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Growth-rate-dependent transcription initiation from the *dam* P2 promoter*

(DNA methylation; lipoic acid ligase; recombinant DNA; gene regulation)

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SUMMARY

Transcription of the *dam* gene in *Escherichia coli* is dependent on growth rate. Using single-copy promoter::*lacZYA* fusions we found that of the five promoter regions which affect *dam* expression, only the P2 promoter shows growth-rate dependence. The determinants for growth-rate control must lie in the region –52 to +27 relative to the transcription start point.

INTRODUCTION

There are at least three cellular processes in *Escherichia coli* which are affected by the level of Dam MTase, a product of the *dam* gene, which modifies the A residue in 5'-GATC sequences in double-stranded DNA (see, for review, Marinus, 1995). First, overproduction of the Dam protein results in a mutator phenotype due to premature

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Abbreviations: aa, amino acid(s); *aro*, genes involved in aromatic aa biosynthesis; *att*, attachment site; β Gal, β -galactosidase; bp, base pair(s); *cde*, constitutive *dam* expression; *cI*, gene encoding λ phage repressor; Dam, DNA adenine MTase; *dam*, gene encoding Dam; Fis, factor for inversion stimulation; IS, insertion sequence(s); kb, kilobase(s) or 1000 bp; *lac*, lactose operon; *lipB*, gene encoding lipoic acid ligase; MTase, DNA methyltransferase; Mut, mutator; nt, nucleotide(s); *P* (*p*), promoter; PCR, polymerase chain reaction; σ^{70} , sigma 70 factor of *E. coli* RNA polymerase; Tn, transposon; ts, temperature sensitive; *urf*, unidentified reading frame(s); ::, novel junction (fusion or insertion).

methylation on newly synthesized DNA, thereby preventing correction of replication errors by the MutHLS system (see, for review, Modrich, 1991). Under these conditions the MutH protein, in complex with the other components, has a much lower affinity to cleave fully methylated DNA rather than hemi-methylated DNA. In wild-type cells, the Dam enzyme level is limiting, leading to transient hemimethylation of DNA trailing the replication fork. Such hemimethylated DNA is the most efficient substrate for MutH-dependent cleavage. Underproduction of Dam MTase is also mutagenic but in this case the reduced methylation leads to the production of unmethylated DNA, in which proper strand discrimination cannot occur, and mutations are introduced into the parental (old) DNA strand.

A second example is the synchronous initiation of multiple origins of chromosome replication (Boye and Løbner-Olesen, 1990). This can be measured by treating growing cells with rifampicin to block further initiation and with cephalixin to inhibit cell division. After a period of incubation to allow completion of chromosome replication cells are harvested, fixed, stained and subjected to flow cytometry. In a cell in which the level of Dam MTase was controlled by temperature through the use of the λ *p_L* promoter and a ts repressor (*cIts857*), synchronous initiation of origins occurred only in a narrow temper-

ature range close to 37°C. At lower or higher temperatures initiation was progressively asynchronous and was maximal at 30°C or 42°C (Boye and Løbner-Olesen, 1990). These results are consistent with a model in which chromosome initiation is dependent on the level of hemimethylated DNA- too much or too little alters initiation frequency.

A third example is the transposition frequency of the transposon Tn10 or its component IS10. Transposition is increased at Dam MTase levels lower than normal (i.e., in a *dam* mutant) and decreased at high MTase levels (Roberts et al., 1985). Part of the explanation for these effects is the inhibition of transcription from the promoter region for the transposase gene by Dam methylation. Since DNA in *E. coli* is almost completely methylated for most of the cell cycle, the transposase promoter is inactive. Transcription is most efficient on the hemimethylated transposase promoter region which occurs only immediately after passage of a replication fork, i.e., once per cell cycle.

Given the above examples, it would seem probable that the cell has a mechanism(s) to control the level of Dam MTase at its normal concentration of 130 molecules per fast-growing cell (Boye et al., 1992).

EXPERIMENTAL AND DISCUSSION

(a) Promoter mapping

In order to study gene regulation it was first necessary to locate the promoter region for the *dam* gene. Using a combination of deletion mapping with promoter::lac fusions and primer-extension experiments, five promoter regions affecting *dam* gene expression were found (Løbner-Olesen et al., 1992). Promoter P2 has the greatest promoter strength: about 70% of the total transcripts traversing the *dam* gene originate from this promoter. The P2 promoter is located about 2.5 kb upstream from the *dam* gene and also affects the *aroK*, *aroB* and *urf74.3* genes which lie between the promoter and *dam*. The P2 promoter has typical σ^{70} hexameric recognition sequences located at -35 and -10 relative to the transcription start point and these are separated by 18 nt (Løbner-Olesen et al., 1992).

(b) Promoter reporter constructs

To more easily monitor the transcription levels from P2 and other *dam* promoters, we have constructed single-copy promoter::lacZYA fusions. An 80-bp fragment containing P2 and spanning the region -52 to +27 (GenBank accession No. M76389, nt 1720-1800) plus *Bam*HI linkers was amplified by PCR from pAL0163 and inserted into the *Bam*HI site of the lacZYA promoter-

cloning vector pRS415 (Simons et al., 1987). After confirming the identity of the insert by sequencing, the P2::lac fusion was integrated in single copy into the λ att site as described by Atlung et al. (1991). A similar approach was used to construct a single-copy *dam* P3::lac fusion containing a 319-bp PCR fragment (GenBank accession No. M76389, nt 3061-3380).

Cells containing these lac fusions were grown with different carbon sources to vary the doubling time and assayed for β Gal activity. The results in Fig. 1 show that the two promoters behave differently; promoter P2 activity is growth-rate-dependent but that of promoter P3 is not. In other experiments we have shown that the remaining *dam* promoters behave like P3.

Transcription initiation from ribosomal RNA P1 and some tRNA promoters is growth-rate dependent (Jinks-Robertson and Nomura, 1987). The underlying mechanism is unknown but expression of these genes is affected by (i) Fis protein; (ii) an A+T-rich region located between -40 and -60; (iii) the ribosomal feedback response; and (iv) stringent control (Jinks-Robertson and Nomura, 1987; Ross et al., 1990;1993). In contrast, the *dam* P2 promoter is not affected by any of these, indicating a different mechanism of growth-rate control (Rasmussen et al., 1994).

(c) Uncoupling growth-rate control and *dam* gene expression

A mutant cell in which *dam* gene expression is unregulated has been isolated following mini-Tn10 mutagenesis

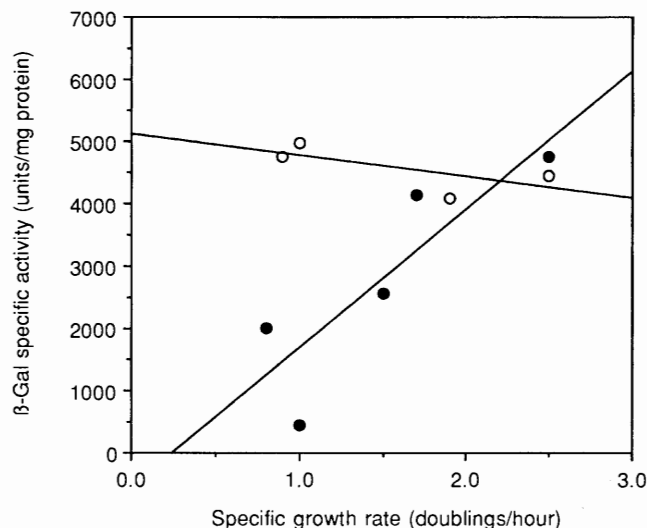


Fig. 1. Relationship of β Gal-specific activity and growth rate. *E. coli* cells containing either a *dam* P2::lac fusion (●) or a *dam* P3::lac fusion (○) were grown in minimal medium with either 0.2% glucose/1% casamino acids or 0.2% glucose or 0.2% glycerol or 1% acetate. β Gal activity was assayed in crude extracts obtained by sonication (Rasmussen et al., 1994). Enzyme levels were determined as an average of five samples taken at different optical densities of the culture.

(Rasmussen et al., 1994). This strain, *cde-4*, shows elevated β Gal activity from a *dam::lacZ* fusion at low growth rates. The mutational defect is in or near *lipB*, the major source of lipoic acid ligase which links lipoic acid to proteins through ϵ -amino groups of lysine. If *cde-4* is indeed allelic with *lipB*, this raises the possibility that this protein modification could have regulatory significance perhaps through differential interaction of a modified regulatory protein with RNA polymerase or the *dam* P2 promoter region or both.

(d) Physiological role for growth-rate control

Growth-rate control of *dam* gene expression may be a reflection of the *E. coli* life cycle. In the colon, *E. coli* cells are expected to grow more rapidly than when they are outside the host. If *dam* gene expression were constitutive (at the level in fast-growing cells), slow growing cells would have too little hemimethylated DNA due to an excess of Dam MTase. A reduction in *dam* MTase level in slow-growing cells would ensure that the same amount of hemimethylated DNA would be present near the replication fork as in fast-growing cells.

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