FINAL

Report sent to Space Biology Program upon termination of NAGW-3046 (for period ending September 30, 1996)

Plus Addendum listing publications to date

Mechanotransduction and the Cytoskeleton

Professor Barbara G. Pickard
Biology Department Washington University
St. Louis MO 63130-4899

By the time the grant was actually funded, I had lost my patch clamper to a postdoctoral position which had current, as well as long-term, funding. At the same time, two intellectual developments in the lab suggested a more powerful approach to the problem defined in the original statement. These were discussed with the "Program Chief", who agreed that an alteration of approach was desirable.

First, still-unpublished experiments by my former patch clamper and by a collaborator in Japan had shown the mode of action of some of the compounds we were interested in testing, and we realized that we could predict the influences of a large number of compounds we were interested in.
thus, we felt challenged to jump ahead and look at the cytoskeletal environment more directly.

Second, expansion of our work on the computational optical sectioning microscope (COSM) which was built by my colleague James G. McNally here at Washington University showed that we had unique capability for observing the cytoskeleton directly, in living cells, during mechanostimulation. In principle, we have a method at hand for locating the channels by this fluorescence microscopy as well. I was able to bring in a postdoctoral associate who was interested in pursuing this study. Also, because of the multiple facets of COSM work, and the immensity of each facet, we applied for a NASA/NASFNET grant in sensory plant biology and received it. Therefore, several studies have gone on in parallel and the people supported by the latter have interacted usefully with the postdoctoral fellow in learning new computer techniques, maintaining fragile equipment, and so on.

Though the coauthors of the forthcoming papers from my lab were supported by different grants, the primary questions addressed by the postdoc supported by this research were: Are there cytoskeletal proteins (besides actin and microtubules) in plants similar to those that make up the known group of cytoskeletal players in animals? How are these distributed in the living cell? How is the distribution influenced by activity of the mechanosensory calcium channel we believe to be responsible for vectorial gravitropic stimulation and for the sensing of mechanical stimuli in general? Additionally, we hoped to visualize the channels with respect to cytoskeletal entities, but the first three questions proved to have such important answers and to require such intensive work to obtain them that we deferred this covisualization for future activities supported by the NASA/ NSF collaborative grant.

We have identified a major here-to-fore unknown cytoskeletal structure in our representative experimental system, the onion epidermal cell, and have named this structure the endomembrane sheath.

The endomembrane sheath appears to anchor at adhesion sites, to which we postulate the gravitropic sensor channels are also tethered. A paper on these adhesion sites has been published in the international journal Protoplasma. The contribution to this paper by

NAGW-3046 (which funded a postdoc) was to extract and separate and immunologically identify the key adhesion protein integrin.

The adhesion sites are postulated to be of importance for the activation of the mechanosensory chanels (see review article on Protoplasma 182:1-9), and they and the endomembrane sheath are presumed important for the internal signalling sequelae that follow activation.

Since this report was filed, one manuscript has been published and one is in press, due out the last issue of 1997. These are:

Reuzeau, C., McNally, J.G., Pickard, B.G. 1997. New Ideas in Cell Biology. The endomembrane sheath: a key structure for understanding the plant cell? Protoplasma 199:□–□.

Reuzeau, C., Doolittle, K.W., McNally, J.G., Pickard, B.G. 1997. Covisualization in living onion cells of putative integrin, putative spectrin, actin, putative intermediate filaments and other proteins at the cell membrane and in an endomembrane sheath. Protoplasma 199:173-197.

Another manuscript is nearly ready to send in but is awaiting an illustration from one of the authors, who left the lab before finishing the work.