Th-AM-SymII-1

PROTEIN ORGANIZATION AND MOBILITY IN CROWDED BIOLOGICAL MEMBRANES. ((James R. Abney[1] and Bethe A. Scalettar[2]), [1]Cardiovascular Research Institute & [2]Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

Biological membranes can contain up to 75% protein and thus should be viewed as nonideal fluids in which protein-protein interactions can profoundly affect both protein organization and mobility. Protein-protein interactions and their effects on membrane properties have been characterized both experimentally and theoretically. The "generic" nonspecific protein-protein force is repulsive and has its origin in short-range excluded-volume forces and long-range electrostatic forces; however, in some cases the protein-protein force may also contain a long-range attractive component that has its origin in protein-induced perturbation of membrane lipid. Over short distance scales, interactions affect organization by creating ordered but dynamic "coordination shells" around each protein; these shells may influence chemical reaction rates and energy transfer efficiencies. Over long distance scales, interactions affect phenomena as diverse as fluctuations in protein density, lateral phase separations, and protein aggregation and crystallization. Protein-protein interactions can also cause membrane proteins to diffuse 100-fold more slowly in biological membranes than in dilute reconstituted membranes. Collisional interactions among mobile proteins can produce a few to ten-fold decrease in the membrane protein diffusion coefficient at physiological protein concentrations. Collisional and binding interactions between mobile and immobile proteins, as well as protein-induced perturbations in membrane viscosity, can further hinder motion, leading to the observed 100-fold slowing of protein diffusion.

Th-AM-SymII-2

ESTIMATION OF EXCLUDED VOLUME EFFECTS UPON MACROMOLECULAR REACTIONS IN THE CYTOPLASM OF E. coli ((S.B.Zimmerman)) NIDDK, NIH, Bethesda, MD 20892.

The potential for excluded volume effects in cells is enormous due to their high macromolecule concentrations. We have estimated the magnitude of these effects in the cytoplasm of E. coli using an experimental analysis of the macromolecular contents coupled with scaled particle theory calculations.

Orders-of-magnitude shifts in rates or equilibria are predicted for a variety of reactions of biological interest. Examples will be given both for several generic types of reactions as well as for the lac operator-repressor interaction. The potential importance of crowding effects on cellular homeostasis will be emphasized.

We are currently studying changes in the properties of DNA caused by the presence of concentrated cytoplasmic extracts. The rate of cohesion between complementary terminal DNA sequences (the "sticky ends" on restriction fragments of lambda DNA) increases greatly. The extracts can cause condensation of the DNA; the mechanism of this condensation and its relation to the rate of cohesion is being examined.

Th-AM-SymII-3

EFFECTS OF VOLUME OCCUPANCY AND CYTOPLASMIC STRUCTURE ON THE TRANSLATIONAL AND ROTATIONAL MOBILITY OF MACROMOLECULES IN CYTOPLASM. ((Katherine Luby-Phelps)) University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235-9040.

The rotational mobility of macromolecules will depend on the solvent viscosity of cytoplasm and should be relatively unaffected by crowding unless volume occupancy is very high. Recent reports by Verkman and colleagues (J. Cell Biol. 112:719, 1991; Mol. Cell Biol. 3:173a, 1992) and results from our laboratory indicate that the solvent viscosity of cytoplasm is not significantly different from water and exhibits no spatial variation. The translational mobility of macromolecules may be affected by both crowding and cytoplasmic structure. The relative diffusion coefficient of inert, fluorescent tracer particles in cytoplasm decreases steeply as a function of the molecular dimensions of the particle (Luby-Phelps et al., PNAS 84:4910, 1987). Linear extrapolation of the data to a particle radius of zero suggests that even the smallest biomolecules may diffuse 3 to 4 times slower in cytoplasm than in water. This was recently confirmed by Abney (Mol. Cell Biol. 3:173a, 1992) and was attributed to collisions with other molecules in a crowded solution. *In vitro* modeling of cytoplasmic structure shows that the size dependence of the relative cytoplasmic diffusion coefficient is not simply due to macromolecular crowding or to percolation through an entangled filament network, but qualitatively resembles the diffusion of tracer particles in an entangled network interpenetrated by a crowded solution of protein-size macromolecules (Hou et al., Biophys. J. 58:31, 1990). Curve fitting and extrapolation of the *in vitro* data quantitatively reproduce the cytoplasmic data to within 10%. The resulting model predicts a cytoplasmic volume fraction of filaments of 0.11 and a weight concentration of background macromolecules of 12.4%. Cytoarchitecture may impose an additional constraint on translational mobility for macromolecules ≥ 26 nm dia. due to steric exclusion from some subcompartments of the cytoplasm.

Th-AM-SymII-4

MACROMOLECULAR CROWDING, CONFINEMENT, STICKINESS, AND THE ORGANIZATION OF CYTOPLASM. ((A.P. Minton)) NIDDK, NIH, Bethesda, MD 20892

The thermodynamic activity, mobility, and reactivity of macromolecules in the cytoplasm of a living cell are greatly influenced by several factors that are ordinarily neglected (but not always negligible) under the conditions of a typical laboratory experiment. The term "crowding" denotes effects arising from the exclusion of soluble macromolecules from volume occupied by other soluble macromolecules. The term "confinement" denotes effects arising from the exclusion of soluble macromolecules from volume occupied by large and relatively immobile structural elements such as fibers or intracellular membranes. The term "stickiness" denotes effects arising from the tendency of proteins and other biological macromolecules to form weak, transient, nonspecific complexes. Semi-quantitative estimates of the effects of each of these factors on the rate and extent of typical reactions taking place in the cytoplasm are presented. Some consequences of these effects for the structure and organization of prokaryotic and eukaryotic cytoplasm are explored.

POTASSIUM CHANNELS V

Th-PM-A1

A NOVEL POTASSIUM CHANNEL GENE FAMILY: *EAG* HOMOLOGS IN *DROSOPHILA*, MOUSE AND HUMAN. ((J.W. Warmke and B. Ganetzky)) Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

The *ether `a-go-go* (*eag*) mutation in *Drosophila* confers repetitive firing of action potentials in motor axons and abnormal release of transmitter at the larval neuromuscular junction (Ganetzky and Wu, *Trends Neurosci.* 8: 322, 1985). Voltage-clamp analysis of *Drosophila* larval muscles revealed that *eag* mutations affect all identified potassium currents (both voltage- and Ca^{2+}-activated) (Zhong and Wu, *Science* 252: 1562, 1991). From sequence analysis of an *eag* cDNA, we proposed that the *eag* locus encodes a structural component of potassium channels that is related to but distinct from all identified potassium channel polypeptides (Warmke *et al., Science* 252: 1560, 1991). Furthermore, the *eag* protein is also related to a family of cyclic nucleotide-gated cation channels and has a putative cyclic nucleotide binding domain (Guy *et al.*, Science 254: 730, 1991). We have recently determined that the *eag* polypeptide is able to form functional potassium channels using the *Xenopus* oocyte expression system (Robertson *et al.*, this volume).

Using a combination of low stringency DNA hybridization and PCR with degenerate oligonucleotide primers, we have found that *eag* is a member of a large conserved gene family. To date, we have identified one *eag* homolog in *Drosophila* whose encoded protein shares 43% identity with the *eag* polypeptide (extending through the hydrophobic core and putative cyclic nucleotide binding domain). In addition, *eag* homologs have been identified in mouse and human. The proteins encoded by two of these genes share 71% and 48% identity respectively with the *Drosophila eag* protein. Together, these genes appear to define three subfamilies of *eag* related genes. Functional analysis of these new *eag* homologs using the *Xenopus* oocyte expression system is in progress.

Th-PM-A2

FUNCTIONAL EXPRESSION OF THE *DROSOPHILA EAG* K^+ CHANNEL GENE. ((Gail A. Robertson[1], Jeffrey W. Warmke[2] and Barry Ganetzky[2]))
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The *eag* gene encodes a polypeptide with homology to the Shaker and Slo families of K^+ channels, especially in the putative pore-forming region (Warmke et al., Science 252:1560, 1991). In addition, it is related to cyclic nucleotide-gated channels and contains a putative nucleotide-binding domain (Guy et al., Science 254:730,1991). Together with previous electrophysiological studies indicating that *eag* mutations affect K^+ currents (Zhong and Wu, Science 252:1562), the sequence data suggest that *eag* encodes a novel type of K^+ channel subunit.

We have expressed *eag* in frog oocytes to determine whether it encodes a polypeptide that can form functional channels, and to characterize the properties of these channels. We observe a voltage-dependent, outwardly rectifying current that rapidly activates and slowly inactivates in response to depolarizing voltage steps. The reversal potential of the tail current varies with the external concentration of K^+ in a manner predicted by the Nernst equation for a K^+-selective channel.

This result suggests that the Eag polypeptide can form a homomeric K^+ channel in Xenopus oocytes. Studies using patch clamp analysis are in progress to further characterize this channel and to examine the effects of cyclic nucleotides on channel function.