

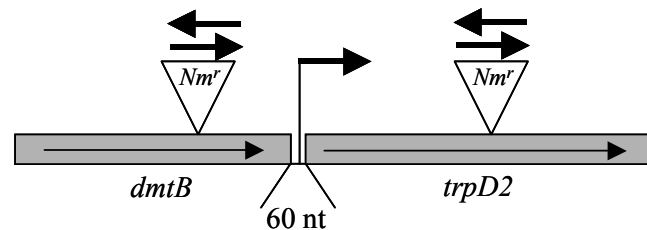
Possible role of a noncoding RNA in the initiation of heterocyst differentiation

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From the onset of nitrogen starvation of *Anabaena* PCC 7120, a series of events unfold culminating about 18 hours later in the appearance of mature, N₂-fixing heterocysts. Hundreds of genes are induced at different times over the course of differentiation (1). Several specific genes important to the process have been identified, most notably *hetR* (2), whose expression is necessary and (in some circumstances) sufficient to trigger heterocyst differentiation. However, despite much effort, no global genetic circuitry has been elucidated of comparable explanatory power to the cascade of sigma factors governing the temporal and spatial expression of genes in during sporulation by *Bacillus subtilis* (3). Whatever circuitry is eventually found promises to represent something new to biology. Serendipity may work where a rational search has failed.

In an attempt to understand the role of DNA methyltransferases in the control of cell cycle and heterocyst differentiation, we cloned and inactivated *dmtB*, encoding a GGCC-specific methyltransferase. Surprisingly, the resulting mutant was unable to initiate heterocyst differentiation. Loss of the methyltransferase itself, however, was



not responsible for this remarkable phenotype, as complementation of the mutant with intact *dmtB* restored DNA methylation but not the ability to differentiate. Moreover, when the interrupting C.K3 cassette (4) was inserted in the opposite orientation (antiparallel to *dmtB*), methylation was lost but heterocyst differentiation was normal. These results pointed to activation by the strong P_{*psbA*} promoter of C.K3 of a downstream gene, perhaps *trpD2*, highly similar to genes encoding anthranilate synthetase (*Anabaena* possesses another similar gene in its *trp* operon). Activation of *trpD2* with the P_{*psbA*} promoter had no effect on differentiation, nor did inactivation of the gene by C.K3 placed in parallel to *trpD2*. However, C.K3 placed antiparallel to the gene produced the same Het⁻ phenotype as did the original *dmtB* knockout. Taken together, these results indicated that expression of some element in or near the small intergenic region blocked heterocyst differentiation. The intergenic sequence is quite interesting. A 17-bp segment flanked by inverted repeats matches almost exactly a segment immediately upstream from *hetRI*, the transcriptional start site closest to *hetR* (5). Furthermore, while the corresponding 17-bp segment in the *dmtB/trpD2* intergenic region of *Nostoc punctiforme* differs in three nucleotides, but two of these differences are found also in *Nostoc's hetRI* region. Finally, the intergenic region appears to be essential for viability, as deletion mutants do not achieve full segregation. These and other findings are most readily explained by the existence of a noncoding RNA transcribed from the intergenic region that regulates the expression of *hetR*.

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