ESTABLISHMENT OF LYSOGENY IN COLIPHAGE 186

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by

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APPENDIX
Neufing, P.J., Shearwin, K.E., Camerotto, J., and Egan, J.B. (1996) The CII protein of bacteriophage 186 establishes lysogeny by activating a promoter upstream of the lysogenic promoter. *Mol. Microbiol.* **21:** 751-761 92

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SUMMARY

Coliphage 186 chooses to develop either lytically or lysogenically. The studies reported in this thesis were aimed at understanding how this phage establishes lysogeny.

The developmental decision in 186 occurs at the level of the lytic (p_R) and lysogenic (p_L) promoters.Transcription from either promoter is at the expense of the other. Thus transcription from p_L results in the production of the CI protein, which represses p_R, while transcription from p_R expresses the Apl protein which can repress p_L (Dodd *et al*., 1990). Evidence from *galK* transcriptional promoter fusions has shown that the lytic promoter (p_R) is considerably more active than the lysogenic promoter (p_L) (Dodd *et al*., 1990). Moreover, as a consequence of the face-to-face arrangement of p_R and p_L, p_L transcription is inhibited by converging p_R transcription. Yet during the establishment of lysogeny, the phage must theoretically progress from a state where p_L is repressed by Apl and interfered with by actively transcribing p_R, to the stable lysogenic state of autogenous control whereby CI repression of p_R allows p_L activity and thereby maintenance transcription of *c*I. The 186 *c*II gene has previously been shown to be required for the establishment of lysogeny and is expected to mediate this transition.

CII contains a potential helix-turn-helix DNA-binding motif suggesting that it may act as a transcriptional regulator. As a prelude to defining the mechanism of action of CII previous work had identified that CII could specifically bind to a minimal 165 bp DNA fragment of 186 which spanned the *apl*/*c*II intergenic region . In the present study, the CII DNA binding site within this region was identified by DNase I footprint. CII was found to bind to inverted repeat sequences separated by two turns of the helix which are located at the 5' terminus of the *c*II gene.

Location of the CII-binding site upstream of p_L suggested that CII may establish lysogeny by activating an alternative lysogenic promoter in this region. Results obtained from transcriptional *lacZ* reporter fusions confirmed that CII functions as a transcriptional activator and primer extension was used to map the start site of this CII dependent (p_E) transcript to the *apl*/*c*II intergenic region. Since results from transcriptional reporter fusions show that the p_E transcript extends into the lysogenic operon past p_R it is presumed that transcription from p_E expresses CI which leads to repression of p_R and relief of the inhibition of p_L by p_R, thus allowing maintenance transcription of *c*I. As the integrase gene (*int*) is part of the same operon as *c*I, it is also presumed that p_E transcription produces Int thus facilitating integration of the phage genome into the bacterial chromosome. It is of interest to note that p_E does not remain fully active throughout establishment since it is subject to direct negative feedback by CI.

In 186 both lysogenic promoters transcribe in the face of p_R yet while the p_R/p_L combination cannot establish lysogeny the p_R/(p_L+p_E) promoter combination is proficient in establishment. Various single copy *lacZ* promoter fusions were constructed to determine why p_E+p_L is more proficient at establishing lysogeny in the face of p_R than p_L alone. Results from this study show that p_E is a significantly stronger promoter than p_L and is less inhibited by converging transcription from p_R. Since the activities of p_L and p_E are additive it was therefore expected that p_E+p_L would be even more proficient than p_E alone in extending transcripts beyond p_R. Contrary to expectation however, the p_E/p_R combination was able to extend a greater number of transcripts beyond p_R than the p_R/(p_L+p_E) combination. Presumably, interfering complexes generated by the opposing transcription from p_R and p_L block the elongation of a small proportion of p_E transcripts.

Promoters initiating converging transcription such as p_R and p_E are expected to interfere with each others activities. p_R transcription has been shown to interfere with p_E but does p_E activity interfere with p_R transcription ? Results from *lacZ* reporter constructs monitoring p_R activity in the presence or absence of active CII indicate that p_E transcription is able to inhibit p_R transcription. This may be an efficient means for 186 to dampen p_R activity before CI represses p_R directly.

Establishment of lysogeny in 186 occurs in the presence of Apl. Since Apl binds in the p_R/p_L region and represses transcription from p_R and p_L it was of interest to determine how Apl would alter the flow of lytic and lysogenic transcription during the establishment of lysogeny. Results from transcriptional reporter studies used to address this question indicate that Apl acts in concert with p_E to increase *c*I transcription, by reducing interfering transcription from p_R.

STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying

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