

retardation in humans, Fragile X Syndrome (FXS). FMRP has been implicated in the translational control of specific mRNAs, and the cognitive symptoms of FXS are thought to stem from the aberrant translation of some of these mRNAs. We have demonstrated that *Drosophila* fragile X mental retardation protein (dFMRP) is required in early embryos for cleavage furrow formation and functions within dynamic cytoplasmic ribonucleoprotein (RNP) bodies during the maternal-to-zygotic transition (MZT). In an effort to identify potential new targets of dFMRP regulation, we have employed a proteomics-based approach, two-dimensional gel electrophoresis. We have discovered forty proteins whose expression differs between control and *dfmr1*-cleavage stage embryos. Twenty-eight of these proteins have been identified by mass spectrometry and will be presented. We are using genetic assays to determine if selected candidates interact with *dfmr1* and affect cleavage furrow formation and biochemical assays to address whether the identified proteins are direct or indirect targets of dFMRP-dependent translational regulation. Characterization of these candidate targets should provide insight into the mechanisms of dFMRP-dependent regulation of cellular morphogenesis in cleavage stage embryos and the etiology of FXS.

doi:10.1016/j.ydbio.2008.05.191

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#### Program/Abstract # 180

##### **Serotonin synthesis is necessary for gastrulation in the sea urchin, *Lytechinus pictus***

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Previous studies in our lab have identified several components of a serotonergic system in blastula and gastrula sea urchin embryos. These studies suggest that serotonin regulates gastrulation. In the present study we examined the role of the serotonin synthetic enzyme, tryptophan hydroxylase (TPH), in sea urchin embryogenesis. Embryos were treated with various concentrations (0.5–20  $\mu$ M) of *p*-chlorophenylalanine methyl ester (PCPA), an inhibitor of TPH, beginning at fertilization or at the hatched blastula stage. Cleavage was not affected, and embryo development stopped prior to gastrulation at the mesenchyme blastula stage with higher concentrations of the drug. Lower concentrations blocked development at the early gastrula stage. The lowest levels of PCPA to exert effects delayed development by delaying the onset of gastrulation beyond the early gastrula stage. Serotonin (100  $\mu$ M) or dibutyl cyclic AMP (1  $\mu$ M), added along with the inhibitor (20  $\mu$ M) at hatching, partially or totally, respectively, rescued embryos from the inhibitory effects of PCPA on gastrulation. PCPA inhibited TPH activity in enzyme assays of embryo homogenates. The nonmethylated inhibitor did not inhibit gastrulation and was also a less potent inhibitor of TPH enzyme activity. This study suggests that PCPA specifically blocks serotonin synthesis, and that serotonin regulates the initiation of both the primary and secondary phases of gastrulation by signaling mechanism(s) that involve cyclic AMP.

doi:10.1016/j.ydbio.2008.05.192

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#### Program/Abstract # 181

##### **Role of serotonin in sea urchin embryo morphogenesis**

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In the sea urchin embryo, pharmacological studies in our lab and others have suggested that serotonin may initiate cell movement processes during gastrulation. To further investigate the role of a serotonergic system in embryogenesis, we measured levels of serotonin and its precursor and metabolites, as well as mRNA levels and enzyme activity of the serotonin synthetic enzyme, tryptophan hydroxylase, in *Lytechinus pictus* blastula- and gastrula-stage embryos and larvae. Serotonin levels peak in mesenchyme blastula embryos, immediately prior to gastrulation, and at mid gastrula and late pluteus larva stages. Alterations in serotonin levels mostly follow the measured fluctuations in serotonin synthesis and metabolism. Preliminary studies suggest that preneural embryos may utilize phenylalanine hydroxylase to synthesize serotonin. Inhibitors of mammalian type 1, 2, 4, 6 and 7 serotonin receptors blocked the initial invagination of the vegetal plate, whereas lower concentrations delayed the initiation of the secondary phase of gastrulation with little effect on subsequent development. Serotonin or dibutyl cyclic AMP, coincubated with ritanserin or chlorpromazine, types 2 and 7 receptor antagonists, at least partially rescued embryos from gastrulation inhibition. Homology searches of the sea urchin genome identified types 1, 2 and 7 receptors, all of which in mammals can increase cyclic AMP levels following activation by serotonin. In sum, sea urchin embryos contain a serotonergic system that may regulate the gastrulation process.

doi:10.1016/j.ydbio.2008.05.193

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#### Program/Abstract # 182

##### **Hydra matrix metalloproteinases are involved in tissue dynamics, patterning process, and morphogenesis**

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Matrix metalloproteinase (MMP) is a family of matrix enzymes that digest extracellular matrix (ECM) molecules. This digestion is necessary when the ECM needs to be remodeled or re-structured to meet the needs of growth and morphogenesis. MMP is well conserved through evolution in multicellular organisms from plant to human. In hydra, a fresh water cnidarian, 10 MMPs, named HMMPs, have been cloned in our lab by searching the hydra genome and EST sequences. They appear as two groups based on sequence characteristics. One group has larger proteins containing the hemopexin domain; the others are smaller containing only the catalytic domain. The two groups showed distinct differences in expression pattern. HMMPs with hemopexin domains, including HMMP, HMMP-A2, and HMMP-A3, appear in tentacles and buds, regions where patterning and morphogenesis happens. Shorter HMMPs, HMMP-B through H, those without a hemopexin domain, appear in the body column, where growth and tissue movement are undertaken. We are currently working on inhibitors to MMPs using zymogram assay and biological approaches. The ultimate goal of this project is to understand each HMMP in terms of its synthesis, substrate, and function during tissue dynamics, pattern formation, and morphogenesis in hydra.

doi:10.1016/j.ydbio.2008.05.194