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The primary goal of this rotation project was the overexpression of the *fla/che/mot* regulatory proteins flhD and flhC from *E. coli*. Already available in the lab was the plasmid pDNA40 (constructed by D. Arnosti) containing the flhD and flhC coding sequences under the control of a T7 RNA polymerase promoter. Also available was the M13 vector mGP1-2 coding for T7 RNA polymerase developed by S. Tabor. The *E.coli* host used for all procedures is XL1-Blue (Stratagene). (Important genotypes for this study: [F' $lacI^{q}Z\Delta M15$ (tet^{r})])

The actual work consisted mostly of the preparation, purification and titre of large quantaties of recombinant bacteriophage following the protocol of Tabor. The mGP1-2 was obtained from the Kustu lab and tested for plaque formation on host cells before use in the preparation of larger quantaties of bacteriophage. (Final preparation on lab notebook "KMS Rotation I" pages 24-30). This procedure was repeated due to low titre of bacteriophage (probably due to low starting titre) in the first preparation and losses in the final purification steps (CsCl gradient centrifugation.) A T1 phage contamination was observed in the first phage preparation, (large turbid plaques) but titred independantly from the mGP1-2 preparation and was probably due to contamination during the titration process. It was not observed in phage fractions in the second preparation. (The presence of CsCl in the final phage preparation did not seem to make a difference in the control expression experiments.)

Preliminary experiments with superinfection of the phage showed arrest of growth (31a-33a) but no apparent (from Coomassie blue staining of polyacrylamide gels) overexpression of proteins with the molecular weight predicted by the flhD and flhC genes. (Gels KMS 1-16,17,18) This analysis was complicated by the lack of an appropriate positive control. Attempts were made to assay the RNA polymerase activity of extracts from infected cells but these experiments experienced some technical difficulties.

About midway through the rotation period a plasmid (pBL-3) coding for the MetR protein (approx 33 kD) under control of the T7 promoter was donated by the Roche Institute to the lab. Host cells were transformed with this plasmid and also retransformed with pDNA40 because the previously transformed cells showed poor growth characteristics. (pg 28-29 and 48)

Coomassie stained gels of cells superinfected with all preparations of mGP1-2 (Prep I (in CsCl), Prep II (dialysed) and a crude supernatant

prepared freshly from the Kustu stock) showed overexpression of a protein at 33 kD in cells containing the pBL-3 plasmid (gel KMS 1-59) but none in cells containing the pDNA40 plasmid (gel KMS 1-56). A pulse-label experiment to assay the incorporation of ^{35}S methionine in the presence of rifampicin showed expression of a 33kD protein (MetR) in pBl-3 transformed cells and considerably lower levals of 13 (flhD) and 22 kD (flhC) proteins in pDNA40 transformed cells.

It is possible that the pDNA40 plasmid contains some nucleic acid sequences upstream of the flhD and flhC coding regions which preclude high levals of overexpression. Some further engineering of the plasmid might be able to overcome this situation. In any case, using this expression system it is possible to make radiolabelled flhD and flhC proteins for preliminary biochemical characterization. (e.g. binding to RNA polymerase.)