The chorion proteins(A, B, C, D, E, HcA and HcB) are synthesized by monolayer of follicular epithelial cells and assembled after secretion into egg shell. They are served as a protective layer of egg; facilitating gas exchange, preventing desiccation. They are composed of 100~150 genes as superfamily in the second chromosome. Chorion gene is the good subjects to study evolutionary and developmental regulation. The 140kb late chorion locus of B. mori contains 15-multigene families (HcA, HcB), which are divergently oriented gene pairs. The chorion proteins of B. mandarina(wild silkmoth) apparently differ from those of B. mori in the structure and morphology, we have studied the chorion late locus of B. mandarina, constructed wild silkmoth genomic library, and obtained the 10 Hc clones with hybridization and PCR. pCH417 (one of the obtained clones) was sequenced characterized. The sequence similarities between B. mori and B. mandarina are 94%, 93% for HcA, HcB respectively. A major sequence difference is founded is C-arm, which consists of (Cys-Gly), (Cys-Gly), meaning the variation is the result of frequent expansion and contraction events, but central domain is highly conserved region. We will study the structural and evolutionary relationship of Hc between B. mori and B. mandarina.

F812

Gene Therapy of Type 1 Diabetes Mellitus: Liposome-mediated DNA Delivery to Murine Skin

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Type 1 diabetes mellitus is caused by severe insulin deficiency secondary to the autoimmune destruction of pancreatic b cells.

We constructed plasmid vector coding insulin gene with keratin 14 promotor and the vectors were liposome-mediated injected into murine skin targeting keratinocytes by the jet injection device. Low-level but sustained expression of the insulin from the murine keratinocytes was achieved in vitro administration of the plasmid vecto and sequencing revealed it was identical to human preproinsulin sequences combined keratin 14 promotor region. In advance of in vivo application, we injected another two kinds of constructed vectors containing LacZ instead of insulin gene. Gene expression was shown in skin as early as 1 week after DNA application and has been sustained until 20 weeks. By PCR and southern blot analyses showed the LacZ gene integrated into chromosomes as we expected. We have taken different approach to study proper insulin gene delivery and its activity in body. Streptozotocin (STZ) selectively destroyed insulin producing beta islet cells of the pancreas providing a model of type 1 diabetes mellitus with remarkable similarity to that of human IDDM patients. So we applied DNA vectors to 4 groups of mice and blood glucose levels were monitored. Both multiple low-dose and single high-dose injections of STZ were sufficient to induce hyperglycemia, increasing the blood g cose levels resulting from loss of pancreatic cells and 6 levels of dose DNA vector treatment decreased 24-68% of blood glucose levels in STZ-induced diabetic mice. Microscopic examination of the X-gal stained region of the gene treated skin tissue explained the promoter activity compared with lacking of the region. And microscopic images of pancreas explained the number of beta cells greatly reduced and almost destroyed at the same time statistical data of vector injection showed sustained normal blood glucose levels. Furthermore keratinocyte-specific gene expression by immunohistochemistry and quantitative analysis of insulin produced from keratinocytes in STZ induced diabetic mice are to be assisted. In this study it was suggested that the skin is a possible target organ for ectopic expression of the insulin gene as a potential treatment modality for type 1 diabetes mellitus and also this system is one of the candidates for gene therapy as gene carrier.

F813

The First Intron of Petunia Actin-Depolymerizing Factor Gene, PADF-1, Is Essential for Gene Expression

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ADF is one of the small actin-binding proteins that regulate actin dynamics in cells. We have previously isolated two cDNA clones, PhADF1 and PhADF2, encoding ADF from Petunia hybrida. Northern and western blot analyses indicated that the gene expressions of PhADFs are regulated at transcriptional level. In addition. immunolocalization exprement confirmed that PhADFs are abundant proteins within the vascular tissues of petunia. characterize the structure and regulation of ADF gene, we have isolated a genomic clone PADF-1, corresponding to PhADF1 from a petunia genomic library. Comparison to cDNA sequence revealed that the coding region of PADF-1 gene is consisted of three exons and two introns. The 1.6 kb of first intron was located immediately 3' of the translation start codon. Promoter/GUS expression study in transgenic Arabidopsis demonstrated that the first intron is the essential element for PADF-1 gene expression.

F814

Expression of Pathogenesis-related Gene by Chemical Inducers and Wounding in Lithospermum erythrorhizon

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cDNA clones encoding pathogenesis-related protein were previously isolated from a cDNA libraries prepared from shikonin-producing cells Lithospermum erythrorhizon (Yu et al., 1999). LePR1 transcripts is predominantly accumulated in the root. However, L:PR1 transcripts is not detected in young leaves. To characterize the gene expression of LePR1 , we investigate the accumulations of LePR1 transcripts by several signal molecules for systemic acquired resistance(SAR) and by wounding in young leaves. LePR1 gene expression is strongly induced by treatment of salicylic acid. Furthermore, accumulation patterns of LePR1 transcripts by salicylic acid analogues, such as acetyl salicylate and benzo(1,2,3)thiadiazole-7-carbothioic S-methylester, are similar to those of salicylic acid treatment. After wounding treatment, accumulation of the LePR1 transcripts is increased until 30 h. LePR1 transcripts is induced by H2O2, after 100 mM H2O2 treatment, reached a peak at 12 h and thereafter gradually decreased. LePR1 gene is induced in treatment with jasmonic acid, linolenic acid, and linoleic acid as well as abscisic acid. LePR1 is accumulated after pathogen infection and is more susceptible to Pseudomonas syringae and Erwinia stewa: 1 i.

F815

Mitochondrial DNA Control Region Polymorphism in a Population from Korea