F308 *Schizosaccharomyces pombe pgr1*$^+$ encoding a glutathione reductase: isolation, characterization and analysis of its expression

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A DNA probe with known conserved regions as primers followed by PCR amplification being used for screening the genomic library, *pgr1*$^+$ encoding a glutathione reductase (GR, EC1.6.4.2) was isolated from the fission yeast, *Schizosaccharomyces pombe*. It consisted of 465 amino acids with deduced molecular weight of 50.2 kDa and showed the characteristic features of typical GR. An intron of 55 bp was shown near the N terminal region. The transcription start point was localized at 239 nt upstream from ATG initiation codon. When the *pgr1*$^-$ was carried on multicopy plasmid, the GR activity and the transcript increased by more than 5-fold. This enabled *S. pombe* cells to resist the external oxidative stresses. The expression of GR had been reported to be induced by menadione (MD), a redox cycling agent [Lee *et al.*, 1995]. These was confirmed by Northern analysis. Other stresses like organic peroxide treatment, high salt, heat shock and starvation also increased the expression of *pgr1*$^-$. Pap1, an AP1 homologue found in this yeast, regulated the expression of *pgr1*$^+$ in gene dosage dependent manner. *pgr1*$^-$ was disrupted by inserting *ura4*$^+$ cassette and diploid cells were transformed with this probe. Surprisingly, only *ura*$^-$ cells survived after dissection. This indicates that the *pgr1*$^-$ is essential for the aerobic growth. This is in contrast to those of budding yeast, *Saccharomyces cerevisiae* and *Escherichia coli*, in which GR was necessary only for protecting the cells against oxidative stresses, suggesting the different roles and contributions of GSH between *S. pombe* and the others discussed.

F309 Promoter Selectivity of RNA Polymerase from *Streptomyces coelicolor*

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RNA polymerases (RNAP) were prepared from log and stationary growth phases in *Streptomyces coelicolor*. Several promoter fragments were prepared for *in vitro* runoff transcription assay of these RNAP from different growth phases. Among promoters tested, *dagA* (agarase) p3, *actIII* (actinorhodin biosynthetic gene) px, *hrdD* (homologue of sigma factor) p2 promoters were strongly selected by stationary phase RNAP. On the other hand, exponential phase RNAP transcribed preferentially from *dagA* p4, p1 and *rrnD* (ribosomal RNA) p2, p3, p4, *glk A* (glucose kinase) p3, *glnR* (glutamine synthetase regulatory gene) p1, *whiB* (sporulation gene) p2. These observations indicated that strict differences exist in promoter selectivity and the associated specificity factor between the two RNA polymerases from different growth phases. The polypeptides which confer on the core RNAP the ability to initiate transcription from specific promoter have been identified with gel elution and reconstitution analysis. *rrnD* p2 transcribing activity corresponded to hrdB, the major sigma factor in *S. coelicolor*. Another sigma factors HrdD and SigE seemed to be specific cognate protein for transcription from *whiB* p2 and *hrdD* p1, *whiB* p2 respectively. Polypeptide with apparent molecular weight of 33 kD directed the transcription from *actIII* px2, *hrdD* p2 promoters. This protein is distinct from the known factors in molecular weight and promoter specificity.