Cellular membranes get organized for signaling

Membranes are fundamental components of all cellular organisms. Not only does the membrane enclose each cell as a separate biological entity, but it performs a myriad of critical functions to maintain the life of the cell with respect to the "extracellular," or outside, world. Despite the enormous variety of life forms, biological membranes have a common basic structure that is composed of a thin (~6.0 nm) film of lipids and proteins. For many years it was thought that the fluid-like behavior of the lipids resulted in a structureless assembly that simply formed an impermeable wall. However, it is now clear that the lipids, in concert with the membrane proteins, can be organized into structures that help the cell perform specific functions.

Fig. 1A schematically depicts current models of membrane structure. The lipids (yellow, green) form a bilayer that incorporates many transmembrane proteins (red and blue), as well as many membrane associated proteins (not shown). Domains of lipids (green areas) may form within the bilayer due to subtle differences in molecular structure. Many believe that these "lipid rafts" may in turn serve to sequester specific groups of proteins that perform specific functions. Others believe that the proteins cluster due to protein-protein interactions and organize the lipids around them. Another component that may add structure to the membrane is the actin cortical cytoskeleton (gray filaments). The role of the cytoskeleton is not clear, but it is has been shown to assist into the complex process of endocytosis at the membrane.

One of the most important functions of the membrane is that of "cellular signaling," the process by which "receptor" proteins in the membrane detect specific biomolecules in the extracellular environment and trigger a response by the cell. In collaboration with the Dept. of Pathology at the University of New Mexico, Sandia is using advanced imaging techniques to map the location of receptor proteins and associated protein partners in the signaling network. Specifically, we use atomic force microscopy (AFM), combined with fluorescence labeling, to map the location of receptor proteins within the topographical structure of the cytoplasmic (interior) side of mast cell membranes. Mast cells are involved in the adaptable immune response that ultimately results in the expression of histamines. The membranes are harvested from the mast cells and laid out on a glass

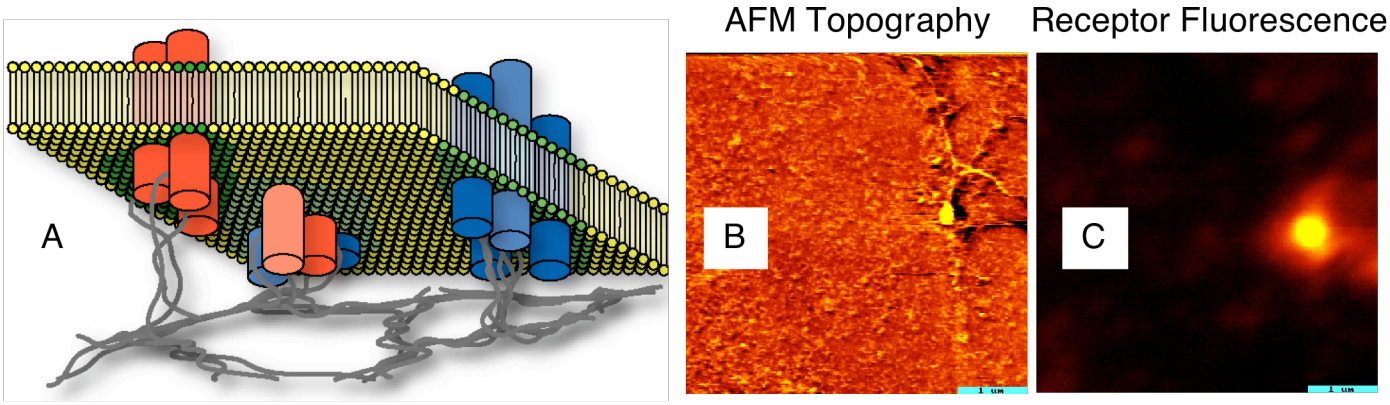

Figure 1: (A) Schematic depiction of a cell membrane showing lipid bilayer, transmembrane proteins (red and blue), and cytoskeleton (grey). (B) AFM image of cytoplasmic (interior) face of mast cells and (C) simultaneous fluorescence image of labeled IgE receptor. The bright cluster of receptors in (C) correlates to the bright topographic feature in (B). Scale bars are 1 micron.

substrate for imaging.

Examples of combined AFM/fluorescence images of cellular membranes are shown in Fig. 1 B,C. Here the IgE receptor protein is fluorescently labeled and seen to form a bright cluster upon activation. The protein cluster is seen as a distinct topographic structure in the AFM image that is100-200 nm in size. Also visible are

remnants of the cytoskeleton. The close association of the IgE receptors and the cytoskeleton are of great interest and may be tied to the endocytosis of the receptor at later stages of the signaling process.

There are many outstanding questions that we seek to answer in our studies. How do the signaling proteins form clusters? Clearly diffusion processes are critical. Is the diffusion driven by lipid interactions or protein-protein interactions? What is the influence of the membrane structure on the formation of the clusters? *Structural* studies such as that provided by AFM are critical in these investigations. Sandia is also engaged in *dynamical* studies to follow protein motion in the same mast cells. In those studies, live cells are imaged with the technique of total internal reflection (TIRF) fluorescence microscopy. TIRF allows one to capture the organization and movement of individual membrane proteins by exclusively exciting fluorophores in the membrane with an evanescent field that extends less than 100 nm into the cell. Thus other parts of the cell do not contribute as background to the image. Sensitive CCD cameras are used acquire time-resolved images of the protein motion. Results so far indicate that the actin cytoskeleton can directly confine the motion of receptor proteins to distinct areas or "corrals."

The cell is the ultimate biosensor. Just a few antigens bound to receptor proteins can induce a cascade of cellular processes which can take many years to deduce. We can learn much about biosensing from fundamental studies in cell membrane structure and function. Moreover, medical advances in cancer research and drug therapies will depend to a large extent on a molecular-level understanding of cellular signaling at the membrane.

Figure 2: Alan Burns earned a PhD in chemical physics at UC Berkeley. He has been a staff member at Sandia since 1980 and is currently in the Biomolecular Interfaces and Systems Dept. based in New Mexico.