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A screen for new genes involved in heart development

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The high degree of conservation of gene function during heart development in many organisms supports the hypothesis that the heart evolved once from a common ancestor. In *Drosophila*, inductive signals from both the ectoderm and signals intrinsic to the mesoderm induce groups of mesodermal cells in each parasegment to become the primordia of the fat body, heart, somatic and visceral muscles. The mesodermal cells migrate bilaterally as a sheath of cells, maintaining their A/P positioning, towards the dorsal side of the embryo. The dorsal-most two rows of cells split from the adjacent somatic mesoderm and join during dorsal closure to form a heart tube. Therefore the *Drosophila* heart provides a useful system for studying the mechanism of cardioblast cell fate determination, cell migration and tubulogenesis, many aspects of which will also be conserved in vertebrates. In order to identify new genes involved in heart development we have examined P-lacZ transposon lines which express β-galactosidase in the heart. Deficiencies in the regions covered by these P-elements have identified five chromosomal regions which may play a role in heart development. The phenotypes of these deficiencies include: an absence of heart and dorsal somatic muscle, consistent gaps in the heart, and an overproduction of posterior heart cells with abnormal somatic muscle. The specificity of these mutants is being further characterized to eliminate heart defects which are secondary to dorsal closure, neurogenic or segmentation defects. The relevant P-lines will then be used to induce excision mutants for all of the interesting deficiency phenotypes identified.

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Extracellular matrix remodeling during intestine regeneration in Holothuria glaberrima

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The sea cucumber,Holothuria glaberrima, regenerates the intestine after evisceration. Regeneration is accomplished by (i) a thickening of the mesenteric edge which will form the coelomic epithelium, muscular and internal connective tissue layers and (ii) invasion of the thickenings by outgrowths of the mucosal epithelium from the esophagus and the cloaca. Extracellular matrix (ECM) degradation, granular tissue formation and reorganization of matrix components are major events associated with wound healing and regeneration. Our goal is to determine if ECM remodeling occurs during intestinal regeneration. We have used monoclonal and polyclonal antibodies against ECM components to observe matrix changes in the regenerating structure. Our results show that ECM components associated with the coelomic epithelium basal lamina become disorganized and/or reduced in expression during early regeneration, while those associated with the luminal epithelium appear to remain unaltered. Other components of the connective tissue layer completely disappear during the regenerative process. Reorganization of the ECM to normal conditions was reestablished after mucosal lumen formation. These results coincide with biochemical analysis of metalloproteases. In collagen gel zymographies, we have identified four phenanthroline sensitive bands. The gelatinolytic activity of the bands increases during the early stages of regeneration, suggesting that metalloproteases are responsible for some of the changes in ECM composition. Funded by EPSCoR, MBRS, RCMI, and UPR.

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Gene expression during intestinal regeneration in the sea cucumber *Holothuria glaberrima*.

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One of the most striking cases of organogenesis in nature is the regeneration of the viscera in holothurians following their evisceration. In the sea cucumber Holothuria glaberrima, intestinal regeneration provides a model experimental system for studying organ formation in adult organisms phylogenetically related to chordates. We have described the regeneration process at the cellular and tissue level by focusing on muscular, epithelial and nervous markers. Our present interest is to identify genes and gene products that are differentially expressed in the intestine of H. glaberrima between non-eviscerated and regenerating specimens. To achieve this objective, cDNA libraries of non-eviscerated and regenerated intestine were prepared and screened by PCR amplification with degenerate primers that recognize conserve sequences of genes, such as Hox genes and transcription factors, known to be important in developmental and morphogenetic processes. The libraries were also used for differential screening to identify genes that are preferentially expressed during the regeneration process. Preliminary analysis of clones has allowed the identification of holothurian genes homologous to vertebrate genes involved in wound repair, oncogenesis and differentiation. These include homeodomain containing genes, serum amyloid precursor proteins, ribosomal proteins, elongation factors, ubiquitin and extracellular matrix proteins. Additional sequences containing long open reading frames that show no homology to known genes in data bases were also identified. Funded by EPSCoR, NASA, MBRS, RCMI and UPR

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TREFOIL PEPTIDES ARE EARLY MARKERS OF EPITHELIAL CELL DIFFERENTIATION IN THE DEVELOPING RAT GASTROINTESTINAL TRACT.

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Trefoil peptides are products of mucus cells and are important regulators of injury in the adult gastrointestinal tract. Recent evidence suggests that they may also be involved in epithelial cell maturation, since in null mutants of the trefoil peptide PS2, there is aberrant epithelial cell differentiation and almost a complete lack of mucus. To begin to study whether trefoil peptides may have a role in epithelial cell differentiation in the developing rat gut, we have examined the expression of trefoils in the fetal rat from 13 days post coitus (dpc) until birth (22 dpc). The gastric and intestinal epithelia are stratified and undifferentiated from 13-18dpc. PS2 and SP are expressed in the embryonic stomach at 15 and 16 dpc, respectively as shown by both RT-PCR and RPA. In the small intestine, ITF is first evident at 15 dpc. Using RIA, SP levels were found to be low but constant (~5pmol/mg protein, about 30% of adult levels) from 17-22 dpc. ITF peptide was 70-80 pmol/mg protein at 17 dpc, rising to 150 pmol/mg protein at 22dpc which is close to adult levels. The trefoil peptides were localised to the apical region of epithelial cells by immunohistochemistry from 17 dpc onwards, while mucus granules and parietal cells were first detected at 18 and 19 dpc respectively. Conclusions: Trefoil peptides are expressed very early in gut development well before other markers of differentiation, and well before the morphological signs of differentiation (polarised, columnar cells) are evident. This suggests a possible role for trefoil peptides in gut epithelial cell differentiation or perhaps mucus cell specification.

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Biliary atresia: is it a result of an imbalance in cell death and proliferation?

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Intrahepatic bile ducts develop from the ductal plate by a process called ductal plate remodelling, which requires a delicate balance between cell proliferation and death. It has been suggested that deregulation of this delicate balance may be involved in the pathogenesis of biliary atresia. Biliary atresia, a condition that affects infants, is characterised by obstructive jaundice and biliary ductular proliferation in the liver. In this project we compared two paediatric conditions with obstructive jaundice and biliary ductular proliferation in the liver; biliary atresia and choledochal cyst with bile flow obstruction. Controls were liver samples from patients who had no evidence of bile flow obstruction. We also included normal fetal liver samples to study the pattern during development. Fluorescent TUNEL technique was used to detect apoptosis and fluorescent anti PCNA antibody was used to detect cell proliferation. Proliferation index (PI) and apoptotic index (AI) were calculated for biliary epithelial cells, mesenchymal cells, and hepatocytes. PI for biliary cells was zero in fetal and control livers, while it was slightly raised in both biliary atresia and choledochal cyst samples. Hepatocyte and mesenchymal cell PI was high in fetal period but fell to low levels in control livers. In the biliary atresia samples, mesenchymal cell PI was significantly raised when compared to the controls($p=<.01$). AI of all three cell types was high during ductal plate remodelling in the fetus and fell to low levels in the control samples. Whereas among the two conditions with obstructive jaundice, choledochal cyst liver showed a very high AI in all cells types compared to the controls ($p=<0.0001$), biliary atresia liver had low levels similar to the controls. Biliary atresia differed from choledochal cyst in its remarkable absence of apoptosis, suggesting that the pathogenesis of the two conditions are different. Biliary atresia may occur as a result of diminution of the apoptotic process, while the mesenchymal proliferation remained active.

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BMP-9 and BMP-10 two novel factoprs involved in heart and liver organogenesis Herbert Neuhaus, Vicki rosen and Scott Thies. Genetics Institute, Inc.

BMP-9 and BMP-10 were originally cloned in low stringency hybridisation screens using probes against the mature regions of BMP-4 and -7. Together with the chicken homolog dorsalin they form a novel subgroup in the TGF-B family. Expression of BMP-9is first detectable at d9.5 p.c.in the mesenchyme surrounding the outgrowing liver bud. At d10.5 expression is spread throughout the entire liver. BMP-9 expression remains restricted to the liver during embryogenesis. It was shown that BMP-9 stimulates the proliferation of HEP G2 cells a liver cell line and adult primary hepatocytes. The effect on embryonic cells is currently under investigation. BMP-10 expression during embryogenesis is restricted to the developing heart. Earliest BMP-10 expression was detected in 9 day old embryos. At this stage, expression was restricted to the endomyocardial cell layer of the heart tube. At later stages, BMP-10 was exclusively expressed in the trabeculated part of the myocardial wall of the heart. A null mutation of the BMP 10 locus was created in ES cells and chimaeras from three independent ES cell lines were generated. These chimaeras are currently bred to obtain germline transmission of the mutation. First homozygotes should be obtained in May. The effect of BMP-10 on heart tissue and cells is currently under investigation. In binding experiments with iodinated protein, I found that both BMPs bind with relatively high affinity to T-ALK, a BMP type II receptor. In situ analysis in d9.5, d10.5 and d14.5 mouse embryos showed that T-ALK is expressed in the heart but not in the liver. Whether BMP-9 shows the same high affinity for other type II receptors which are expressed in the liver is not known at the moment