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Commentary

Glycosylation of $\alpha_2\delta_1$ subunit: a sweet talk with $Ca_v1.2$ channels

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Commentary to: Identification of glycosylation sites essential for surface expression of the $Ca_v\alpha_2\delta_1$ subunit and modulation of the cardiac $Ca_v1.2$ channel activity (J. Biol. Chem. 2016, pp. 4826-4843)

Key words: Calcium channel – $Ca_v1.2$ channel – Ancillary subunit – $\alpha_2\delta_1$ subunit – Glycosylation — Trafficking

Voltage-gated $Ca_v1.2$ calcium (Ca^{2+}) channels are expressed in a variety of tissues where they serve important physiological functions. In nerve cells, they are predominantly expressed on the cell body and spines where they shape neuronal firing and support Ca^{2+}-dependent gene transcription processes (Lichvárová and Lacinová 2015; Ortner and Striessnig 2016). In the heart, they supply Ca^{2+} influx that activates type 2 ryanodine receptors, allowing rapid mobilization of the sarcoplasmic reticulum Ca^{2+} store and the increase in myoplasmic Ca^{2+} concentration that triggers contraction. In neuroendocrine cells, they support an excitation-secretion process that account for the release of a number of hormones and neurotransmitters. Finally, the essential role of $Ca_v1.2$ channels is further exemplified by their implication in a number of pathophysiological conditions, essentially linked to an alteration of the channel protein expression and function (Zamponi et al. 2015).

The $\alpha_2\delta$ subunit is an integral component of the channel complex, which is essential for cell surface trafficking and functional expression of the Ca_v pore-forming subunit (Dolphin 2012; Geisler et al. 2015). While $\alpha_2\delta$ is the product of a single gene, it undergoes a number of post-translational modifications that have important consequences on the fate and behavior of the protein. First, a proteolytic cleavage generates α_2 and δ peptides, which remain associated by disulphide bonds. Second, a glycosylphosphatidylinositol (GPI) moiety is attached to the C-terminal region of the δ domain, and contributes to the anchoring of the protein to the plasma membrane (Davies et al. 2010). Third, $\alpha_2\delta$ is extensively glycosylated, which accounts for about a third of its molecular weight (Marais et al. 2001). However, the functional role of $\alpha_2\delta$ glycosylation remains largely unknown.

Asparagine (N)-linked glycosylation, which refers to the enzymatic attachment of glycan moieties to newly synthetized proteins, has emerged as an essential mechanism controlling ion channel function (Lazniewska and Weiss 2014). For instance, N-linked glycosylation of $Ca_v1.2$ and $Ca_v3.2$ channels is necessary for effective surface trafficking and functional expression of the channel protein (Weiss et al. 2013; Park et al. 2015; Ondacova et al. 2016), and more generally affects expression of other ion channel families (Penuela et al. 2014). In a recent study published in the *Journal of Biological Chemistry*, Tétreault and colleagues assessed the role of asparagine-linked glycosylation on the trafficking of $\alpha_2\delta_1$ and its consequence on $Ca_v1.2$ channel function (Tétreault et al. 2016). In order to assess to role of N-glycosylation in the trafficking of $\alpha_2\delta_1$ to the cell surface, the authors used site-directed mutagenesis to eliminate consensus N-glycosylation sites (N-X-S/T) by replacing the asparagine residue (N) to a glutamine (Q). Expression of glycosylation-deficient $\alpha_2\delta_1$ mutants in HEK 293 cells revealed that glycosylation at some specific loci is essential for surface expression of the protein. For instance, glycosylation at asparagine N633 was found essential for expression of $\alpha_2\delta_1$ at the cell surface, and consistent with the role of $\alpha_2\delta_1$ in the functional expression of $Ca_v1.2$ channels, L-type currents were abolished. In contrast, while disruption of glycosylation at asparagine N812 resulted in a 50% decrease of the surface expression of $\alpha_2\delta_1$, the L-type current density was not dra-

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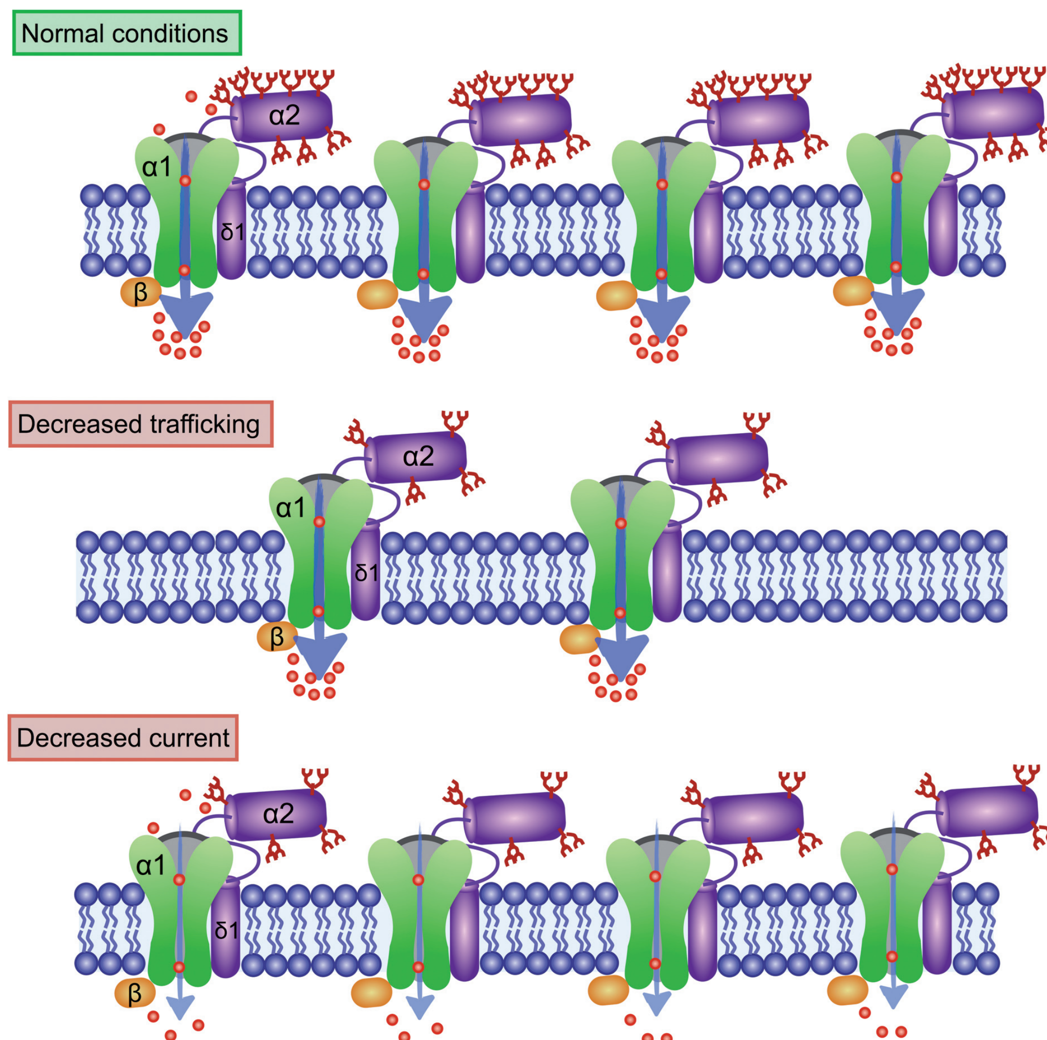

Figure 1. Modulation of $Ca_v1.2$ channel by $\alpha_2\delta_1$-dependent glycosylation. Glycosylation of $\alpha_2\delta_1$ controls functional expression of $Ca_v1.2$ channels *via* two distinct mechanisms: (1) a trafficking-dependent pathway that involves the chaperone role of $\alpha_2\delta_1$ in the sorting of the channel to the cell surface (middle panel); and (2) a trafficking-independent regulation mediated by the direct modulation of the channel gating at the surface (bottom panel).

matically affected. Analysis of multiple mutant combinations also revealed a less essential implication of some of the other glycosylation loci in the expression of $\alpha_2\delta_1$. Altogether, the authors demonstrated that N-glycosylation of $\alpha_2\delta_1$ at some specific loci is essential for the biogenesis and surface expression of the protein, and influences functional expression of the $Ca_v1.2$ channel, but stopped short in demonstrating the implication of $\alpha_2\delta_1$ glycosylation in the surface expression of the $Ca_v1.2$ channel protein.

The novel and important findings of Tétreault and colleagues raise interesting questions about the role of $\alpha_2\delta_1$ glycosylation in the functioning of the calcium channel complex. Alteration of the L-type Ca^{2+} conductance upon expression of the glycosylation-deficient $\alpha_2\delta_1$ subunit may result either from a decreased surface expression of the $Ca_v1.2$ channel protein, or from an alteration of the functioning of the channel (Figure 1). Previous reports indicated that glycosylation at asparagines N136 and N184 influence functional expression of $Ca_v2.2$ channels (Sandoval et al. 2004). In the study by Tétreault and colleagues, these sites did not play a role in modulating surface expression of $\alpha_2\delta_1$ suggesting a direct action on the functioning of the channel protein independently of the surface trafficking of the channel. It is noteworthy that the N348 locus is located in the VWA domain that contributes to the interaction of $\alpha_2\delta_1$ with the Ca_v subunit (Gurnett et al. 1997; Wu et al.

2015). Therefore, it is likely that glycosylation of $\alpha_2\delta_1$ plays a role in the interplay with the channel protein. In addition, a number of glycosylation sites surround the molecular determinants of antiepileptic/analgesic gabapentin drug binding onto $\alpha_2\delta_1$ (Marais et al. 2001; Field et al. 2006) and may have important influence on the pharmacology of the calcium channel.

Overall, the findings of Tétreault and colleagues provide novel insights into the molecular biology of $\alpha_2\delta_1$, and establish glycosylation as a key post-translational modification in regulating functional expression of the calcium channel complex. Considering that expression of $\alpha_2\delta_1$ was found altered in a number of pathological conditions (Bauer et al. 2009; Lana et al. 2014; Nieto-Rostro et al. 2014; D'Arco et al. 2015), and that glycosylation has been reported to support glucose-dependent increase of ion channel expression (Orestes et al. 2013; Weiss et al. 2013), the notion that the expression of $\alpha_2\delta_1$ is largely influenced by its degree of glycosylation may have important pathological implications, for instance in the development of painful neuropathy that arise from diabetes. Further study will certainly uncover some deeper physiological and pathological implications of the glycosylation of $\alpha_2\delta_1$.

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