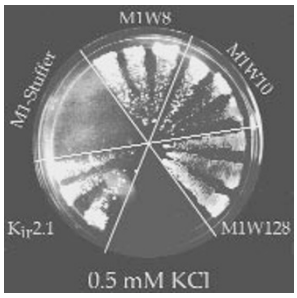
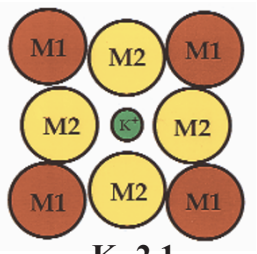
# Predicting channel structures with genetics

No one doubts the power of genetics. Nevertheless, the use of genetic techniques in structural research — specifically, for predicting the exact three-dimensional structures of proteins — is not so widespread. Now, Minor, Masseling, Jan and Jan (*Cell* **96**: 879-891) have verified that genetic approaches can indeed contribute handsomely to structural knowledge, notably in the often intractable setting of the membrane. They predict the transmembrane structure of an inwardly rectifying potassium channel ($K_{ir}$), a member of a superfamily of eukaryotic channels that are responsible for regulating many cellular functions (for example, cell excitability, vascular tone, heart rate, renal salt flow, and insulin release) by modulating membrane potential. The results of Minor and colleagues indicate that the $K_{ir}$ channel structure is distinct from that of the only known X-ray crystal structure of a potassium channel (KcsA) — despite contrary expectations, which arise from the fact that these channels, at first glance, have the same transmembrane topology.

$K_{ir}$ channels are made up of four subunits, each with two transmembrane domains, M1 and M2. To address how these domains are arranged in the active channel, Minor and colleagues constructed libraries of $K_{ir}$ proteins with randomly mutagenized M1 or M2 domains. From these libraries, they selected functional $K_{ir}$ channels that could rescue a potassium transport-deficient strain of yeast (left image). Significantly, positive clones from these selection experiments displayed wild type electrophysiological properties, indicating that the wild-type channel structure was present. The results of these experiments show that, like other transmembrane domains, M1 and M2 are extremely tolerant to sequence changes, but only at specific positions, and preferences are observed with respect to side chain shape and chemistry.

The patterns of allowed amino acid substitutions suggest that both M1 and M2 are helices and hint at the protein–protein, protein–lipid, and protein–water interaction surfaces. The specific arrangement of transmembrane helices in the $K_{ir}$ channel was determined by second-site suppressor experiments. In these studies, mutations that result in nonfunctional $K_{ir}$ channels were made at conserved positions in one transmembrane domain, and suppressor mutations that restored channel activity were identified from libraries containing randomized mutations in the other domain. Together, the results suggest that each M1 helix interacts with two M2 helices, thus yielding a helical bundle model for the $K_{ir}$ transmembrane channel structure, with M1 and M2 forming the outer and inner helices, respectively (right image). This arrangement appears to be shared by all $K_{ir}$ family members, based on sequence comparisons. Such a clear result indicates that genetic approaches should be added to every structural biologist's list of tools. *Boyana Konforti*




**$K_{ir}$ 2.1**
Adapted from Minor *et al.*



# Early crystals

A number of innovations are dramatically reducing the amount of time required to determine three-dimensional structures of biomolecules by X-ray crystallography. For example, on page 458 of this issue of *Nature Structural Biology,* Perrakis and colleagues present an automated method for model building into an electron density map. However, the first steps in any such enterprise are purification of the molecule of interest and crystallization, and despite the availability of crystallization 'kits', obtaining high-quality crystals can still be rate-limiting in many cases.

In his recently published book *Crystallization of biological macromolecules* (Cold Spring Harbor Laboratory Press; 1999), Alexander McPherson outlines the science of protein crystallization during its infancy. Perhaps not surprisingly, the first documented crystallization was of hemoglobin from the blood of earthworms, which was accomplished by F.L. Hünefeld in 1840. Other proteins, such as the reserve proteins of plant seeds and hen egg albumin, were also crystallized during the late 1800s, but some of the more notable achievements occurred during the early 1900s. One such accomplishment was that of J.B. Sumner, who was intent on purifying an enzyme to prove that enzymes were indeed proteins — a subject of debate at the time. Success came in 1926 when he crystallized, and thus purified, jack bean urease, and proved its protein nature. (Interestingly, in 1919 Sumner had already crystallized another protein, concanavalin B, which was not known to have enzymatic activity at that time but is now known to be a chitinase.) In 1927, insulin was crystallized by J.J. Abel and colleagues and in 1937, lysozyme was crystallized by E.P. Abraham and R. Robinson. Both proteins were destined to become, along with hemoglobin and myoglobin, among the first proteins with known three-dimensional structures. During the 1930s and 1940s, other enzymes such as trypsin and trypsinogen, chymotrysin and chymotrypsinogen, pepsin and pepsinogen, were crystallized by J.H. Northrup, R.M. Herriott, and M. Kunitz. For their work on crystallizing enzymes, Sumner and Northrup shared the 1946 Nobel prize along with W.M. Stanley, who crystallized tobacco mosaic virus in 1935.

Scientists in the field of X-ray diffraction took advantage of their colleagues' high quality crystals to study protein structures. For example, in 1934, J.D. Bernal and Dorothy Crowfoot (later Dorothy Hodgkin, see the book review on page 412 of this issue) presented X-ray photographs of pepsin, and a year later, Crowfoot published similar work with insulin. Today, although technological advances are making it possible to obtain useful diffraction data from previously unusable crystals, the science of crystallization will still play a major role in the success of future large scale efforts, such as structural genomics projects. *Tracy Smith*