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Isolation of Maternal Genes Expressed during Ascidian Oogenesis B.J. Swalla, K.W. Makabe, W.R. Jeffery University of California-Davis, Zool.Dept; BML, Bodega Bay, CA94923

Two closely related ascidian species were used to define maternal genes that specify ascidian larval tissues. *Molgula oculata* develops into a typical ascidian tadpole larva with a head and a tail. In contrast, *Molgula occulta*, a sympatric species, develops into an anural (tailless) larva that lacks a brain pigment cell, notochord, and tail muscle. Interspecific hybrids between *M. occulta* eggs and *M. oculata* sperm can make a brain pigment cell and a small tail containing notochord, but lack differentiated muscle cells. The lack of muscle cell differentiation in *M. occulta* or hybrid embryos may be due to the elimination or modification of maternal muscle determinants. We have identified a cDNA clone encoding a novel egg cytoskeletal component, p58, which is localized in the myoplasm of tailed ascidian species (but not localized in anural species) that is similiar to the *Drosophila* gene *enhancer-of-split*. Both proteins have a c-terminal region homologous to yeast cdc-4 and transducin. We also used a subtractive hybridization procedure to isolate three cDNA clones encoding mRNAs that are specific to or enriched in *M. oculata* oocytes. Two of these clones may encode DNA binding proteins, and one is a member of the tyrosine kinase family. One of the putative DNA binding proteins contains a nuclear localization signal, and a zinc cluster motif. The other contains a leucine zipper, suggesting it may function as a dimer. These genes are candidates for maternal factors that control the development of the ascidian tadpole larva.

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The Pattern of Ovariole Formation and Germ Cell Production in Puparia of Drosophila melanogaster. E.F. Beckemeyer and P.D. Shirk, USDA-ARS Insect Attractants, Behavior and Basic Biology Res. Lab., Gainesville, FL 32604.

We have used immunofluorescent staining and confocal imaging to examine ovariole morphogenesis in whole ovaries of Oregon-R Drosophila during pupal and pharate adult stages. Antisera to vasa was used to distinguish the germ cells from the somatic tissues of the ovaries. We have analyzed the allocation of germ cells into ovarioles and the divisions of germ cells within ovarioles. Greater numbers of germ cells appear to be present initially in each ovariole than suggested as necessary by current stem-cell hypotheses for production of cystoblasts.

The previously described concentric arrangement of ovarioles within young adult ovaries appears to be generated from a looping of the initial array of ovarioles. Comparison of ovariole morphogenesis and germ cell production among orders of insects with the polytrophic, meroistic type of ovarioles (Diptera, Hymenoptera and Lepidoptera) indicates diverse strategies in the establishment of ovarioles and in the sequence and timing of germ cell divisions. The monospecific, polyclonal vasa antiserum was a gift of Drs. B. Hay and Y.N. Jan.

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Ultrastructural localization of vasa protein during early oogenesis. W. L. Diehl-Jones and P. F. Lasko. Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1 (membership applied for, April 1992).

The vasa gene is a member of the posterior group of Drosophila maternal-effect genes, and is required for embryonic germ cell formation. Its predicted protein sequence suggests that vasa may be an ATP-dependent RNA-binding protein involved in post-transcriptional gene regulation. vasa protein is found in the germaria of adult ovaries and in nurse cell chambers during early oogenesis. The ultrastructural localization of vasa protein in germaria and egg chambers was examined with immunogold labelling. During early oogenesis (stage 1-4 follicles), vasa protein labels diffusely in the germ cell cytoplasm. More localized signal is detected in three regions: within a 200 nm band of the cytoplasmic face of the perinuclear zone; over perinuclear nuage material, and in close association with outer mitochondrial membranes. We will also discuss the EM localization of vasa protein in ovaries from females carrying a weak temperature-sensitive mutant allele of vasa, and from flies mutant for related loci.

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Mutational analysis of the vasa protein. L. Liang, C. Wei and P. F. Lasko. Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1 (Spon. by G. Maclachlan).

The vasa gene is a member of the posterior group of Drosophila maternal-effect genes, and is required for embryonic germ cell formation. Its predicted protein sequence contains all the motifs of the D-E-A-D family of ATP-dependent RNA-binding proteins. By direct sequencing of PCR-amplified genomic DNA from hemizygous mutants, we have identified unique amino acid changes in six ethyl methane sulfonate induced alleles. Three of the mutations alter conserved residues in a small region of the protein near the consensus A-motif for ATP binding. Two other mutations, both pheno-typically very strong, lead to glutamic acid substitutions for conserved glycine residues near the C-terminal end of the D-E-A-D protein consensus. The final mutation leads to a non-conservative substitution (serine for arginine) in the N-terminal unique region of the vasa protein. Two-dimensional gel analysis of the wild-type and mutant vasa proteins will also be presented.

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Crosstalk between MPF and protein kinase C in the reorganization of the cortical cytoskeleton. J.H. Olson and D.G. Capco. Molecular and Cellular Biology/Zoology, Arizona State University, Tempe, Arizona 85287-1501.

What mechanisms regulate cytoskeletal organization and function? To address this question we have employed the Xenopus oocyte/egg system which undergoes an extensive reorganization of the peripheral actin-myosin cytoskeleton as the oocyte is converted to the fertilization-competent metaphase II egg. The oocyte periphery cannot undergo contraction, while the egg can. In a previous study we have shown that activation of protein kinase C (PKC) can induce oocytes to undergo contraction in a two phase process (J. Cell Biol. 115:45a). Since maturation promotion factor (MPF) becomes active during the conversion of the oocyte to the metaphase II eggs we also tested the potential of this signal transducer by injecting oocytes with MPF to determine whether this signal transducer could also induce contractile ability in the cell periphery. Surprisingly, immediately after MPF injection the cell periphery acquired contractile ability in 100% of the eggs. However this contractile ability could be inhibited (i.e., reduced to 15%) if the oocytes were first treated with antagonists of PKC, suggesting that MPF acts through PKC. In addition, lysates were made from oocytes treated with PKC stimulators (i.e., PKC-lysate) and microinjected into other oocytes. The PKC-lysate could induce injected oocytes to immediately acquire contractile potential. Controls demonstrate that this is not due to carryover of PKC stimulator. PKC inhibitors could block the contraction inducing ability of the PKC-lysate. Our results suggest that MPF and PKC act in concert to induce the cytoskeletal reorganization and allow for the acquisition of contractile ability. Supported by NIH grant HD 27151.

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Actin and Myosin II in Unfertilized Zebrafish Eggs: Filamentous and Non-filamentous Actin Domains in the Cortex. N.H. Hart and K.A. Becker, Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855

Reorganization of the cytoskeleton leads to extensive restructuring of the egg cortex upon fertilization. To study the underlying organization of the cytoskeleton in the unfertilized egg that facilitates transformation of the cortex, we have used SDS-PAGE, immunoblotting, and fluorescence microscopy to identify and map the location of actin and non-muscle myosin II. Electrophoresed lysates of unfertilized B. rerio (zebrafish) eggs revealed single bands that comigrated with chicken skeletal muscle actin and myosin protein standards. Western blots of transferred proteins showed a single band upon incubation with either monoclonal anti-actin antibody (Amersham, Inc.) or platelet anti-myosin antibody (gift of J. Sellers). Fixed whole eggs, cortical fragments of eggs, or frozen sections were probed with either rhodamine-conjugated phalloidin (RhPh) to identify filamentous actin, anti-actin antibody to label total cellular actin, or platelet anti-myosin to localize myosin II. RhPh staining produced a continuous, thin band of fluorescence subjacent to the plasma membrane. This was resolved into strongly-staining punctate foci, that spatially correlated with surface microplicae, and an interconnected network of weaker-staining filaments. By contrast, eggs probed with anti-actin antibody showed staining throughout the cortex, including the region of cortical granules, and in cytoplasm between yolk platelets. These observations indicate that the unfertilized egg cortex contains two distinct domains of actin, one filamentous and the other non-filamentous. Myosin II antibody codistributed with the filamentous actin domain and appeared as strongly fluorescing dots. The presence of actin and non-muscle myosin suggests that these proteins may function in establishing the mechanical properties of the egg cortex as well as in regulating changes in egg shape upon fertilization.