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Multiple motifs regulate the trafficking of $GABA_B$ receptors at distinct checkpoints within the secretory pathway

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γ-Aminobutyric acid type B receptors ($GABA_B$) are G-protein-coupled receptors that mediate GABAergic inhibition in the brain. Their functional expression is dependent upon the formation of heterodimers between $GABA_BR1$ and $GABA_BR2$ subunits, a process that occurs within the endoplasmic reticulum (ER). However, the mechanisms that regulate receptor surface expression remain largely unknown. Here, we demonstrate that access to the cell surface for $GABA_BR1$ is sequentially controlled by an RSR(R) motif and a LL motif within its cytoplasmic domain. In addition, we reveal that msec7-1, a guanine-nucleotide-exchange factor (GEF) for the ADP-ribosylation factor (ARF) family of GTPases, critical regulators of vesicular membrane trafficking, interacts with $GABA_BR1$ via the LL motif in this subunit. Finally, we establish that msec7-1 modulates the cell surface expression of $GABA_B$ receptors, a process that is dependent upon the integrity of the LL motif in $GABA_BR1$. Together, our results demonstrate that the cell surface expression of the $GABA_BR1$ subunit is regulated by multiple motifs, which act at distinct checkpoints in the secretory pathway, and also suggest a novel role for msec7-1 in regulating the membrane trafficking of $GABA_BR1$ subunits.
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Introduction

$GABA_B$ receptors are GPCRs that mediate GABAergic inhibition in the brain. They exert their effects mainly by regulating voltage-gated Ca^{2+} channels and G-protein activated inwardly rectifying K^+ channel (Calver et al., 2002; Couve et al., 2000). Two $GABA_B$ receptor subunits, $GABA_BR1$ and $GABA_BR2$, have been identified. In addition, several splice variants have been described for $GABA_BR1$ (Calver et al., 2002; Couve et al., 2000; Kaupmann et al., 1997). Gene knock-out studies have revealed that $GABA_BR1$ and $GABA_BR2$ are essential components of CNS receptors (Gassmann et al., 2004; Prosser et al., 2001; Schuler et al., 2001; Thuault et al., 2004). Functional $GABA_B$ receptors are heterodimers formed by $GABA_BR1$ and $GABA_BR2$ subunits. Interestingly, heterodimerization has been shown for many GPCRs, but to date, $GABA_B$ receptors are unique in their absolute requirement for heterodimerization for functional expression (Couve et al., 2000; Nelson et al., 2002).

The regulation of $GABA_B$ receptor assembly and delivery to the plasma membrane are likely to be of major significance in controlling the efficacy of synaptic inhibition, as in contrast to most other GPCRs, $GABA_B$ receptors do not undergo agonist-dependent internalization and exhibit long cell surface half-lives (Fairfax et al., 2004; Perroy et al., 2003). Studies on other oligomeric transmembrane proteins have revealed that the cell surface expression is controlled at multiple checkpoints along the secretory pathway to ensure that only properly folded and assembled receptors access the plasma membrane (Ma et al., 2002; Ren et al., 2003). Indeed, proteins synthesized in the ER travel between the various intracellular compartments using different membrane vesicle systems, such as COPI, COPII, and clathrin-coats, that can modulate surface expression (Barlowe, 2000; Kirchhausen, 2000). However, proteins involved in the $GABA_B$ receptors trafficking are largely unknown. To address these issues, recombinant expression has been primarily utilized.

Abbreviations: $GABA_B$, γ-aminobutyric acid type B receptors; GPCR, G-protein-coupled receptors; ER, endoplasmic reticulum; LL, di-leucine; GEF, guanine-nucleotide-exchange factor; ARF, ADP-ribosylation factor; TGN, trans-Golgi network; PH, pleckstrin homology domain.

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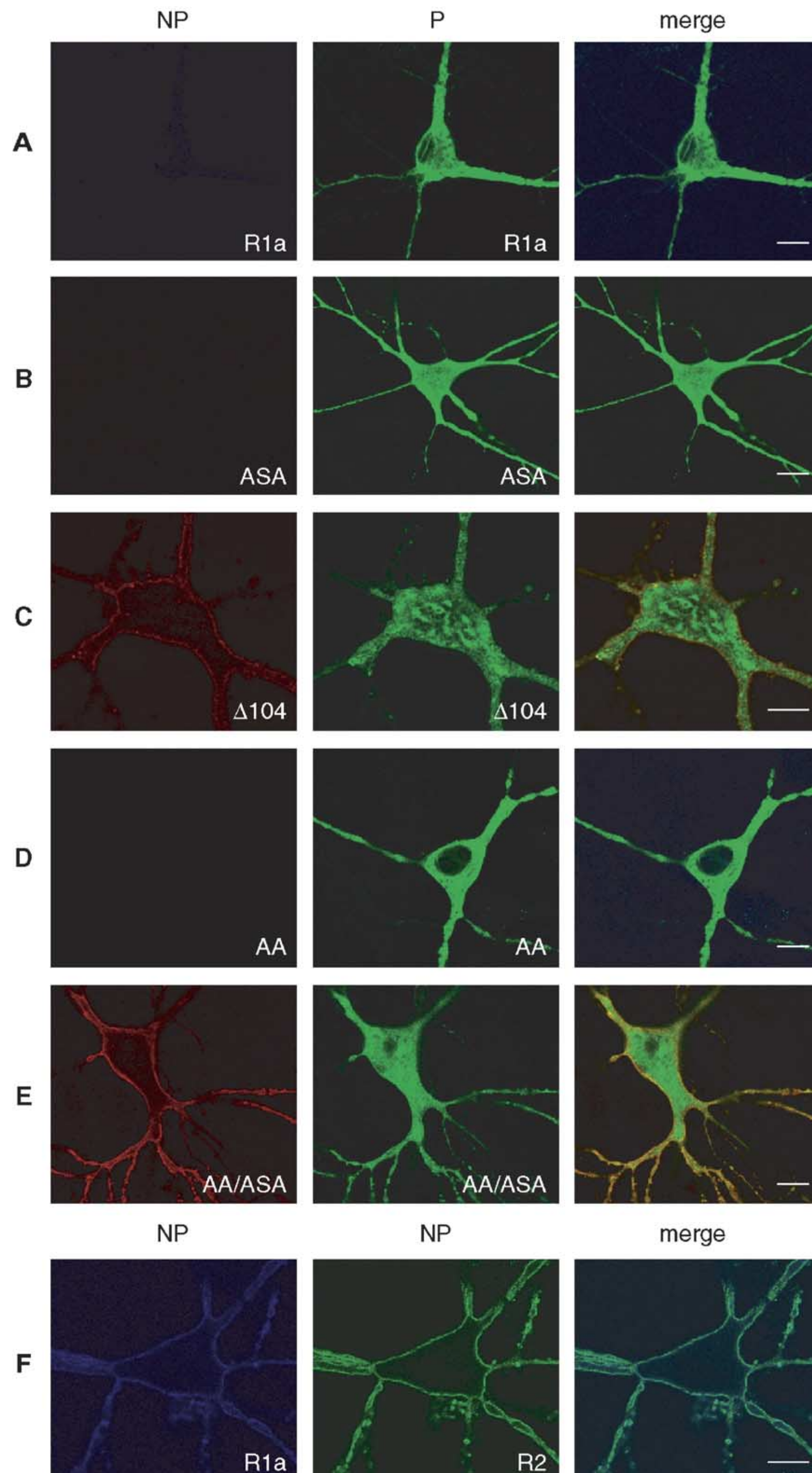

Fig. 1. Cell surface expression of wild type and mutant $GABA_BR1$ constructs in hippocampal neurons. Cultured hippocampal neurons were microinjected with $GABA_BR1$ (A), $GABA_BR1$-ASA (B), $GABA_BR1$-Δ104 (C), $GABA_BR1$-AA (D), $GABA_BR1$-AA/ASA (E), or $GABA_BR1$ and $GABA_BR2$ (F). For A to E, 30 h after microinjection neurons were stained under non-permeabilized (NP) conditions with 9E10 antibody and a Texas red conjugated secondary antibody. Neurons were then permeabilized (P) and stained with 9E10 antibody and a secondary conjugated to FITC. For F, neurons were stained under non-permeabilized (NP) conditions with 9E10 antibody, followed by a Cy-5 secondary antibody for $GABA_BR1$ and HA antibody followed by a Texas red secondary antibody for $GABA_BR2$. The scale bar = 10 μm.

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