F101

Molecular Characterization of Allatotropin Gene and its Expression in the Silk Worm *Bombyx mori*

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Allatotropin is an insect neuropeptide originally isolated from the head of pharate adult from the sphinx moth *Manduca sexta*. It is known that the allatotropin of *M. sexta* has a principal function to stimulate juvenile hormone *in viro* by the corpora allata of retrocerebral complex. The allatotropin has also cardioacceleratory action on the pharate adult heart of *M. sexta*. In this investigation, allatotropin cDNA encoding *M. sexta* allatotropin was cloned and sequenced in *B. mori* using RT-PCR. It shows highly homology sequence with that of allatotropin from *M. sexta* in the allatotropin peptide precursor of 130 amino acides described recently. This allatoropin gene was also expressed in the various tissues including the midgut by northern blotting. The distribution of allatotropin neuropeptide was also confirmed in the central and enteric nervous system using an immunocytochemical methods.

F102

Adaptive Response Induced by Heat in HeLa S_3 and CHO Cells

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The present study has performed to elucidate heat induced adaptive response in HeLa S_3 and CHO cells. Three assays were employed in this study; isolated DNA ladder pattern by DNA gel electrophoresis, morphological assessment (apoptotic cell rates) of apoptosis by acridine orange/ ethidium bromide (AO/EB) staining and expression of Hsp 70 by western blot analysis. DNA ladder patterns were clearly detectable in HeLa S_3 and CHO cells heated for 30 mins at 45℃ than that in cells pre-heated for 5 mins at 43℃ and subsequently heated for 30 mins at 45℃. Apoptotic cell rates was decreased in HeLa S_3 and CHO cells pre-heated for 5 mins at 43℃ and subsequently heated for 30 mins at 45℃ than that in cells heated for 30 mins at 45℃. As the results obtained by western blot, the expression of Hsp 70 was increased in HeLa S_3 and CHO cells pre-heated for 5 mins at 43℃ and subsequently heated for 30 mins at 45℃ than that in cells heated for 30 mins at 45℃.

F103

Fine Mapping at 7q11.23 Using RAG Mouse Cell - WS/SVAS Lymphoblastoid Cell Hybrids

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Williams syndrome (WS) is a contiguous gene syndrome caused by hemizygosity for a chromosomal deletion at 7q11.23. The range of phenotypes includes mental retardation, dysmorphic facies, congenital heart disease (supravalvular aortic stenosis; SVAS), short stature, a specific cognitive profile, hyperacusis, and infantile hypercalcaemia. One of well-known causative genes deleted in the critical region is elastin (ELN), and hemizygosity for ELN causes SVAS but not any other features of WS. The number of genes mapped to the WS deletion have been steadily increasing over the past 5 years.

These include LIMK1, STX1A, WBSCR1, RFC2, FZD3, GTF2I, and etc. No definitive role in the WS phenotype has been yet assigned to any of these genes. In this study, we present the results of deletion mapping in classic WS patients and SVAS patients with deletion but no other features of WS. To investigate the microdeletion at 7q11.23 in 8 WS and 9 SVAS patients, FISH was performed using 244H3 BAC clone that had been previously localized into the 500-kb region commonly deleted in WS. In FISH analysis, loss of heterozygosity (LOH) at ELN locus was found in eight WS patients and two SVAS patients. In addition, patial deletion in one SVAS patient, and no remaining deletion in six SVAS patients were also observed. RAG mouse cells were hybridized with lymphoblastoid cells of WS and SVAS patients to allow their deletion breakpoints to be more finely mapped. Genomic PCRs were carried out to test the sequence tagged site (STS) content of the hybrids using primer pairs designed from the microsatellite markers and genes on chromosome 7. Hybrids containing a chromosome 7 with deletion at the critical region from each patient were investigated for the presence of ELN, LIMK1 RFC2, D7S1870, and D7S489 by another round of PCR. These results make the genotype-phenotype matching possible and, therefore, provide valuable informations to fully understand the WS and SVAS pathogenesis.

F104

Construction of a Plasmid Vector Ensuring Site-Specific Integration and Stable Gene Expression

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Insertion of reporter constructs into the mammalian genome leads to variable gene expression due to position effects at the site of integration. This random integration has limited the gene therapy of human genetic disorders by its undesirable effects. We report here the newly constructed plasmid vector (pIRES-neo-YJ) based on the concepts of homologous recombination and position-independent promoter enhancing of beta-globin matrix attachment region (Glb-MAR). Chromosome 7 centromere-specific alpha satellite (alphoid) DNA sequence was cloned into pIRES-neo-YJ for homologous recombination of the cloned gene with the centromeric region of chromosome 7, which is genetically silent. Beta Glb-MAR sequence that allows high levels of transcription independent of the chromosomal site of integration was also inserted into pIRES-neo-YJ to ensure the stable and higher expression of the cloned genes. We expect that pIRES-neo-YJ would provide a valuable tool to eliminate random integration of cloned genes into the undesirable chromosomal region and their short-lived expression which often encounters during the construction of transgenic animals and human gene therapy.

F105

Xist Expression in Male-derived OTF9 Embryonal Carcinoma(EC) Cells

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The Xist (X inactive specific transcript) gene that resides at the putative X inactivation center (XIC) of the X chromosome is sorely expressed in female, but not in male somatic cells. Most researchers generally accept the fact that only the Xist allele on the inactive X chromosome is transcriptionally active. But it