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Characterization of Regulatory Sequencesof the *Fgf-8* Promoter in *Xenopus laevis*

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Fgf-8(Fibroblast growth factor-8) is one of the key signaling molecules implicated in various developmental processes such as formation of central nervous system, limb, heart and liver. In developing embryos, *Fgf-8* is expressed in various regions including neural fold, spinal cord, midbrain-hindbrain junction, tail tip, pharyngeal clefts, and heart mesoderm. To characterize the expression of *Fgf-8* during developmental processes in urodeles, we isolated its 5'upstream sequences from a genomic library of *Xenopus laevis*. The cloned DNA fragment is approximately 18-kb long and contains the promoter, 5'-UTR, the first and second exons, and an intron in between. As determined by nucleotide sequencing, the promoter of *Fgf-8*shows apparent lack of the TATA box. However, it has 1 kb-long GATA repeat sequences that contain 11 nested GACA repeats. Serial deletions constructed with the 2.2-kb *Fgf-8* promoter were fused to luciferase reporter gene to analyze the effects of individual sequence elements on transcriptional activity. Two regions of the *Fgf-8*promoter appeared to exert strong effects on transcription, of which the putative -1989 ~ -1893 region played a predominant role. Point mutations introduced into these regions further demonstrated that SOX family and AP-4 were two of the most responsible elements supporting transcriptional activation of *Fgf-8*.

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Differentiation of Embryonic Stem Cells into Endodermal Cells and Hepatocytes

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Embryonic stem (ES) cells differentiate into various lineage of cells and thus can serve as a source of cell therapy to cure disordered tissues or genetic diseases. In an effort to extend the potential use of stem cell therapy to liver disease, we utilized embryoid bodies (EBs) derived from cultured ES cells to identify and enrich endodermal cells in vitro and differentiated hepatocytes. Various cell types exist in EBs were determined by hematoxylin and eosin staining and immunohistochemistry. Several endoderm-related genes were expressed preferentially in 2-4 week-old EBs. Endodermal layer cells strongly stained with anti-a-fetoprotein and anti-GATA-4 antibodies were located outer surface of EBs. The endodermal cells could be separated from the EB by trypsin treatment and the majority of the isolated cells were identified by RT-PCR and stained with anti-a-fetoprotein antibody. These endodermal cells were maturated to hepatocytes in the presence of growth factors and Matrigel Matrix. Our results demonstrate that mouse ES cells can differentiate in vitro into a mixed cell population including endodermal cells and hepatocytes. This suggests that isolation of endodermal cells via EB formation and differentaition to hepatocytes could be an easy and practical way of using ES cells to cure endodermal tissue disorder such as liver disease.

E705

Role of Cholesterol in Development of *Caenorhabditis elegans*

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Defects in cholesterol biosynthesis result in disordered development. A cholesterol auxotroph, *Caenorhabditis elegans* was used as an model organism to investigate the effects of cholesterol starvation and overfeeding on development at both embryonic and post-embryonic stages by examining brood size, embryonic lethality, growth rate, worm size and life span. The brood sizes of worms grown with cholesterol 50 mg/L (overfeeding) were reduced to 25% and worms grown without cholesterol to 42% compared to the control group grown with cholesterol 5mg/L. Embryonic lethality was detected either 1.1% with cholesterol overfeeding or 3.0% without cholesterol as compared to 0.2% of the control group. The percent development from an embryo to an adult was not noticeably affected with cholesterol overfeeding but lowered by an average of 11.2% without cholesterol. The growth rate and worm size were also affected by the cholesterol concentrations. Worms grown without cholesterol were 82% of the normal size and their life spans were extended by 1.32 times. Interestingly, the cholesterol effect was temperature-dependent. Furthermore, the extended life span without cholesterol at 20℃ was not detected by moving worms to 25℃. These results suggest that cholesterol overfeeding may be able to ameliorate the developmental hardship at 25℃. Thus, cholesterol appears to be required for the entire developmental stages of *C. elegans* and the proper amount of cholesterol (5 mg/L) is required for the normal development at 20℃ and cholesterol overfeeding seems to protect the developmental process of *C. elegans* at 25℃.

E706

Expression of the *Drosophila* p38b Gene Promoter during Development and in the Immune Response

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p38 MAPK have been extensively studied as a stress-responsive kinase and recently known its role in development. However, the mechanisms by which p38 gene expression is regulated remains unknown. In this study, to investigate expression of the *Drosophila* p38b (D-p38b) gene, we established transgenic flies bearing the D-p38b-lacZ fusion genes containing the D-p38b promoter region (-901 to +208 with respect to the transcription initiation site). Levels of the D-p38b mRNA examined by RT-PCR and analyses of expression patterns of the D-p38b-lacZ in transgenic flies indicated that the D-p38b gene is expressed throughout development. Expression of the D-p38b-lacZ gene during embryogenesis was similar to expression of the D-p38b mRNA in situ reported by other group. The results indicated that the promoter region is sufficient for endogenous expression of the D-p38b gene. Strong expression of the D-p38b-lacZ gene was detected in the brain and ganglion, imaginal disc, salivary gland, gut and fat body among larval tissues and in the gut, fat body and reproductive systems of adult female and male. Interestingly, upregulated expression of the D-p38b-lacZ gene in the fat body and gut by injury was detected. Transgenic flies bearing the D-p38b-lacZ fusion gene promise to be useful for further studies on the mechanisms of regulation of the D-p38b gene expression during development and in the immune response.