13.1.7 Drug Screening on Ion Channels

The center-stage role of ion channels in many physiological responses has been stressed by functional studies in cells, organs, and animals, by the emerging channelopathies as well as by the successful use of ion channel modulating drugs and naturally occurring toxins. Current drugs only target a dozen of the known channel members, while most of the other 400 types are currently all being investigated as potential drug targets by the pharmaceutical industry. Drug-discovery projects today depend strongly on large-scale blind-screening for finding new chemical lead molecules. High-throughput binding assays can be used for drug screening, but provides no functional information and is dependent on high-affinity ligands. Other options are ion flux assays which utilize specific ions such as rubidium, thallium, and lithium and measures their flux through K^+ and Na^+ channels, respectively. Fluorescent readouts from assays using voltage-sensitive dyes that indirectly measures ion channel activity by responding to changes in membrane potentials or by dyes responding to changes in ion concentrations (e.g., Ca^{2+}) can also be applied. Fluorescent, ion flux, and binding assays deliver high-throughput screening, but to some degree indirectly assay ion channel activity and do not allow direct control of voltage-dependent ion channel activity. The only "semi" high-throughput, high-quality technology to be used for screening on every ion channel type is the automated patch–clamp technique. With this method parallel recordings are performed by a robot on up to 384 arrays of ion channel expressing cells positioned on silicon chips, thereby enabling screening of large compound libraries (~30,000) in a matter of days.

13.1.8 Structure of Voltage-Gated Ion Channels

The superfamily of voltage-gated ion channels encompasses more than 140 members and is one of the largest families of signaling proteins, following the G-protein-coupled receptors and protein kinases. The pore-forming α-subunits of voltage-gated ion channels build upon common structural elements and come in four variations. The simplest version is composed of two transmembrane (TM) segments connected by a membrane-reentrant pore-loop with N- and C-termini on the inside (Figure 13.3). Four of such subunits form the channel. This architecture is typical for the so-called inward-rectifying K^+ channels (K_{ir}). It is found in a number of bacterial channels, suggesting it is the ancestor of the family. The second type, the two-pore potassium channel (K_{2P}) is made by a concatenation of two such subunits (4-TM), and the channel is formed by two double constructs. The third type is the 6-TM subunit, in which four extra membrane-spanning N-terminal domains including a voltage-sensor have been added to the basic 2-TM pore unit. Four of these 6-TM units form a channel. The group of 6-TM channels is rather large and includes the voltage-gated K^+ channels (K_V), the calcium-activated K^+ channels (K_{Ca}), the cyclic nucleotide-gated channels (CNG), the hyperpolarization-gated channels (HCN), the cation channel sperm-associated protein (CatSper), and the transient receptor potential channels (TRP). Finally, the fourth channel structure type is

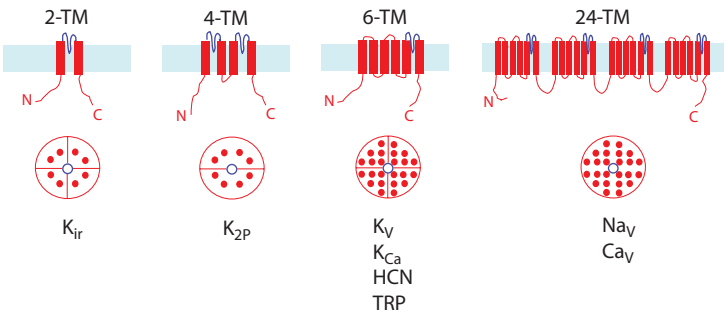

FIGURE 13.3 Topology of voltage-gated cation channels.