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Opinion

Gatekeepers Controlling GPCR Export and Function

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Regulated export of G protein-coupled receptors (GPCRs) from intracellular stores involves chaperones and escort proteins, which promote their progression to the cell surface, and gatekeepers, which retain them in intracellular compartments. Functional γ-aminobutyric acid ($GABA)_B$ receptors, the paradigm of this phenomenon, comprise GB1 and GB2 subunits forming a heterodimer. GB1 is retained in the endoplasmic reticulum (ER) in the absence of GB2. A specific ER-resident gatekeeper, prenylated Rab acceptor family 2 (PRAF2), is involved in GB1 retention and prevents its progression into the biosynthetic pathway. GB1 can be released from PRAF2 only on competitive interaction with GB2. PRAF2 is ubiquitous and belongs to a subgroup of the mammalian Ypt-interacting protein (Yip) family. Several other GPCRs are likely to be regulated by Yip proteins, which might be involved in the pathophysiology of human diseases that are associated with impaired receptor targeting to the cell surface.

Trends

Functional $GABA_B$ receptors are obligate heterodimers of the GB1 and GB2 subunits.

The agonist binding GB1 is retained inside the cells in the absence of association with GB2.

GB2 competitively releases GB1 from PRAF2, a specific ER-resident gatekeeper.

The stoichiometry of PRAF2 relative to GB1 and GB2 is a key parameter of $GABA_B$ function *in vivo*.

Molecular Mechanisms Regulating Receptor Cell Surface Density

The number of receptors present at the cell surface that can be reached and activated by cognate ligands is evidently essential in terms of downstream signaling outputs. Most studies addressing regulation of GPCRs have focused on desensitization, the termination of activated receptor signaling, GPCR endocytosis and recycling, and the transcriptional control of GPCR-coding genes. For a long time it has been assumed that, apart from translation, the only level of regulation of native receptor proteins engaged in the secretory pathway was to successfully pass through quality-control checkpoints that prevent unfolded proteins reaching the cell surface, redirecting them to the degradation pathway. The regulated export of nascent GPCRs from intracellular stores is a concept that has emerged recently. It is based on the observation that, in primary cells, several GPCRs are only marginally expressed at the cell surface, whereas abundant stores exist both in the ER and in the Golgi apparatus. One of the pioneering observations of this phenomenon was that, in both primary and transfected cells, an important proportion of the PAR1 thrombin receptor was intracellular and colocalized with Golgi markers. Whereas surface PAR1 was internalized and subsequently targeted to lysosomes on thrombin activation, the intracellular pool was translocated to the plasma membrane, leading to the recovery of thrombin responsiveness [1]. Subsequently, a series of independent observations coincided to suggest that the regulated export of GPCRs from the Golgi apparatus and the ER was a much wider phenomenon than previously anticipated (see [2,3] for a historical overview). It also appeared that receptors are released from intracellular compartments on appropriate extracellular signals and/or association with various chaperones or escort proteins (reviewed in [2,3]). However, some key issues of this novel paradigm of regulation, such as the identification of the molecular tethers that retain GPCRs in the intracellular compartments or the characterization of the mechanisms of their release from these compartments, remained poorly understood.

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The $GABA_B$ Receptor Paradigm

The metabotropic $GABA_B$ receptor has been one of the most intensively investigated GPCRs over the past three decades, because of the very unusual regulation of its cell surface export. It belongs to the class-C family of GPCRs, which also comprises the metabotropic glutamate receptor, the calcium-sensing receptor, and the taste T1R, which is characterized by a large extracellular Venus flytrap (VFT) domain involved in ligand recognition. Together with the ionotropic $GABA_A$ receptor, $GABA_B$ responds to GABA, the major inhibitory neurotransmitter in the central nervous system (CNS). Presynaptic $GABA_B$ receptors reduce neurotransmitter release, whereas postsynaptic receptors cause hyperpolarization of neurons [4]. By inhibiting synaptic transmission, $GABA_B$ plays a key role in modulating neuronal activity, and impaired function of $GABA_B$ has been associated with multiple neurological and psychiatric diseases [5–7]. Peripheral $GABA_B$ receptors have also been identified, particularly in the gastrointestinal tract [8]. Baclofen, a $GABA_B$ agonist, is currently used to treat spasticity of various pathophysiological origins and alcoholism, whereas peripherally restricted $GABA_B$ agonists devoid of CNS side effects are under investigation to treat gastroesophageal reflux disease [9]. In addition, recent studies indicate that peripheral $GABA_B$ receptors might represent a pharmacological target for tumor therapy [10] and for improving pancreatic β cell survival [11].

In addition to its functional roles, the $GABA_B$ receptor occupies a special position among GPCRs, since several paradigms established for the GPCR family are based on pioneering studies based on $GABA_B$ as receptor model. Unlike the other class-C GPCRs, which form homodimers, functional $GABA_B$ receptors are obligate heterodimers comprising one GB1 and one GB2 subunit [12–14]. Mice lacking GB1 do not exhibit any detectable electrophysiological, biochemical, or behavioral response to agonists [15,16]. Similarly, $GB2^{-/-}$ mice display spontaneous seizures, hyperalgesia, hyperlocomotor activity, and severe memory impairment, analogous to $GB1^{-/-}$ mice, although atypical electrophysiological $GABA_B$ responses are present in a few areas of the brain [17]. The loss of normal $GABA_B$ function *in vivo* and *in vitro* when either GB1 or GB2 is missing is explained by the particular distribution of key functional tasks between receptor subunits. GB1 is responsible for ligand recognition through its VFT domain, whereas GB2 does not bind to any known $GABA_B$ ligand [18]; its VFT domain behaves only like an allosteric modulator of the GB1 VFT, enhancing its agonist affinity [19]. The transmembrane region of GB2, instead, is responsible for G-protein coupling [20,21] and also facilitates cell surface expression of GB1 [22].

A Missing Mechanistic Aspect in the Regulated Delivery of $GABA_B$ to the Cell Surface

The cell surface density of functional heterodimeric $GABA_B$ receptors is controlled by a unique mechanism of delivery from the biosynthetic compartments to the plasma membrane. Early studies reported that recombinant GB1 subunits fail to reach the cell surface when expressed in heterologous systems or overexpressed in ganglion neurons [23]. The explanation of this phenomenon is that GB1 is retained in the ER and does not reach the cell surface in the absence of GB2 [12–14]. Subsequent investigations identified an arginine-based ER retention/retrieval 'RSRR' signal in the carboxy-terminal tail of GB1 [22]. This signal is a variant of a more general RXR motif (two arginine residues separated by any amino acid) found in various ER-retained subunits of multimeric protein complexes, such as the octameric ATP-sensitive K^+ channels [24] or N-methyl-D-aspartate (NMDA) receptors [25]. The alanine substitution of the arginine residues of the RXR motif allowed the corresponding GB1-AXA subunit to reach the cell surface even in the absence of GB2. It was then proposed that only the shielding of this retention signal via a coiled-coil interaction with the C terminus of GB2 might allow the GB1–GB2 heterodimer to progress along the secretory pathway delivering functional $GABA_B$ receptors to the cell surface [22]. A second key di-leucine (LL) motif was subsequently identified upstream of the RXR motif in the carboxy-terminal tail of GB1, which also controls the cell surface export of