F312 Molecular analysis of RNA polymerase alpha subunit gene from *Streptomyces coelicolor* A3(2)

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The *rpoA* gene, encoding α subunit of RNA polymerase, was cloned from *Streptomyces coelicolor* A3(2). It is preceded by *rpsK* and followed by *rplQ*, encoding ribosomal proteins S11 and L17, respectively, similar to the gene order in *B.subtilis*. Using T7 expression system, we overexpressed *S.coelicolor* α protein in *E.coli*. A small fraction of this protein was found to be incorporated into *E.coli* RNA polymerase. Antibody against *S.coelicolor* α protein crossreacted with that of *B.subtilis* more than with *E.coli* α subunit. The ability of recombinant α protein to assemble b and b' subunits into core enzymes *in vitro* was examined by measuring core enzyme activity. Maximal reconstitution was obtained at $\alpha_2 : \beta + \beta'$ ratio of 1:2.3, indicating that the recombinant α protein is fully functional for subunit assembly. Similar results were also obtained for natural α protein. Limited proteolysis with endoproteinase Glu-C revealed that *S.coelicolor* α consists of tightly folded N terminal domain and relatively unstructured C terminal domain, the latter being more protease-sensitive than that of *E.coli* α.

F313 Isolation and characterization of *zwfl*$^+$ encoding glucose-6-phosphate dehydrogenase from *Schizosaccharomyces pombe.*

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A genomic clone encoding G6PD was isolated and sequenced from fission yeast *Schizosaccharomyces pombe*. From known G6PD genes, several primers were designed and PCR was done using genomic DNA as a template. Obtained 3 species of PCR products were found to have high homolgy with other isofunctional enzymes. With this PCR product as a radioactive probe, λEMBL3 genomic libray was screened, and 10 independent clones were isolated. About 5 kb *Eco*RI fragment containing *zwfl*$^+$ was identified and subcloned into pTZ18R plasmid. The primary structure of *S. pombe*'s *zwfl*$^+$ was determined. Its amino acid sequence was deduced and shown to be highly conserved in comparison with those of human, *E. coli, and S. cerevisiae.* G6PD was known to act as an antioxidant defence enzyme. According to this we measured the changes of enzyme activity and transcript upon treatment of oxidants.