F137

Homeodomain Protein Msx1에 의한 당단백질 Subunit 유전자의 전사 억제 기작

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Msx1 protein은 homeodomain protein으로 cellular proliferation 및 differentiation의 조절에 중요한 기능을 가지는 것으로 알려져 있는데 뇌하수체 전엽의 thyrotrope cell에도 이 단백질이 다량 존재하는 것으로 알려져 있다.본 연구에서는 Msx1 protein에 의한 당단백질 subunit 유전자 발현 조절 기작을 조사하고자 하였다. Thyrotrope 기원 세포주인 TSH에 인간 당단백질 subunit 유전자 construct를 Msx1 expression vector와 cotransfection 함으로써 Msx1이 당단백질 subunit 유전자를 억제하고 있음을 관찰하였으며 이러한 결과를 Msx1을 도입한 세포와 그렇지 않은 세포로부터 Real-Time PCR을 통하여 당단백질 subunit 유전자의 RNA 수준에서의 변화량을 비교하여 검증하였다. Msx1에 의한당단백질 subunit 유전자의 전사 억제 기능이 Msx1의 homeodomain DNA 결합 자리와는 무관하며 TBP와의 직접적인 상호작용에 의해 발생된다는 사실을 TBP antibody를 이용한 GST pull down assay를 통하여 증명하였다. 당단백질 subunit 유전자 promoter의 mutant construct 와 Msx1의 homeodomain 부위의 mutant를 이용한 cotransfection 실험으로 재확인 되었다. 특히 Msx1에 의해 억제되었던 당단백질 subunit 유전자의 활성이 과량의 TBP의 도입에 의해 원상태로 복원되는 결과들은 Msx1이 TBP와 상호작용 한다는 가설을 다시 한번 지지해 주고 있다.

F138

Mass Expression and Purification of the Tetrameric Recombinant Ly-6E.1 Protein

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Ly-6A/E(Sca-1) is wide used as a cell surface marker for hematopoietic stem cells (HSCs). Ly-6A/E(Sca-1), anchored on cell surface by a GPI linkage, is expressed in HSC and differentially expressed during T lymphocytes development. The protein was proposed to function in cell-cell adhesion and T lymphocyte activation, but precise role of Ly-6A/E(Sca-1) was not known yet. A couple of possible candidates of Ly-6A/E ligand was reported and the existence of other ligands were propsed. In this study to find new Ly-6E.1 ligand, Ly-6E.1/streptavidin tetramer was generated by enzymatic biotinylation of the recombinant Ly-6E.1 (Ly-6BH) with His6X tag to obtain high binding avidity to its ligand by using the baculoviral expression system. Ly-6BH would be useful tool for identifying Ly-6E.1 ligand and studying the function of Ly-6A/E(Sca-1) in hematopiesis and T lymphocytes development.

F201

cDNA Cloning, Charaterization, and Expression of Lipase from Rice (*Oryza sativa* cv. Dongjin)

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Lipases are useful enzymes that are primarily responsible for the hydrolysis of acylglycerides in lipid processing. Lipase can be employedin the production of pharmaceuticals, cosmetics, leather, detergents, foods, perfumery, medical diagnostics, and other organic synthetic materials. A cDNA clone of the lipase was isolated from rice seed coat cDNA library(*Oryza sativa* cv. Dongjin). The cloned lipase cDNA was consists of 1,445 bp long encoding 361 amino acid residues. The sequence showed no extensive homology to mammalian and microbial lipases, but a number of plant lipases, including rice and arabidopsis lipase gene, were found to be related to this gene. The deduced nucleic acid sequence shows 78% and 52% identity to lipase of other *Oryza sativa* and *Arabidopsis thaliana*, respectively. To estimate the copy number of lipase genes in rice bran(*Oryza sativa* cv. Chuchung) genome, Southern hybridization analysis indicated that rice bran has a single copy of the lipase gene. *Oryza sativa* lipase gene was also expressed in *Escherichia coli* under the control of lacZ promoter. So we used pET expression vector system(Novagen). Lipase activity in the *E. coli* clone was found mainly in the pellet fraction. In SDS/PAGE analysis, the size of lipase was about 40kDa.

F202

Influence of Nitrogen Source against Cadmium Effect on *in vitro* Growth, Chlorophyll and Rubisco/Rubisco Activase in Tobacco Leaves

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Influence of nitrogen source against the effect of Cd on *in vitro* growth, chlorophyll and rubisco/rubisco activase was investigated in tobacco grown MS medium. *In vitro* growth reduced by 0.2 mM Cd was recovered by KNO3 and NH4NO3, and this recovery was most significant when tobacco was treated with 80 mM KNO3 and NH4NO3, respectively. The recovery effect at 80 mM KNO3 was significantly higher than that at NH4NO3. Chlorophyll levels were also recovered by 80 mM KNO3 and NH4NO3. Rubisco content at 80 mM KNO3 and NH4NO3 was significantly increased than that at other treatments. Rubisco activity showed patterns of change similar to rubisco content. These data suggest that rubisco content was associated with activity of rubisco, and that the activation and induction of rubisco reduced by Cd were recovered by nitrogen source. The degree of intensity of 55 and 15 kD polypeptides identified as the large and small subunit of rubisco by SDS-PAGE analysis at 80 mM KNO3 and NH4NO3was significantly higher than that at other treatments. Under the assumption that effects of nitrgen source on rubisco may be related to rubisco activase, its content and activity were determined. The rubisco activase content at 80 mM KNO3 and NH4NO3 was more increased than other treatments. A similar change pattern was also observed in activity of rubisco activase.