaperone system in GPCR folding. The Hsp70 family ER luminal protein BiP/GRP78 is the master regulator of the ER. Assisted by Hsp40 family co-factors, BiP facilitates translocation of nascent chains in the ER lumen, participates in protein folding and oligomerization and contributes to the retro-translocation of misfolded proteins to ERAD[4]. Some GPCRs, such as thyrotropin-releasing hormone receptor (TRH receptor) and lutenizing hormone receptor (LSH receptor), were reported to interact with BiP[6,7]. LSH receptor, was also found to interact in the ER with GRP94, a member of the Hsp90 family and Bip cofactor[7]. GRP94 likely interacts with more advanced folding intermediates than BiP, since it binds some substrates that have been released from BiP[4]. N-glycosylation of the aminoterminal region or of extracellular loops (which are luminal in the ER) is common among GPCRs. Cotranslational addition of a $Glucose_3Mannose_9N\text{-}acetylglucosamine_2$ chain to asparagine residues by the oligosaccharyltransferase provides binding sites for carbohydrate-binding lectin chaperones such as calnexin and calreticulin. After the removal of the 2 terminal glucoses by a glucosidase, monoglucosylated nascent proteins interact with lectin chaperones, the interaction being terminated by the cleavage of the last glucose by glucosidase II. Once released, correctly folded glycoproteins can exit the ER. In contrast, incorrectly or incompletely folded glycoproteins are re-glucosylated by glucosyltransferase promoting a renewed association with calnexin and calreticulin. Cycles of glucosylation and de-glucosylation continue until the glycoprotein has either reached its native conformation or is targeted for degradation[3]. As expected from the GPCR glycosylation profile, multiple reports illustrate the interaction between GPCRs and carbohydrate-binding chaperones[6-12]. Interestingly, ER-retained receptor mutants were found to display enhanced interaction with both carbohydrate-binding chaperones and/or BiP[9,13]. Finally, several reports documented the degradation of wild type or mutant GPCRs by ERAD[12,14,15]. The developing field of the proteomic analysis of GPCR-associated protein complexes[16] will likely confirm that interaction with the general chaperone system is a common feature for all receptors.

Post ER trafficking

Exit of proteins from the ER occurs at ER exit sites, where buds are formed and coated with the COPII coat under the control of the Sar1p GTPase. Proteins released from the ER quality control machinery accumulate in these buds[17]. A recent study provided

experimental evidence that ER exit of GPCRs is indeed mediated through Sar1-dependent COPII-coated vesicles[18]. Signals in the cytoplasmically exposed C-terminal tails of transmembrane protein cargos (i.e. the transmembrane proteins that are being transported in the secretory pathway) are likely involved in direct binding with components of COPII[19]. These signals comprise di-acidic motifs (DXE or similar) and pairs of aromatic (FF, YY or FY) or bulky hydrophobic (LL or IL) amino acid residues[17]. Cargo receptors for soluble secretory proteins present in ER buds also possess these motifs in their carboxyterminal tail. Many GPCRs contain similar ER export motifs [20], suggesting that they might interact directly with COPII complex proteins, although experimental evidence for this is lacking.

In mammalian cells, protein traffic moving from ER-exit sites to the Golgi complex passes through the ER-Golgi intermediate compartment (ERGIC). ERGIC is a site of anterograde and retrograde sorting under the control of COPI coat proteins, Rab and Arf GTPases[21]. Vesicles exiting from this compartment are either directed to the Golgi or back to the ER, depending on the cargo, the ARF-GTPase isoform involved in coat recruitment, and on Rab effectors. ERGIC mainly harbors two Rabs that have opposing functions. Rab2 likely promotes the formation of vesicles returning to the ER[22], whereas Rab1 isoforms are involved in ER to ERGIC and ERGIC to cis-Golgi transport[23]. Indirect evidence for GPCR trafficking through the ERGIC has been provided by studies analyzing the effects of Rab1 and Rab2 proteins on receptor export. Forward trafficking of both the angiotensin AT_1 receptor and the ß2-adrenoceptor were impaired by siRNA-mediated knockout of Rab1b and overexpression of dominant-negative Rab1a[24]. Surprising, trafficking of the α_{2B}-adrenoceptor was not affected, suggesting receptor-specific pathways. The surface expression of both ß2- and α_{2B}- adrenoceptors was perturbed by Rab2 mutants or siRNA-promoted inhibition of Rab2 [25].

The Golgi complex is composed of stacks of flattened cisternae. Each layer of the stack, from cis-Golgi to the trans-Golgi network (TGN) contains glycosyltransferases. ER to Golgi carriers join the Golgi stack by fusing with *cis* cisternae[26]. The transport mechanisms of cargo and enzymes through the Golgi stack has not been completely elucidated and lead to conflicting models[27]. Once in the Golgi, cargo proteins may be sorted to the plasma membrane, the endosomal system or the ER. Retrograde transport to the ER is likely involved in re-targeting misfolded proteins to the ERAD[28]. Other defective proteins are targeted to lysosomal degradation after sorting to endosomes[28]. Studies of δ-opioid (DOP) receptor glycosylation demonstrated that its *O*-glycosylation (on Ser or Thre residues) and final processing of N-linked oligosaccharides occur in different compartments of the Golgi[29]. Perturbing the function of the Rab6 GTPAse, which regulates vesicular transport in the Golgi[30], inhibited the anterograde transport of *Drosophila* rhodopsin[31] and of mammalian GPCRs [25]. There is no evidence for GPCR sorting from the Golgi to endosomes or ER and it

remains unknown whether GPCR targeting to the cell surface from the Golgi is regulated. However, a recent report indicates that the N-terminus of the α_{2B}-adrenoceptor may contain a signal to exit from the Golgi. Indeed, a receptor mutant, in which adjacent Tyr and Ser amino acid residues were substituted, was totally trapped in this organelle[32].

GPCR oligomerization might control receptor maturation and cell surface translocation

Most GPCRs may exist as either homodimers or heterodimers. Dimerization seems to occur in the ER where it could have an important role in biosynthesis and quality control of newly synthesized receptors[33]. Heterodimerization can mask retention signals present in the sequence of some receptors, such as the $GABA_{B(1)}$[34], which are constitutively trapped in the ER in the absence of maturation partners. In contrast, the mechanism by which GPCR homodimerization might affect ER exit remains to be elucidated. A plausible hypothesis is that homodimerization might help receptor folding. Association of nascent polypeptides with chaperones prevents unproductive interactions with the environment that result in protein aggregation[35]. Hydrophobic regions (such as membrane spanning domains of GPCRs) are particularly prone to non-specific aggregation. Thus, the ordered association of two nascent GPCR polypeptides via their transmembrane regions (often constituting the dimerization interface) could hide a significant proportion of the exposed hydrophobic surface and facilitate correct folding. The hypothesis that in a GPCR dimer receptor protomers may serve as folding chaperones one to each other, is consistent with the fact that functional GPCR heterodimers have been obtained in reconstituted cell models containing receptors, which do not "meet" in real life. In these artificial conditions, where two distinct GPCR polypeptides are forced to enter simultaneously in the ER, if they display sufficient structure-driven propensity to assemble, they may form heterodimers. Homodimerization might also contribute to quality control. Dimeric receptors are likely to be structurally symmetric. Random mutations affecting the overall structure of one protomer, may generate asymmetry within the dimer. Checking for symmetry could represent a simple method for ER quality-control mechanisms to recognize and retain nascent mutations, for disposal via the ERAD. Consistent with this model, mutant ER-retained GPCRs generally display dominant negative effect of over wild type forms in heterozygous individuals or in reconstituted cellular models[33].

Changing Paradigms

GPCRs displaying regulated translocation to the plasma membrane from intracellular stores.

It is commonly believed that, in the absence of agonist-promoted endocytosis, GPCRs are mainly expressed at the cell surface, but this may not always be the case. The

protease-activated receptor (PAR) family represents a well-known example. Thrombin receptors (PAR1 and PAR2) are irreversibly activated by cleavage, internalized and degraded in lysosomes. A large pool of intracellular receptor, mostly localized in the Golgi apparatus and protected from activation by thrombin, is translocated to the plasma membrane upon activation of cell surface receptors. Replenishment of plasma membrane thrombin receptors is correlated with recovery of thrombin responsiveness[36].

Similarly, regulated pools of intracellular dopamine D1 receptors exist in tubular renal cells. In these cells, receptor recruitment from cytosolic stores to the plasma membrane is elicited by agonist activation of cell surface receptors[37] or via atrial natriuretic peptide-dependent heterologous activation[38]. An analogous phenomenon was also reported for α_{1A}-adrenoceptors in response to neuropeptide Y stimulation[38], leading to the concept that receptor recruitment to the plasma membrane might be a mechanism for receptor sensitization. Selective up-regulation of D1 receptor was subsequently reported in neuronal dendritic spines upon NMDA receptor activation and increased intracellular calcium[39]. The recruitment of renal D1 receptor seems to occur via Golgi-derived vesicles and requires an intact microtubular network[40].

Another model of regulated cell surface GPCR delivery, is represented by DOP receptor in neuronal cells[41]. Only a small fraction of DOP receptors is localized at the neuronal plasma membrane[42], consistent with their low physiological involvement in acute pain response[43]. Sustained stimulation of μ-opioid receptors can redistribute DOP receptors to neuronal plasma membranes *in vivo* and improve DOP-dependent antinociceptive effects[44,45]. It was suggested that cell surface translocation of DOP receptor from intracellular compartments might account for the enhanced effect of DOP-targeting drugs during chronic pain[41]. Several stimuli may elicit DOP receptor translocation, including the rise in intracellular calcium by either release from intracellular stores or direct opening of ion channels[39].

Several other GPCRs inefficiently expressed at the plasma membrane, such as the odorant receptors[46], the human GnRH receptor[47], the α_{1D}-adrenoceptor[48] and the LSH receptor[49], might represent other candidates for regulated translocation to the cell surface.

The increasing number of non-conventional chaperones and escorts assisting GPCR translocation to the plasma membrane (Table 1).

Some proteins may necessitate the specialized assistance of specific chaperones in the ER to fold properly. These so-called “private” chaperones assist nascent proteins in various ways. Outfitters[50], are chaperones or enzymes that directly participate in the folding of their cognate proteins. A few private chaperones involved in GPGR folding have been reported. NinaA and RANBP2, two cyclophilin type II proteins displaying peptidyl-prolyl *cis*-

trans isomerase activity, function as chaperones for *Drosophila* and vertebrate rhodopsin, respectively[51, 52]. Rhodopsin also interacts with HSJ1b, a protein member of the DnaJ/Hsp40 chaperone family[53]. In neurons, HSJ1 proteins, which function at the cytosolic face of the ER, facilitate the transfer of "client" proteins onto Hsc70 chaperones and their subsequent ubiquitylation and sorting to the proteasome[54]. Thus, HSJ1 isoforms likely participate in ERAD and protect neurons against cytotoxic protein aggregation. Another group of GPCR private chaperones falls in the category of escort proteins[50], which bind nascent proteins in the ER and escort them to the Golgi complex and the plasma membrane.

During the past ten years, a vast array of membrane-associated or cytoplasmic proteins has been identified, which constitutively interact with GPCRs within intracellular compartments and facilitate their cell surface expression. These proteins functionally behave like GPCR chaperones or escorts although they often display other biological roles. They were often identified by expression-cloning approaches or two-hybrid screens aimed to identify accessory factors helping the functional expression of "difficult to study" receptors.

The first example of non-conventional escort proteins for GPCRs is represented by receptor-activity-modifying-proteins (RAMPs[55]). RAMPs are type-I single-transmembrane domain proteins with a large N-terminal extracellular domain and a short C-terminus. They were initially described as obligatory interacting partners for the cell surface expression of a Class-B[56] GPCR, the calcitonin-like receptor. RAMPs remain associated with the receptor at the cell surface. Interestingly, depending on the associated RAMP, the ligand binding properties of the calcitonin-like receptor vary, RAMP1 inducing affinity for the calcitonin gene-related peptide (CGRP) whereas RAMP2 determines an adrenomedullin receptor phenotype[57]. Subsequent studies uncovered that RAMPs also aid in the constitutive plasma membrane translocation of a Class-C GPCR, the calcium-sensing receptor (CaS)[58]. However, some observations suggest that being an escort protein for GPCRs is not the principal physiological function of RAMPs. First, RAMPs are almost ubiquitous, contrasting with the restricted tissue distribution of calcitonin-like[57] and CaS receptor[58]. Second, the cell surface expression of other RAMP-interacting Class-B GPCRs (VPAC, parathyroid hormone, glucagon and glucagon-like peptide receptors), is not affected by their association with RAMPs[55].

For a long time, the study of odorant receptors has been hampered by the lack of functional cell surface expression in heterologous cells, raising the hypothesis that odorant tissue must contain specific auxiliary factors regulating their plasma membrane trafficking. Single transmembrane proteins named RTP1, RTP2 (Receptor Transporting Protein 1 and 2) and REEP1 (Receptor Expression Enhancing Protein 1) were finally found to permit functional cell surface targeting of odorant receptors in fibroblasts[46], reminiscent of previous studies in *C. elegans*, in which odorant receptor localization to olfactory cilia required

interaction with the ODR-4 transmembrane protein[59]. Members of the RTP and REEP families display a much more diffuse distribution than the olfactory epithelium, suggesting that these proteins may regulate other GPCRs and/or exhibit additional functions. In particular, RTP and REEP mRNAs were detected in human circumvallate papillae and testis, which are the sites of bitter taste (TAS2) receptor expression. Experiments in heterologous cells confirmed the enhancement of TAS2 receptor cell surface targeting upon interaction with RTP3-4 and REEPs[60].

The mammalian vomeronasal organ, a small sensory organ located near the base of the nasal septum and involved in the detection of pheromones, contains specific Class-C GPCRs, which function as pheromone receptors. These vomeronasal receptors require the association with M10s proteins for proper traffic to the plasma membrane[61]. M10s belong to the superfamily of MHC class-I molecules, but are exclusively expressed in the vomeronasal organ. As classical MHC molecules, M10s contain an open peptide-binding cleft and associate with ß2-microglobulin. It was proposed that M10s, in addition to their escort role, might modulate the ligand specificity of vomeronasal receptors (similar to RAMPs) or participate in neuronal plasticity[62].

As for odorant receptors, it has been very difficult to obtain functional expression of exogenous adrenocorticotropin MC_2 receptor, except in cells of adrenocortical origin, suggesting that MC_2 receptor expression may require an adrenal-specific accessory factor. In a genetic disease, the familial glucocorticoid deficiency (FGD), the adrenal cortex is resistant to adrenocorticotropin. By investigating patients with FGD and normal MC_2 receptor, mutations were identified in a gene encoding a 19-kDa single-transmembrane domain protein, named MC_2 receptor accessory protein (MRAP). MRAP was found to interact with MC2R and to regulate its trafficking from the endoplasmic reticulum to the cell surface[63, 64]. This example illustrates particularly well the physiopathological relevance of the interaction between a GPCR and a “private” escort protein.

Another similar example comes from studies in mice. Abnormal serotonin signaling has been implicated in the pathophysiology of depression. Cell surface density and function of the serotonin 5-HT_{1B} receptors are decreased in knockout mice for p11[65] (also known as calpactin I- or annexin II-light chain) a member of the S100 EF-hand calcium-dependent signaling modulators[66]. These mice exhibit a depression-like phenotype. The distribution of p11 mRNA in the brain resembles that of 5-HT_{1B} receptor mRNA, p11 specifically interacts with 5-HT_{1B} receptors (not with other serotonin or dopamine receptors), and colocalizes with the receptor at the cell surface of transfected cells[65]. Interestingly, p11 is increased in the brain of mice treated with antidepressants and reduced in depressed patients.

As for p11, other GPCR-interacting proteins may control plasma membrane receptor targeting without traveling to the cell surface. A well-known example is the ER-membrane-

associated protein, DRiP78. Overexpression or down-modulation of this putative two-transmembrane domain protein leads to ER retention of D_1 receptors, reduced ligand binding, and impaired kinetics of receptor glycosylation[67]. DRiP78 binds to a FXXXFXXXF motif found in the C-terminus of various GPCRs, supporting the hypothesis that DRiP78 may function as a chaperone for several receptors. Accordingly, a subsequent study indicated a role of DRiP78 in the maturation of the AT_1 angiotensin II receptor[68]. Noteworthy, DRiP78 also specifically interacts with Gγ subunits of heterotrimeric G proteins, protecting them from degradation until a stable partner (cognate Gβ subunit) is provided. These results suggest a chaperone role of DRiP78 in the assembly of Gβγ subunits[69].

A membrane-associated Golgi protein, ATBP50 (for AT_2 receptor binding protein of 50 kDa) was reported to bind to the cytoplasmic carboxyterminal tail of the angiotensin AT_2 receptor and to control its cell surface expression, as demonstrated by receptor retention within intracellular compartments after inhibition of ATBP50 expression[70]. ATBP50 and two splice variants of the same gene share two myosin-like coiled-coil regions and form homo and hetero-dimers *in vitro.* These proteins display a much broader distribution than the AT_2 receptor, consistent with additional functions or with a more general role of escort protein for other GPCRs.

Another example of intracellular protein, interacting with the carboxyterminal tail of a GPCR (and also the first intracellular loop) and regulating its cell surface expression, is represented by RACK1 (from Receptor for Activated C-Kinase 1)[71]. RACK1 is an ER protein that constitutively binds the thromboxane A2 receptor (TPß). The cell surface expression of TPß was directly correlated with the concentration of RACK1: in cells with low RACK1 after specific siRNA treatment, TPß was retained in the ER. Interestingly, RACK1 displays selectivity for GPCRs since its expression level was able to affect the cell surface distribution of the chemokine receptor CXCR4 but not that of the ß2-adrenoceptor or prostanoid DP receptors.

Comparable specificity was documented for GEC1, a 117-residue protein, member of the microtubule associated protein (MAP) family[72]. GEC1 interacts in the Golgi and the ER with the C-terminus of κ-opioid (KOP) receptors, but not with that of μ or δ subtypes. GEC1 expression enhanced the level of mature fully glycosylated forms of KOP receptors, and facilitated trafficking of KOP receptor to the cell surface. GEC1 levels appear to be tightly regulated, as indicated by a toxic effect of overexpression. Moreover, because of its broad tissue distribution, GEC1 might participate in cell trafficking of other membrane proteins[72].

Finally a number of GPCR-interacting proteins, such as the dynein light-chain subunit TcTex, Homer proteins and Filamin A, were reported to connect rhodopsin[73], glutamate receptors[74] and dopamine[75] receptors, respectively, to the cytoskeleton, participating by this mean to their final subcellular localization. At least in the case of the visual receptor, this

targeting function is also associated with an escort/chaperone-like role, as indicated by toxic effects of rhodopsin mutations, which inhibit the interaction with TcTex[73].

Proteins that negatively regulate GPCR export via retention.

A simple explanation of GPCR retention within intracellular compartments in the absence of appropriate signals of forward export or in the case of a lack of "private" chaperones or escort proteins, might be the persistent interaction with proteins of the general quality-control machinery. Although this hypothesis remains plausible, some observations argue for the existence of specific retention mechanisms.

For example, the second extracellular loop of PAR2 was shown to interact with the N-terminal domain of the Golgi-resident type I transmembrane protein p24A. PAR2 is trapped in the Golgi because of this interaction. Upon activation of cell surface PAR2, the small G protein ARF1 is recruited in its GDP-bound form, to Golgi membranes, where a specific exchange factor activates ARF1. This process results in the dissociation of PAR2 from p24A and receptor sorting to the plasma membrane[76]. During development, a GPCR-retaining protein was reported to control the surface receptor availability of Frizzled (FZD), a GPCR, which promotes caudalizing signals. This ER-resident protein, Shisa, is specifically expressed in head ectoderm, where it binds to and inhibits cell surface trafficking of FZD. Shisa-mediated receptor retention thus constitutes a mechanism to control head-tail polarity[77]. Although evidence for receptor-specific retention mechanisms is still limited for GPCRs, other recent examples exist for growth-factor receptors[78], suggesting that this field may rapidly evolve in the near future.

Concluding remarks and perspectives.

The emerging picture of GPCR trafficking from biosynthetic compartments to the plasma membrane appears much more sophisticated than expected, particularly if the recent hypothesis of large signaling complexes containing GPCRs, G proteins and effectors being assembled during maturation[79], is confirmed by future studies.

Several receptors are retained within intracellular compartments waiting for external signals that control their release from molecular tethers. In most cases, the pathways connecting signaling events with receptor export and the entity of the tethers have not been identified yet. Moreover, the subcellular location of retained receptors is not unique, implicating at least the ER and the Golgi complex.

Many GPCRs are constitutively associated with a long list of "private" chaperones or escort proteins, which are necessary for their proper targeting to the plasma membrane. How general this phenomenon may be, what the mechanisms involved in receptor retention and release are and the potential connection between this phenomenon and signal-regulated

transport remain to be elucidated. Moreover, these private chaperones, which often display other cellular functions, might actually assist multiple GPCRs and possibly other integral membrane proteins in their trafficking to the cell surface.

Several conserved motifs have been identified in the sequence of many GPCRs, which may have some role in their forward trafficking. These motifs represent a molecular code determining the association with proteins that retain or assist GPCR in their journey through biosynthetic compartments. Deciphering the code is still a task that lies ahead.

Finally, the issue of GPCR maturation and trafficking to the cell surface is already an important issue in human health, as several receptor mutants leading to intracellular retention have been identified, which cause disease via impaired signaling[80]. Maturation and trafficking of these mutated receptors can be improved by membrane-permeant small molecules, which bind to retained receptors and induce export-competent conformational changes[81]. Thus, improving our knowledge on the routing regulation of this important class of membrane receptors will probably elicit the development of new therapeutical approaches to control the targeting of GPCRs at the plasma membrane.

Acknowledgements

Thank you to Michel Bouvier and Ralf Jockers for the critical reading of the manuscript. This work was supported by the Sidaction grant N° 23005-09-00/AO17-2 to SM.

References

1. Higy, M., et al. (2004) Topogenesis of membrane proteins at the endoplasmic reticulum. Biochemistry 43, 12716-12722
2. Krebs, M.P., et al. (2004) Quality control of integral membrane proteins. Trends Biochem Sci 29, 648-655
3. Ellgaard, L., and Helenius, A. (2003) Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 4, 181-191
4. Hebert, D.N., and Molinari, M. (2007) In and out of the ER: protein folding, quality control, degradation, and related human diseases. Physiol Rev 87, 1377-1408
5. Tsai, B., et al. (2002) Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. Nat Rev Mol Cell Biol 3, 246-255
6. Siffroi-Fernandez, S., et al. (2002) Association of the thyrotropin receptor with calnexin, calreticulin and BiP. Efects on the maturation of the receptor. Eur J Biochem 269, 4930-4937
7. Mizrachi, D., and Segaloff, D.L. (2004) Intracellularly located misfolded glycoprotein hormone receptors associate with different chaperone proteins than their cognate wild-type receptors. Mol Endocrinol 18, 1768-1777
8. Rozell, T.G., et al. (1998) Association of gonadotropin receptor precursors with the protein folding chaperone calnexin. Endocrinology 139, 1588-1593
9. Morello, J.P., et al. (2001) Association of calnexin with wild type and mutant AVPR2 that causes nephrogenic diabetes insipidus. Biochemistry 40, 6766-6775
10. Lanctot, P.M., et al. (2006) Role of N-glycan-dependent quality control in the cell-surface expression of the AT1 receptor. Biochem Biophys Res Commun 340, 395-402
11. Rosenbaum, E.E., et al. (2006) Calnexin is essential for rhodopsin maturation, Ca2+ regulation, and photoreceptor cell survival. Neuron 49, 229-241
12. Lu, M., et al. (2003) Endoplasmic reticulum retention, degradation, and aggregation of olfactory G-protein coupled receptors. Traffic 4, 416-433
13. Fan, J., et al. (2005) A point mutation in the human melanin concentrating hormone receptor 1 reveals an important domain for cellular trafficking. Mol Endocrinol 19, 2579-2590
14. Petaja-Repo, U.E., et al. (2001) Newly synthesized human delta opioid receptors retained in the endoplasmic reticulum are retrotranslocated to the cytosol, deglycosylated, ubiquitinated, and degraded by the proteasome. J Biol Chem 276, 4416-4423
15. Robben, J.H., et al. (2005) Characterization of vasopressin V2 receptor mutants in nephrogenic diabetes insipidus in a polarized cell model. Am J Physiol Renal Physiol 289, F265-272
16. Daulat, A.M., et al. (2007) Purification and identification of G protein-coupled receptor protein complexes under native conditions. Mol Cell Proteomics 6, 835-844
17. Barlowe, C. (2003) Signals for COPII-dependent export from the ER: what's the ticket out? Trends Cell Biol 13, 295-300
18. Dong, C., et al. (2008) Endoplasmic reticulum export of adrenergic and angiotensin II receptors is differentially regulated by Sar1 GTPase. Cell Signal 20, 1035-1043
19. Aridor, M., et al. (1998) Cargo selection by the COPII budding machinery during export from the ER. J Cell Biol 141, 61-70
20. Dong, C., et al. (2007) Regulation of G protein-coupled receptor export trafficking. Biochim Biophys Acta 1768, 853-870
21. Appenzeller-Herzog, C., and Hauri, H.P. (2006) The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function. J Cell Sci 119, 2173-2183
22. Tisdale, E.J. (2003) Rab2 interacts directly with atypical protein kinase C (aPKC) iota/lambda and inhibits aPKCiota/lambda-dependent glyceraldehyde-3-phosphate dehydrogenase phosphorylation. J Biol Chem 278, 52524-52530

23. Moyer, B.D., et al. (2001) Rab1 interaction with a GM130 effector complex regulates COPII vesicle cis--Golgi tethering. Traffic 2, 268-276
24. Wu, G., et al. (2003) Distinct pathways for the trafficking of angiotensin II and adrenergic receptors from the endoplasmic reticulum to the cell surface: Rab1-independent transport of a G protein-coupled receptor. J Biol Chem 278, 47062-47069
25. Dong, C., and Wu, G. (2007) Regulation of anterograde transport of adrenergic and angiotensin II receptors by Rab2 and Rab6 GTPases. Cell Signal 19, 2388-2399
26. Trucco, A., et al. (2004) Secretory traffic triggers the formation of tubular continuities across Golgi sub-compartments. Nat Cell Biol 6, 1071-1081
27. Pfeffer, S.R. (2007) Unsolved mysteries in membrane traffic. Annu Rev Biochem 76, 629-645
28. Arvan, P., et al. (2002) Secretory pathway quality control operating in Golgi, plasmalemmal, and endosomal systems. Traffic 3, 771-780
29. Petaja-Repo, U.E., et al. (2000) Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human delta opioid receptor. J Biol Chem 275, 13727-13736
30. Jordens, I., et al. (2005) Rab proteins, connecting transport and vesicle fusion. Traffic 6, 1070-1077
31. Shetty, K.M., et al. (1998) Rab6 regulation of rhodopsin transport in Drosophila. J Biol Chem 273, 20425-20430
32. Dong, C., and Wu, G. (2006) Regulation of anterograde transport of alpha2-adrenergic receptors by the N termini at multiple intracellular compartments. J Biol Chem 281, 38543-38554
33. Bulenger, S., et al. (2005) Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. Trends Pharmacol Sci 26, 131-137
34. Margeta-Mitrovic, M., et al. (2000) A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron 27, 97-106.
35. Gething, M.J., and Sambrook, J. (1992) Protein folding in the cell. Nature 355, 33-45
36. Hein, L., et al. (1994) Intracellular targeting and trafficking of thrombin receptors. A novel mechanism for resensitization of a G protein-coupled receptor. J Biol Chem 269, 27719-27726
37. Brismar, H., et al. (1998) Dopamine-induced recruitment of dopamine D1 receptors to the plasma membrane. Proc Natl Acad Sci U S A 95, 5573-5578
38. Holtback, U., et al. (1999) Receptor recruitment: a mechanism for interactions between G protein-coupled receptors. Proc Natl Acad Sci U S A 96, 7271-7275
39. Scott, L., et al. (2002) Selective up-regulation of dopamine D1 receptors in dendritic spines by NMDA receptor activation. Proc Natl Acad Sci U S A 99, 1661-1664
40. Kruse, M.S., et al. (2003) Recruitment of renal dopamine 1 receptors requires an intact microtubulin network. Pflugers Arch 445, 534-539
41. Cahill, C.M., et al. (2007) Trafficking of delta-opioid receptors and other G-protein-coupled receptors: implications for pain and analgesia. Trends Pharmacol Sci 28, 23-31
42. Cahill, C.M., et al. (2001) Immunohistochemical distribution of delta opioid receptors in the rat central nervous system: evidence for somatodendritic labeling and antigen-specific cellular compartmentalization. J Comp Neurol 440, 65-84
43. Kieffer, B.L. (1999) Opioids: first lessons from knockout mice. Trends Pharmacol Sci 20, 19-26
44. Cahill, C.M., et al. (2001) Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception. J Neurosci 21, 7598-7607
45. Morinville, A., et al. (2003) Regulation of delta-opioid receptor trafficking via mu-opioid receptor stimulation: evidence from mu-opioid receptor knock-out mice. J Neurosci 23, 4888-4898
46. Saito, H., et al. (2004) RTP family members induce functional expression of mammalian odorant receptors. Cell 119, 679-691

47. Janovick, J.A., et al. (2006) Regulation of G protein-coupled receptor trafficking by inefficient plasma membrane expression: molecular basis of an evolved strategy. J Biol Chem 281, 8417-8425
48. Uberti, M.A., et al. (2005) Heterodimerization with beta2-adrenergic receptors promotes surface expression and functional activity of alpha1D-adrenergic receptors. J Pharmacol Exp Ther 313, 16-23
49. Pietila, E.M., et al. (2005) Inefficient maturation of the rat luteinizing hormone receptor. A putative way to regulate receptor numbers at the cell surface. J Biol Chem 280, 26622-26629
50. Herrmann, J.M., et al. (1999) Out of the ER--outfitters, escorts and guides. Trends Cell Biol 9, 5-7
51. Baker, E.K., et al. (1994) The cyclophilin homolog NinaA functions as a chaperone, forming a stable complex in vivo with its protein target rhodopsin. Embo J 13, 4886-4895
52. Ferreira, P.A., et al. (1996) Cyclophilin-related protein RanBP2 acts as chaperone for red/green opsin. Nature 383, 637-640
53. Chapple, J.P., and Cheetham, M.E. (2003) The chaperone environment at the cytoplasmic face of the endoplasmic reticulum can modulate rhodopsin processing and inclusion formation. J Biol Chem 278, 19087-19094
54. Westhoff, B., et al. (2005) HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. Curr Biol 15, 1058-1064
55. Parameswaran, N., and Spielman, W.S. (2006) RAMPs: the past, present and future. Trends Biochem Sci 31, 631-638
56. Horn, F., et al. (1998) GPCRDB: an information system for G protein-coupled receptors.
Nucleic Acids Res 26, 275-279
57. McLatchie, L.M., et al. (1998) RAMPs regulate the transport and ligand specificity of the calcitonin- receptor-like receptor. Nature 393, 333-339
58. Bouschet, T., et al. (2005) Receptor-activity-modifying proteins are required for forward trafficking of the calcium-sensing receptor to the plasma membrane. J Cell Sci 118, 4709-4720
59. Dwyer, N.D., et al. (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell 93, 455-466
60. Behrens, M., et al. (2006) Members of RTP and REEP gene families influence functional bitter taste receptor expression. J Biol Chem 281, 20650-20659
61. Loconto, J., et al. (2003) Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. Cell 112, 607-618
62. Olson, R., et al. (2006) MHC homologs in the nervous system — they haven't lost their groove. Current Opinion in Neurobiology 16, 351-357
63. Metherell, L.A., et al. (2005) Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. Nat Genet 37, 166-170
64. Cooray, S.N., et al. (2008) The melanocortin 2 receptor accessory protein exists as a homodimer and is essential for the function of the melanocortin 2 receptor in the mouse y1 cell line. Endocrinology 149, 1935-1941
65. Svenningsson, P., et al. (2006) Alterations in 5-HT1B receptor function by p11 in depression-like states. Science 311, 77-80
66. Donato, R. (1999) Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim Biophys Acta 1450, 191-231
67. Bermak, J.C., et al. (2001) Regulation of transport of the dopamine D1 receptor by a new membrane- associated ER protein. Nat Cell Biol 3, 492-498.
68. Leclerc, P.C., et al. (2002) A polyaromatic caveolin-binding-like motif in the cytoplasmic tail of the type 1 receptor for angiotensin II plays an important role in receptor trafficking and signaling. Endocrinology 143, 4702-4710

69. Dupre, D.J., et al. (2007) Dopamine receptor-interacting protein 78 acts as a molecular chaperone for G-gamma subunits before assembly with G-beta. J Biol Chem 282, 13703-13715
70. Wruck, C.J., et al. (2005) Regulation of transport of the angiotensin AT2 receptor by a novel membrane-associated Golgi protein. Arterioscler Thromb Vasc Biol 25, 57-64
71. Parent, A., et al. (2008) RACK1 regulates the cell surface expression of the G protein-coupled receptor for thromboxane A(2). Traffic 9, 394-407
72. Chen, C., et al. (2006) GEC1 interacts with the kappa opioid receptor and enhances expression of the receptor. J Biol Chem 281, 7983-7993
73. Tai, A.W., et al. (1999) Rhodopsin's carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1. Cell 97, 877-887
74. Ango, F., et al. (2000) Dendritic and axonal targeting of type 5 metabotropic glutamate receptor is regulated by homer1 proteins and neuronal excitation. J Neurosci 20, 8710-8716
75. Lin, R., et al. (2001) Dopamine D2 and D3 receptors are linked to the actin cytoskeleton via interaction with filamin A. Proc Natl Acad Sci U S A 98, 5258-5263.
76. Luo, W., et al. (2007) p24A, a type I transmembrane protein, controls ARF1-dependent resensitization of protease-activated receptor-2 by influence on receptor trafficking. J Biol Chem 282, 30246-30255
77. Yamamoto, A., et al. (2005) Shisa promotes head formation through the inhibition of receptor protein maturation for the caudalizing factors, Wnt and FGF. Cell 120, 223-235
78. Couturier, C., et al. (2007) Silencing of OB-RGRP in mouse hypothalamic arcuate nucleus increases leptin receptor signaling and prevents diet-induced obesity. Proc Natl Acad Sci U S A 104, 19476-19481
79. Dupre, D.J., et al. (2006) Seven transmembrane receptor core signaling complexes are assembled prior to plasma membrane trafficking. J Biol Chem 281, 34561-34573
80. Conn, P.M., et al. (2007) G protein-coupled receptor trafficking in health and disease: lessons learned to prepare for therapeutic mutant rescue in vivo. Pharmacol Rev 59, 225-250
81. Bernier, V., et al. (2004) Pharmacological chaperone action on G-protein-coupled receptors. Curr Opin Pharmacol 4, 528-533

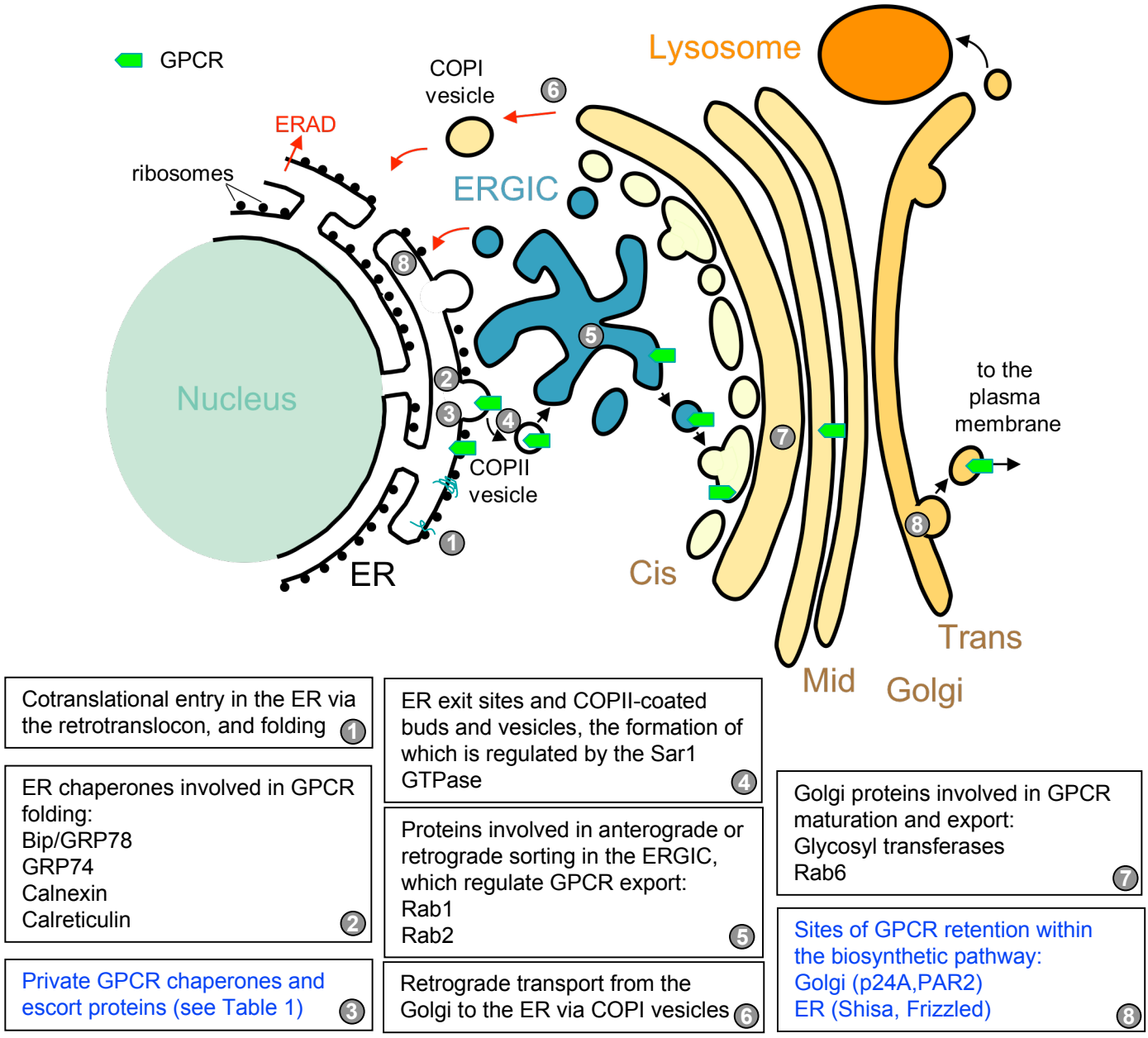

Figure 1. Subcellular events involved in GPCR maturation and cell surface export.
The scheme represents a step-by-step outline of GPCR synthesis and transport along the secretory pathway. Numbered boxes refer to either general mechanisms of protein transport (in black) or to GPCR-specific events (in blue). The proteins indicated in the boxes are described in the text and have all been shown to specifically contribute to GPCR transport.
ER: endoplasmic reticulum; ERGIC: ER-Golgi intermediate compartment; ERAD:ER-associated degradation pathway; COPII: coat protein II (or coatomer), involved in the transport of proteins from the rough endoplasmic reticulum to the Golgi apparatus. COPI: coat protein I, found on Golgi membrane at steady state, and involved in the formation of vesicles leaving the Golgi, including those of the retrograde transport to the ER.

Table 1. Non-classical chaperones and escorts, which assist GPCR translocation to the plasma membrane.

Name	Type of protein	Other functions Tissue distribution	Chaperone (C) or Escort (E)	GPCR(s)	References
RAMP (1-3)	Type 1, 1-TM	broad	E	AMY, CGRP CaS	52-54 55
RTP (1-4)	Type 1, 1-TM	broad	E	Odorant Taste	43 56
REEP	Type 1, 1-TM	broad	E	Odorant Taste	43 57
ODR4	Type 1, 1-TM	olfactory neurons	E	*C elegans* Odorant	56
M10s	Type 1, 1-TM	ß2-µglobulin-associated MHC class I vomeronasal organ	E	Vomeronasal	58, 59
MRAP	Type 1, 1-TM	adrenal brain	E ?	MC_2	60, 61
P11	Ca-binding EF-hand (helix-loop-helix motif) protein	Ca-dependent signaling modulator	C ?	$5\text{-}HT1_B$	62
DRiP78	2-TM	broad Gβγ subunit assembly	C ?	D1 AT_1	64 65
ATBP50	myosin-like coiled-coil regions	broad	C ?	AT_2	67
RACK1	7 WD repeat predicted propeller structure	ubiquitous receptor for activated C-kinase 1	C ?	TPß CXCR4	68 68
GEC1	microtubule associated Protein (MAP)	broad	C ?	KOP	69

Abbreviations: AMY, amylin; CaS, calcium-sensing;CGRP, calcitotonin gene-related peptide; TM, transmembrane
"?" denotes a possible, but unconfirmed property