STUDIES ON THE CONTROL OF LATE GENE TRANSCRIPTION IN COLIPHAGE 186

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SUMMARY

STUDIES ON THE CONTROL OF LATE GENE TRANSCRIPTION IN COLIPHAGE 186.

This thesis describes studies carried out in an investigation of the control of late gene transcription in temperate coliphage 186. The specific aims of this study were to identify the phage functions involved in activating late gene transcription and to investigate the mechanism of control of late gene transcription.

The location and characterization of a 186 late promoter was chosen as the first step in investigating the control of late transcription in 186. Polarity and marker rescue studies had indicated that the *Bam*HI-*Eco*RI (10.0% to 13.3%) region was likely to encode the promoter for the transcription unit encoding late genes *VUTSRQ* (Hocking and Egan, 1982c; Finnegan and Egan, 1979). In this work the DNA sequence of the *Bam*HI-*Eco*RI (10.0% to 13.3%) region was determined. Computer assisted analysis of the DNA sequence led to the prediction that two divergent genes were located on this restriction fragment. The rightward gene was identified as the *V* gene and the leftward gene was designated *CP12*.

The opposite directions of *V* and *CP12* indicated the likelihood that divergent transcription initiated between these genes. Extensive homology was found in this region with the corresponding late promoters of the related phage P2, and led to the prediction that gene *V* was transcribed from a rightward late promoter p_V, while *CP12* was predicted to be transcribed from a leftward late promoter p_{12}. Primer extension analysis of *in vivo* RNA confirmed the *in vivo* activities of these promoters and demonstrated that the activity of both promoters was dependent upon the *B* gene product, consistent with the requirement of the *B* gene product for late gene transcription as demonstrated by Finnegan and Egan (1981).

An assay system for transcription from a 186 late promoter was established by placing the *galK* gene under control of the late promoter p_V on a multicopy plasmid. Consistent with the need for phage functions for activation, p_V showed no activity in the absence of phage functions *in vivo*. Late gene transcription has been shown to be dependent on the functions of gene *B* and replication gene *A* (Finnegan and Egan, 1981). In this work, the *B* gene product was shown to be the only phage function required directly

for activation of p_V transcription and to be required at high concentration for activation. Phage replication had previously been concluded to provide a template toplogy necessary for B protein to activate late transcription (Kalionis *et al.*, 1986b), however late transcription was shown to occur in the absence of phage replication and ruled out the need of a specific template toplogy. Studies indicated that the role of replication in activation of late gene transcription was to increase *B* gene dosage and to potentially derepress *B* gene transcription. These studies also indicated a protocol for the isolation of host mutants unable to activate late gene transcription.

Kalionis *et al.* (1986b) concluded that the *B* gene product autogenously controlled its own transcription in the lysogenic state, either directly or indirecly. Control of *B* gene transcription was investigated since there was no evidence of activation of late transcription in a lysogen, even though B protein was present and active in repressing its own transcription in this state. In this work the control of *B* gene transcription was shown not to be direct, but to be indirect and require the involvement of the *c*I gene product for repression of transcription from the *B* promoter. The inability of the prophage *B* gene to activate late transcription was concluded to be a direct consequence of repression of *B* gene transcription and not due to the *c*I gene product interfering with *B*-dependent activation directly.

The *c*I gene product is normally involved in repression of lytic transcription by binding to a primary operator site overlapping the early lytic promoter (Lamont *et al.*, 1988). However, the presumptive operator site for the repressor of *B* gene transcription (Kalionis *et al.*, 1986b) shows no homology to the primary CI operator. Since the autogenous control of *B* gene transcription was indirect in the lysogenic state, this indicated that either the expression of the *c*I gene was under the control of B protein or that B protein modified the activity of the CI protein. Expression of the *c*I gene does not appear to be under *B* gene control and indicates that the control of *B* gene transcription is likely to be by a mechanism in which the B protein alters the DNA-binding specificty of the CI protein so that it represses transcription from the *B* promoter.

The work presented in this thesis provides the basis for further studies in elucidating the mechanism of late promoter activation and control of *B* gene transcription in coliphage 186.