TITLE:

The identification of intronic S/MAR sequence of bovine *TH* gene and the nuclear matrix proteins interacting with it.

ABSTRACT:

Tyrosine hydroxylase (TH) is a key enzyme in the catecholamines biosynthesis. *TH* undergoes multilevel regulation which provides species-, development- and tissue-specific activity of the gene. It was shown that interactions of nuclear matrix (NM) proteins and S/MAR sequences affect spatial chromatin organization of individual genes and thereby control their transcription. Our hypothesis is that NM is involved in a complex regulatory system of *TH* gene expression anchoring the S/MAR sequences of human and bovine *TH* genes. The purpose of this work was to: (i) map the minimal region of the bovine *TH* first intron which possesses S/MAR feature and (ii) identify the NM *trans* factors binding the intronic fragment of the *TH* gene.

Two bovine tissues and two human cell lines differing in *TH* activity were used. NM proteins were obtained from the bovine liver and human hepatoma cell line (HepG2), in which *TH* is transcriptionally inactive, and the bovine adrenal medulla and SH-SY5Y human neuroblastoma cells, in which *TH* gene remains active. *In vitro* binding assay and electromobility shift assay (EMSA) were performed to localize a minimal S/MAR sequence. Southwestern technique was used to reveal NM protein profiles and to characterize proteins interacting with molecular probes.

The results revealed sequence specific interactions of bovine *TH* first intron and the bovine or human NM proteins. Two non-isotopic molecular probes 829 bp and 428 bp covering part of bovine *TH* gene (+770/+1598 and +766/+1193 respectively) bound NM proteins isolated from both bovine tissues during *in vitro* binding experiments. Shorter sequence (+766/+1193) is sufficient for binding to NM structure and it contains the S/MAR element. Moreover, the molecular probes showed a higher affinity for NM proteins from bovine adrenal medullae than for bovine liver ones.

Southwestern assay allowed to indicate NM polypeptides responsible for binding both 829 bp and 428 bp probe. Probes were attached by similar number of NM proteins from adrenal medulla and liver. Hovewer, some interacting proteins were tissue-specific. The Southwestern experiments with NM proteins from human tumor lines also disclosed interactions with both probes. A comparative analysis of the binding profiles revealed that the bovine *TH* intronic fragment was bound by both bovine and human NM proteins including tissue/cell-specific polypeptides as well as proteins that could be evolutionary conserved between bovine and human.

Finally, EMSA method was used to map the minimal sequence possessing S/MAR feature. The technique enabled to narrow down the bovine *TH* intronic S/MAR. It was shown that 210 bp

(+807/+1016) fragment localized in the first intron of bovine *TH* gene is bound by specific NM proteins or protein complexes.