THE FACTORS OF TISSUE-SPECIFIC EXPRESSION OF THE BOVINE β-CASEIN GENE

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Tissue-specific expression of most milk protein genes is know to be determined by sequences of 5 flanking (-500 b.p.) regions. An important role of the 3-nontranslated region (3-NTR) in tissue-specific expression of the bovine β-casein gene in transgenic animals has been established. A site for the CCAAT/enhancer binding protein (c/EBP) has been identified in the 3-NTR sequence. Analysis of functional significance of this site in experiments on transfection of HC11 cells has demonstrated a stimulating effect of c/EBP factor overexpression.

Sites for c/EBP are also localized in sequences of the promoter region of bovine β-and k-casein genes. Southwestern blot of nuclear extracts from lactating mammary gland (MG) reveals only one protein with the c/EBP binding site of the β-casein gene with a molecular weight of 42 kDa. In these experiments, the nuclear factor 1 (NF1) site of the-casein gene binds with 3 proteins of 80, 34 and 32,5 kDa. In the β-casein gene promoter the c/EBP binding site tightly juxtaposed to a binding site for NF1. This suggests their cooperative action in the regulation of expression of the bovine β-casein gene. Within c/EBP sequences, it is possible to identify a binding site for the nuclear factor Oct1 which may be a possible competitor of c/EBP in c/EBP-NF1 complex.

The level of expression of c/EBP, NF1 and NFkB1 in cells of pregnant MG is higher than in liver and it is regulated in the course of MG development. The concentration of mRNA for c/EBP decreases and for NFkB1 increases from the 1st to the 16th day of lactation whereas NF1 remains constant and reduces only on the 1st day of lactation.

The stimulating effect of c/EBP cannot determine the tissue-specific character of gene expression. To determine a factor responsible for this effect, a Western-south blot analysis of proteins from liver and MG extracts has been performed using b-NTR RNA. It has been established that the cytoplasm contains a total of 7 different proteins with molecular sizes 116, 84, 80, 75, 49,5, 44 and 42 kDa which bind to this 3-NTR.

It has been established that the 75 kDa protein may be specific for 3 NTR of β-casein mRNA. Data with RNA including a mutant c/EBP site suggest that the CAAU sequence of the c/EBP motif is involved in binding with 3-end-casein mRNA and tissue specific stability. Screening of the MG cDNA library has been carried out, clones encoding RNA-binding proteins have been obtained. Now their analysis is underway.

In the course of realization of the project *Milk sterility* clones of the antibacterial factor of mammary gland cells (MAP) have been produced. Vectors for expression of MAP and the lysozyme gene have been constructed for the purpose of generating transgenic animals producing these proteins in milk.