Pattern formation is a crucial event that occurs at all scales during embryonic development. Sea urchin larvae possess a skeleton which is secreted by the primary mesenchyme cells (PMCs). However, skeletal patterning information is localized within the ectodermal cells, and is detected by thin filopodia extended from the PMCs. SB23580 (SB) and NiCl2 treatments provoke opposite ectodermal effects, since SB dorsalizes, while nickel ventralizes the ectoderm. However, transient exposure to either perturbant induces dramatic skeletal patterning defects via ectodermal perturbation. We predicted that ectodermal patterning genes are absent in both nickel- and SB-treated embryos, and are a minority cohort of co-regulated genes, while the majority of genes are reciprocally regulated by SB and nickel. We therefore used RNA-seq analysis of control, SB-, and nickel-treated embryos to identify genes mutually down-regulated by both perturbants. This group of genes represents 1.5% of scaffolds, compared to ~20% of scaffolds affected by each single perturbant. 72 candidates, corresponding to mutually-downregulated genes that encode surface proteins, were identified. These conserved genes include Reelin, BMP5-8, Notch2, Mindbomb, ST-14, 5-LOX, Prestin, SVEP, MLD, RECK. Functional characterization demonstrates that these candidates are specifically required for skeletal patterning and do not impact ectodermal specification. Morphant phenotypes reveal novel and dramatic skeletal patterning defects which are reflected in defective PMC migration patterns. Thus, patterning of the sea urchin skeleton reflects a functional convergence of genes that, in vertebrates, control diverse processes ranging from neocortical patterning, auditory amplification, vasculogenesis, and metastasis. Interestingly, sea urchin larva lacks a neocortex, a vasculature, audition, and cancer. Thus, sea urchin skeletal patterning provides a surprising and unexpected glimpse of the ancestral functions for this cohort of genes.

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**Program/Abstract #467**
**Identification of the gene responsible for the wings apart phenotype in *Drosophila melanogaster***
Ginny R. Morriss, Carmelita T. Jaramillo, Richard M. Cripps
*University of New Mexico, Albuquerque, NM, USA*

The Drosophila wings apart (wap) locus contains a semi-lethal gene that when mutated leads to the absence of the Tergal Depressor of Trochanter (TDT) muscle. wap has been mapped to the proximal X chromosome but it is unclear what gene is mutated to produce the wap phenotype. The aspect of muscle development disrupted in wap mutants leading to TDT loss is also unknown. To identify the wap gene, we performed complementation mapping of wap mutants crossed with known X chromosome deletions. We sectioned thoraces of progeny from these crosses to observe if these flies exhibit the TDT phenotype associated with wap. Results of mapping analysis and phenotypic characterization suggest the most likely candidate for the wap gene is DIP1. PCR of DIP1 underway in wild-type and wap mutant flies to detect the mutation leading to the observed phenotype has shown an alanine to threonine amino acid substitution in the DIP1 coding region in wap mutants. Loss- and gain-of-function assays are in progress to determine if loss of DIP1 reproduces the wap mutant phenotypes and if over-expression of DIP1 rescues the wild-type phenotype. The impact of the wap mutation will be analyzed by determining at which step in development TDT muscle formation is disrupted. Initial experiments have shown that wap mutants lack TDT specifying founder cells, suggesting wap is necessary for early TDT specification. The broad goal of this research is to identify mechanisms of muscle formation in the Drosophila adult. Since similar developmental mechanisms are used in vertebrate and invertebrate muscle formation, this study can aid in understanding processes which may impact vertebrate muscle formation and whose mis-regulation may lead to muscular diseases.

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**Program/Abstract #468**
**Live imaging of stomatal determinants reveals dynamic interaction among precursor cells**
Kylee Peterson[a], Amanda Rychel[a], Keiko Torii[b]
[a]*Dept of Biology, University of Washington, Seattle, WA, USA*
[b]*Dept of Biology, University of Washington and PREST, JST, Tokyo 102-0075, Japan*

Stomata, watertight valves on the plant surface, allow land plants to survive in dry conditions by facilitating gas exchange while moderating water loss. Their distribution on the plant epidermis is even and nonrandom; stomata are not found adjacent to one another. The bHLH transcription factors SCREAM (SCRM) and SCRM2 are robustly expressed throughout stomatal development and have been shown to interact with the bHLHs SPEECHLESS, MUTE, and FAMA. These heterodimers specify three sequential cell-state transitions critical to stomatal differentiation. Live time-lapse imaging of a translational fusion of SCRM with green fluorescent protein (GFP) under its native promoter shows that SCRM-expressing cells arise in pairs on a germinating cotyledon. As a rule, one cell differentiates into a stoma, while one divides asymmetrically away from it, maintaining the one-cell spacing rule. Computational analysis of fluorescence intensity determines the relationship of GFP–SCRM expression level to cell fate in unperturbed cotyledons, signaling mutant backgrounds, and cell ablation contexts leading to possible cell fate change.

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