784 GDNF-induced Expression of Tyrosine Hydroxylase
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Glial cell line-derived neurotrophic factor (GDNF) was first identified as a protein with specific neuro-trophic activity to the dopaminergic neurons in nigra-striatum of brain. Several lines of studies have demonstrated that GDNF plays an important role in the survival and/or differentiation of dopaminergic neurons as well as motor neurons. The signal transduction from GDNF has been known to be GFRα-1 and Ret dependent. The MAP kinase pathway is functional in GDNF-induced some cellular reactions. Although c-jun was found to be phosphorylated following GDNF stimulation in several experiments, the downstream genes of c-jun in the case of GDNF stimulation remain unknown.

To elucidate the mechanism on GDNF-induced survival of dopaminergic neurons, we examined the states of some genes involved in cell growth, apoptosis and dopamine metastasis. Here we report that tyrosine hydroxylase (TH), the rate limiting enzyme of dopamine biosynthesis, is induced by GDNF in a neuroblastoma cell line, and this induction occurs at a transcriptional level. We will show the data that parallelled with GDNF-induced neurite outgrowth, TH-messenger RNA induced soon after GDNF treatment and continuously expressed thereafter. The elevated level of TH-protein was also observed but with slower time course. The results from promoter analysis showed that the GDNF-responsive DNA sequence in TH promoter is located within the 2.0 kb proximal promoter region, and AP1 site in this region is quite contributive to promoter activation. DNA binding experiments indicated that a new DNA-protein complex forms in AP1 site following GDNF stimulation. Our work also evidenced that MAP kinase cascade is the main pathway transducing the signal from GDNF to TH. Further work is undergoing to understand the detail about GDNF-induced TH expression.

785 THE ELEVATED EXPRESSION LEVEL IN CYCLIN-DEPENDENT KINASE INHIBITOR GENES IN TUMOR-BEARING MICE LIVER
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The expression level of cyclin-dependent kinase inhibitor (CDKI) genes, p21 and p16 were determined by a quantitative RT-PCR (QPCR) method in BALB/c mouse liver transplant-ed with methylcholanthrene-induced fibrosarcoma. Effect of bleomycin administration was studied on these mRNAs. The mRNA of the specific gene was amplified by RT-PCR technique using the specific primers for the gene, the amount of cDNA thus obtained was quantitatively determined by QPCR method. Results indicate that the expression level in p21 and p16 genes 3-5 times increased in tumor-bearing mouse liver than those found in normal liver. Bleomycin induced, in normal mouse liver, a 3-fold increase in p21 expression level, but it decreased p16 level to one half of the initial level. On the other hand, tumor-bearing mouse liver showed no obvious changes in p21 and p16 expression by the stimulation with bleomycin. These results suggest that these genes related to the cell cycle regulation are fully expressed in tumor-bearing animal by unknown mechanisms, and these highly expressed genes would not respond to the stimuli initiated from DNA damage.

786 From Gene to Protein in Hours not Days
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The need for a fast and convenient protein expression system is ubiquitous. Nearly every property that characterizes a living organism is affected by proteins. Many diseases are caused by dysfunction of proteins and the number of genes coding for unknown proteins rises enormously especially by sequencing more and more whole genomes. In the following an in vitro protein expression system, called RapidTranslationSystem, RTS, is presented which can produce up to 500 µg native protein in a 1 ml reaction within 24 hours. The system is based on a coupled transcription/translation reaction. T7-RNA polymerase is used for transcription, while all necessary components for the translation are provided by an E. coli lysate. In order to maintain protein synthesis for at least 24 hours the reaction solution is continuously supplied with substrates and energy components through a semi-permeable membrane. The broad applicability of the system is demonstrated with a variety of template genes. Even large eukaryotic or cell-toxic proteins have been synthesized efficiently. Co-expression of two different template genes is also possible. Slightly changing the reaction conditions enabled the expression of functional active recombinant plasminogen activator (tPA), a protein with 9 disulfide bonds. The use of PCR-generated linear templates avoiding the time-consuming steps of cloning is under evaluation, as well as a method for analysis and optimization of mRNA secondary structures, which can strongly influence the expression efficiency.

787 Bilirubin and Activation of CYP1A1 Expression Under Ultrasound Action
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CYP1A1 is a polycyclic aromatic hydrocarbon-responsive monooxygenase enzyme with little known endogenous inducers or substrates. We investigated level of CYP1A1 mRNA and protein expression adn 7-ethoxyresorufin-O-deethylase activity under experimental rising of bilirubin level in a blood rat and under ultrasound contact action on area of a liver at 0.4 W/sm² and duration of 10 minutes. The increased levels of free hemoglobin and of unconjugated bilirubin in the plasma blood of rat subjected to ultrasound contact action were shown. The rising of bilirubin level in rat blood plasma achieving after intravenous administration of bilirubin led to elevation of CYP1A1 mRNA, protein levels and 7-ethoxyresorufin-O-deethylase activity. The analogous changes were observed after ultrasound exposure of rat. There data, when compared, suppose that bilirubin induces CYP1A1 and may be intermediary in activation of CYP1A1 expression under ultrasound action.