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# Modulation of $Ca_v1.3$ $Ca^{2+}$ channel gating by Rab3 interacting molecule

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ABSTRACT

Neurotransmitter release and spontaneous action potentials during cochlear inner hair cell (IHC) development depend on the activity of $Ca_v1.3$ voltage-gated L-type $Ca^{2+}$ channels. Their voltage- and $Ca^{2+}$-dependent inactivation kinetics are slower than in other tissues but the underlying molecular mechanisms are not yet understood. We found that Rab3-interacting molecule-2α (RIM2α) mRNA is expressed in immature cochlear IHCs and the protein co-localizes with $Ca_v1.3$ in the same presynaptic compartment of IHCs. Expression of RIM proteins in tsA-201 cells revealed binding to the β-subunit of the channel complex and RIM-induced slowing of both $Ca^{2+}$- and voltage-dependent inactivation of $Ca_v1.3$ channels. By inhibiting inactivation, RIM induced a non-inactivating current component typical for IHC $Ca_v1.3$ currents which should allow these channels to carry a substantial window current during prolonged depolarizations. These data suggest that RIM2 contributes to the stabilization of $Ca_v1.3$ gating kinetics in immature IHCs.



## Introduction

Depolarization-induced $Ca^{2+}$ entry through voltage-gated $Ca^{2+}$ channels (VGCC) into electrically excitable cells is a key process regulating numerous physiological processes. Ten $Ca^{2+}$ channel isoforms within three classes ($Ca_v1$–3) with different biophysical properties and subcellular localizations (Catterall et al., 2005) accomplish these diverse functions. Among isoforms gating is further fine-tuned by alternative splicing (Lipscombe and Raingo, 2007; Singh et al., 2008), accessory α2-δ and β-subunits (Davies et al., 2007; Dolphin, 2003) as well as by other channel associated proteins (Calin-Jageman and Lee, 2008; Dai et al., 2009). Among the high voltage activated $Ca^{2+}$ channels $Ca_v2$ channels predominantly control presynaptic neurotransmitter release in neurons whereas postsynaptic $Ca^{2+}$ influx through $Ca_v1$ (L-type) $Ca^{2+}$ channels (LTCCs) modifies gene transcription and synaptic plasticity (Gomez-Ospina et al., 2006; Zhang et al., 2006). However, presynaptic neurotransmitter release at ribbon synapses from sensory cells of retinal photoreceptors and the cochlea is under the control of $Ca_v1$ rather than $Ca_v2$ channels. Tonic neurotransmitter release in response to light- or sound-evoked graded changes in membrane potential between −60 and −40 mV requires unusually slow inactivation of L-type $Ca^{2+}$ currents in photoreceptors (Rabl and Thoreson, 2002) and cochlear inner hair cells (Grant and Fuchs, 2008; Johnson and Marcotti, 2008; Lee et al., 2007) and maintenance of window currents over prolonged time periods (McRory et al., 2004). Slow inactivation in IHCs is also a prerequisite to produce $Ca^{2+}$ signals and spontaneous action potentials during IHC development (Marcotti et al., 2003).

In VGCCs inactivation during depolarizations is driven by the $Ca^{2+}$ concentration sensed at the inner channel mouth ($Ca^{2+}$-dependent inactivation, CDI) and by transmembrane voltage (voltage-dependent inactivation, VDI). To prevent efficient inactivation by these processes, LTCCs in photoreceptors developed special strategies. Photoreceptor L-type currents are largely carried by $Ca_v1.4$ LTCCs which auto-inhibit their own calmodulin (CaM)-dependent CDI by an intramolecular protein interaction within their C-terminus and their remaining VDI is intrinsically slow (Singh et al., 2006). $Ca_v1.3$ channels in the heart and brain display pronounced CDI and fast VDI (Koschak et al., 2001; Mangoni et al., 2003; Yang et al., 2006) but both processes are very slow in $Ca_v1.3$ channels of cochlear inner hair cells (Grant and Fuchs, 2008; Johnson and Marcotti, 2008; Marcotti et al., 2003). Strongly reduced CDI in IHCs can be explained by $Ca^{2+}$ binding proteins, such as CaBP1 and CaBP4 (Cui et al., 2007; Lee et al., 2007; Striessnig, 2007; Yang et al., 2006), which compete with CaM binding and $Ca^{2+}$ sensing to the $Ca_v1.3$ α1 subunit. $Ca_v\beta2$ was recently shown to slightly affect CDI in $Ca_v\beta2$ deficient IHCs, however VDI remained unaltered (Neef

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et al., 2009). Even if CDI is completely inhibited by these proteins, the remaining VDI of $Ca_v1.3$ currents in IHCs is still much slower than the VDI of $Ca_v1.3$ currents in heart, brain or heterologously expressed $Ca_v1.3$ channel complexes (Koschak et al., 2001; Mangoni et al., 2003; Platzer et al., 2000). So far the molecular basis for this physiologically relevant difference is unclear. The underlying mechanisms could be

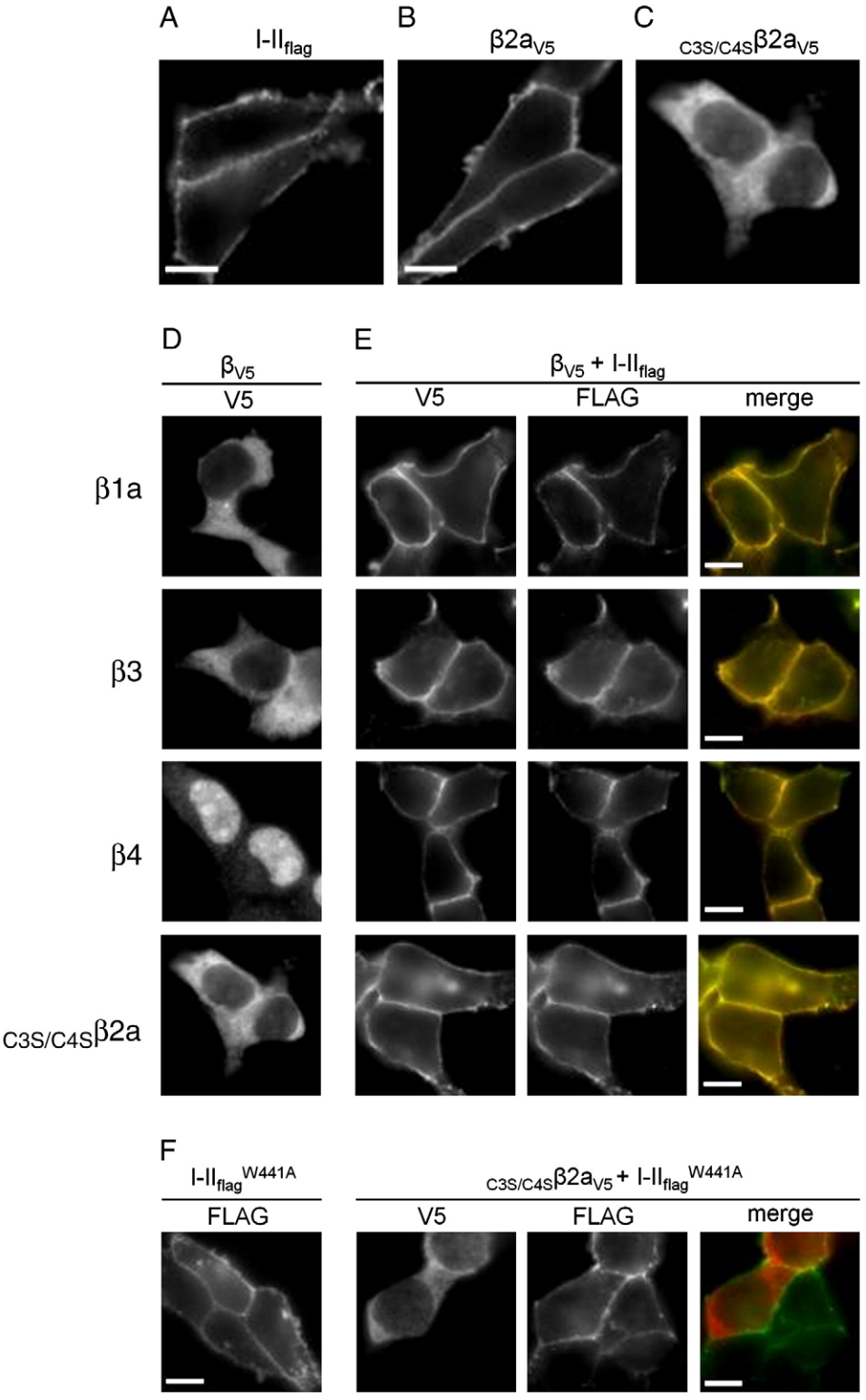


**Fig. 1.** A flag-tagged I–II-linker of $Ca_v1.3$ channels targets β subunits to the plasma membrane in tsA-201 cells. Immunofluorescence images of C-terminally V5-tagged β subunits and C-terminally flag-tagged I–II-linker of $Ca_v1.3$ ($I–II_{flag}$) heterologously expressed in tsA-201 cells. (A–C) Expression of $I–II_{flag}$ (A), palmitoylated $\beta2a_{V5}$ (B), and the palmitoylation deficient $_{C3S/C4S}\beta2a_{V5}$ (C). (D) Expression of only $\beta1a_{V5}$, $\beta3_{V5}$, $\beta4_{V5}$, and $_{C3S/C4S}\beta2a_{V5}$. Note that $\beta4_{V5}$ shows nuclear localization as expected from previous studies. (E) Co-expression of the flag tagged I–II-linker and the indicated C-terminally V5 tagged β-subunits. (F) Expression of either $I–II_{flag}{}^{W441A}$ alone or together with $_{C3S/C4S}\beta2a_{V5}$, respectively. Representative cells from 3 independent experiments are shown. Scale bar = 10 µm.