CONTEXT-DEPENDENT UTILIZATION OF NOTCH ACTIVITY IN *DROSOPHILA* GLIAL DETERMINATION

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Activation of Notch promotes differentiation of many different cell types in *Drosophila* embryonic peripheral nervous system (PNS). How these different outcomes of Notch signaling are controlled is not understood. Here, we show that Notch activity to promote gliogenesis is entirely context-dependent in the embryonic PNS. In the dorsal bipolar dendritic (dbd) sensory lineage, asymmetric cell division of the dbd precursor produces a neuron and a glial cell. Within the dbd lineage, Notch is specifically activated in one of the daughter cells, and is required for a glial fate. Ectopic Notch activation can direct gliogenesis in a subset of embryonic PNS lineages, suggesting that Notch-dependent gliogenesis is supported in certain developmental contexts. We present evidence that POU-domain protein Nubbin is one of the factors that provide such context.

COMPARING THE GENE EXPRESSION PROFILES OF VISUAL NEURONS BETWEEN PLANARIAN AND MOUSE RETINA BY SINGLE CELL PCR ANALYSIS

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Planarian eyes are composed of a single type of neural cells, which receive light and transmit the signal to the brain. On the other hand, the vertebrate retina contains diversed cell types, one glia and six neuronal cells that develop from a single progenitor cell to process light signal transduction. This suggests that during evolution the functions were divided among different cells and resulted in the formation of multi-layered complex retina. To investigate this hypothesis, we have compared gene expression profiles between planarian visual cells and mouse retinal cells at single cell level. We prepared cDNA form a single-cell corrected by FACS sorting after cell dissociation, and profiled gene expression by PCR anlyses with gene-specific primers. Here we will show the gene expression profile at single cell level and compared it among planarian and mouse retinal neurons.

INTRODUCTION AND EXPRESSION OF FOREIGN GENES INTO EGGS AND EMBRYOS OF THE LAMPREY, *LAMPETRA JAPONICA*

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The lamprey occupies a position as a key animal in the study of vertebrate evolution. We have attempted to introduce exogenous gene constructs into fertilized eggs or embryos of the Japanese lamprey, *Lampetra japonica*. Eggs are injected with gene constructs in which a coding sequence of reporter genes such as LacZ or GFP is connected to virus promoters or 5'upstream regions of vertebrate actin genes. Expression of reporter genes are observed starting two days after injection and continued for more than one week. Tissue-restricted expression of these reporter genes has been also achieved by applying electric pulses immediately after microinjection of gene constructs into the targeted tissue of the embryo. These techniques would enable us to analyze detailed cell lineage of lamprey embryos. Another important application would be to introduce developmental genes of the lamprey or other animals into lamprey embryos; this would provide us with information on evolutionary changes in functions of genes or gene cascades.

ROLE OF HGF/C-MET SIGNALING IN SOMITE MYOGENESIS

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For myogenesis, HGF (also known as Scatter Factor) is an indispensable molecule that plays a key role in the regulation of migration via its receptor, c-MET. Our previous studies have demonstrated that HGF markedly enhances the dispersion and emigration of somitic myoblasts in culture. The present study was carried out to assess the significance of HGF in somite myogenesis by means of morphological, immunocytochemical and biochemical methods using in-vitro model of somitic myoblast motility. In addition, the spatiotemporal distribution of HGF-related, muscle-specific, and cell adhesion proteins in the developing chick embryos were examined by whole-mount immunohistochemical techniques. Our observations are revealing that both HGF and c-MET are localized to myogenic cells and that HGF may act in an autocrine/paracrine fashion. These results suggest possible role of HGF in chemotactic response of myoblasts, as a potent regulator of somite myogenesis.

CONTROL OF MOUSE MELANOCYTE DIFFERENTIATION BY MELANOCORTIN RECEPTOR 1

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α-melanocyte stimulating hormone (MSH) supplemented to a serum-free culture medium from the initiation of primary culture of 0.5-day-old epidermal cell suspensions of C57BL/10JHir mice (black) induced the differentiation of melanoblasts into melanocytes. Pigment-producing differentiated melanocytes appeared around 2-4 days, and almost all cells differentiated around 7-9 days of culture. After 12-14 days, almost all keratinocytes died, and pure cultures of differentiated melanocytes were obtained, but no stimulation of melanocyte proliferation was observed. To make clear whether the expression of MSH receptor (MC1R) gene is stimulated by MSH at the time of differentiation, RT-PCR analysis of the gene was performed. The results showed that strong expression of MC1R gene was already observed before addition of α-MSH in the culture system, and no stimulation was observed after the addition of α-MSH. Moreover, strong expression of the MC1R gene was observed in the epidermis of 0.5-day-old skin. These results suggest that the expression of MC1R gene initiates just after birth, and the gene plays an important role in the regulation of murine melanocyte differentiation.

STRUCTURE AND ALTERNATIVE SPLICING OF THE FAST SKELETAL MUSCLE TROPONIN T GENE

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Troponin T (TnT) is a tropomyosin-binding subunit of the troponin complex, which is responsible for regulation of vertebrate striated muscle contraction. Vertebrate TnT is encoded by three genes that are differentially expressed in fast, slow, and cardiac muscles. The fast skeletal muscle troponin T (fTnT) gene generates developmentally regurated and tissue-specific multiple isoforms. The mechanism that regulates alternative splicing of the fTnT gene is poorly understood. In this study, we determined the complete genomic sequence of the chicken fTnT gene and 3' genomic sequences of *Xenopus* and zebra fish fTnT genes, and compared the sequences with one another. The chicken fTnT gene of ca.34 kb included 27 exons. The 3 kb 3'-sequence of the *Xenopus* fTnT gene included 3 exons. The 1.5 kb 3'-sequence of the zebra fish fTnT gene included 4 exons. Introns of the zebra fish fTnT gene were smaller in size than those in other species. Mutually exclusive exons existed in the chicken and *Xenopus* fTnT 3'-sequences. The zebra fish fTnT gene had only an exon corresponding to exon 16 of the chicken fTNT gene.

CLONING OF CHICKEN ENDOTHELIN AND THE FUNCTION ON PIGMENT CELL DIFFERENTIATION

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We elucidated the effects of endothelins (ET-1, 2, 3) on pigment cell proliferation and differentiation in chickens using synthetic peptides derived from mouse cDNA. By supplement with ET-1, 2, or 3, melanoblasts from 3-day embryos showed distinct growth and differentiation to melanocytes. As a first step to analyze the expression and the functions of endogenous ETs in development, we tried to clone chicken ETs cDNA. Using embryo tissues of black silky, white silky, white leghorn and barred plymouth rock, fragments of ET cDNA were isolated by RT-PCR method. A fragment from black silky was extended by 5'RACE to 1010 bp and it contained a functional domain that was specific for each ET type. Deduced amino acid sequence from the obtained fragment showed quite high homology with mouse and human endothelin 3. Therefore, obtained chicken ET cDNA was determined as ET-3. From the results, it was clearly shown that we are able to rule out the species specificity of ET in culture system but the chicken cDNA would be a useful tool to determine the role and function of ET precisely in chicken pigment cell differentiation.

GENE EXPRESSION OF ErbB4 RECEPTOR IN CTGF TRANSGENIC AND IN *Cbfa1* null mutant mice

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It has already been reported that *ErbB4* has a possibility of a receptor of connective tissue growth factor (CTGF). To clarify the relationship between CTGF and *ErbB4*, the gene expression patterns of *ebrB4* in a CTGF transgenic (TG) mice and *cbfa1* null mutant(-/-) mice during late development were analyzed by in situ hybridization. Generally, *erbB4* gene expresses on neurons in the central nervous system and the myocardium as a receptor of Neuregulin 1. Same expression patterns were also found in a TG mouse and in a -/- mouse. Whereas, *erbB4* gene expression was found in hypertrophic chondrocytes in endochondral ossification except for -/- mice. Certain osteoporosis was resulted in CTGF TG mice, however no difference of gene expression of *ErbB4* was observed in comparison with control mice. In *Cbfa1*