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The role of ARP2/3 and Scar/WAVE complexes in polar growth of the apical cell of the moss Physcomitrella

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Beyond Model Organisms

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The role of ARP2/3 and Scar/WAVE complexes in polar growth of the apical cell of the moss *Physcomitrella*

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The components required for moss filaments to undergo polar extension growth, and to perceive and respond to orienting vectors such as light and gravity, are localized in the single apical cell. To identify genes that function in these polar processes, the moss *Physcomitrella* patens has the unique ability among plants to undergo efficient homologous recombination. RNAi methodology is also available for this purpose. Actin microfilaments are required for these polar responses in *P. patens*. When the ARP2/3 complex member arpc4 is deleted, the null mutant (Δarpc4) is viable and clearly undergoes normal morphogenesis of filaments into leafy shoots. However, we observe a striking reduction of tip growth of the apical cell and a defect in its response to polarized white light. Insertion of YFP-ARPC4 into Δarpc4 rescues the mutant phenotypes and localizes ARPC4 exclusively to the tip of the apical cell, the site of actin dynamics and polarized extension. Using RNAi, we show that when another member of the ARP2/3 complex (i.e. ARPC1) is lacking, a similar but more severe phenotype is seen. We are able to rescue this RNAi line by ARPC1 overexpression. When BRK1(Scar/WAVE) is deleted, the resulting null mutant (Δbrk1) is viable and responds normally to polarized light. Δbrk1 completes the morphogenetic transition from filamentous growth to leafy shoots but displays a striking reduction of apical cell growth. Insertion of BRK-YFP into Δbrk1 rescues the mutant phenotypes, and localizes BRK1 exclusively to the tip of the apical cell. However, Δbrk1 is unable to localize both ARPC4 and the cell wall proteoglycan AGP to the tip of the apical cell. Our results are consistent with a model that the Wave/SCAR complex is required to localize Arp2/3, which is an essential component for orienting polar tip growth. Supported by the NSF (IBN-00112461).

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The derived Hox gene zen is required for function, but not specification, of the extraembryonic serosal membrane in *Oncopeltus fasciatus*

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The Hox3 orthologue of insects, zen, does not have a Hox-like role in conferring segment identity but instead is expressed extraembryonically. Fruit fly zen and beetle zen1 are required in this domain to establish the identity of extraembryonic membranes; in their absence the presumptive extraembryonic tissue acquires an embryonic identity. However, functional studies of other zen genes suggest tissue specification is not the only function of zen. We have been investigating the role of zen in the milkweed bug, *Oncopeltus fasciatus*, by maternal RNA interference, and by comparison of wild type zen expression with the dynamics of extraembryonic membrane function. Oncopeltus zen (Of-zen) is specifically expressed in the outer extraembryonic membrane, the serosa. However, Of-zen is expressed relatively late in this tissue, precluding a role in specification. In early development the serosa surrounds yolk and embryo. Halfway through embryogenesis, the movements of katatrepsis change this physical arrangement: the serosa contracts toward the anterior pole of the egg, causing the embryo to emerge from the yolk. Knockdown of Of-zen has no effect on the formation of the serosa but renders it inert, resulting in a failure of katatrepsis and thus confining the embryo within the yolk such that it completes development inside out. Highest levels of Of-zen expression correlate well with the dynamics of katatrepsis, including patterns of actin polymerization. We are pursuing these observations as we elucidate the mechanism of zen function and its molecular .context, including downstream targets.

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Cell or organism? Insights the Mermaid's Wineglass (*Acetabularia acetabulum*), a classic unicellular model, can contribute to modern biology

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Acetabularia acetabulum (a.k.a. *A. mediterranea*) is a classic unicellular model for development: in the 1930s,

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