D117 Cloning and Expression of Estrogen-regulated Genes in Mouse Uterus in Delayed Implantation process by Differential Display

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Implantation consists of complicate and yet precise series of interaction between embryo and uterine epithelial cells, and this event is under control of ovarian steroids, estrogen and progesterone. However, little is known about genetic regulation of implantation process. In the present study, we employed the differential display RT-PCR (DD RT-PCR) technique to clone estrogen-regulated genes at the point of embryo implantation using delayed implantation model. Total RNA was extracted from whole uterine horns of estrogen-treated and control groups. By DD RT-PCR, we found 120 putative estrogen-regulated genes. To verify the expression pattern, mRNA levels of 120 genes were simultaneously analyzed by reverse Northern blotting. Twenty of 120 genes were changed at 24 hours after estrogen treatment, but the others showed no change or could not be detected. Then, the time course change in these 20 genes was examined by Northern blot analysis. mRNA level of putative clone (5C71) was upregulated 24 hours after estrogen treatment and some (2C41, 1A101 and 2C81) was rapidly induced by estrogen treatment. Sequencing and homology search are in progress, to further analyze estrogen-regulated genes in the mouse uterus.

D118 Regulation of heat shock protein 25 expression in early mouse development

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In the present study, we examined the temporal and spatial expression of mouse small heat shock protein (hsp 25) during the mouse preimplantation embryo development. A reverse transcription-coupled polymerase chain reaction (RT-PCR) method showed that there were multiple mRNA signals including authentic hsp 25 and others (possible hsp 25 variants). Hsp 25 was detected at 1-cell stage as a maternal transcript. Its mRNA transcript slightly decreased at 2-cell stage but increased again after morula stage. We also examined cellular localization of hsp 25 at the protein level by an immunofluorescence-immunocytochemistry. Hsp 25 was more diffusely distributed in the cytoplasm under normal conditions. Heat shock (at 43 °C for 30 min) did not lead to any significant change in subcellular localization in the developing mouse embryos. However, in the case of chronic heat shock (at 43 °C for 3 to 24 h), the nucleus was more densely immunostained by anti-hsp 25 antibody than the cytoplasm, indicating a possible stress-induced nuclear translocation of hsp 25.