Voltage-gated sodium (Na\textsubscript{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\textsubscript{v}1.5, the principal Na\textsubscript{v} channel in the heart, was previously known to cause cardiac arrhythmia and the retention of Na\textsubscript{v}1.5 in an intracellular compartment in cardiomyocytes. Conclusive evidence is now provided that direct interaction between Na\textsubscript{v}1.5 and ankyrin-G is necessary for the expression of Na\textsubscript{v}1.5 at the cardiomyocyte cell surface.

Although consistent with a requirement for a direct interaction with ankyrin-G for Na\textsubscript{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\textsubscript{v}1.6 through interaction with sodium channel β subunits (Ratcliffe et al., 2001). Similarly, the Brugada mutation of Na\textsubscript{v}1.5 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\textsubscript{v}1.5 in neonatal as well as adult cardiomyocytes. The study further demonstrates that loss of cell surface Na\textsubscript{v}1.5 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\textsubscript{v}1.5 also abolishes the ability to restore cell surface Na\textsubscript{v}1.5. Lowe et al. (2008) also take the localization of ankyrin-G and Na\textsubscript{v}1.5 to the ultrastructural level with the demonstration by immunogold labeling of coclusters of Na\textsubscript{v}1.5 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\textsubscript{v}1.5 and ankyrin-G colocalize at high resolution in cardiomyocytes and coimmunoprecipitate from heart tissue; (2) Na\textsubscript{v}1.5 localization in cardiomyocytes is lost with (a) a point mutation of Na\textsubscript{v}1.5 that abolishes binding to ankyrin-G, (b) depletion of ankyrin-G, and (c) mutation of ankyrin-G that abolishes binding to Na\textsubscript{v}1.5; and (3) mutation of Na\textsubscript{v}1.5 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\textsubscript{v}1.5 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\textsubscript{v}1.6 (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 manner and the retention of Na\textsubscript{v}1.5 in an intracellular compartment in cardiomyocytes. Conclusive evidence is now provided that direct interaction between Na\textsubscript{v}1.5 and ankyrin-G is necessary for the expression of Na\textsubscript{v}1.5 at the cardiomyocyte cell surface.
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\textsubscript{1.5} to the cell surface? One possibility is that ankyrin-G and spectrin act as a scaffold that retains Na\textsubscript{1.5} 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 \(\beta2\) 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\textsubscript{1.5}-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\textsubscript{1.5} 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.

J. Healy is supported by a predoctoral grant from the American Heart Association.

Submitted: 18 December 2007
Accepted: 19 December 2007

References

Chung, H.J., Y.N. Jan, and L.Y. Jan. 2006. Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains. Proc. Natl. Acad. Sci. USA. 103:8870–8875.

Cohen, S.A. 1996. Immunocytochemical localization of rH1 sodium channel in adult rat heart atria and ventricle. Presence in terminal intercalated disks. Circulation. 94:3083–3086.

Garrido, J.J., P. Giraud, E. Carlier, F. Fernandes, A. Moussif, M.P. Fache, D. Debanne, and B. Dargent. 2003. A targeting motif involved in sodium channel clustering at the axonal initial segment. Science. 300:2091–2094.

Jenkins, S.M., and V. Bennett. 2001. Ankyrin-G coordinates assembly of the spectrin-based membrane skeleton, voltage-gated sodium channels, and L1 CAMs at Purkinje neuron initial segments. J. Cell Biol. 155:739–746.

Kizhatil, K., J.Q. Davis, L. Davis, J. Hoffman, B.L. Hogan, and V. Bennett. 2007a. Ankyrin-G is a molecular partner of E-cadherin in epithelial cells and early embryos. J. Biol. Chem. 282:26552–26561.

Kizhatil, K., W. Yoon, P.J. Mohler, L.H. Davis, J.A. Hoffman, and V. Bennett. 2007b. Ankyrin-G and beta2-spectrin collaborate in biogenesis of lateral membrane of human bronchial epithelial cells. J. Biol. Chem. 282:2029–2037.

Lemailliet, G., B. Walker, and S. Lambert. 2003. Identification of a conserved ankyrin-binding motif in the family of sodium channel alpha subunits. J. Biol. Chem. 278:27333–27339.

Lowe, J.S., O. Palygin, N. Bhasin, T.J. Hund, P.A. Boyden, E. Shibata, M.E. Anderson, and P.J. Mohler. 2008. Voltage-gated Na\textsubscript{+} targeting in the heart requires an ankyrin-G–dependent cellular pathway. J. Cell Biol. 180:173–186.

Malhotra, J.D., K. Kazen-Gillespie, M. Hortoch, and L.L. Isom. 2000. Sodium channel beta subunits mediate homophilic cell adhesion and recruit ankyrin to points of cell-cell contact. J. Biol. Chem. 275:11383–11388.

Mohler, P.J., I. Rivolta, C. Napolitano, G. LeMaileit, S. Lambert, S.G. Priori, and V. Bennett. 2004. Nav1.5 E1053K mutation causing Brugada syndrome blocks binding to ankyrin-G and expression of Nav1.5 on the surface of cardiomyocytes. Proc. Natl. Acad. Sci. USA. 101:17533–17538.

Pan, Z., T. Kao, Z. Horvath, J. Lemos, J.Y. Sul, S.D. Cramon, V. Bennett, S.S. Scherer, and E.C. Cooper. 2006. A common ankyrin-G-based mechanism
retains KCNQ and NaV channels at electrically active domains of the axon. *J. Neurosci.* 26:2599–2613.

Rasmussen, H.B., C. Frøkjær-Jensen, C.S. Jensen, H.S. Jensen, N.K. Jørgensen, H. Misonou, J.S. Trimmer, S.P. Olesen, and N. Schmitt. 2007. Requirement of subunit co-assembly and ankyrin-G for M-channel localization at the axon initial segment. *J. Cell Sci.* 120:953–963.

Ratcliffe, C.F., R.E. Westenbroek, R. Curtis, and W.A. Catterall. 2001. Sodium channel β1 and β3 subunits associate with neurofascin through their extracellular immunoglobulin-like domain. *J. Cell Biol.* 154:427–434.

Scriven, D.R., P. Dan, and E.D. Moore. 2000. Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. *Biophys. J.* 79:2682–2691.

Zhou, D., S. Lambert, P.L. Malen, S. Carpenter, L.M. Boland, and V. Bennett. 1998. AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J. Cell Biol.* 143:1295–1304.