Ankyrins

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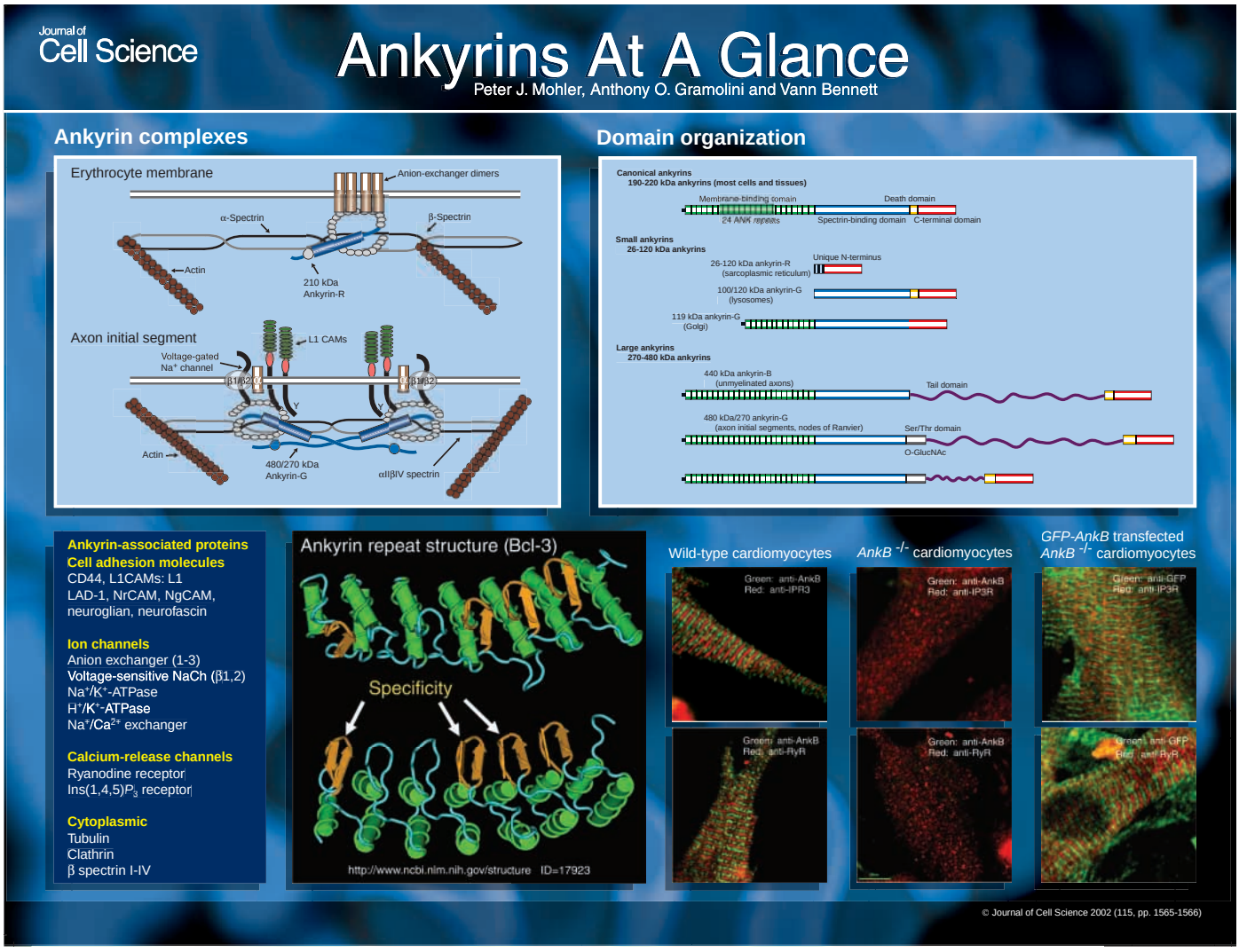
Ankyrins are a ubiquitously expressed family of intracellular adaptor proteins involved in targeting diverse proteins to specialized membrane domains in both the plasma membrane and the endoplasmic reticulum (Bennett and Baines, 2001). Vertebrate ankyrin polypeptides fall into three classes, each containing multiple alternatively spliced variants: ankyrins-R (R for restricted distribution, and the prototypic ankyrin first characterized in erythrocytes; also expressed in a subset of neurons and striated muscle) encoded by *Ank1* on human chromosome 8p11; ankyrins-B (B for broadly expressed; first characterized in brain, but now recognized in most cell types) encoded by *Ank2* on human chromosome 4q25-27; and ankyrins-G (G for giant size and general expression, first characterized as a 480 kDa polypeptide in the nervous system; expressed in most cell types) encoded by *Ank3*, on human chromosome 10q21. Although ankyrin genes are not present in the completed genomes of yeast or plants, simple metazoans including *Drosophila melanogaster* (*Dank1*, *Dank2*) and *Caenorhabditis elegans* (*unc44*) express ankyrin(s) that exhibit a high level of similarity to their vertebrate orthologues. Therefore, ankyrins appear to have evolved early in metazoan evolution and might perform functions unique to multicellular animals.

Canonical ankyrins are 190-220 kDa proteins expressed in most tissues and cell types and comprise a membrane-binding domain (MBD) of 24 *ANK* repeats, a spectrin-binding domain, a death domain and a C-terminal domain. Whereas death domains in other proteins may function in activation of NF-κB, caspase proteases and cell death, this domain has no known role within ankyrins. Ankyrin expression is regulated by both tissue- and developmental-stage-specific cues that also give rise to numerous ankyrin polypeptides due to alternative splicing. 440 kDa ankyrin-B and 480 kDa ankyrin-G polypeptides result from an



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insertion of a 220 kDa random coil between the spectrin-binding domain and the death domain, which results in a predicted extended length of up to 0.5-0.6 μm. These giant isoforms have specialized functions in unmyelinated axons (ankyrin-B) and in the targeting of voltage-dependent Na^+ channels to axon initial segments and nodes of Ranvier (480 kDa and 270 kDa ankyrin-G also contain a 40 kDa serine/threonine-rich domain glycosylated with GlucNAc monosaccharide residues). Finally, small ankyrin isoforms lacking large portions of canonical ankyrins are localized to specialized membrane sites, for example, 119 kDa ankyrin-G (Golgi), 100/120 kDa ankyrin-G (lysosomes) and 26 kDa ankyrin-R (sarcoplasmic reticulum).

Current views of ankyrin function are based on co-localization and biochemical interactions of ankyrin with other proteins. Ankyrin associates with a variety of membrane proteins including ion channels (Na^+/K^+ ATPase, H^+/K^+ ATPase, anion exchangers AE 1-3, voltage-sensitive Na^+ channels, Na^+/Ca^{2+} exchanger), calcium-release channels [ryanodine receptor, inositol (1,4,5)-trisphosphate receptor], cell adhesion molecules [CD44, L1CAMs (L1, NgCAM, neurofascin, LAD-1, NrCAM, neuroglian)], as well as cytoplasmic proteins, including clathrin and tubulin (Bennett and Baines, 2001). Many of these interactions are mediated by ANK repeats within the MBD, although the Na^+/K^+ ATPase and H^+/K^+ ATPase associate at least in part with the spectrin-binding domain. Finally, ankyrin phosphorylation is important for regulating the affinity of ankyrin for specific proteins, including spectrin.

ANK repeats are 33-residue motifs involved in protein recognition and are found in more than 325 unrelated human proteins [>5800 repeats in more than 1400 predicted proteins in the non-redundant sequence database (http://smart.embl-heidelberg.de)], including tankyrase, p53-binding protein (53BP2), transcriptional regulators GABPβ and NF-κB inhibitory protein IkBα, and the TRP family of ion channels (Sedgwick and Smerdon, 1999). ANK repeats fold into stacks of antiparallel α-helices interconnected by exposed loops arranged perpendicular to the α-helices. The specificity for ANK-repeat–protein interactions is likely to be conferred by non-conserved residues that flank each ankyrin repeat, located at the tips of exposed loops. Ankyrin MBDs with 24 ANK repeats are multivalent and can accommodate multiple protein interactions; thus, they may assemble multiprotein complexes at specific cellular sites.

Physiological roles of ankyrin-G and ankyrin-B in targeting proteins to specialized membrane domains have been demonstrated by gene-knockout studies in mice. Mice with cerebellar-specific loss of ankyrin-G display coordinate loss of voltage-gated Na^+ channels, β-IV spectrin and neurofascin at the plasma membrane of axon initial segments, decreased ability of Purkinje neurons to fire action potentials, and progressive ataxia (Jenkins and Bennett, 2001; Zhou et al., 1998). Ankyrin-B-null mice die at birth with multisystem disorders including degeneration of long axon tracts, myopathy and degeneration of the thymus (Tuvia et al., 1999). Ankyrin-$B^{-/-}$ cardiomyocytes display downregulation and mis-sorting of calcium-release channels [ryanodine and inositol (1,4,5)-trisphosphate receptors] within the endoplasmic reticulum in cardiomyocytes that can be rescued by transfection with cDNA encoding ankyrin-B. Both ankyrin-G and ankyrin-R are expressed in cardiomyoctes, but cannot compensate for loss of ankyrin-B. Rescue studies with ankyrin-B/G chimeras have identified the C-terminal domain of ankyrin-B as the defining domain in specifying ankyrin-B activity (Mohler et al., 2002). A working hypothesis to explain the cellular basis for these phenotypes is that ankyrins play roles as chaperones or guides that direct vesicle transport of a variety of ion channels to sites in the plasma membrane as well as the endoplasmic reticulum.

Ankyrins have been implicated in human disease (Bennett and Baines, 2001). Hereditary spherocytosis results from decreased expression and/or mutated forms of ankyrin-R. A similar model (*nb/nb*) is observed in mice due to a near loss of ankyrin-R (210 kDa) in multiple cell types, which causes anemia, degeneration of a set of Purkinje neurons and cerebellar dysfunction. In addition, ankyrin-B has been mapped to human chromosome 4q25-27, the linkage site for human type 4 long QT syndrome (Schott et al., 1995). This disorder results in bradycardia and can cause cardiac arrhythmias leading to loss of consciousness or sudden death. As ankyrin-B is highly expressed in cardiac tissue, further examination of both ankyrin-$B^{+/-}$ and ankyrin-$B^{-/-}$ mice may reveal a similar cardiac phenotype. More generally, ankyrin mutations could result in improper localization and, therefore, activity, of ion channels identified with ankyrin-binding domains and thus result in a variety of functional 'channelopathies'.

References

Bennett, V. and Baines, A. J. (2001). Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. *Physiol. Rev.* **81**, 1353-1392.

Jenkins, S. M. and Bennett, V. (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.

Mohler, P. J., Gramolini, A. O. and Bennett, V. (2002). The ankyrin-B C-terminal domain determines activity of ankyrin-B/G chimeras in rescue of abnormal inositol 1,4,5-trisphosphate- and ryanodine receptor distribution in ankyrin-B (–/–) neonatal cardiomyocytes. *J. Biol. Chem.* **7**, 7.

Schott, J. J., Charpentier, F., Peltier, S., Foley, P., Drouin, E., Bouhour, J. B., Donnelly, P., Vergnaud, G., Bachner, L., Moisan, J. P. et al. (1995). Mapping of a gene for long QT syndrome to chromosome 4q25-27. *Am. J. Hum. Genet.* **57**, 1114-1122.

Sedgwick, S. G. and Smerdon, S. J. (1999). The ankyrin repeat: a diversity of interactions on a common structural framework. *Trends Biochem. Sci.* **24**, 311-316.

Tuvia, S., Buhusi, M., Davis, L., Reedy, M. and Bennett, V. (1999). Ankyrin-B is required for intracellular sorting of structurally diverse Ca^{2+} homeostasis proteins. *J. Cell Biol.* **147**, 995-1008.

Zhou, D., Lambert, S., Malen, P. L., Carpenter, S., Boland, L. M. and Bennett, V. (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.

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