Pas de Deux or More: The Sulfonylurea Receptor and K^+ Channels

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Pancreatic islets of Langerhans are miniature fuel sensors, secreting insulin and glucagon appropriately during the fed and fasted states. Glucose causes electrical activity in the ß cells of the islet, which appears as bursts of action potentials upon plateaus of depolarizations and occurs promptly after glucose uptake and its metabolism (*1*). These bursts of action potentials lead to transient elevations of intracellar calcium ($[Ca^{2+}]_i$) that cause insulin secretion (*2*). Both processes are regulated by an ion channel, the adenosine triphosphate (ATP)–sensitive K^+ channel (K_{ATP}), which couples nutrient metabolism to the membrane potential (*3*, *4*). The K_{ATP} current (I_{KATP}) sets the ß cell resting membrane potential, but is blocked by a glucose-induced increase in the cytosolic ratio of ATP to ADP. The resulting membrane depolarization activates voltage-dependent L-type Ca^{2+} channels and triggers $[Ca^{2+}]_i$ release, initiating insulin secretion (*5*).

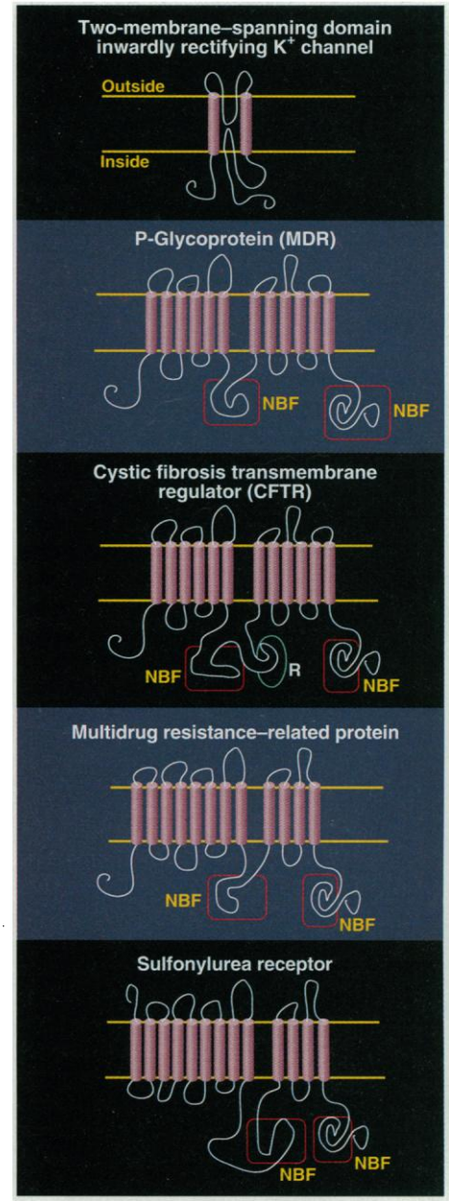
Sulfonylureas are insulin secretagogues used to treat diabetes mellitus, which depolarize ß cells by blocking K_{ATP} channels (*3*, *6*). Cloning of the receptor for these important drugs and the clinical result of its malfunction are described in two reports in this issue of *Science* (*7*), which also shed new light on the nature of the K_{ATP} channel in islets.

K_{ATP} channels are also present in cardiac myocytes, where they were first described (*8*), and in muscle, neuronal, and mitochondrial membranes (*9*). These channels participate in a multitude of processes that link energy metabolism to regulation of the membrane potential. Modulated by G proteins and protein kinases, K_{ATP} channels have a rich pharmacology; medicinal applications include hypertension, asthma, heart disease, diabetes, and epilepsy (*10*). The differences in single-channel conductance, ATP sensitivity, and pharmacology are such that it is likely that K_{ATP} channels are encoded by a family of related genes (*9*).

K_{ATP} itself, the ion permeation subunit, may belong to a new family of K^+ channel genes encoding inward rectifier–like K^+ channels (*11*). Their transmembrane topology, unique among ion channels, seems to include only two membrane-spanning domains and an intervening region homologous to the pore domain of *Shaker*-like channels (Fig. 1A) (*12*). Three such genes are candidate K_{ATP} channels: ROMK1, rcKATP-1/CIR, and uK_{ATP}-1 (*11*, *13*). ROMK1 has special importance in the renal tubule but seems to generate a current dissimilar to the ß cell I_{KATP}. The cardiac rcKATP-1 and related genes (KATP-2, GIRK3) are the likely potential channel subunit genes. However, rcKATP-1/CIR is critical for a different K^+ current, the G protein–coupled inwardly rectifying K^+ channel (I_{KACh}) (*13*). I_{KACh} thus seems to be heteromultimeric, possibly with a large number of subunits, comprised of both rcKATP-1/CIR along with GIRK1, originally thought to encode this channel by itself (*13*). The rcKATP-1/CIR channel messenger RNA is expressed in brain and other tissues, and the protein may heteromultimerize to generate several K^+ currents (*11*, *14*). None of the two-membrane–spanning domain channels has the sensitivity to sulfonylureas seen in native ß cell K_{ATP} channels. Now we see that these or similar channels may associate in some way with a separate protein that does bind sulfonylureas, newly described in the reports in this issue (*7*).

The sulfonylurea receptor (SUR) has been wooed and won by a more traditional strategy: The protein was purified through the use of covalently bound sulfonylurea derivatives and partially sequenced. Complementary DNA (cDNA) probes were then employed to screen multiple cDNA libraries to obtain the entire cDNA. The two reports in this issue identifying the cDNA encoding SUR from Aguilar-Bryan *et al.* and Thomas *et al.* are thus the products of almost 10 years of work, begun in the laboratory of Chris Boyd. Surprisingly, they have found that the binding protein (no ion permeation has yet been found with the expressed protein) is a member of the growing family of ATP-binding cassette proteins. This family includes the P-glycoprotein and multidrug resistance (MRP) proteins, which cause chemotherapeutic drug resistance when overexpressed, and the CFTR protein, mutations of which cause cystic fibrosis [see figure (*14*)]. These proteins all have multiple membrane-spanning domains and two nucleotide-binding folds; at least some of them are ATP-hydrolyzing pumps, while the CFTR behaves as a channel. A surprising feature of the SUR is the predicted orientation of the amino-terminal domain in the extracellular space, in the absence of an identifiable leader sequence. A cryptic cleavage site may explain why the mass of 177 kilodaltons predicted from the SUR cDNA sequence differs markedly from the apparent mass of 140 kilodaltons for the photolabeled protein, as the similar MRP protein has a highly sensitive carboxyl-proteolysis site (*15*, *16*).

The report by Thomas and co-workers shows that a well-known, if rare, disease of newborns, familial hyperinsulinemic hypo-



Topologies of the inward rectifier K^+ channel family and ATP-binding cassette proteins. NBF, nucleotide-binding fold; R, regulatory domain.

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glycemia of infancy (FHHI), is associated in several families with mutations in the second nucleotide-binding domain of SUR, suggesting that this fold may have something to do with insulin secretion by analogy with a similar CFTR mutation (*7*, *17*). The mechanism of this defect, a putative increase in the ability of ATP to close K_{ATP} channels, suggests that the ß cells would be chronically depolarized and therefore hypersecretory. If the SUR mutations actually cause a loss of open K_{ATP} channels, this explains why the syndrome is autosomal recessive, because only a few open K_{ATP} channels are required to set the resting membrane potential (*18*). [It also explains the well-known leucine sensitivity of these patients, because leucine stimulates ß cell oxidative phosphorylation, which would produce more ATP to further depolarize the membrane (*19*).] Gene therapy may therefore afford an interesting opportunity for treatment of FHHI. Mental retardation in FHHI, ascribed to repeated hypoglycemia, should now be reconsidered to be complicated by defective SUR expression in the nervous system, and cardiac abnormalities might be uncovered in these patients as well. Insulin hypersecretion due to an SUR mutation raises the possibility that insulin hyposecretion syndromes related to defects in SUR might also exist, although diabetes genes have not been localized to the region of FHHI.

Whither K_{ATP}? Inward rectifier–related channels and SUR certainly seem destined for each other, assuming the SUR is not itself a channel, but the right match remains elusive. An exhaustive combinatorial approach with pairs of inward rectifier–like channels may be illuminating. Additional bridging subunits may yet turn up to couple sulfonylurea binding to channel inhibition. The isolation of cDNAs for these putative membrane proteins will, we sense, fuel future studies of metabolic coupling to cell excitability.

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Adaptive Mutation: Who's Really in the Garden?

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Neo-Darwinists teach that mutations arise independently of biological needs. Physical insults, chemical fluctuations, and replication errors lead to stochastic changes in DNA sequences. Random mutations mean that the evolutionary watchmaker is blind (*1*). To bolster their argument, neo-Darwinists cite an experiment by Luria and Delbrück in which bacterial mutations occur prior to selection for the mutant phenotype (*2*). However, this neo-Darwinist position has been challenged for over a decade by the discovery that certain mutations occur much more frequently when they are selected, and thus adaptively useful, than they do during normal growth (*3*–*6*). The principal difference between these so-called "adaptive mutations" and the phage resistance mutations studied by Luria and Delbrück is that selection for adaptive mutations is not lethal. Thus, nonmutant cells can survive to undergo DNA changes under selective conditions. Not surprisingly, the evolutionary significance of adaptive mutation is highly controversial, and there is great curiosity as to its mechanism, a curiosity that will be stimulated by two new reports in this issue of *Science* (*7*, *8*).

A popular system in which to study adaptive mutation uses the lacI-Z33 mutation, a fusion of lacI (encoding repressor) and lacZ (encoding ß-galactosidase), which prevents bacterial growth on lactose medium because it includes a frameshift mutation that blocks lacZ translation (*9*). Strains carrying lacI-Z33 display delayed kinetics of Lac^+ colony appearance when plated on lactose medium, indicating that many more frameshift events occur after selection than during prior growth, that is, adaptive mutation (*9*). Several laboratories have begun unraveling the molecular basis of lacI-Z33 reversion. RecA and RecBCD homologous recombination functions are necessary for adaptive lacI-Z33 reversion but not for background reversions (*9*, *10*). Moreover, adaptive frameshifts are different in sequence from those occurring nonselectively (*11*).

The two reports in this issue of *Science* add spice to the lacI-Z33 pot by demonstrating that plasmid transfer functions are also required for adaptive reversion (*7*, *8*). The lacI-Z33 mutation used in the studies described above happens to be carried on an F_{lac} plasmid. Peters and Benson have recently shown that F_{lac} transfer is frequent under conditions comparable to selection for lacI-Z33 reversion (*12*). While investigating the significance of these facts for adaptive mutation, Radicella and co-workers (*7*) find that selection-induced reversion and plasmid transfer are inseparable: A chromosomal lacI-Z33 mutation (in which transfer does not happen) displays a 25-fold reduction in selection-induced reversion, adaptive Lac^+ revertants carry plasmids that have been transferred, and nontoxic inhibition of plasmid transfer blocks selection-induced reversion. Most striking is the finding that streptomycin-sensitive lacI-Z33 bacteria can produce adaptive Lac^+ revertants in the presence of lethal concentrations of streptomycin if and only if they can transfer the F_{lac} plasmid to streptomycin-resistant recipient cells. In the accompanying report, Galitski and Roth demonstrate that a regulatory molecule that inhibits expression of F_{lac} transfer functions also blocks adaptive lacI-Z33 reversion (*8*). They also find that

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