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Short communication

DNA methylation influences *trpR* promoter activity in *Escherichia coli* K-12

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Summary. Methylation of adenine in the GATC-sequence of the −35 region of the *trpR* promoter decreases activity by 2–3 fold.

Escherichia coli promoter sequences contain two conserved regions located 10 and 35 pairs before the transcription start site (Hawley and McClure 1983). The "−35" region of the *trpR* promoter contains the sequence 5′-GATC-3′ (Singleton et al. 1980; Gunsalus and Yanofsky 1980) which is a substrate for the DNA adenine methylase specified by the *dam* gene (for review see Marinus 1984). Since the methyl group protrudes into the major groove of DNA, it might hinder transcription initiation by influencing RNA polymerase binding compared to unmethylated DNA.

To test this possibility, use was made of a lambda phage, GB9 (Bogosian and Somerville 1984), which contains a *lacZ* operon fusion to the *trpR* promoter (*trpR*::*lacZ*). This phage was used to construct two strains: GM 1481 (*trpR55 lacI22 lacZ118* (Oc) *proB48 met-90 trpA9605*(Am) *his-85* (Am) *rpsL177 azi-9 relA19* (λGB9)) and GM1482 (as GM1481 but *dam-13*::Tn*9*). β-Galactosidase production in these strains should be a reflection of transcription initiation at the *trpR* promoter. As shown in Table 1, the *dam* mutant strain produced 2-3 times as much enzyme as its *dam*$^+$ isogen. This difference in enzyme activity is reflected in the coloration of the colonies of these strains on MacConkey-Lactose plates. GM1482 (*dam*$^-$) produces uniformly dark red colonies whereas those from GM1481 (*dam*$^+$) have a dark red center surrounded by a whitish halo.

Introduction of pMQ148, a plasmid containing the *dam*$^+$ gene, into GM1482 (*dam*$^-$) results in decreased β-galactosidase production (Table 1) and a concommitant change in colony coloration identical to GM1481 (*dam*$^+$). This control experiment also eliminates the possibility that the strains differ in prophage copy number. Since *dam*$^-$ strains display a hyper-recombination phenotype (Marinus 1984) a concern would be that increased β-galactosidase activity in Table 1 is due to increased recombination to form the wild type *lacZ* sequence. To test this several hundred non-lysogenic survivors of prophage induction were isolated from strain GM1482. All were Lac$^-$. It was shown previously (Marinus and Morris 1974) that there

Table 1. β-Galactosidase activity in *trpR*::*lacZ* lysogens

Strain	β-Galactosidase activity
GM1481 (*dam*$^+$)	631–747
GM1482 (*dam*$^-$)	1,385–2,231
GM1482/pMQ148 (*dam*$^+$/*dam*$^-$)	739–868
GM1482, cured of λGB9	0.8–1.1

β-Galactosidase was measured as described by Miller (1972) in logarithmic phase cells. The values represent the variation between four independent cultures of each strain

is no difference in the rate of β-galactosidase production in *dam*$^-$ versus *dam*$^+$ strains. Also, because the prophage is *ind*$^-$ the increased rate of spontaneous phage induction in *dam*$^-$ strains (Marinus 1984) cannot account for the increased β-galactosidase activity. The simplest explanation for the observed results is that methylation of the 5′-GATC-3′ sequence in the −35 region of the *trpR* promoter decreases transcription initiation.

Other promoters which contain -GATC- in the −35 region include *sulA* (Cole 1983), *dnaA* P2 and origin B (Hawley and McClure 1983). Of these only *sulA* has been tested and it shows a 6-fold increase in activity in *dam* mutants (Peterson et al. 1985). The results in this communication suggest that only part of this increase (2–3 fold) is due to the direct effect of methylation at the promoter and the remainder is due to its derepression as an SOS gene.

Two other examples are known where *dam* methylation affects gene regulation. Methylation of the *mom* gene of phage Mu is required for expression by an unknown mechanism (Kahmann 1984) and methylation of a *dam* site overlapping the −10 region of the inward promoter of Tn*10* ihibits activity (D. Roberts and N. Kleckner, unpublished data).

The physiological relevance of DNA methylation on promoter activity is unclear since all measurable *dam* sites in non-replicating DNA are methylated in vivo (see Marinus 1984). Perhaps promoter activity is enhanced on hemimethylated templates which may exist at the replication fork. In this context, the *trpR* gene is between the origin of replication and the *trp* operon perhaps to ensure that a burst of repressor synthesis occurs before replication of the *trp* operator. For the purification of products specified by genes with *dam* promoter sequences, however, an easy

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2–3 fold amplification may be achieved by preparing material from *dam* mutant strains.

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