HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET

Tiedekunta/Osasto — Fakultet/Sektion	Laitos — Institution
Faculty of Science	Department of Genetics

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Työn nimi — Arbetets titel: Mapping of regulation regions for transcription activity in rat catechol-O-methyltransferase (COMT) gene.

Oppiaine — Läroämne: Genetics

Työn laji — Arbetets art	Aika — Datum	Sivumäärä — Sidoantal
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Catechol-O-methyltransferase (COMT, E.C.2.1.1.6.) catalyzes the O-methylation of catecholamines by transferring a methyl group of SAM to the phenolic group of the acceptor catechol substrate.

Two forms of COMT proteins occur: S-COMT (24 kDa and 25 kDa, rat and man, respectively) and MB-COMT (28 kDa and 30 kDa, rat and man, respectively).

The rat COMT gene spans a region of 13 kb, containing five exons, the first of which is non-coding. The translation initiation codons for the two protein forms is located in the second exon 129 bp apart. The transcription of rat COMT gene yields 1.6 kb and 1.9 kb mRNAs, for S-COMT and MB-COMT, respectively. The transcription is controlled by two promoters, P1, a tissue-specific and P2, a housekeeping promoter. Both of the promoter have several putative consensus binding sites for common transcription factors.

In this work the purpose was to study the DNA-protein interactions in the two promoters with the recombinant transcription factors and the nuclear proteins from rat liver and brain and HeLa cells. The labelled probes for the DNaseI protection assays covered the region between the two ATGs (+3 to +129) and -109 - +1 in P1 and from -136 to +59 in P2. For the P2 promoter the regions -104 - -75, -70 - -41, +11 - -+28' and +11 - +40 were further studied with the EMS assays based on the protections in the DNaseI protection assays.

The ability of three P1 promoter regions to initiate transcription in a promoterless vector system were studied. The regions were located 542 bp, 978 bp and 1637 bp upstream of the second initiation codon and they were constructed in reporter plasmids.

In the DNaseI protection assays of the P1 promoter the CAAT box and a putative AP-2 site were protected when assayed with rat liver nuclear proteins. With rat brain nuclear proteins the same sites and also the TATA box were protected. No tissue-specific protections could be detected with the DNaseI protection assays. The region -190 - +1 showed protections with AP-1, AP-2 and Sp1 recombinant factors. This region was not studied with the nuclear proteins and thus the functionality of these sites remains to be solved.

The reporter plasmid constructions showed that the 1.64 kb construct had somewhat stronger ability to initiate transcription in the promoterless vector than the 0.54 kb and 0.98 kb constructs. These two constructs did not differ from each other in the CAT assays. Thus the important region for P1 transcription initiation lies upstream of -849.

In the P2 promoter five functional binding sites were detected, containing two Sp1 sites and three AP-2/Sp1 sites. The long protection in the footprinting assays at +10 - over +50 could not be shown to be tissue-specific site in the EMS assays. The EMS assays showed binding of several factors to all these sites.

Avainsanat — Nyckelord: COMT, promoter, transcription factor, DNaseI protection, EMSA

Säilytyspaikka — Förvaringsställe: Library at the Department of Genetics

Muita tietoja — Övriga uppgifter: