W-AM-Symi-1

OVERVIEW: INTRODUCTION TO MITOCHONDRIAL CHANNELS. ((Joyce J. Diwan)) Biology Department and Center for Biophysics, Rensselaer Polytechnic Institute, Troy, NY 12180.

Historically, the first ion channels to be characterized were those of plasma membranes of nerve and muscle cells. Various channel types are now known to mediate ion fluxes across intracellular membranes, including, e.g., membranes of mitochondria, endoplasmic reticulum, the nuclear envelope, synaptic vesicles, chloroplasts, and vacuoles. Some intracellular channels have been characterized in detail. Others have been discovered only recently. The symposium on Intracellular Ion Channels will focus on just a few of the known channels. Two classes of channels to be described mediate release of Ca^{++} from the endoplasmic reticulum, including the sarcoplasmic reticulum of muscle. Some of these Ca^{++}-release channels are activated by Ca^{++} itself. Others are activated by the second messenger inositol triphosphate (IP_3). Of the mitochondrial channels to be discussed, the outer membrane porin channel (VDAC) has been most thoroughly characterized; yet important questions remain. An inner mitochondrial membrane channel of about 100 pS conductance in the presence of 150 mM KCl is found to be voltage-gated. Electrophysiological studies have identified a mitochondrial megachannel as responsible for the permeability transition activated under conditions of matrix calcium loading. Another mitochondrial channel exhibits conductance increments of about 40 pS in the presence of 150 mM KCl and is cation-selective (see abstract by G. Costa, R. Murphy, & J. Diwan).

W-AM-Symi-2

THE MITOCHONDRIAL MULTIPLE CONDUCTANCE CHANNEL ACTIVITY. ((K.W. Kinnally)) Dept. of Biol. Scie., SUNYA, Albany, NY 12222

Multiple conductance channel (MCC) activity observed by patch-clamping mitoplasts designates a class of conductance levels of the inner mitochondrial membrane (Kinnally et al. J Bioenerg Biomembr 24 99. MCC activity is detected in the presence of the voltage dependent MCS (Mitochondrial Centi-picoSiemen) channel, a marker of the inner mitochondrial membrane. The levels range from ~40 to over 1000 pS and have similar features, e.g. activation by Ca^{2+} and voltage, sensitivity to inhibitors. It is notable that activation and inhibition often proceed in steps corresponding to these same levels. MCC may be present at junctions of the inner and outer membranes since preparations enriched in contact sites exhibit activity (Moran et al. 1990 JBC 265 908) which we consider characteristic of MCC. Parallel studies of the permeability transition pore (PTP) in mitochondrial suspensions and MCC with patch-clamping suggest that the two are the same (Szabó & Zoratti 1992 J Bioenerg Biomembr 24 111). The channel is inhibited selectively by cyclosporine A and by a variety of agents also affecting other channels (e.g. MCS) including antimycin A and ligands of the mitochondrial benzodiazepine receptor. We have found that high concentrations of some inhibitors activate rather than inhibit MCC. Studies aided by NSF grant MCB9117658.

W-AM-Symi-3

MITOCHONDRIAL PERMEABILITY THROUGH ION CHANNELS. ((C. Ballarin, O. Moran* and M.C. Sorgato)) Dipartimento di Chimica Biologica, Università di Padova (Italy) and *Istituto CNR di Cibernetica e Biofisica, Genova (Italy)

Mitochondria possess high conductance ion pathways in either the outer and inner membrane. The best known ion channel of the outer membrane is the VDAC (voltage-dependent anion channel) which has been characterized in detail in planar bilayers. However, the in situ analysis of the integral outer membrane with the patch clamp technique has revealed that the membrane has an electric behaviour quite different from that displayed in planar bilayers. In integral mammalian outer membranes smaller conductances with fast kinetics, cationic selectivity and asymmetric voltage gating, are found. By patch clamping the cap regions (which are made of remnants of the outer membrane) of Saccharomyces cerevisiae mitoplasts, conductance steps of around 700 pS are found, which resemble in magnitude the conductance expected for VDAC, but from which they differ with respect to kinetics and voltage dependence. The direct observation of yeast inner membranes has also been carried out. So far, two channels have been identified, one of around 30 pS, open preferentially at negative (matrix) potentials, one around 50 pS, mostly open at positive (matrix) potentials.

W-AM-Symi-4

INTRACELLULAR CALCIUM RELEASE CHANNELS OF MUSCLE (CRC). ((Sidney Fleischer)) Dept. Mol. Biol., Vanderbilt Univ., Nashville, TN 37235.

The CRC are a new class of channels characterized by their large size and four-fold symmetry. They serve a key role in excitation-contraction coupling. The ryanodine receptor (Ry Rec) CRC was first isolated from skeletal muscle and heart, and the IP_3 Rec (IP_3 Rec) was later isolated from smooth muscle. The Ry Rec has a mass of 2.3 million consisting of four subunits of 565KD; the IP_3 Rec is 1.25 million with 313 KD subunits. The 3-dimensional structure of the Ry Rec has been obtained by image enhancement techniques of electron micrographs. The Ry Rec has dimensions of 26 x 26 x 16 nm; the size of the IP_3 Rec is ~25 x 25 x 10 nm. The IP_3 Rec appears to be present in most, if not all, eukaryotic cells. The Ry Rec has been identified in several tissues other than muscle. Recently, we found that heart contains an IP_3 Rec which has a different intracellular localization than the Ry Rec. The IP_3 rec is localized at the intercalculated discs, whereas the Ry Rec is localized at the triad/dyad junction throughout the myocyte. Several aspects of intracellular signalling involving CRC's are at the forefront. 1) How is the CRC modulated? 2) What is the significance of new findings that an immunophilin (FK binding protein) is tightly associated with the Ry Rec? 3) Is there cross-talk between the Ry Rec and IP_3 Rec in tissues such as heart and brain where both exist in the same cell? The participation of numerous co-workers and collaborators is gratefully acknowledged, especially R.J. Boucek Jr., C.C. Chadwick, J. Frank, L. Hymel, M. Inui, Y. Kijima, A.R. Marks, M. Mayrleitner, E.M. Ogunbunmi, A. Saito, H.G. Schindler, P. Tempst, A.P. Timerman, T. Wagenknecht and J. Wall. (NIH HL32711; Muscular Dystrophy Association)

W-AM-Symi-5

ELECTROPHYSIOLOGY OF ENDOPLASMIC RETICULUM CALCIUM-RELEASE CHANNELS. ((Barbara E. Ehrlich)) Depts. of Medicine and Physiology, Univ. of Connecticut, Farmington, CT 06032.

Two classes of intracellular calcium release channels have been identified in the endoplasmic reticulum, the inositol 1,4,5-trisphosphate ($InsP_3$)-gated calcium channel and the ryanodine receptor/channel (RyaR). The channels coexist in most cells, but they may play different roles within the cell. We have compared the functional properties of these channels after reconstitution into planar lipid bilayers. They can be distinguished by single channel conductance and by the compounds that activate and inhibit channel activity. For example, polyanions such as heparin inhibit the $InsP_3$-gated channel and activate the RyaR by distinct mechanisms. Similarly, calcium modulates the activity of the two channel types, but there are characteristic differences in the interactions. Both channel types show positive and negative regulation of channel function by calcium, but over different concentration ranges. For the $InsP_3$-gated channel, activation and inhibition are complete within the physiological range of cytosolic calcium, whereas for the RyaR, only activation occurs over this range of calcium. The ability of these and other cellular compounds, such as ATP, to modulate the activity of both channel types may alter channel function to meet a variety of physiological and pathological challenges.

W-AM-SymII-1

cAMP-DEPENDENT PROTEIN KINASE PROVIDES A STRUCTURAL FRAMEWORK FOR THE PROTEIN KINASE FAMILY.

((Susan Taylor, Jianhua Zheng, Janusz Sowadski, Wei Wen, Friedrich Herberg, Dominic Fantozzi, and Wes Yonemoto)) Dept. of Chemistry, Univ. of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0654.

cAMP-dependent protein kinase (cAPK) is one of the simplest members of a family of enzymes that play critical regulatory roles in the eukaryotic cell. cAPK, being one of the smallest protein kinases, has served as a prototype for the entire family of over 200 enzymes. The catalytic (C) subunit of cAPK is itself also subject to posttranslational modifications, both phosphorylation and myristylation. The crystal structure of a ternary complex containing the C-subunit, an inhibitor peptide, and MgATP shows how key conserved residues converge at the active site. It also describes the sites of posttranslational modification. Two stable phosphorylation sites exist and replacing these phosphates leads to a destabilized or inactive enzyme that, in some cases, is unable to recognize the regulatory (R) subunit. Phosphorylation at Thr197 is particularly critical and a homologous residue, critical for activation, is found in other protein kinases such as the cell division cycle kinases, cdc2. Myristylation stabilizes the enzyme by folding into a hydrophobic pocket. The inhibition of the C subunit by either the type I R-subunit or a heat stable protein kinase inhibitor (PKI) depends on the synergistic high affinity binding of MgATP. Mutant C-subunits that are catalytically intact but unable to be regulated by either PKI or R, we can demonstrate that different regions of the enzyme surface are important for the high affinity binding of these two physiological inhibitors. The dissociated C-subunit is free to migrate between the cytoplasm and the nucleus while association with either R or PKI prevents access to the nucleus. Microinjecting directly into the nucleus shows that free C-subunit can move in both directions while R and the holoenzyme cannot escape from the nucleus.

W-AM-SymII-2

MOLECULAR PROPERTIES OF MYOSIN LIGHT CHAIN KINASES.

((Stull, J.T., Herring, B.P., Gallagher, P.J., Gao, Z.-H. and Zhi, G.)) Department of Physiology, UT Southwestern, Dallas, TX 75235.

Ca^{2+}/calmodulin (CaM)-dependent myosin light chain kinases (MLCK) are dedicated protein kinases that phosphorylate a Ser near the N-terminus of regulatory light chains (RLC) of vertebrate myosins. RLC phosphorylation plays an important role in cytokinesis, receptor capping, initiation of smooth muscle contraction, and potentiation of striated muscle contraction. MLCK in smooth and non-muscle cells has catalytic and physicochemical properties that distinguish it from the enzyme found in striated muscles. However the structures and relative organization of functional domains are similar, including a catalytic core, an autoinhibitory region, and a calmodulin-binding domain. The structural basis for intrasteric activation by CaM and catalysis has been examined by site-directed mutagenesis and chemical cross-linking procedures. Basic residues in the autoinhibitory region bind to specific acidic residues on the surface of the larger lobe of the catalytic core, thereby preventing RLC binding. Upon activation by CaM, RLC binds to the larger lobe of the catalytic core and is phosphorylated in the active site. Residues on both sides of the phosphorylatable Ser in the RLC are important for catalysis. Studies demonstrate similarities, but also important differences in the molecular properties of these 2 types of MLCKs.

W-AM-SymII-3

DECODING Ca^{2+} SIGNALS BY MULTIFUNCTIONAL Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE? Howard Schulman, Phyllis Hanson, Tobias Meyer, Melanie MacNicol and Lubert Stryer. Stanford University School of Medicine, Stanford, CA 94305-5332.

Multifunctional Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase) is one of the major protein kinases coordinating cellular responses to hormones and neurotransmitters. CaM kinase has structural/functional properties that may facilitate its response to changes in Ca^{2+} that are transient or pulsatile. Substrate phosphorylation and autophosphorylation are stimulated when calmodulin binds and disrupts the autoinhibitory/calmodulin-binding domain. After calmodulin dissociates from an autophosphorylated subunit, the kinase no longer deactivates because its autoinhibitory domain is disrupted by the anionic phosphate moiety. The effects of transient rises in Ca^{2+} are potentiated by the conversion of CaM kinase to this Ca^{2+}-independent form. The frequency of Ca^{2+} oscillations or spikes may be decoded by CaM kinase. The affinity of CaM kinase for calmodulin increases more than 100-fold by autophosphorylation which traps calmodulin by increasing its off-rate from less than a second to several seconds. Trapping is a cooperative process that is inefficient at low occupancy of calmodulin on the multimeric kinase. Cooperativity results from the mechanism of autophosphorylation which involves a subunit phosphorylating its neighbor in the holoenzyme, each with bound calmodulin. Simulations of kinase activation at limiting calmodulin show that repetitive Ca^{2+} pulses lead to recruitment of calmodulin to the holoenzyme, which further stimulates autophosphorylation and trapping. This cooperative positive feedback loop potentiates the response of the kinase to sequential calcium spikes and establishes a threshold frequency at which the enzyme becomes highly active. The biochemical properties of CaM kinase provide for molecular potentiation of calcium signals and frequency detection.

W-AM-SymII-4

STRUCTURE: FUNCTIONAL ANALYSIS OF THE EPIDERMAL GROWTH FACTOR RECEPTOR.

((G.N.Gill)) UCSD,La Jolla,CA92093-0650.

The EGFR consists of 4 major subdomains: an amino terminal ligand binding ectodomain, a membrane spanning domain, a cytoplasmic tyrosine kinase catalytic core and a regulatory carboxyl terminal domain. Signal transduction depends on the intrinsic protein tyrosine kinase (PTK) activity of the receptor. To identify structural features that distinguish tyrosine from serine protein kinase (PSK), a molecular model of the kinase domain of EGFR was constructed by substituting its amino acid sequence for that of the catalytic subunit of cyclic AMP-dependent protein kinase (cAPK) in a 2.7 A refined crystallographic model containing bound ATP and a peptide inhibitor. General folding and the configuration of invariant residues at the active site were conserved. The unique kinase nucleotide binding site was conserved with most contact provided by the small lobe. Two sequence motifs that distinguish PTK and PSK correspond to loops that converge at the active site. An arginine in the catalytic loop is proposed to interact with the γ phosphate of ATP. The second loop provides a binding surface that positions the tyrosine of the substrate. A positively charged surface provides additional sites for substrate recognition. The regulatory C' terminus contains 5 identified sites of tyrosine self-phosphorylation. Self-phosphorylation activates the enzyme, provides sites for assembly of SH2 domain proteins and exposes multiple endocytic codes which act combinatorially to attenuate signal transduction.

POTASSIUM CHANNELS III

W-PM-A1

VOLTAGE-DEPENDENT K^+ CHANNELS REGULATE ARTERIAL SMOOTH MUSCLE MEMBRANE POTENTIAL AND TONE.

((Harry J. Knot, Blair E. Robertson, Joseph E. Brayden and Mark T. Nelson)) University of Vermont, Dept. of Pharmacology, Colchester Medical Research Facility, Colchester, VT 05446-2500. (Spon. by J.M. Quayle) BER is an International Research Fellow of the AHA. Supported by the NSF and NIH.

Voltage-dependent K^+ channels have been identified in many different tissues and have been traditionally thought to be involved in the repolarization of the action potential. In contrast, the role of these K^+ channels in the control of steady-state membrane potential, particularly in arterial smooth muscle, is unclear. We tested the hypothesis that steady-state K^+ efflux through voltage-dependent K^+ channels is involved in the regulation of arterial smooth muscle membrane potential. Both 4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP) inhibited whole-cell, voltage-dependent K^+ currents in smooth muscle cells isolated from rabbit cerebral arteries. 4-AP and 3,4-DAP produced half-maximal inhibition at concentrations of 1 mM and 0.5 mM, respectively. To minimize the contribution of the Ca^{2+}-activated K^+ channel, intracellular Ca^{2+} was buffered to 20 nM, and TEA^+ or iberiotoxin and nimodipine were included in the bath solution. Elevation of intravascular pressure constricts and depolarizes smooth muscle cells in small cerebral arteries from about -65 mV to -40 mV resulting in myogenic tone. We used isolated mid-cerebral arteries (110 ± 24 µm) that depolarize and develop myogenic tone in response to pressure. Blockers of voltage-dependent K^+ channels. 3,4-DAP (300 µm) and 4-AP (1 mM) depolarized the arteries by about 19 mV. The compounds constricted these arteries by 27 and 55 µm when subjected to a transmural pressure of 30 and 50 mm Hg respectively. The depolarization to 4-AP and 3,4-DAP was unaltered by blocking calcium channels (which dilated the arteries and closed K_{Ca} channels) and by the K_{ATP} channel inhibitor, glibenclamide. We, therefore, propose that steady-state K^+ efflux through 4-aminopyridine-sensitive, voltage-dependent K^+channels plays an important role in the regulation of membrane potential of arterial smooth muscle and consequently myogenic tone.

W-PM-A2

RESCUE OF LETHAL SUBUNITS INTO FUNCTIONAL HETEROMULTIMERIC VOLTAGE-DEPENDENT K^+ CHANNELS.

((M. Taglialatela, J.P. Payne*, J.A. Drewe, H. Hartmann and A.M. Brown)) Depts. Molecular Physiology and Biophysics and *Internal Medicine, Baylor College of Medicine, One Baylor Plaza, 77030 Houston, TX.

We have shown that injections of cRNAs of a chimeric K^+ channel (CHM) (Hartmann et al., *Science* 251:942, 1991) having mutations at positions 369 and 374 expressed currents in *Xenopus* oocytes with altered channel conductance, ion selectivity and TEA block. Further mutagenesis of these sites has shown that not all CRNAS expressed macroscopic or single channel currents. The function of one nonexpressing mutation, CHM V369L, was recovered by coinjection with the cRNA of a potent expressor, CHM L374V. Coinjection produced a new heteromeric phenotype in addition to the parent CHM L374V homomer. The single channel outward K^+ conductance of CHM L374V was significantly different from the heteromultimer in asymmetric conditions (4.5±0.2 Ps, n=16 vs. 14.8±0.7 Ps, n=16). Gating also differed substantially (mean open time for CHM L374V was 5.4±0.3 ms vs. 13.3±1.8 ms for the heteromultimer and mean closed time for CHM L374V was 32.4±8 ms vs. 5.1±1.6 ms for the heteromultimer). The single channel outward Rb^+ conductance, however, was nearly identical for the two phenotypes (13.9±1.5 Ps for L374V vs. 13.1±1.1 Ps for the heteromultimer). The stoichiometry of the heteromultimer was determined by coinjection of different ratios of the CRNAS of CHM L374V and CHM V369L. Binomial analysis suggested that the heteromultimer comprised of three CHM L374V subunits and one CHM V369L subunit was an active form of the channel. (Supported by the NS23877 grant to AMB and by the CNR 215.24 grant to MT).