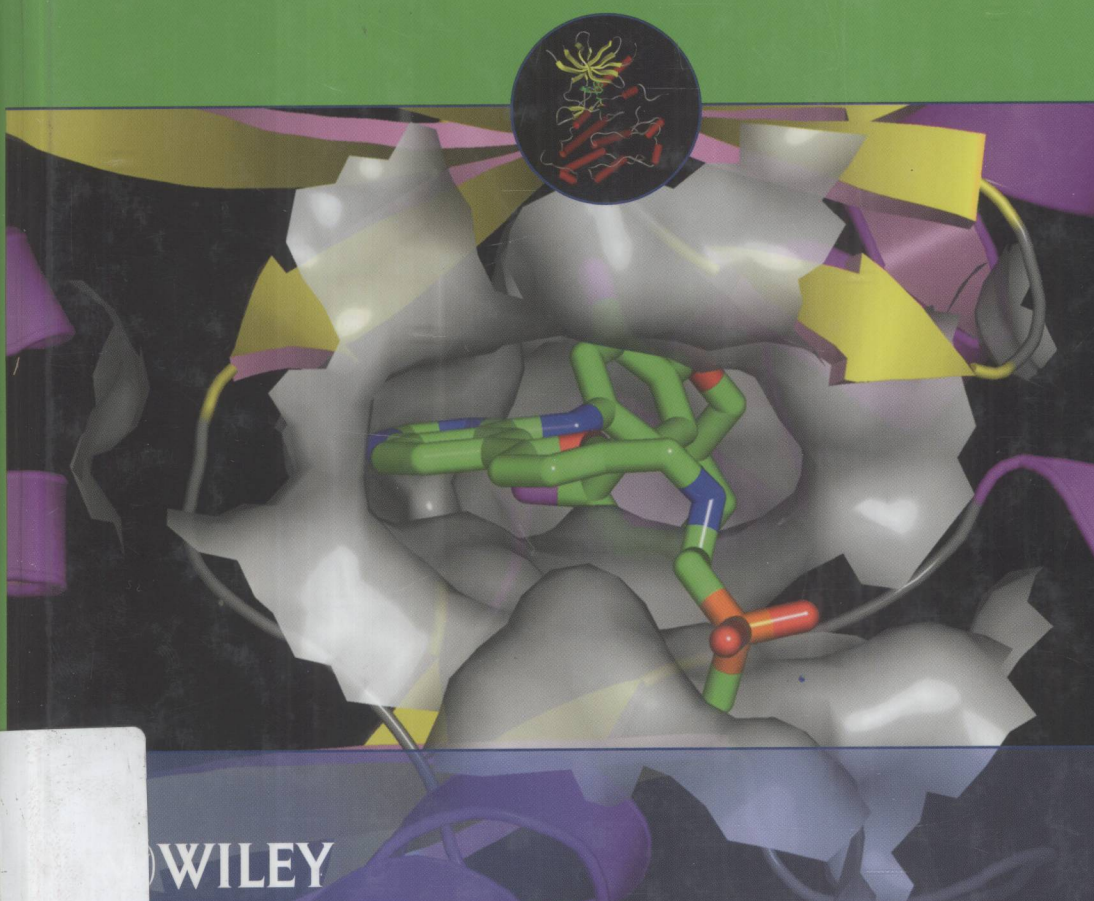
GENE FAMILY TARGETED MOLECULAR DESIGN

EDITED BY
KAREN E. LACKEY



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PREFACE

Approximately half of the anticipated small-molecule drug targets fall into just six gene families: G-protein-coupled receptors (GPCRs), protein kinases, zinc metallopeptidases, serine proteases, nuclear hormone receptors, and phosphodiesterases. A system-based research approach groups proteins into classes based on sequence and common motifs that form 3D space receptive for small-molecule interactions. The goal of small-molecule drug discovery is to modulate the activity of a biological target via interactions with an externally administered molecule at optimal drug intervention points in disease pathology to afford the maximum therapeutic index. Key to achieving this mechanistic approach to drug discovery is the design, synthesis, and evaluation of biologically effective compounds. Working within a system of related targets allows scientists to apply learning from one member to accelerate identification of ligands for other disease-associated targets.

The purpose of this book is to provide a description of compound design methods for generating small molecules that interact with important biological targets in the following major gene families: G-protein-coupled receptors/7-transmembrane receptors, ion channels, integrins, kinases, proteases, protein–protein interactions, transporters, and nuclear receptors. Each chapter will cover affinity for the intended target, the mechanism of the interaction, small molecule examples, and ways to change the molecule to attenuate the activity. We hope to provide a solid foundation of information that allows readers to then approach more expert technical literature with a greater understanding. At the end of the introductory chapter, I have summarized some books that provide an in-depth coverage of the functional areas of drug discovery that contribute to this stage of research.

The reader will readily come up to speed in a new area of research or be able to compare their work across other gene families. Nature has developed classes of related proteins and has borrowed similar motifs to keep all of the different functions of a cell and complex system working. The selectivity achieved by nature is impressive, and these are the lessons we can exploit to repair, enhance, diminish, or eliminate an activity using a small molecule. By understanding the major gene families, the scientist can design molecules that target the intended protein and minimize interactions with the other protein classes. Beyond the scope of this book, but nonetheless important in selection of targets and molecule design, are the subsequent steps of drug development. The stages of drug generation beyond discovery include extensive toxicity measurements, development, clinical evaluation, registration, and commercialization and marketing of effective medicines for specific disease treatments or prevention.

Synthetic, structural, computational, and medicinal chemists in academia, biomedical companies, and the pharmaceutical industry will benefit from a gene family-focused description of molecular design. There is a tremendous amount of literature on the topic and yet very little work has been done to condense the information into a manageable format as practical guidance for a chemist to get started in the area of designing compounds that intervene in important points of disease pathology. Also, overwhelming amounts of information are available in each research area making it a daunting task to do cross-comparisons of the different gene families. The pros and cons of different discovery methods (i.e., use of high-throughput screening, protein–ligand crystal structures, transient transfection assays, etc.) are included to help the reader understand the value and context of the biological evaluation of compounds currently available within each gene family.

Another benefit added to this book is the biographies of the contributing authors compiled in Appendix. By reviewing the educational backgrounds and careers of each of the experts in the field, the reader can peruse the many paths of study one can take in a scientific journey for drug discovery. The reader can also learn how the scientific fields are integrated to design molecules for drug discovery. I sincerely hope you enjoy reading the fascinating way by which the scientists have managed to create small molecules that effectively modulate their intended biological targets in the major target classes covered in the chapters of this book.

April 2008 KAREN E. LACKEY

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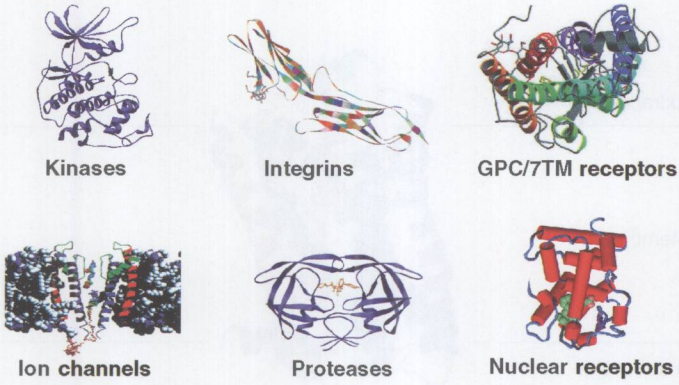

FIGURE 1.1 The gene families of proteins are classified by function and common structure motifs as can be seen by the representative structures for kinases, integrins, GPCRs, ion channels, proteases, and nuclear receptors.

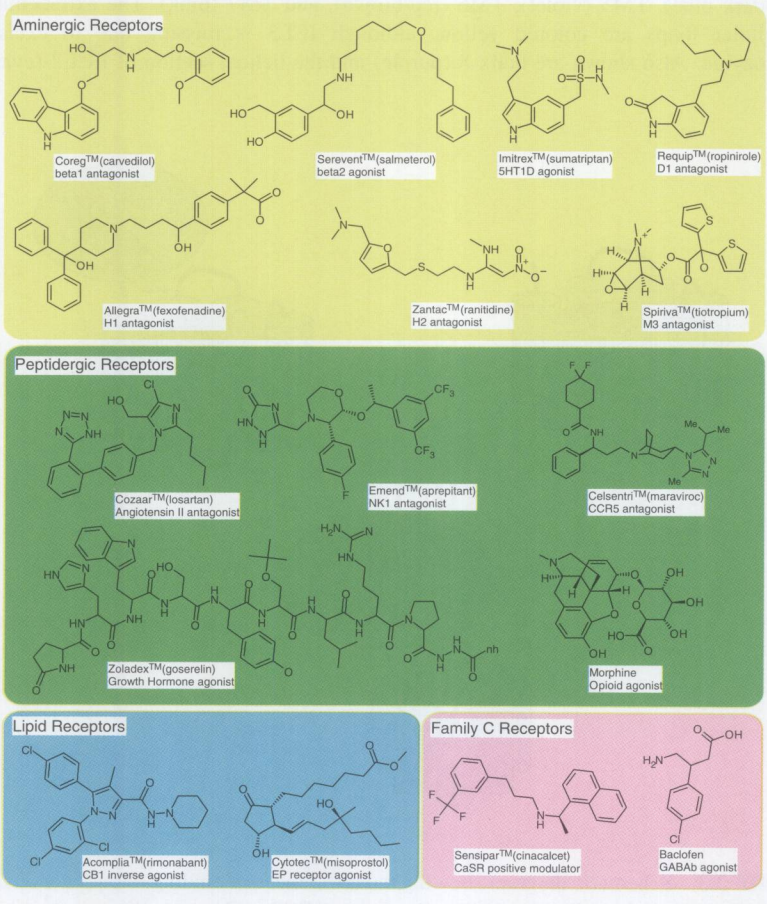

FIGURE 2.1 Examples of marketed drugs targeting GPCRs.

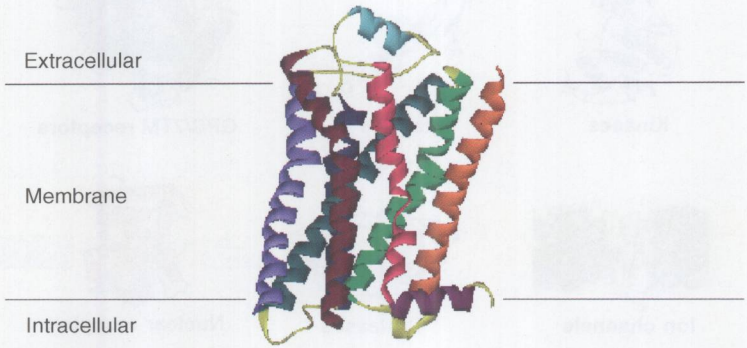

FIGURE 2.2 Semischematic representation of a G-protein coupled receptor. The picture is generated from the coordinates of the β2-adrenergic receptor crystal structure 2rh1 with transmembrane helices colored as follows: TM1 (orange), TM2 (green), TM3 (light blue), TM4 (dark blue), TM5 (violet), TM6 (red/brown), and TM7 (pink). The extracellular and intracellular loops are colored yellow, although ICL3 is missing due to excision of T4-lyzozyme. Also shown are helix 8 (purple) and the helical section of ECL2 (cyan).

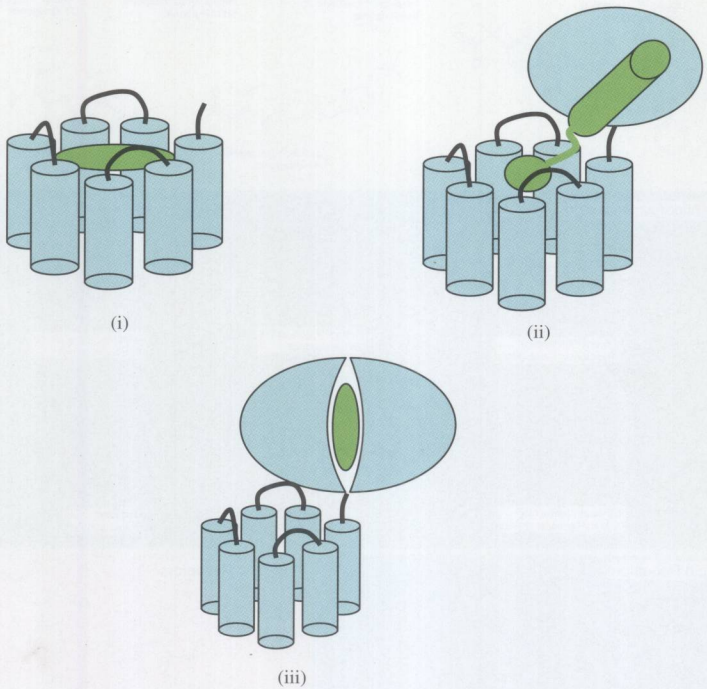

FIGURE 2.3 Schematic diagram showing the differences in binding of endogenous ligands by Family A (i), Family B (ii), and Family C (iii) GPCRs. The approximate position of the orthosteric site is shown in green.

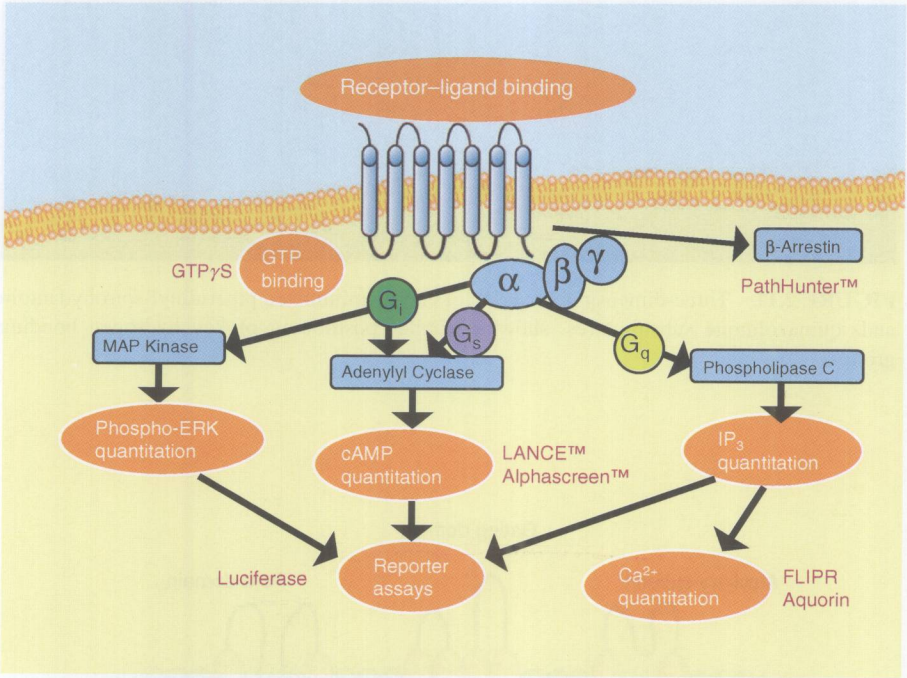

FIGURE 2.4 G-protein signal transduction pathway steps (orange ellipses) and typical associated assay formats (magenta text).

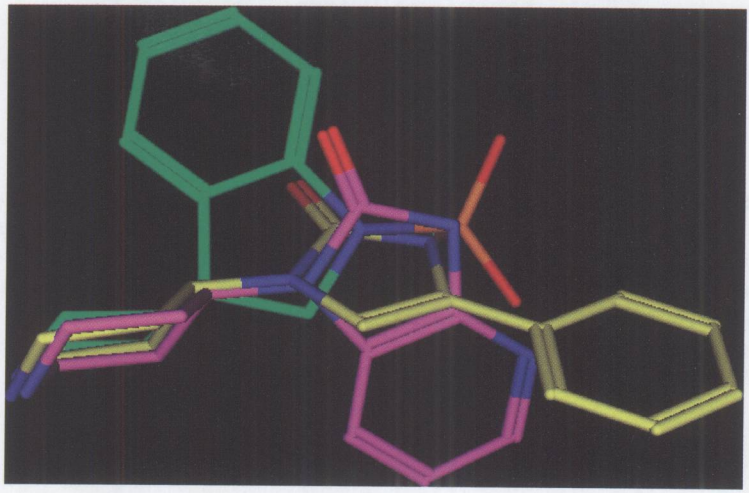

FIGURE 2.7 Three-dimensional overlay of aryl piperidine privileged structures, showing incremental positioning of aryl and hydrogen bonding groups.

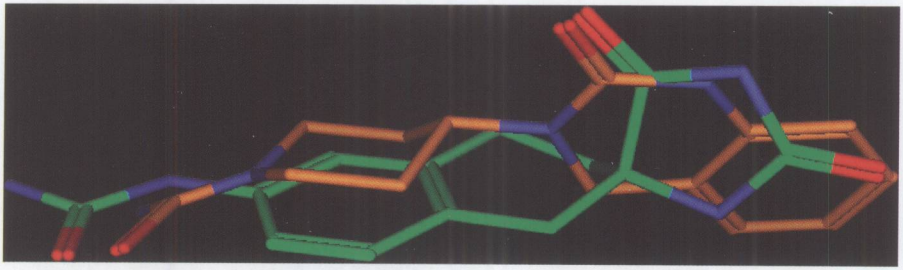

FIGURE 2.11 Three-dimensional overlay of energy minimized piperidinyl-spirohydantoin and -quinazolinone substructures, showing similar positioning of key hydrogen bonding groups.

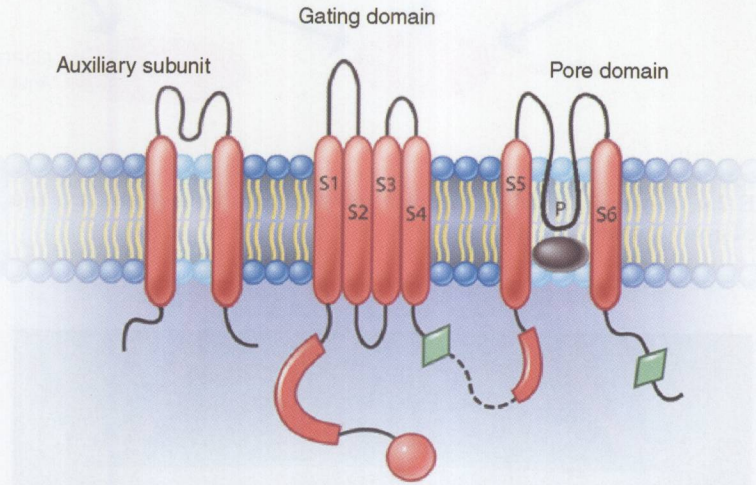

FIGURE 3.1 Voltage-dependent ion channels possess similar architectural features. Ion selectivity resides within the pore domain. When a gating domain is covalently attached, different functional properties result. Functional diversity also occurs if auxiliary subunits associate with the ion channel. Other mechanisms, such as phosphorylation (green diamonds), contribute to the regulation of ion channel function. From Garcia and Kaczorowski, Potassium channels as targets for therapeutic intervention, *Sci. STKE* 2005, pe46 (2005).

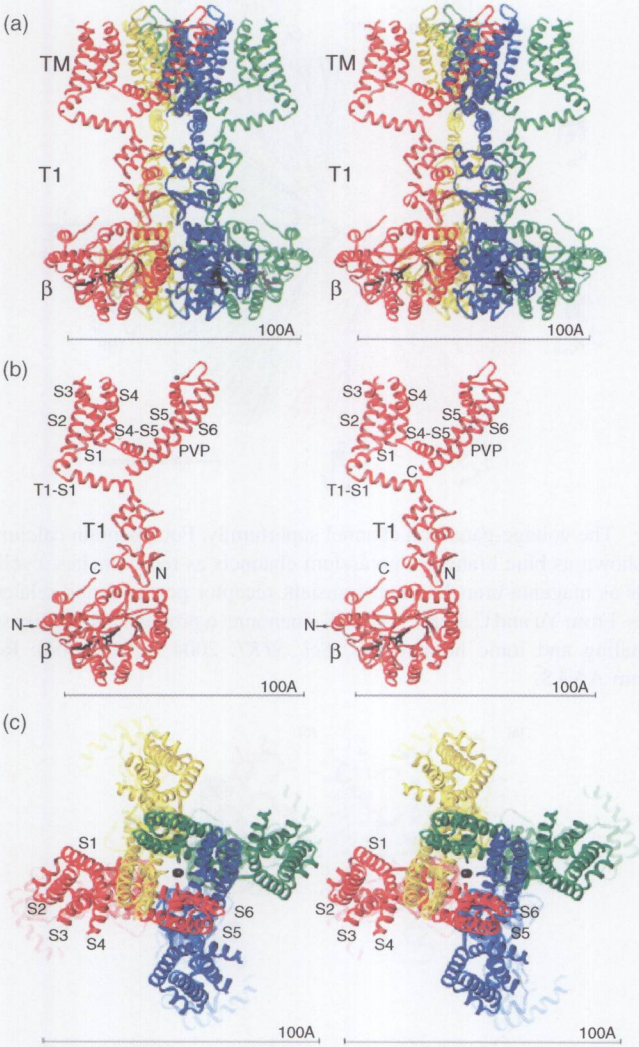

FIGURE 3.4 Crystal structure of a mammalian voltage-gated potassium channel. Stereo views of the Kv1.2–β2 subunit complex. The four subunits are colored differently. In (a), TM indicates the integral membrane component of the complex. (b) A single subunit of the channel and β subunit are viewed from the side. (c) A view from the extracellular side of the pore. From Long et al. Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. *Science* 309: 897–903 (5 August 2005). Reprinted with permission from AAAS.

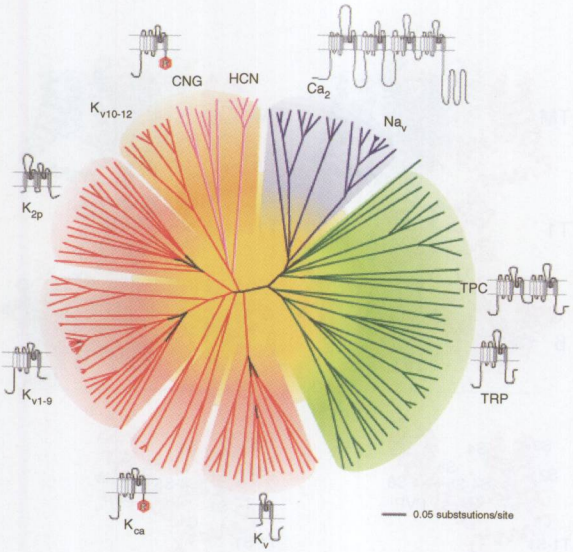

FIGURE 3.2 The voltage-gated ion channel superfamily. Four-domain calcium and sodium channels are shown as blue branches, potassium channels as red branches, cyclic nucleotide-gated channels as magenta branches, and transient receptor potential and related channels as green branches. From Yu and Catteral, the VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci. STKE* 2004, re15 (2004). Reprinted with permission from AAAS.

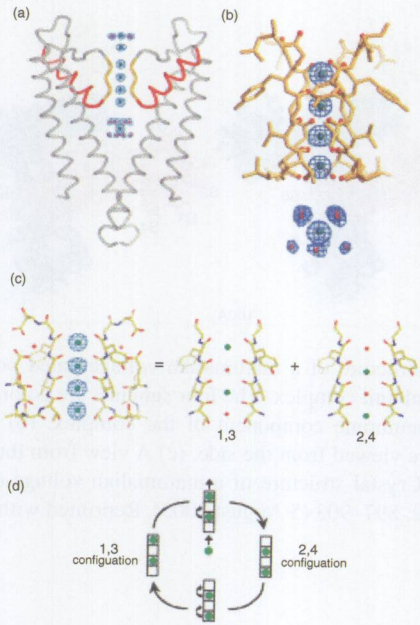

FIGURE 3.3 Ion conduction in potassium channels. (a) Two of the four pore domain subunits of a potassium channels are shown. (*See text for full caption.*)

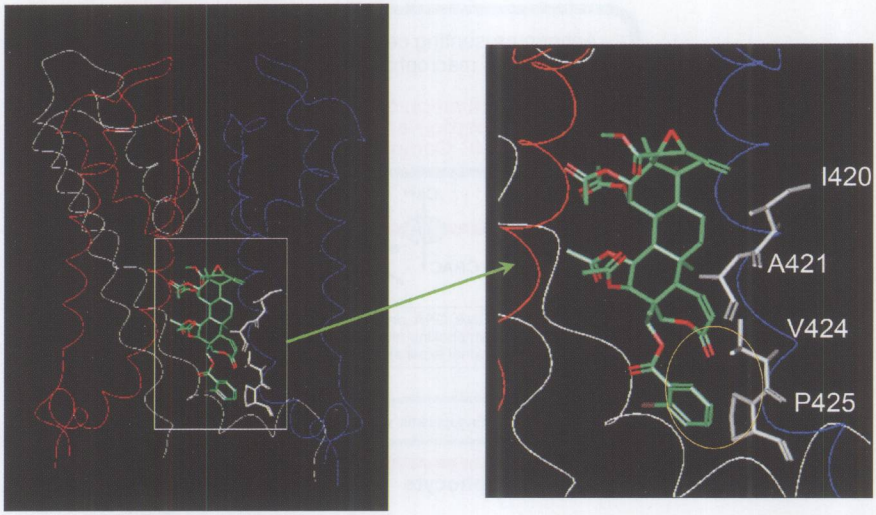

FIGURE 3.8 Docking of correolide C in Kv1.3. A model of Kv1.3 with a bent S6 helix was generated using the crystal structure of the KcsA channel as a template and modified using experimental data constraints. In the displayed orientation, the 3-keto group of the E-ring ester points to the selectivity filter, the saturated hydrocarbon face of the molecule interacts with the hydrophobic wall of the channel, and the other face, with four acetyl groups, lies in the water-filled cavity. The bromobenzyl group provides binging energy through van de Waals interaction with Pro425 in the channel.

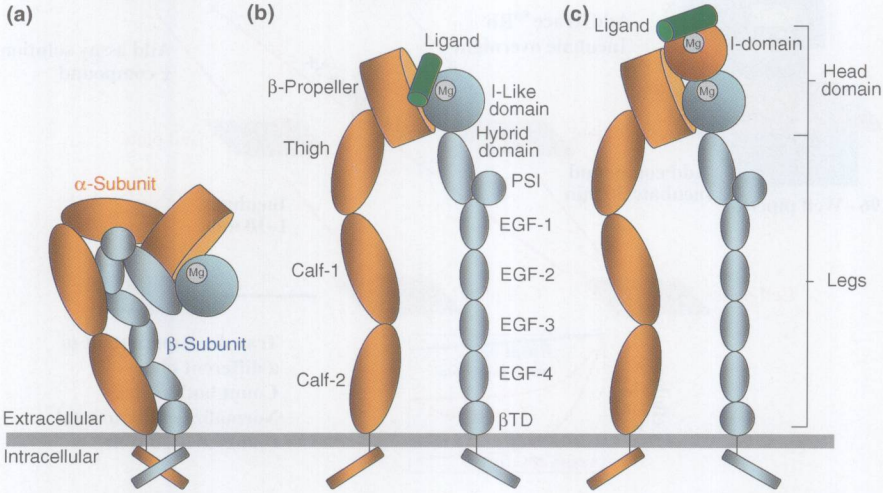

FIGURE 4.2 A generic integrin structure. (a) Bent, "inactive" conformation. (b) Extended "active" conformation. (c) Integrin containing I-domain in the α-subunit. βTD, β-terminal domain; EGF, epidermal growth factor domain; PSI, plexins, semiphorins, and integrins domain; Mg, metal ion-dependent adhesion site (MIDAS).

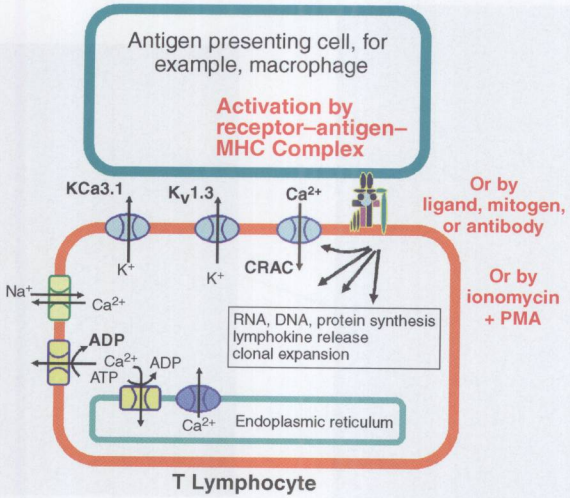

FIGURE 3.5 Human T cell activation. Sustained Ca^{2+} influx through Ca^{2+}-release-activated calcium (CRAC) channels is required for lymphokine release and T cell proliferation. Kv1.3 channels hyperpolarize the membrane and facilitate Ca^{2+} entry through CRAC channels. Blockade of Kv1.3 with peptides or small molecules prevents T cell activation *in vitro* and *in vivo*.

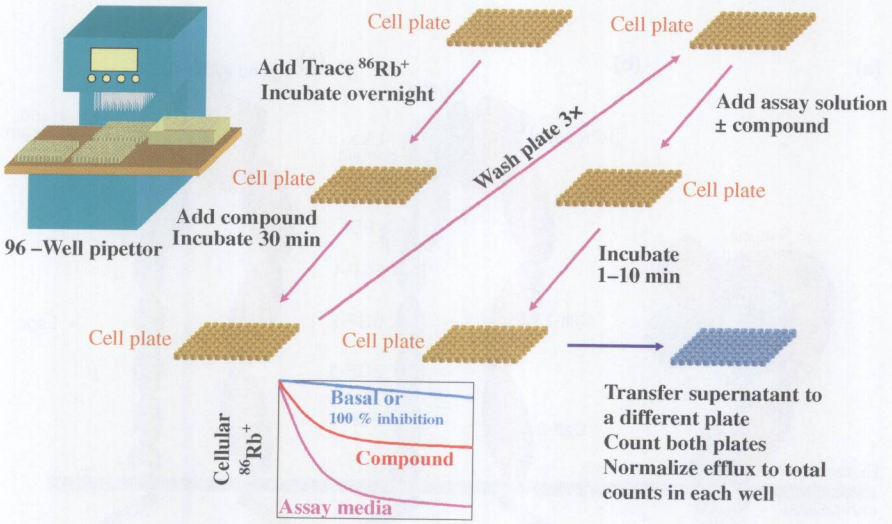

FIGURE 3.6 Scheme of a functional Rb^+ efflux assay used for identifying potassium channel modulators.

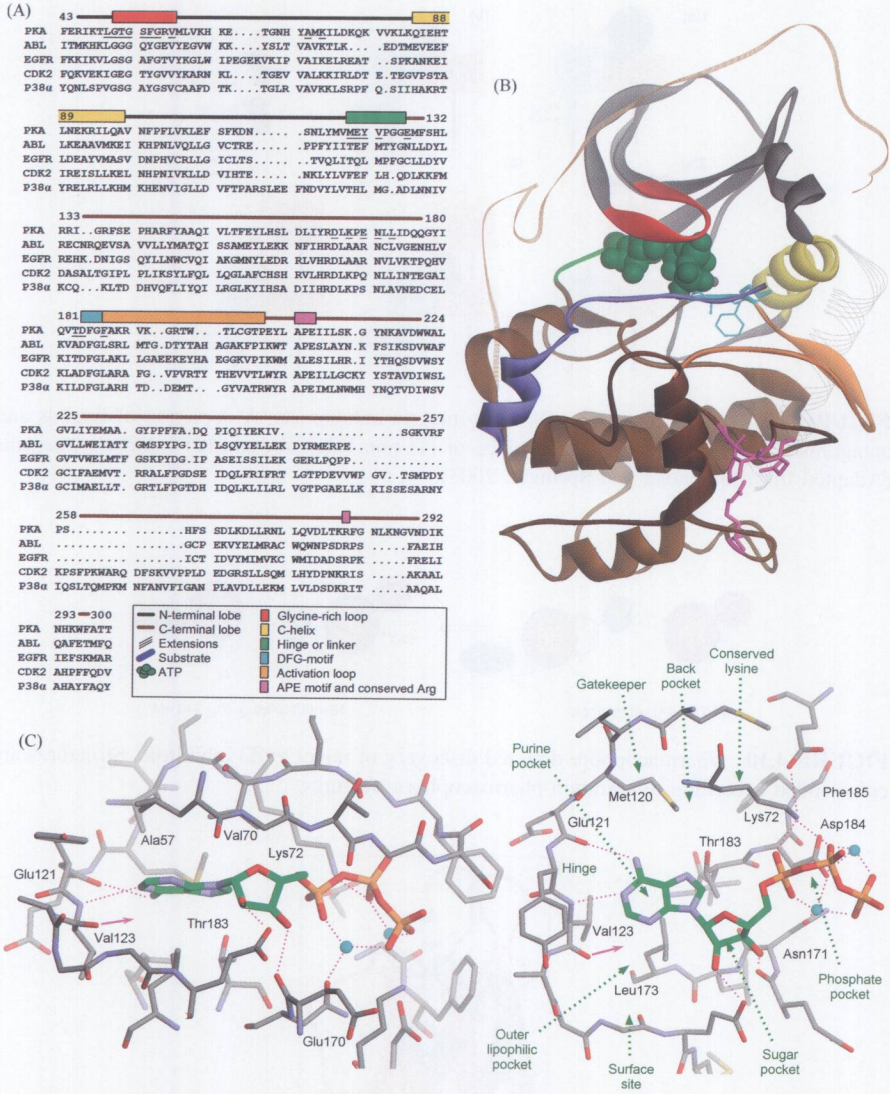

FIGURE 5.1 (a) Kinase sequence alignment highlighting key structural features. Residues close to ATP are underlined. (b) Ribbon representation of PKA using the same color scheme as (a). (c) Interactions of ATP in the binding site of PKA.