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2 **Gatekeepers controlling GPCR export and function**

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11

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13 GABA_B receptor; endoplasmic reticulum gatekeeper; Prenylated Rab Acceptor

14 Family 2 (PRAF2); regulated export

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17

18 **Abstract**

19 Regulated export of G protein-coupled receptors (GPCRs) from intracellular
20 stores involves chaperones and escort proteins, which promote their progression to
21 the cell surface, and gatekeepers, which retain them in intracellular compartments.
22 Functional GABA_B receptors, the paradigm of this phenomenon, are composed of
23 GB1 and GB2 subunits forming a hetero-dimer. GB1 is retained in the endoplasmic
24 reticulum (ER) in the absence of GB2. A specific ER-resident gatekeeper, PRAF2, is
25 involved in GB1 retention and prevents its progression into the biosynthetic pathway.
26 GB1 can be released from PRAF2 only upon competitive interaction with GB2.
27 PRAF2 is ubiquitous and belongs to a subgroup of the mammalian Ypt-interacting
28 protein (Yip) family. Several other GPCRs are likely regulated by Yip proteins, which
29 might be involved in the pathophysiology of human diseases that are associated with
30 impaired receptor targeting to the cell surface.

31

32

33 *Molecular mechanisms regulating receptor cell surface density*

34 The number of receptors present at the cell surface that can be reached and
35 activated by cognate ligands is evidently essential in terms of downstream signaling
36 outputs. Most studies addressing regulation of G protein coupled receptors (GPCRs)
37 have focused on desensitization, the termination of activated receptor signaling, on
38 GPCR endocytosis and recycling, and on the transcriptional control of GPCR-coding
39 genes. Indeed, for a long time it has been assumed that, apart from translation, the
40 only level of regulation of native receptor proteins engaged in the secretory pathway
41 was to successfully pass through quality-control check points, which prevent
42 unfolded proteins to reach the cell surface, redirecting them to the degradation
43 pathway. The regulated export of nascent GPCRs from intracellular stores is a
44 concept that has emerged recently. It is based on the observation that in primary
45 cells several GPCRs are only marginally expressed at the cell surface, whereas
46 abundant stores exist both in the endoplasmic reticulum (ER) and in the Golgi
47 apparatus. One of the pioneering observations of this phenomenon was that in both
48 primary and transfected cells, an important proportion of the PAR1 thrombin receptor
49 was intracellular and co-localized with Golgi markers. Whereas surface PAR1 was
50 internalized and subsequently targeted to lysosomes upon thrombin activation, the
51 intracellular pool was translocated to the plasma membrane, leading to the recovery
52 of thrombin responsiveness [1]. Subsequently, a series of independent observations
53 coincided to suggest that the regulated export of GPCRs from the Golgi apparatus
54 and the ER was actually a much wider phenomenon than previously anticipated (see
55 [2, 3] for an historical overview). It also appeared that receptors are released from
56 intracellular compartments upon appropriate extracellular signals and/or association
57 with a variety of chaperones or escort proteins (reviewed in [2, 3]). However, some

58 key issues of this novel paradigm of regulation, such as the identification of the
59 molecular tethers that retain GPCRs in the intracellular compartments or the
60 characterization of the mechanisms of their release from these compartments
61 remained poorly understood.

62

63 *The GABA_B receptor paradigm*

64 The metabotropic GABA_B receptor has been one of the most intensively
65 investigated GPCRs over the past three decades, because of the very unusual
66 regulation of its cell surface export. It belongs to the class-C family of GPCRs, also
67 comprising the metabotropic glutamate receptor, the calcium sensing receptor and
68 the taste T1R, which is characterized by a large extracellular Venus flytrap (VFT)
69 domain involved in ligand recognition. Together with the ionotropic GABA_A receptor,
70 GABA_B responds to the γ -aminobutyric acid (GABA), the major inhibitory
71 neurotransmitter in the central nervous system (CNS). Presynaptic GABA_B receptor
72 reduces neurotransmitter release, whereas postsynaptic receptor causes
73 hyperpolarization of neurons [4]. By inhibiting synaptic transmission, GABA_B plays a
74 key role in modulating neuronal activity and impaired function of GABA_B has been
75 associated with multiple neurological and psychiatric diseases [5-7]. Peripheral
76 GABA_B receptors have also been identified, particularly in the gastrointestinal tract
77 [8]. Baclofen, a GABA_B agonist, is currently used to treat spasticity of various
78 pathophysiological origins and alcoholism, whereas peripherally-restricted GABA_B
79 agonists devoid of CNS side effects are under investigation to treat
80 gastroesophageal reflux disease [9]. In addition, recent studies indicate that
81 peripheral GABA_B receptors might represent a pharmacological target for tumor
82 therapy [10] and for improving pancreatic β -cell survival [11].

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

100

101 *A missing mechanistic aspect in the regulated delivery of GABA_B to the cell surface*

102 The cell surface density of functional hetero-dimeric GABA_B receptors is
103 controlled by a unique mechanism of delivery from the biosynthetic compartments to
104 the plasma membrane. Early studies reported that recombinant GB1 subunits fail to
105 reach the cell surface when expressed in heterologous systems or overexpressed in
106 ganglion neurons [23]. The explanation of this phenomenon is that GB1 is retained in
107 the ER and does not reach the cell surface in the absence of GB2 [12-14].

108 Subsequent investigations identified an arginine based ER retention/retrieval “RSRR”
109 signal in the carboxyterminal tail of GB1 [22]. This signal is a variant of a more
110 general RXR motif (two arginine residues separated by any aminoacid) found in a
111 variety of ER-retained subunits of multimeric protein complexes, such as the
112 octameric ATP-sensitive K⁺ channels [24] or the N-methyl-D-aspartate (NMDA)
113 receptors [25]. The alanine substitution of the arginine residues of the RXR motif
114 allowed the corresponding GB1-AXA subunit to reach the cell surface even in the
115 absence of GB2. It was then proposed that only the shielding of this retention signal
116 via a coiled-coil interaction with the C-terminus of GB2 might allow the GB1-GB2
117 hetero-dimer to progress along the secretory pathway delivering functional GABA_B
118 receptors to the cell surface [22]. A second key di-leucine motif was subsequently
119 identified upstream of the RXR motif in the carboxyterminal tail of GB1, which also
120 controls the cell surface export of the receptor in hippocampal neurons [26]. It was
121 observed that in the absence of co-expressed GB2 the GB1-AA-ASA mutant,
122 encompassing the mutation of both the RXR and the di-leucine motifs, was much
123 better exported to the cell surface than the wild type GB1 or the single GB1-ASA
124 mutant. Because the GB1-ASA mutant was partially accumulated within the trans-
125 Golgi network (TGN) and because this accumulation was rescued by the double
126 mutation, a two-step sequential mechanism regulating GABA_B export at distinct
127 checkpoints in the ER and the Golgi was postulated [26]. However, a missing
128 mechanistic aspect of the complex regulation of the GABA_B hetero-dimer delivery to
129 the cell surface has remained for long time: the nature of the tether(s) that in the
130 absence of association with GB2 retain(s) GB1 in the ER and in the TGN. This tether,
131 which is responsible for the predominant ER localization of the GB1 subunit in
132 primary neurons [27], might play an essential role in GABA_B receptor physiology.

133 *PRAF2, the missing ER gatekeeper controlling GABA_B delivery to the cell surface*

134 The coiled-coil domain of the carboxyterminal tail of the GB1, containing both
135 RXR and LL motifs, is presumably involved in the interaction with a putative ER-
136 resident “gatekeeper”, which regulates the delivery of the GABA_B heterodimer to the
137 cell surface.

138 During the past ten years, multiple GB1 interacting proteins have been
139 reported, which regulate GABA_B expression in some way, often interacting with the
140 GB1 coiled-coil domain. However, under physiological conditions, none of these
141 proteins appears to retain GB1 in the ER via a direct interaction. The cytoplasmic
142 scaffolding protein 14-3-3 σ can dissociate GABA_B receptor hetero-dimers present at
143 the plasma membrane [28] [29] and does not interact with GB1 in the ER [29, 30].
144 The cytoplasmic coatamer COP1 might rather be involved in the retrieval of
145 monomeric GB1 from the cis-Golgi to the ER [30]. Msec-7, an ARF1, 3,6 guanine-
146 nucleotide exchange factor, which specifically interacts with the GB1 tail di-leucine
147 motif, enhances GABA_B trafficking from the Golgi to the cell surface, after its exiting
148 from the ER. GISP, another scaffolding protein of the AKAP family, was reported to
149 enhance GABA_B receptor function possibly by slowing down receptor desensitization
150 and lysosomal sorting [31]. The transcription factor CCAAT/enhancer-binding protein
151 homologous protein CHOP is markedly overexpressed upon ER stress, for example
152 during cerebral ischemia. In this context, CHOP interacts with both GB1 and GB2 in
153 the ER preventing or disrupting hetero-dimerization, and forward trafficking of the
154 receptors to the plasma membrane [32]. Because of its marginal level of expression
155 under physiological conditions and the fact that it is not an ER resident protein, it is
156 unlikely that CHOP is responsible for GB1 retention in this compartment. In
157 conclusion, none of the GB1-interacting partners identified so far displayed the

158 expected biochemical and/or functional properties for a GB1 gatekeeper in the ER.

159 Ultimately, Prenylated Rab Acceptor Family 2 (PRAF2), an ER-resident 4-
160 transmembrane domain protein, was identified recently as a major GABA_B
161 gatekeeper [27]. PRAF2 interacts with the GB1 subunit of GABA_B but not with the
162 GB2 subunit, this interaction involving both RXR and LL motifs of the carboxy-
163 terminal tail of GB1. PRAF2-bound GB1 remains trapped in the ER and its release
164 only occurs upon competitive displacement of PRAF2 by GB2. Thus, the local
165 concentration of PRAF2, relative to that of GB1 and GB2, tightly controls cell surface
166 receptor density and thus impacts on GABA_B function in neurons or in transfected
167 cells (Figure 1). Interestingly, minor (less than two-fold) modifications of PRAF2
168 concentration in a localized region of mouse brain, the ventral tegmental area (VTA),
169 caused major behavioral effects in animal models (Figure 2). These effects could be
170 attributed to the resulting local changes in cell surface GABA_B receptor expression
171 [27].

172 Although identified ten years ago as an interacting partner of the CCR5
173 chemokine receptor in a two-hybrid screen [33], PRAF2 had no clearly identified
174 functions so far; it was found enriched in human brain [34], overexpressed in multiple
175 cancers [35, 36] being correlated with poor prognosis [37]. PRAF2 belongs to a
176 family of three ubiquitous 4-transmembrane domain proteins [35], localized either in
177 the ER (PRAF2 and 3) or in the Golgi apparatus (PRAF1) (Table 1). Similarly to the
178 function of PRAF2 on GABA_B export, PRAF3 was reported to delay the ER exit of the
179 Na⁺-dependent glutamate transporter Excitatory Amino-Acid Carrier 1 (EAAC1) [38,
180 39]. PRAF1, the first identified member of the PRAF family, was proposed to activate
181 the dissociation of prenylated Rab proteins from the GDP dissociation inhibitor GDI,
182 thus facilitating the association of prenylated Rabs with target Golgi membranes [40].

183 The property of PRAF1 (also known as PRA1) to interact with numerous prenylated
184 Rab GTPases [41] led to classify PRAF family members among mammalian Yips
185 (Ypt-interacting proteins, Ypts being the yeast Rab homologues) [42]. Mammalian
186 Yips (Table 1) also comprise 5-transmembrane proteins of the YIPF (YIP1 domain
187 family, 1 to 7) and YIF1 (Yip1 interacting factor homolog A and B) groups, as well as
188 the 2-transmembrane protein family REEP (receptor expression enhancing proteins,
189 1 to 6). Collectively, Yips are involved in the control of cellular vesicle trafficking
190 between the ER and the Golgi network, REEPs displaying the additional function of
191 adapter proteins for cargo receptors [43].

192

193 *PRAF gatekeepers might regulate other GPCRs in various tissues*

194 Although the mechanistic aspects of PRAF2-dependent regulation have only
195 been elucidated for the GABA_B receptor, it is quite plausible that PRAFs might
196 collectively regulate several other GPCRs. Indeed, as indicated above, PRAF2 was
197 originally identified in two-hybrid screens as an interaction partner of the chemokine
198 CCR5 receptor, regulating in some way its membrane localization [33]. PRAF3, on
199 the other hand, was reported to restrict the subcellular localization of the β 2-
200 adrenoceptor and the D2-dopaminergic receptor to the ER [39]. In preliminary
201 studies, we found that PRAF gatekeepers associate (in BRET and co-IP
202 experiments) with other chemokine receptors, such as CCR2 and CCR7, and with 5-
203 HT2 serotonergic receptors (unpublished data). Finally, both PRAF2 [35] and
204 PRAF3 [38, 44] are expressed in most tissues, where they coexist with multiple
205 GPCRs.

206 In addition to the GABA_B receptor, several other GPCRs undergo regulated
207 translocation to the plasma membrane from ER or Golgi stores [2], where they might

208 possibly be retained by PRAFs or PRAF-like gatekeepers. In tubular renal cells,
209 intracellular dopamine D1 receptors are recruited to the plasma membrane by
210 agonist activation of cell surface receptors [45] or via atrial natriuretic peptide-
211 dependent heterologous activation [46]. Similarly, the sustained stimulation of μ -
212 opioid receptors can redistribute δ -opioid receptors (DOP) to the plasma membrane
213 *in vivo*, improving DOP-dependent anti-nociceptive effects [47]. Several other
214 GPCRs poorly expressed at the plasma membrane, such as the odorant receptors
215 [48], the human GnRH receptor [49], the α_{1D} -adrenoceptor [50], the LH receptor [51],
216 and the CCR5 chemokine receptor [52] represent other candidates for gatekeeper-
217 controlled translocation to the cell surface.

218 Although the functional organization of the GABA_B receptor as an obligatory
219 hetero-dimer is quite unique among GPCRs, it is currently admitted that most if not
220 all the receptors of this family are expressed at the cell surface as homo-dimers [53].
221 Several observations indicate that, among other functions, homo-dimerization might
222 also contribute to quality control of newly synthesized receptors [54]. An interesting
223 hypothesis in this context is that the homo-dimerization of two correctly folded
224 receptors might displace the interaction with resident gatekeepers similarly to hetero-
225 dimerization. The GABA_B model might thus represent an atypical example of a more
226 general phenomenon.

227

228 *Potential involvement of PRAFs in human disease*

229 Similar or even larger changes of PRAF2 than those we observed inducing
230 phenotypic effects in animals can either be detected physiologically, during neuronal
231 differentiation for example, or in pathological samples, such as human cancer tissues
232 [36] and brain areas of persons deceased after excessive alcohol intake [55]. These

233 findings indicate a potential causative connection between the concentration of PRAF
234 gatekeepers and pathological changes in humans. In addition, although not
235 functionally investigated so far, several variants of PRAF2 have been reported (see
236 the link www.nextprot.org/db/entry/NX_O60831/sequence), which are located in
237 various domains of the protein, including within the regions that are likely at the
238 interface with GB1 [27]. Therefore, both qualitative and quantitative changes of these
239 gatekeepers might have an impact on GPCR receptivity and consequently be
240 involved in human disease.

241 Finally, the new concept of GPCR gatekeepers might lead to revisit already
242 known pathophysiological mechanisms. For example, in the kidney, where both
243 PRAF2 and PRAF3 are present, variants of the V2 vasopressin receptor (V2R) are
244 poorly expressed at the cell surface of tubular cells, impairing water reabsorption and
245 causing nephrogenic diabetes insipidus [56]. Membrane-permeable pharmacological
246 chaperones have been reported, which bind to intracellular receptors and facilitate
247 their cell surface export, with a partial rescue of receptor function and of excessive
248 diuresis [57]. It would be interesting in this context to examine the association of wild-
249 type and variant V2Rs with PRAFs, as an increased affinity of the variant receptor for
250 its cognate gatekeeper, might well explain its retention in the ER.

251

252 **Concluding remarks**

253 The identification of the resident gatekeeper, which retains the GB1 subunit of the
254 GABA_B receptor in the ER upon interaction with both the di-leucine and RXR motifs
255 of the carboxyterminal tail of GB1 until it is displaced by the GB2 subunit, completes
256 the picture of the complex mechanism regulating the delivery of functional GABA_B
257 receptors to the cell surface. PRAFs were already reported to interact with and/or

258 regulate several other GPCRs and plasma membrane transporters. In addition,
259 among mammalian Yips, REEP family proteins, which were initially considered as
260 GPCR escorts, might also behave like gatekeepers regulating the ER exit of some
261 receptors [43]. Therefore, for many GPCRs and more generally for multi-span
262 plasma membrane proteins, the gatekeeper-regulated release from intracellular
263 stores might become a new parameter to be considered when analyzing the
264 effectiveness of receptor response in physiological conditions, during agonist
265 treatment or in the case of pathological export defects. These considerations raise a
266 number of new questions* in the field, such as how gatekeepers are themselves
267 regulated, how many other GPCRs are potentially regulated by these types of
268 gatekeepers, in which organs and for which functions, and are changes in
269 gatekeeper concentration or point mutations at the interface with receptors involved
270 in human disease. We anticipate that these questions are sufficiently important to
271 promote additional investigations in the years to come.

272

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278

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448
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451

452 **Figure Legends**

453

454 **Figure 1. Retention and release of GB1 is governed by the relative**
455 **concentrations of GB1, GB2 and PRAF2.** (A) In neurons GB2 and PRAF2 compete
456 for binding to GB1. Hetero-dimerization of GB1 with GB2 allows the release from the
457 ER and the export of the functional GABA_B receptor to the cell surface. Association
458 with PRAF2 maintains the GB1 subunit in the ER. (B) The heterologous expression
459 of GB1 in transfected cells, which contains endogenous PRAF2 and no GB2,
460 reproduces the conditions existing in neurons of GB2-KO mice. GB1 is retained in
461 the ER and no GABA binding sites can be measured at the cell surface. (C) If PRAF2
462 concentration is decreased (i.e. siRNA treatment) in transfected cells expressing
463 exogenous GB1, or GB1 markedly overexpressed, the available PRAF2 molecules
464 are completely saturated by GB1. Unbound GB1 is not retained and can reach the
465 cell surface. Surface GABA binding sites can be measured in these conditions
466 corresponding to surface GB1. However, because of the absence of GB2 subunits,
467 functional GABA_B receptors capable of transducing a signal are lacking.

468

469 **Figure 2. Locomotor activity in mice expressing exogenous PRAF2 in the VTA.**

470 To examine whether perturbing the stoichiometry of GB1, GB2 and PRAF2 might
471 affect GABA_B-dependent regulations *in vivo*, PRAF2 concentration was enhanced (2-
472 3 folds) in the ventral tegmental area (VTA), a brain area containing GABA_B-
473 expressing neuron, by stereotaxic injection of an adenovirus driving the local
474 expression of the exogenous gatekeeper. Activation of the dopaminergic circuits from
475 the VTA to the nucleus accumbens (NAcc) produces locomotor activity, which is
476 inhibited by GABA_B activation (blue-boxed panels). In case of loss of surface GABA_B

477 receptors in VTA dopaminergic neurons, caused by PRAF2-mediated GB1 retention,
478 dopamine release is increased and locomotor activity enhanced (red-boxed panels).
479 The unilateral change of PRAF2 concentration in the VTA led to a remarkable
480 contralateral turning phenotype (see supplemental videos in [26]).
481

Name	Other Names	Yeast homologue	Gene ID	AA number	TM	Localization / Functions	Regulated Receptors/Transporters
YIPF5 (Yip1 domain family, member 5)	Yip1; YPT-interacting protein 1A; Yip1A; FinGER5; SMAP5; SB140	Yip1p	81555	257	5	ER exit sites, ER to Golgi Transport [58, 59]	
REEP1 (receptor expression-enhancing protein1)	Yip2a	Yip2, Yop1	65055	201	2	ER morphology and dynamics[60]	Odorant receptors [48]; bitter taste receptor [61]; α_2c AR [43]
REEP2	Yip2d		51308	252	2	ER resident [43]	Odorant receptors [48]; sweet receptor [62]; α_2c AR [43]
REEP3	Yip2b		221035	255	2	clearing ER from metaphase chromatin [63]	Odorant receptors [48]
REEP4	Yip2c		80346	257	2	clearing ER from metaphase chromatin [63]	
REEP5	Yip2e		7905	189	2		
REEP6	Yip2f		92840	184	2	ER resident [43]	α_2c AR [43]
RABAC1,	Yip3; PRA1, PRAF-1	Yip3	10567	185	4	Golgi [44]; Prenylated Rab acceptor [40]	
PRAF-2	Yip6a; JM4		11230	178	4	ER [35]	CCR5 [33], GABA _B [27]
ARL6IP5	JWA; PRA2; jmx; hp22; PRAF-3 ; DERP11; HSPC127; addicsin; GTRAP3-18; Yip6b		10550	188	4	ER [44]; control of ER exit [39]	EAAC1 [38]; EAAT1,2,4; β_2 AR; $\alpha_2\beta$ AR; D ₂ R [39]; DOR [64]
YIPF6	FinGER6; MGC21416; Yip4	Yip4	286451	237	5	Golgi [65]	
YIPF1	FinGER1; DJ167A19.1, Yip5a	Yip5	54432	306	5	Golgi [65]	
YIPF3	KLIP1; FinGER3; C6orf109; dJ337H4.3; Yip5b		25844	350	5	Cis-Golgi [66] [65]	
YIPF2	FinGER2; Yip5c		78992	316	5	Golgi [65]	
YIF1A (Yip1 interacting factor homolog A)	54TM; YIF1; YIF1P; FinGER7	Yif1	10897	293	5	ER to Golgi transport; delivery into dendrites [67]	
YIF1B	FinGER8		90522	314	5	Golgi [65]; ER-Golgi trafficking [68]	5-HT1AR [68]
YIPF7	FinGER9, Yip1B		285525	280	5	ERGIC; cisGolgi; skeletal muscle [69]	
YIPF4	FinGER4; Nbla11189		84272	244	5	Cis-Golgi [66]	

482

483 **Table 1. Classification and nomenclature of PRAFs and mammalian Yips**

484 ***Outstanding Questions Box**

485

486 - How many GPCRs undergo a similar regulation of their cell surface export by
487 PRAFs, other Yips or functionally equivalent additional ER and Golgi gatekeepers?

488 - What is the molecular basis of gatekeeper selectivity for cargo receptors?

489 - How are ER and Golgi gatekeepers coordinated to regulate the export of a specific
490 receptor?

491 - Is GPCR oligomerization a more general mechanism controlling receptor export to
492 the cell surface?

493 - How are PRAFs and more generally Yips themselves regulated? How this type of
494 regulation might be connected with receptor function and regulation?

495 - Are there pathological conditions associated with mutations or quantitative changes
496 of receptor gatekeepers?

497 - Can diseases associated with impaired cell surface targeting of GPCR variants be
498 explained by enhanced interaction between receptors and ER or Golgi gatekeepers?

499 - Is it possible to target receptor – gatekeeper interfaces with chemical compounds to
500 enhance receptor export and treat diseases?

Highlights

Functional GABA_B receptors are obligate hetero-dimers 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, endoplasmic reticulum-resident gatekeeper

The stoichiometry of PRAF2 relatively to GB1 and GB2 is a key parameter for GABA_B function *in vivo*

Figure 1

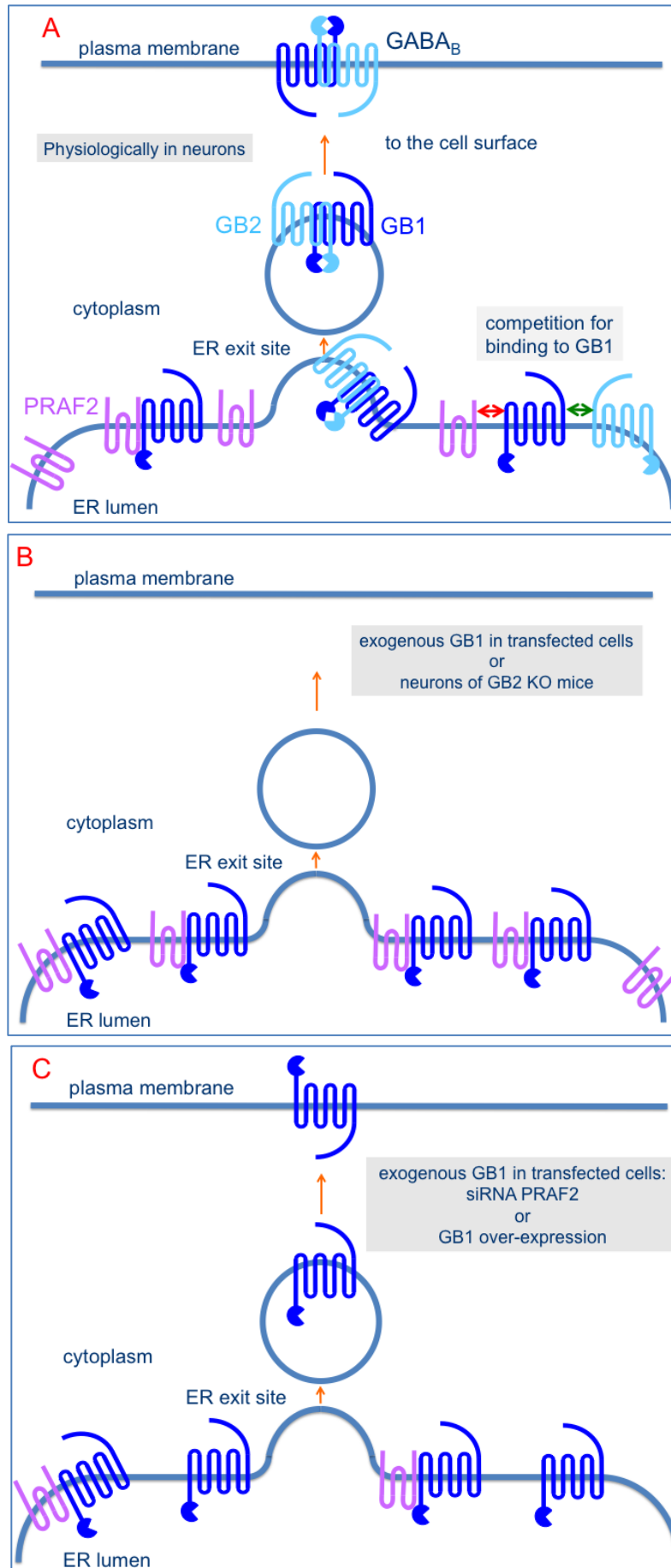


Figure 1

Figure 2

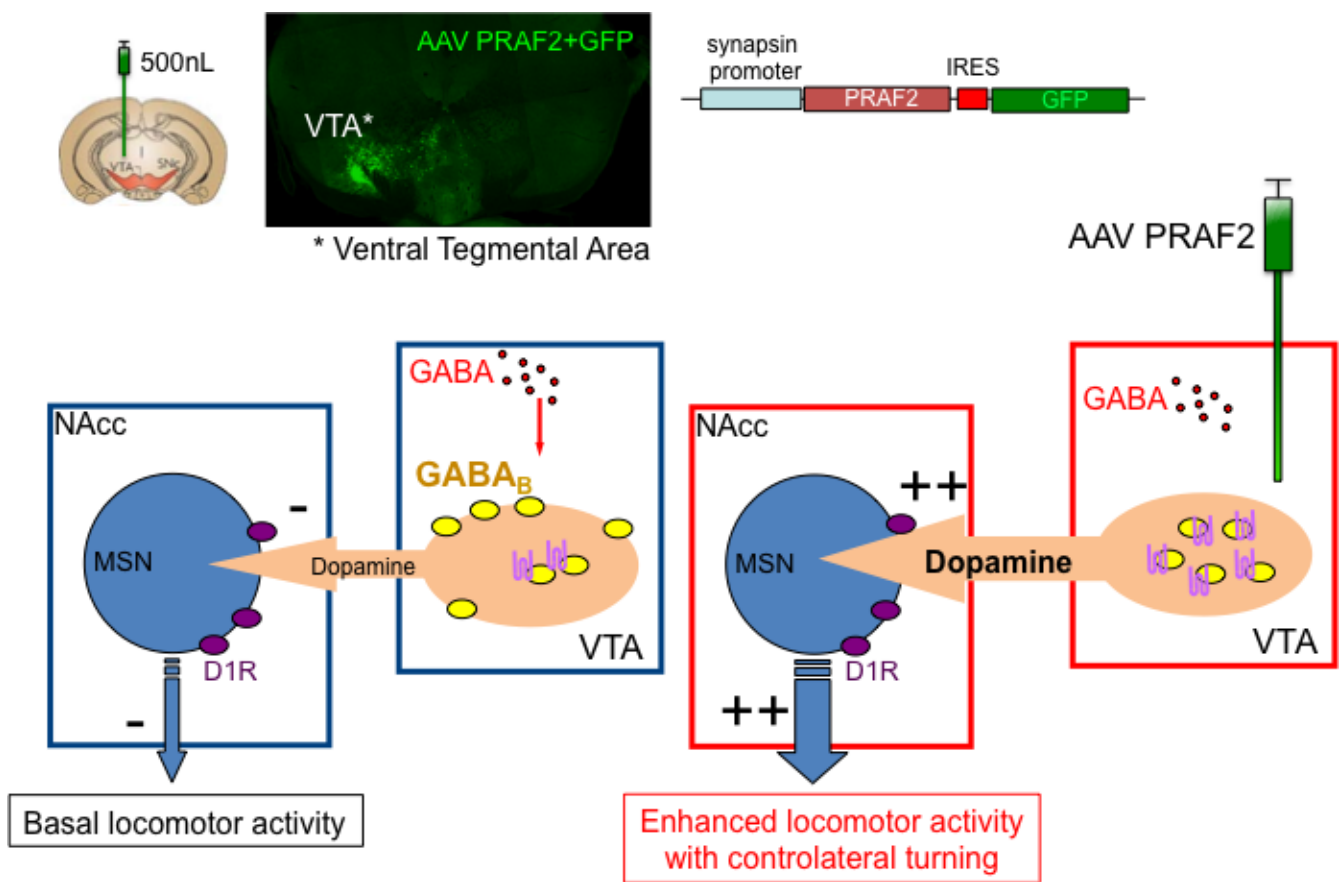


Figure 2