A MECHANISM FOR TRANSCRIPTIONAL INTERFERENCE BETWEEN CONVERGENT PROMOTERS IN THE DEVELOPMENTAL SWITCH OF BACTERIOPHAGE 186

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THESIS SUMMARY

Convergent transcription defines a situation where two promoters are arranged face-to-face, leading to a partial 5' overlap between their transcripts. Multiple examples of convergent promoters have been reported in a wide range of organisms (including bacteriophage, *E. coli*, plants, yeast and humans). In both eukaryotes and prokaryotes the simultaneous *in vivo* activity of such promoters generally leads to repression of transcription from the opposing promoter, a phenomenon termed transcriptional interference. Interference is often asymmetric, with a strong (the aggressive) promoter reducing the expression of a weak (the sensitive) promoter. This interaction has been used in various ways to effect regulation of gene expression.

A number of studies have investigated this phenomenon in both *E. coli* and eukaryotic systems however the exact mechanism(s) remain speculative. Theoretically, a wide range of molecular mechanisms of interference are possible. This study uses largely *in vivo* methods to eliminate some of these possibilities and sets out to further understand the mechanism operating for one set of converging promoters.

The convergent promoters used in this study are those of the lysis-lysogeny switch from bacteriophage 186. The bacteriophage 186 lytic promoter, *pR*, is ten times stronger than the lysogenic promoter, *pL*, found 62 bp downstream in a convergent orientation. In chapter 2 a single copy, promoter, reporter system was established to measure convergent promoter activity. It was shown that the stronger promoter reduced the transcriptional activity of the weaker promoter 5.6 fold, and that this interference is not reciprocal. No variation in interference was seen when different host strains were used.

In Chapter 3, this promoter system was used to determine what action of *pR* causes interference of *pL*. A minor role for *pR* bound RNAP during interference was demonstrated by the following observations: (i) Increasing the spacing between the promoters an extra 100 bp brought no loss in interference but rather a slight increase. This is inconsistent with a steric hindrance model of interference where RNAP bound at *pR* inhibits RNAP binding at *pL*. (ii) Placing the promoters in a close divergent orientation completely abolished interference, which is contrary to a competitive

inhibition model of interference. And (iii) The placement of a unidirectional intrinsic terminator between *pR* and *pL* significantly reduced interference from 9.2 fold to 3.3 fold. This is contrary to a mechanism of interference where RNAP bound at *pR* acts as a roadblock.

A major role of elongation from *pR* over *pL* during interference was shown by the observations that (i) Divergent promoters that also actively transcribe the 62 nt of 5' antisense transcript did not restore interference, contrary to a model of interference that involves antisense transcription. And (ii) that terminating elongation from *pR* prior to transcription over *pL* dramatically reduced interference.

The nature of interference caused by elongation over *pL* was then investigated in Chapter 4. Based on the *in vitro* observation that open complexes formed at *pL* were slow to escape and clear the promoter and that the activity of these complexes was reduced by collisions with converging elongating polymerase from *pR*. A 'sitting duck' mechanism of interference is proposed in which *pR* convergent transcription over *pL* negatively affects promoter initiation intermediates that form at *pL*.

In Chapter 5 the validity of this mechanism was supported by an investigation of transcriptional interference between another example of convergent promoters found in the developmental switch of the related bacteriophage, P2. The strong lytic promoter was shown to reduce the activity of the weak lysogenic promoter only 2.2 fold (contrary to the 30 fold interference found in the literature). The difference in interference with that of 186 promoters was shown to be partly due to the differences in sensitivity of the two lysogenic promoters. The *in vitro* rate of escape of open complexes formed at the P2 lysogenic promoter was shown to be much faster than that of 186 *pL*. The lack of interference for P2 promoters could therefore be explained by a reduced potential of its lysogenic promoter to form 'sitting ducks'. Thus, interference in the 186 system occurs because RNAP complexes at *pL* that are waiting to clear are sensitive to passing elongating polymerase from *pR*. This 'sitting duck' mechanism is likely to be important for promoters that are close together. Mechanisms involving collisions between elongating polymerases and promoter occlusion and a general mechanism for transcriptional interference by convergent promoters discussed in Chapter 6.