Summary of Research for:

Fucus as a Model System to Study the Role of Auxin Transport and the Actin Cytoskeleton in Gravity Response

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The overarching goal of this proposal was to examine the mechanisms for the cellular asymmetry in auxin transport proteins. As auxin transport polarity changes in response to reorientation of algal and plant cells relative to the gravity vector, it was critical to ask how auxin transport polarity is established and how this transport polarity may change in response to gravity stimulation. The experiments conducted with this NASA grant fell into two categories. The first area of experimentation was to explore the biochemical interactions between an auxin transport protein and the actin cytoskeleton. These experiments used biochemical techniques, including actin affinity chromatography, to demonstrate that one auxin transport protein interacts with the actin cytoskeleton. The second line of experiments examined whether in the initially symmetrical single celled embryos of *Fucus distichus*, whether auxin regulates development and whether gravity is a cue to control the morphogenesis of these embryos and whether gravi-morphogenesis is auxin dependent. Results in these two areas are summarized separately below. As a result of this funding, in combination with results from other investigators, we have strong evidence for an important role for the actin cytoskeleton in both establishing and change auxin transport polarity. It is also clear that *Fucus distichus* embryos are auxin responsive and gravity controls their morphogenesis.

Biochemical characterization of an auxin transport protein:

One of the very important questions about the auxin efflux carrier protein complex is how is this protein localized to the membrane along the lower side of cells that transport auxin, to thereby control the directional polarity of auxin movement. Interaction with the actin cytoskeleton may be a mechanism that either targets the protein to this membrane location and/or fixes the protein in this location. To explore this possibility we have been testing whether an auxin transport protein interacts with the actin cytoskeleton. We have been specifically exploring one protein that is part of an auxin efflux carrier complex, the naphthylphthalamic acid (NPA) binding protein, which is the site of action of auxin transport inhibitors, including NPA. Treatments that fragment the actin cytoskeleton release NPA binding activity from the cytoskeleton and reduce auxin transport (Butler et al., 1998). NPA binding protein is retained by F-actin affinity columns, indicating a direct interaction between NPA binding protein and the actin cytoskeleton (Hu et al., 2000). Currently, efforts are focused on characterizing the interaction between the NPA binding protein and other proteins that make up the auxin efflux carrier complex.

We have constructed two models for how the polar distribution of auxin transport proteins is established and how actin-fragmenting drugs may reduce auxin transport. First, the actin interaction may function to localize proteins once they have reached the appropriate position in the membrane (Muday, 2000). The second possibility is that actin tracks that allow cycling of this protein and other proteins that mediate IAA efflux between the endosome and the basal membrane (Muday and Murphy, 2002; Muday et al. 2003). For either of these models fragmentation of the actin cytoskeleton would result in reduction in polar distributions of an auxin efflux carrier and thereby reduced polar auxin transport.

Role of auxin transport in Fucus embryonic development and gravity induced polarity

A number of recent reports have implicated the plant hormone auxin and its polar transport in plant embryo and vascular development, using both genetic mutants and auxin transport inhibitor treatments. Several Arabidopsis mutants have been identified that have altered embryo development and appear to have a primary defect in auxin response or homeostasis. We have asked whether auxin transport is also important during the first embryonic division and are using embryos of the brown algae, *Fucus* to test this possibility. First, it was necessary to determine if these embryos contained auxin and exhibited auxin transport (Basu et al. 2002). Indole-3-acetic acid (IAA) was identified in *Fucus* embryos and mature tissues by gas chromatography-mass spectroscopy. IAA movements into and out of embryos were observed using radiolabeled IAA and IAA efflux was regulated by NPA. *Fucus* embryos normally develop with a single unbranched rhizoid, but growth in the presence of excess concentration of the auxin, IAA, or the auxin transport inhibitor, NPA, leads to formation of embryos with either branched and multiple rhizoids when there are limiting amounts of light. These embryo alterations are induced by as little as 30 minutes of exposure to these compounds immediately after fertilization and the effects are complete within the first 6 hours, suggesting that auxin plays a role in initial stages of development. These results suggest that early stages of *Fucus* embryo development including orientation of polarity and developmental pattern are interconnected with auxin transport (Basu et al. 2002).

Additional studies have examined the role of auxin transport in the formation of polarity in response to environmental stimuli. Rhizoids normally form on the shaded site of embryos that are given unilateral light. We have found that *Fucus* rhizoid development will also orient in response to gravity (Sun et al. in review). Clinorotation, which is continuous rotation of the embryos to randomize the gravity vector, will reverse this polarization, as will treatment with auxin transport inhibitors. Auxin transport inhibitors block development of photopolarization, as well. Current experiments are focused on understanding the mechanisms by which auxins regulate development. As NPA and IAA affect photopolarization as early as four hours after fertilization, similar to the timing for developmental effects, we are examining other developmental events that occur in this time frame. The earliest changes in cellular structures that have been identified during development of other species of brown algae are rearrangements in the actin cytoskeleton. We have found similar early actin patches in *Fucus* that form in response to light gradients on the shaded side of the embryo and that auxin transport inhibitors do not prevent formation of these patches, but do randomize the location. It addition to an important role of polarized actin patches in embryo polarity development, localized vesicle secretion also plays an important role. Other experiments that are underway to examine the timing of vesicle secretion in these embryos relative to the action of auxin transport inhibitors. Finally, inhibitors that alter actin filament organization prevent gravity induced polarization (Sun et al. 2003). These experimental results clearly indicate that auxin transport and the actin cytoskeleton are integrally linked in the response of the embryos to gravity and light signals.

Articles published with support of this grant

Sun, H, Basu, S, Brady, SR, Luciano, RL, and Muday, GK (In review) Interactions between auxin transport and the actin cytoskeleton in developmental polarity of *Fucus distichus* embryos in response to light and gravity. Development

Muday, GK, Peer, WA, Murphy, AS (2003) Vesicular cycling mechanisms that control auxin transport polarity. Trends in Plant Science. 8:301-303.

Basu, S., Brian, L., Quatrano, R.L., and Muday, G.K. (2002) Early embryo development in *Fucus* is auxin dependent. Plant Physiology. 130: 292-302

Muday, G.K. and Murphy, A.S. (2002) Insight: An emerging model of auxin transport regulation. Plant Cell: 14: 293-299

Muday, G.K. and DeLong, A. (2001) Polar Auxin Transport: Controlling where and how much. Trends in Plant Science. 6: 535-542

Muday, G.K. (2001) Auxin and Tropisms. Journal of Plant Growth Regulation. 20:226-243

Muday, GK (2000) Maintenance of asymmetric cellular localization of an auxin transport protein through interaction with the actin cytoskeleton. J Plant Growth Reg 19: 385-396

Hu S, Brady, SR, Kovar, D, Staiger, C, Clark, G, Roux, S, and Muday, GK. (2000) Identification of plant actin binding proteins by F- actin affinity chromatography. Plant Journal 24: 127-137

Muday, GK, Hu, S, and Brady, SR (2000) The actin cytoskeleton may control the polar distribution of an auxin transport protein. Gravitational and Space Biology Bulletin. 13: 5-83

Butler, JA, S Hu, S Brady, MW Dixon and GK Muday (1998) In vitro and in vivo evidence for actin association of the NPA binding protein. Plant Journal 13, 291-301.

Inventions:

No inventions resulted from this work.