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Abstracts

Symposium 1: RNA, localization, translational and regulation

Program/Abstract # 2
Localized RNAs, localized translation, and developmental asymmetry

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Intracellular mRNA localization establishes protein asymmetries for polarization of somatic cells, oocytes, and embryos. In both invertebrates and vertebrates, mRNA localization during oogenesis generates asymmetric distributions of maternal determinants to establish the embryonic body plan. Localization of bicoid and nanos mRNAs to opposite ends of the *Drosophila* oocyte provides the sources for protein gradients that pattern the anterior–posterior body axis of the embryo. Using a system for fluorescent tagging of mRNAs in vivo, we have been able to visualize movement of *bicoid* and *nanos* mRNAs in living oocytes. Results from cell biological studies show that these mRNAs are localized concurrently by different localization pathways and reveal new complexities in mRNA localization mechanisms. Biochemical assays have begun to identify components of a localization-competent nanos RNA–protein complex and current efforts aim to determine how localization of nanos and its translation are coupled through these factors. The importance of mRNA localization and translational control to nanos function in the nervous system will also be discussed.

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Program/Abstract # 3
Switching from repression to activation: Post-transcriptional regulation of BMP2 synthesis

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Elevated BMP2 synthesis is associated with increased lung cancer malignancy and poor patient prognosis. Increased BMP2 levels also are involved in other pathologies, such as vascular calcification. Using transgenic and cell culture *BMP2* expression models, we discovered that an ultra-conserved post-transcriptional *cis*-regulatory element in the *BMP2* 3′untranslated region (UTR) markedly repressed *BMP2* expression in cells which do not synthesize BMP2 (e.g., non-transformed lung cells or pluripotent mesenchymal cells). In contrast, the ultra-conserved sequence markedly stimulated *BMP2* expression in cells that express BMP2 (e.g., transformed lung cells). Biochemical methods demonstrated that cytoplasmic nucleolin binds the *BMP2* 3′ UTR. Overexpression of nucleolin in transformed lung cells blunted the activating effect of the ultra-conserved sequence in these cells. Conversely, siRNA knockdown of nucleolin in non-transformed lung cells dramatically induced BMP2 protein without changing the *BMP2* mRNA level. Thus nucleolin normally inhibits BMP2 synthesis in normal lung cells *via* ultra-conserved sequence. Furthermore, increased nucleolin levels can inhibit abnormal BMP2 synthesis. Our studies have revealed novel mechanisms that naturally down-regulate the *BMP2* gene in tissues where BMP2 expression is dynamically regulated (developing heart) or pathologically induced (transformed lung cells, aorta and coronary vasculature).

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Program/Abstract # 4
Pattern formation by small RNA signals

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Plant stem cells not only serve as a persistent source of cells from which lateral organs arise, they also produce signals important for the patterning of lateral organs. Our work focuses on small regulatory RNAs as potential signals. Leaves of higher plants exhibit a varying degree of asymmetry along their dorsoventral axis. This asymmetry is specified via the polarized expression of class III homeodomain-leucine zipper (hd-zipIII) genes, which specify dorsal cell fate. Through detailed in situ hybridization analyses we were able to show that this polarized hd-zipIII expression is set up by the graded expression pattern of a 21-nucleotide microRNA, miR166. More recently, we have shown that this miR166 gradient is generated through the opposing activity of a different small regulatory RNA, the transacting siRNA tasiR-ARF. A further twist to the story is added by the observation that tasiR-ARF in turn is regulated by a third small RNA, miR390. Our observations identify a novel mechanism of pattern formation in which cell fates along a developmental axis are specified through the opposing activities of distinct small RNAs. miR390 and tasiR-ARF define the dorsal side of the leaf by restricting the expression domain of miR166, which in turn delineates the ventral side by restricting expression of the HD-ZIPIII transcription factors. Importantly, comparison of the expression patterns of small RNA precursor transcripts to those of the mature small RNAs provides evidence that small regulatory RNAs

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