Seminar Series Kicks-Off

On June 19, 2001, Dr. Geoffrey Goodhill of Georgetown University, spoke on "Computational Models in Neural Development" as the first speaker in our new series, entitled Seminars in Molecular Medicine and Biotechnology. Dr. Goodhill spoke on two aspects of his work: the creation of a new experimental approach to control mechanisms for determining the direction of neuron growth and a theoretical model of development of maps in the visual cortex.

One control mechanism for direction of growth is centered on a cell's ability to detect gradients. Dr. Goodhill's laboratory has devised a technique using a double-layered gel to create specific and stable gradients. Using this technique, they have discovered that cells can detect as little as a .37% change in nerve growth factor. Previous techniques put the level of detection at 1%. The rigor of this new assay should allow Dr. Goodhill to test more complex gradients with multiple factors which had not been possible before.

The last part of Dr. Goodhill's talk centered on modeling the development of ocular dominance (OD) and orientation (OR) maps within the visual cortex. During development, cells within the visual cortex are alternately assigned to the left or right eye and can be mapped. Using an elastic net algorithm, the model can predict relative order of development from the final OR and OD periodicities. The model, along with experimental values, predicts that OR develops first in the cat while OD develops first in macaques. However, when a strabismal cat, whose vision has the left eye disassociated from the right eye, was compared to the monkey then the pattern indicated that OD developed first.

While the experimental model was well received, it was the modeling of OR and OD maps within the visual cortex that elicited the most questions, especially the change in the cat. Dr. Lederer and others speculated that the patterns might be indicative of a specie's habits, i.e. a carnivore pattern or an herbivore pattern. Dr. Goodhill gave us an excellant start to this series.

CaaX Proteins Discussed

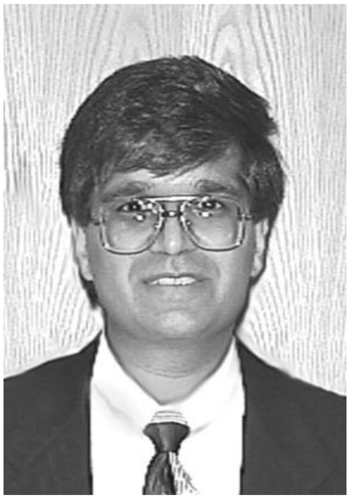
Dr. Walter K. Schmidt from the Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine was the speaker on June 21 for the Seminars in Molecular Medicine and Biotechnology series. His topic, "Post-Translational Processing of Prenylated Proteins" centered on the CaaX class of proteins. These rather ubiquitous proteins are involved in many cellular functions. They are characterized by the CaaX sequence on the C-terminal end. Dr. Schmidt has concentrated on the components that control the C-terminal modifications necessary to process CaaX precursors. Using yeast and looking at the a-factor mating pheromone, he has determined the localization and function of several of the components that control the process.

One of the most important and interesting components characterized by Dr. Schmidt was Ste24p. He determined that this was a zinc metalloprotease, localized in the endoplasmic reticulum. The localization to the ER was quite surprising as CaaX proteins are not generally found there. Besides its surprising location, Ste24p is a rather unique protein in that it has two distinct functions, C-terminus and N-terminus cleavages, and uses two different cleavage sites to perform those functions. While other proteases are known to have multiple functions they usually use only one cleavage site. Dr. Schmidt hopes to investigate how Ste24p can have two different cleavage sites and how they are controlled.

The processing of a-factor has homology with a number of pathways in the human, notably RAS. The characterization of Ste24p and the other components with RAS pathway homologs may lead to a new class of anti-cancer drugs which target C-terminus or N-terminus processing.

Dr. Schmidt's beautifully presented talk was warmly received and the discussion which followed demonstrated the interest the topic engendered.

Sparks Fly at Dr. Jafri's Talk



The third seminar was on a topic dear to hearts of many in the audience, calcium sparks. Dr. Saleet Jafri of the Department of Mahematical Science at the University of Texas at Dallas spoke on "Modeling the Mechanism of Calcium Sparks in the Heart." The activation of Ca^{2+} sparks is fairly well understood. Ryanodine receptors (RyRs) are Ca^{2+} -release channels that are activated by Ca^{2+} itself (calcium-induced Ca^{2+} release, CICR). The trigger Ca^{2+} activates a tightly packed cluster of RyRs and the opening of these channels release Ca^{2+} from the sacroplasmic reticulum (SR). This release produces a Ca^{2+} spark. The problem, however, is how does the release process terminate.

Dr. Jafri proposed a "Sticky Cluster" model to explain the termination of Ca^{2+} sparks. The model took into consideration three main factors that contribute to RyR gating: 1) The large number of Ryanodine receptors within a cluster, 2) the concentration of Ca^{2+} within the SR and 3) the "coupling" of RyRs to each other, that is, the state of a single RyR being influenced by the state of the other RyRs in the cluster. This coupling imparts the "stickiness" noted in the model's name. Thus, as a spark is initiated, the calcium level in the subspace between the array of receptors and the T-tubule wall containing the L-channels increases dramatically. This activates RyRs via CICR and the Ca^{2+} release channels open leading to an even higher Ca^{2+} in the subspace and even more complete activation of RyRs. This "positive feedback" would keep the RyRs activated unless some other element was involved. The modeling shows that decreasing the open probability of RyRs as SR Ca^{2+} falls, in combination with coupled gating, enables the positive feedback to be overcome. Experimental support for the models comes from an examination of Ca^{2+} sparks following a drug that decreases "stickiness."

As with most good models, Dr. Jafri's "Sticky Cluster" model suggests some very provocative and interesting hypotheses that will be tested in the next few years.

Perplexing Prion Proteins

Dr. Ilia Baskakov from the Institute for Neurodegenerative Diseases, University of California at San Francisco, the fourth participant in our new series, spoke to MBC on "Conformation Transition of the Prion Protein: Exception or Rule in Protein Folding." Prions, associated with "Mad Cow Disease" among other infectious neurodegenerative diseases, are small, mysterious, self-replicating proteins whose normal functions within cells have not been discovered. They are known to be glycosylated and membrane bound and have been found only in the brain to date, though there is a report of a homologous protein in the testis. However, in the disease state, whether infectious or non-infectious, they form amyloid-like plaques that disrupt normal cellular function. Many different proteins can form plaques, large, organized conglomerations, though not all of these cause disease.

The prion protein can fold into two different, stable configurations: the normal or α helical monomer form or a misfolded oligomer form of β sheets. The prion plaques are composed of the misfolded β oligomer form of the protein. Dr. Baskakav's work suggests that an unfolded prion protein can go into either an α monomer state, which forms quickly and is under kinetic control, or into the β oligomer form, which is thermodynamically stable but has a high energy barrier to its formation. Because the β form is thermodynamically more stable, one would suppose that it would be the "normal" state, which is not the case, hence the question in the title. The research is complicated by the lack of cellular tags to study the native protein *in vivo*, as well as, the long, strain-dependent disease development time in mice. Even simple experiments can take up to two years.

The complexities of dealing with a protein whose basic function is unknown, as well as what controls its ability to replicate in the disease state, were well brought out by Dr. Baskakov's thought-provoking talk.

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