Differentially Expressed Genes in Bm5 Cell Line Induced with Tunicamycin for Studies of Unfolded Protein Response (UPR)

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For studies of unfolded protein response (UPR), we isolated differentially expressed genes in Bm5 cell line induced with treatment of tunicamycin, the synthesis inhibitor of N-linked oligosaccharides in cells and constructed the subtractive cDNA library enriching UPR-related genes. An UPR-related genes enriched subtractive cDNA pool was generated through the course of suppression subtractive hybridization (SSH) and selective PCR amplification. The subtracted library was screened by hybridization using T and D cDNA mixture as probes, respectively. The positive clones, which produced stronger signal when probed with T than with D, were sequenced and their sequence homologues in GenBank database were searched with BLASTx by internet. The analysis of subtraction efficiency showed that the differentially expressed genes in T comparing to in D were enriched significantly. A total of 459 subtractive clones were randomly selected for generating expressed sequence tags (ESTs) and compared against an GenBank database by BLASTx. The BLASTx search of 459 clones revealed that 334 (73%) were homologous to functionally known genes and 125 clones had no matches in the database. Among known genes, 39 (11.7%) clones showed significant similarity to the previously reported genes from *B. mori*. One hundred-fourty-one (42.2%) clones similarity to genes from insects other than *B. mori*. Finally, 147 clones (44%) showed similarity to the genes from other kingdoms, including microorganisms (11.1%), plants (2.7%) and vertebrates (30.2%). Functional categorization of the database-matched clones through PEDENT database search indicated that 60 UPR-related (transcriptional induction of molecular chaperone and foldase, immune, ER-associated degradation and translational attenuation) genes (18%) and 4 glycosylation-related genes (1.2%) as well as genes involved in the metaboic pathways and in translation of mRNAs were most abundantly represented in subtractive cDNA library constructed from Bm5 cell treated with tunicamycin.