Being there: cellular targeting of voltage-gated sodium channels in the heart

Vann Bennett[1,2,3] and Jane Healy[1,2,3]

[1]Howard Hughes Medical Institute, [2]Department of Cell Biology, and [3]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

Voltage-gated sodium (Na_v) channels in cardiomyocytes are localized in specialized membrane domains that optimize their functions in propagating action potentials across cell junctions and in stimulating voltage-gated calcium channels located in T tubules. Mutation of the ankyrin-binding site of $Na_v1.5$, the principal Na_v channel in the heart, was previously known to cause cardiac arrhythmia and the retention of $Na_v1.5$ in an intracellular compartment in cardiomyocytes. Conclusive evidence is now provided that direct interaction between $Na_v1.5$ and ankyrin-G is necessary for the expression of $Na_v1.5$ at the cardiomyocyte cell surface.

Being at the right place at the right time is a well-established principle of success for people that also applies to ion channels. Proper localization is especially important for vertebrate Na_v channels, which must be clustered at high density to generate and/or propagate action potentials. In cardiomyocytes, Na_v channels (principally $Na_v1.5$) are clustered at intercalated discs, where, together with gap junctions, they transmit action potentials between cells, and are also clustered at T tubules, where they activate voltage-sensitive calcium channels (Fig. 1; Cohen, 1996; Scriven et al., 2000). Several findings have implicated the ankyrin family of membrane adaptors in Na_v channel clustering and localization in excitable membranes of both neurons and the heart. Vertebrate Na_v channels share a conserved ankyrin-binding motif (Garrido et al., 2003; Lemaillet et al., 2003). Moreover, Na_v β subunits also exhibit ankyrin-binding activity (Malhotra et al., 2000). Knockout of ankyrin-G in the postnatal mouse cerebellum results in the loss of $Na_v1.6$ from Purkinje neuron axon initial segments (Zhou et al., 1998; Jenkins and Bennett, 2001). $Na_v1.5$ in the heart colocalizes and coimmunoprecipitates with ankyrin-G (Mohler et al., 2004). Furthermore, E1053K mutation in the ankyrin-binding motif of the cardiac $Na_v1.5$ channel abolishes ankyrin binding and causes Brugada Syndrome, a cardiac arrhythmia caused by the loss of function of $Na_v1.5$ (Mohler et al., 2004). The same E1053K mutation also prevents delivery of $Na_v1.5$ to the cardiomyocyte plasma membrane (Mohler et al., 2004).

Correspondence to Vann Bennett: v.bennett@cellbio.duke.edu

Although consistent with a requirement for a direct interaction with ankyrin-G for Na_v channel localization in neurons and cardiomyocytes, other interpretations of these experiments are also possible. For example, knockdown of ankyrin-G in the cerebellum also affected the localization of neurofascin (Zhou et al., 1998; Jenkins and Bennett, 2001), which potentially could stabilize $Na_v1.6$ through interaction with sodium channel β subunits (Ratcliffe et al., 2001). Similarly, the Brugada mutation of $Na_v1.5$ could perturb an interaction with other ankyrins or unrelated proteins.

Lowe et al. (see p. 173 of this issue) address these issues in the heart with the demonstration that siRNA-mediated knockdown of ankyrin-G but not ankyrin-B abolishes the surface expression of $Na_v1.5$ in neonatal as well as adult cardiomyocytes. The study further demonstrates that loss of cell surface $Na_v1.5$ in ankyrin-G–depleted neonatal cardiomyocytes can be reversed by rescue with a version of ankyrin-G that is resistant to siRNA. Moreover, mutation of ankyrin-G that abolishes the binding activity for $Na_v1.5$ also abolishes the ability to restore cell surface $Na_v1.5$. Lowe et al. (2008) also take the localization of ankyrin-G and $Na_v1.5$ to the ultrastructural level with the demonstration by immunogold labeling of coclusters of $Na_v1.5$ and ankyrin-G in adult cardiomyocyte membranes. These data, together with previous observations (Mohler et al., 2004), satisfy the equivalent of Koch's postulates for physiological interactions between proteins: (1) $Na_v1.5$ and ankyrin-G colocalize at high resolution in cardiomyocytes and coimmunoprecipitate from heart tissue; (2) $Na_v1.5$ localization in cardiomyocytes is lost with (a) a point mutation of $Na_v1.5$ that abolishes binding to ankyrin-G, (b) depletion of ankyrin-G, and (c) mutation of ankyrin-G that abolishes binding to $Na_v1.5$; and (3) mutation of $Na_v1.5$ in an organism (in this case humans) causing the loss of ankyrin binding results in a phenotype that is consistent with the loss of $Na_v1.5$ function (i.e., Brugada Syndrome).

These findings raise the question of whether the ankyrin-G pathway is used by other components of intercalated discs and T tubules. In axon initial segments, ankyrin-G is required for the localization of KCNQ2/3 channels and neurofascin in addition to $Na_v1.6$ (Jenkins and Bennett, 2001; Chung et al., 2006; Pan et al., 2006; Rasmussen et al., 2007). Interestingly, each of these proteins has independently evolved an ankyrin-binding motif (Pan et al., 2006). It is possible that multiple, unrelated proteins in the heart also could engage the ankyrin-G machinery for coordinated localization in the same

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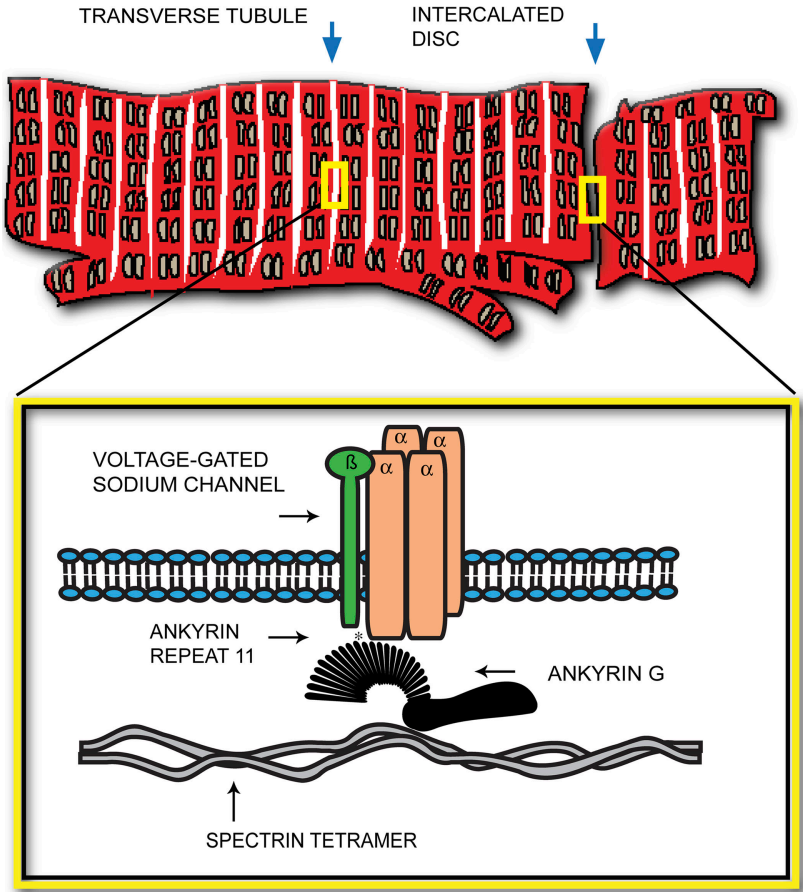

Figure 1. **Schematic model depicting the association of Na_v channels with ankyrin-G/spectrin at intercalated discs and T tubules in cardiomyocytes.** Evidence for this scheme is that $Na_v1.5$, the predominant Na_v channel in the heart, binds to ankyrin-G, requires ankyrin-G for cell surface expression, and, at steady state, colocalizes with ankyrin-G.

specialized domain. Given that T tubules and intercalated discs both contain ankyrin-G yet have distinct proteins, additional mechanisms must exist for fine-tuning the composition of these domains.

What is the role of ankyrin-G in the delivery and/or retention of $Na_v1.5$ to the cell surface? One possibility is that ankyrin-G and spectrin act as a scaffold that retains $Na_v1.5$ after delivery and prevents endocytosis. However, studies of ankyrin-G in epithelial cells suggest a more complex mechanism (Kizhatil et al., 2007a,b). Ankyrin-G in these cells collaborates with β2 spectrin in formation of the lateral membrane and in exit of epithelial cadherin from the trans-Golgi network (Kizhatil et al., 2007a,b). It will be important to evaluate the role of ankyrin-G in the assembly of intercalated discs and $Na_v1.5$-enriched domains of T tubules in cardiomyocytes. A current technical challenge is that these cell surface domains are not fully differentiated in neonatal cardiomyocytes, whereas adult cardiomyocytes frequently lose their morphology and viability after several days in culture. Ultimately, it will be of great interest to resolve the cell biology underlying the targeting of $Na_v1.5$ as well as other membrane-spanning proteins, such as gap junction subunits and calcium channels, whose localization in differentiated cardiomyocytes is key to their physiological function.

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