

Novel growth rate control of *dam* gene expression in *Escherichia coli*

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Summary

Transcription of the *dam* gene in *Escherichia coli* is growth rate regulated by a mechanism distinct from that used for ribosomal RNA gene promoters. Single-copy operon fusions to *lacZ* indicated that the major promoter, P2, is responsible for most or all of the growth rate dependence. Promoter P2 is a typical σ^{70} promoter with 18 bp spacing between the –10 and –35 hexamers. Primer extension analysis was used to show that there was no inhibition of transcription from promoter P2 in cells induced for the stringent response. Beta-galactosidase specific activity from a single-copy *dam::lacZ* fusion was unaffected by either excess *rrnB* RNA or the level of Fis protein. Thus growth rate control of *dam* gene expression differs from that of the rRNA and tRNA genes by its lack of response to stringent control, ribosomal feedback and enhanced transcription by Fis protein. We devised a procedure for selection of mutant cells in which *dam* gene expression was unregulated. One such mutant (*cde-4*), obtained by miniTn10 insertion, showed the same level of β -galactosidase activity at all growth rates tested. In contrast, growth rate-dependent expression of the *rrnB* gene was unaffected by *cde-4* confirming the different modes of regulation. The *cde-4::miniTn10* insertion is located close to kilobase 670 on the physical map in or near the *lipB* gene.

Introduction

The *dam* gene of *Escherichia coli* encodes a DNA methyltransferase which modifies the adenine residue in the

sequence -GATC- in double-stranded DNA (Lacks and Greenberg, 1977; Hattman *et al.*, 1978). DNA methylation at the replication fork lags behind synthesis such that DNA is in a hemi-methylated configuration for a short time (Campbell and Kleckner, 1990). During this interval the hemi-methylated DNA is subjected to *dam*-directed mismatch repair for removal of replication errors (Modrich, 1991). Certain promoters, such as that for the transposase of Tn10, are more active in a hemi-methylated configuration thus linking their expression to the cell cycle (Roberts *et al.*, 1985). Hemi-methylated origins (*ori*) of replication of certain plasmids and *E. coli* minichromosomes are incapable of initiation *in vivo* (Russel and Zinder, 1987). The *oriC* region remains hemi-methylated for about 30% of the cell cycle (Ogden *et al.*, 1988; Campbell and Kleckner, 1990) thus preventing premature initiations. *Dam* methylation also ensures that initiation of all *oriC* regions in the cell occurs simultaneously (Boye and Løbner-Olesen, 1990).

The time during which the DNA is in a hemi-methylated state must be strictly controlled since prolongation or reduction of this time can lead to an increased mutation rate (Marinus and Morris, 1974; Marinus *et al.*, 1984), asynchronous initiation of chromosome replication (Boye and Løbner-Olesen, 1990) or an alteration in gene expression (Roberts *et al.*, 1985). These effects are due directly to an alteration in the level of Dam methyltransferase from its normal level of 130 molecules in fast-growing cells (Boye *et al.*, 1992). The Dam methyltransferase is encoded by the *dam* gene located at minute 74 on the *E. coli* genetic map. The *dam* gene is part of a transcriptional unit that includes at least four genes: *aroK*, *aroB*, *urf74.3* and *dam*. Five transcription initiation sites have been mapped in this region but it is not known how, or if, they are subject to regulatory control (Løbner-Olesen *et al.*, 1992). In this communication, we show that the major promoter (P2) of the *dam* transcriptional unit is subject to growth rate control by a mechanism distinct from that used for ribosomal RNA gene promoters.

Results

Expression of the dam gene at different growth rates

Initial studies showed that a bacterial strain (ALO838) with *lacZYA* fused to the *dam* gene at its normal chromosomal location produced more β -galactosidase at high growth

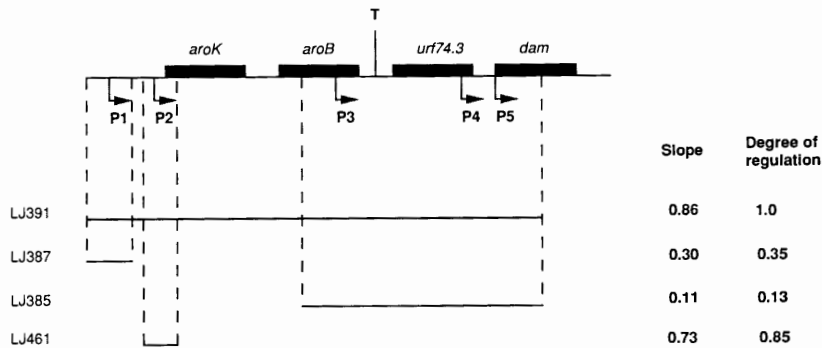


Fig. 1. Schematic outline of the *dam* operon. The coding regions of the *aroK*, *aroB*, *urf74.3* and *dam* genes are shown by black boxes. T indicates a transcriptional terminator region. The individual promoters are denoted P1, P2, P3, P4 and P5, respectively, and the direction of transcription from these are indicated by arrows. The solid lines represent the sequences fused transcriptionally to *lacZ*. Broken vertical lines indicate the borders of the cloned fragments. The numbers in the 'Slope' column are the slopes of linear regression lines obtained from β -galactosidase specific activities from cells grown at different growth rates. The column marked 'Degree of regulation' represents the slope of the particular promoter fusion divided by that from LJ391.

rates than low growth rates (data not shown). In order to determine which of the five known promoter regions was responsible for growth rate control of *dam* gene expression, we constructed plasmid-borne *lacZ* transcriptional gene fusions containing various amounts of chromosomal DNA upstream of the *dam* gene (as described in the *Experimental procedures*). The gene fusions were subsequently integrated as single copies at the *attB* site on the chromosome. The structures of the gene fusions used are shown in Fig. 1.

When strain LJ391, which contains all five *dam* promoters fused to *lacZ*, was grown at different growth rates, the β -galactosidase specific activity increased four-fold when the growth rate of the culture increased from 0.2 to 2 doublings per hour. As shown in Fig. 1, these data yielded a slope of 0.86 when plotted against each other after transformation to allow for comparison of promoters of different strengths (Dickson *et al.*, 1989). The slope of 0.86 is comparable to that for strain ALO838 with the *dam::lacZ* fusion in its normal chromosomal location (data not shown). A similar result to that of the operon fusions (slope=1.0) was obtained with a *dam::lacZ* protein fusion in *attB* (data not shown), indicating that there is no contribution from a translational component or differential mRNA stability to the growth rate control mechanism. The slope of *dam* expression with changing growth rate is less dramatic than that observed with rRNA promoters (Dickson *et al.*, 1989) suggesting different control mechanisms.

Figure 1 shows the DNA segments which were used to localize the region responsible for growth rate control. The DNA fragments containing promoters P1 or P3–5 showed little or no growth rate-dependent expression (Fig. 1) suggesting that it is principally the activity of promoter P2 that is affected. Indeed a 240 bp fragment bearing only promoter P2 showed growth rate dependence (strain LJ461 in Fig. 1). An 80 bp sequence containing promoter P2 and spanning the region -52 to $+27$ (relative to

the start site of transcription) also shows growth rate dependence (data not shown).

Primer extension analysis of transcripts initiated from P2 in slow- and fast-growing cells indicated that there was a three- to fourfold difference in abundance of the 5'-end of the message as determined by densitometric scanning (data not shown). This confirms that the change in Dam methyltransferase activity at different growth rates occurs at the level of transcription.

Expression of the dam gene is not enhanced by Fis protein and is not subject to stringent control or the ribosomal feedback response

Expression of the ribosomal RNA operons is (i) enhanced by binding of Fis protein to an upstream activator sequence; (ii) intrinsically high owing to the presence of an AT-rich region between -40 and -60 ; (iii) stringently regulated; and (iv) under the control of the ribosomal feedback response (Jinks-Robertson and Nomura, 1987; Nilsson *et al.*, 1990; 1992; Ross *et al.*, 1990; 1993). We measured β -galactosidase activity in *fis*⁺ and *fis*⁻ strains containing a *lacZ* transcriptional fusion to the *dam* gene at its normal chromosomal location. Enzyme activity was found to be independent of Fis protein during steady-state growth (Table 1). The slight decrease in *dam* gene expression in the *fis* mutant strain is not due to the slower growth rate of the *fis* mutant but can be explained by the low DNA to mass ratio in cells with a filamentous phenotype (U. von Freiesleben and K. V. Rasmussen, in preparation).

In order to test whether the *dam* P2 promoter was regulated by the stringent response, starvation for isoleucine was induced by the addition of a high concentration ($200 \mu\text{g ml}^{-1}$) of valine to an exponentially growing culture (Lamond and Travers, 1985). Cell growth (as measured by the change in optical density at 450 nm) stopped immediately, indicating that stable RNA synthesis had stopped.

Table 1. Expression of *dam* in a *fis* mutant strain and effect of the ribosomal feedback mechanism.

Strain	Promoter fusion	Beta-galactosidase specific activity
ALO838 (wt)	P1-P5	181
LJ428 (<i>fis</i>)	P1-P5	166
LJ391/pBR322	P1-P5	196
LJ391/pNO1301	P1-P5	201
LJ383/pBR322	P1-P5 (protein)	1.7
LJ383/pNO1301	P1-P5 (protein)	1.5
LJ513/pBR322	<i>rrmB</i> P1	2903
LJ513/pNO1301	<i>rrmB</i> P1	1713
LJ514/pBR322	<i>rrmB</i> P1 (C-1T, C-15G)	790
LJ514/pNO1301	<i>rrmB</i> P1 (C-1T, C-15G)	1025

Beta-galactosidase was assayed in crude cell extracts; the specific activity is expressed as units per OD₄₅₀. In all experiments cells were grown in minimal medium supplemented with glucose and casamino acids. Ampicillin (100 µg ml⁻¹) was added to the growth medium for cells containing the plasmids pBR322 and pNO1301.

The mRNA transcripts originating from the P2 *dam* gene promoter were still present in large amounts 20 min after isoleucine starvation (Fig. 2). This was not due to an extraordinarily long half-life of the 5' end of the mRNA, as this had been shown to decay with a half-life of 1–2 min after new synthesis had been blocked by the addition of rifampicin to the culture (Fig. 2).

Induction of the stringent response results in a concomitant increase in cellular ppGpp concentration and a rapid reduction in transcription of *rrmB* promoters (Cashel and Rudd, 1987). That P2 *dam* promoter activity is not

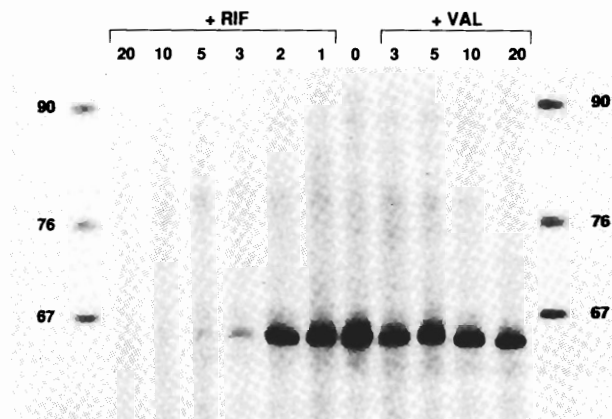


Fig. 2. 5'-end message stability after induction of the stringent response. Strain LJ24/pMS2 was grown exponentially in minimal medium supplemented with glucose. At OD₄₅₀ = 0.35, the 0 min sample was taken for RNA extraction. The culture was then split in two. To one half of the culture rifampicin was added to a final concentration of 300 µg ml⁻¹ and samples were taken for RNA preparation at 1, 2, 3, 5, 10 and 20 min, respectively. To the other half valine was added to a final concentration of 200 µg ml⁻¹ and samples were taken for RNA preparation at 3, 5, 10 and 20 min, respectively. The primer-extended products were run next to a labelled *MspI* digest of pBR322. The numbers indicate the size of the labelled fragments and are given in base pairs.

inhibited by stringent response induction indicates that it is not affected by physiological concentrations of ppGpp.

The ribosomal feedback response is observed as a repression of transcription from a chromosomal *rrmB-lacZ* gene fusion when the *rrmB* operon is introduced into the cell in multiple copies (Jinks-Robertson *et al.*, 1983). This is demonstrated in Table 1 where an *rrmB* P1 fusion strain (LJ513) with the *rrmB*-overproducing plasmid (pNO1301) contains less β-galactosidase than the same strain with the vector plasmid (pBR322) although the growth rate of the two strains is the same. As a control for a promoter that does not respond to repression by overproduction of rRNA we used strain LJ514 in which the mutant promoter *rrmB* P1 (C-1T C-15G) is not repressed by pNO1301 (Gaal and Gourse, 1990). From the data shown in Table 1, it is clear that neither transcription from the *dam* operon fusion in strain LJ391 (P1-P5) nor translation of the *dam* protein fusion in strain LJ383 (P1-P5) was affected by the presence of multiple copies of *rrmB*.

We conclude that the expression of the *dam* gene is not enhanced by Fis protein nor is it subject to the stringent or ribosomal feedback responses.

Isolation of a mutant strain affected in *dam* gene expression

In order to understand the mechanism underlying the growth rate-dependent regulation of the *dam* gene, we isolated mutants that showed constitutive *dam* gene expression. A strain with a chromosomal *lacZ* amber mutation and bearing the low-copy-number plasmid pALO163 (which contains the five *dam* promoters fused to *lacZ*) forms white colonies with red centres on MacConkey-lactose indicator plates. Mutants that formed red colonies on this medium were isolated following miniTn10 mutagenesis (Way *et al.*, 1984). The mutants were designated *cde* (constitutive *dam* expression) and were screened for increased β-galactosidase specific activity at slow growth rates which would indicate the elimination of a negative effector of *dam* gene expression. A mutant strain with this property was identified and designated *cde-4*.

At slow growth rates the *cde-4* mutant shows increased β-galactosidase specific activity compared to the wild type (Table 2). We were unable to obtain values for β-galactosidase activities at intermediate growth rates since the *cde-4* strain grew poorly in minimal medium supplemented with glucose with or without casamino acids. In L-broth, however, the *cde-4* mutant strain grows at the same rate as the wild type and, as expected, both strains show similar β-galactosidase specific activities (data not shown). This also suggests that L-broth contains a component(s) that stimulates the growth of the *cde-4* strain.

Table 2. Expression of *dam::lacZ* in a *cde-4* mutant strain.

μ^b	Beta-galactosidase Specific Activity ^a	
	wild type	<i>cde-4</i>
0.2	378	985
0.3	502	804
0.7	735	853

a. Beta-galactosidase specific activity is expressed as units per mg of protein. Values have not been corrected for gene dosage.

b. μ is the growth rate of the culture given as doublings per hour at 37°C.

Table 3. Expression of *dam* and *rrnB lacZYA* promoter fusions in a *cde-4* mutant strain.

<i>dam</i> P1-P5				<i>rrnB</i> P1			
wild type		<i>cde-4</i>		wild type		<i>cde-4</i>	
μ^a	s.a. ^b	μ	s.a.	μ	s.a.	μ	s.a.
0.5	1105	0.3	3133	0.7	14 139	0.3	10 796
0.9	1640	0.6	3241	0.9	18 418	0.6	12 030
1.2	3024	0.7	2142	1.0	46 491	0.8	17 721
1.6	4620			1.5	76 690		

a. μ is the growth rate of the culture given as doublings per hour at 30°C.

b. s.a. = β -galactosidase specific activity, is expressed as units per mg of protein. Values have not been corrected for gene dosage.

Table 3 shows that expression of the *rrnB::lacZYA* fusion is not constitutive in the *cde-4* strain at low growth rates but is reduced to the level expected for normal growth rate regulation. This result confirms that *dam* and *rrnB* do not share the same mechanism of growth rate dependence. Beta-galactosidase specific activity of the *dam::lacZYA* fusion is higher in Table 3 than Table 2. This is due to an uncharacterized effect of temperature on *dam* gene expression in which β -galactosidase activity is higher at 30°C compared to 37°C.

The miniTn10 insertion causing the *cde-4* mutation was mapped by Hfr matings to a position between 10 and

20 min on the genetic map (data not shown). Subsequent mapping by P1 transduction showed that *cde-4* was 28% co-transducible with a Tn10-*kan* situated at 14.50 min (*zbd-3104*) and 63% co-transducible with a Tn10-*kan* at 15.75 min (*zbd-3105*) (Singer *et al.*, 1989). This places *cde-4* at approximately 15.40 min on the genetic map (Wu, 1966).

A fragment from Tn10 was used as a probe to locate the position of the *cde* gene by hybridization (Southern, 1975) to DNA isolated from the *cde-4* mutant and digested with some of the enzymes used to construct the map produced by Kohara *et al.* (1987). Figure 3 shows that the insertion occurred in the region bounded by the *Bgl*I site at 667.5 kb and the *Eco*RI site at 671.5 kb. This region contains the *lipAB* genes and three open reading frames (ORFs) of unknown function (Reed and Cronan, 1993). The *cde-4* mutation does not result in lipoic acid auxotrophy suggesting that *cde* could be either *lipB* or one of the ORFs. Mapping the exact location of the insertion at the DNA sequence level is in progress.

Discussion

Our results indicate that the *dam* P2 promoter is subject to growth rate control and that the *cde-4* allele uncouples this regulation. The mechanism of growth rate dependence of *dam* P2 appears to be distinct from that of other previously described *rrn* and tRNA gene promoters in its lack of response to stringent control, ribosomal feedback and Fis protein. To our knowledge all other growth rate-controlled promoters respond to at least one of these conditions.

The molecular mechanism underlying growth rate-dependent expression of ribosomal and transfer RNA genes is unknown. The recent isolation of a dominant lethal mutation affecting the σ^{70} subunit of RNA polymerase, which has the properties expected of an unregulated growth rate-control mutant, suggests a direct role for this enzyme (Keener and Nomura, 1993). This confirms

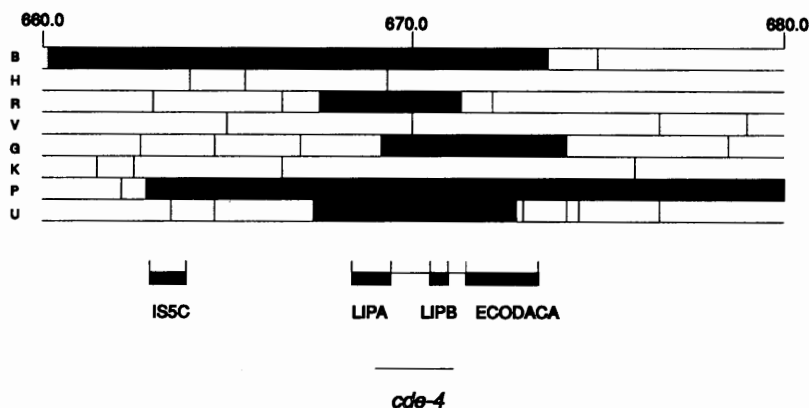


Fig. 3. Location of the *cde-4* insertion on the physical map (Kohara *et al.*, 1987). The shaded restriction fragments hybridized to the radioactive probe from Tn10. Digests with *Hind*III, *Eco*RV and *Kpn*I were not tested. B, H, R, V, G, K, P and U denote *Bam*HI, *Hind*III, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I and *Pvu*II respectively. The locations of ISSC and ECODACA are described by Rudd (1992), and of LIPA and LIPB by Reed and Cronan (1993).

previous data indicating that for the *rrnB* gene, the P1 promoter itself harbours the growth rate-control determinants (Gourse *et al.*, 1986). Mutational analysis of this promoter indicated that increased spacing between the -10 and -35 hexamers or specific base changes including those in the -35 region eliminated growth rate control (Gaal *et al.*, 1989; Dickson *et al.*, 1989).

The growth rate determinants for the *dam* P2 promoter are confined to the region encompassing -52 to +27 relative to the start site of transcription. If it is the P2 promoter *per se* which is responsible then a comparison with the growth rate-regulated *rrn* promoters (Fig. 4) shows no obvious similarities with regard to spacing between the conserved hexamers or the nucleotide sequence in the promoter region other than the -10 and -35 regions. Within the -35 region the TT nucleotides are found in all promoters and the -10 region is generally close to consensus (a blatant exception is the first base of the *dam* P2 -10 region). The *dam* P2 region between -40 and -60 contains 11 GC and 9 AT base pairs making it unlikely that an UP element exists (Ross *et al.*, 1993). Mutational analysis of the *dam* P2 promoter region should identify the sequence determinants for control.

As too much or too little Dam methyltransferase has been shown to increase mutagenesis, cause asynchronous initiation of chromosomes and alter the frequency of transposition (Marinus and Morris, 1974; Marinus *et al.*, 1984; Roberts *et al.*, 1985; Boye and Løbner-Olesen, 1990), we speculate that the level of Dam methyltransferase in the cell has to be adjusted to the growth rate of the culture to ensure that these processes occur efficiently. For example, shifting fast-growing cells to a slow-growth mode would lead to an excessive concentration of the Dam methyltransferase, and a subsequent overmethylation of DNA at the replication fork, unless *dam* transcription were down-regulated. Similarly, shifting slow-growing cells to a fast-growth mode requires up-regulation of *dam* transcription to prevent undermethylation of DNA.

We can express the idea stated in the preceding paragraph in more quantitative terms by relating *dam* gene expression to the rate of fork movement (i.e. nucleotide synthesis which reflects the rate at which GATC sequences are formed). This can be done by plotting each of these as a function of growth rate (Fig. 5). As we

have shown in this paper, *dam* gene expression is coupled to growth rate (Fig. 5). Cooper and Helmstetter (1968) and Kubitschek and Newman (1978) found that the rate of fork movement is related to growth rate as shown in Fig. 5. The number of replication forks is not important in this graph since the number of forks per cell mass is independent of growth rate (Bremer and Dennis, 1987).

In slow-growing cells, the curves in Fig. 5 indicate that the level of transcription (and by extension, Dam methyltransferase) is adjusted to the rate of fork movement until the maximal rate is reached. At high specific growth rates, however, the level of Dam methyltransferase is increased relative to replication fork movement (Fig. 5). We suggest that the rate of methylation of new GATC sequences following passage of the replication fork is therefore increased (Campbell and Kleckner, 1990). This adjustment of *dam* gene expression to growth rate in fast-growing cells ensures that new GATC sequences are methylated before passage of the next replication fork, thereby preventing the formation of excessive amounts of unmethylated DNA.

An alternative possibility for the growth rate dependence of *dam* gene expression is that it is an indirect consequence of its location and that some other gene(s) in the transcriptional unit is required to be regulated in this manner. It is not immediately obvious why the *aroK* and *aroB* genes, whose products function early in the aromatic amino acid biosynthetic pathway, should require growth rate control. The function of the other gene in the transcriptional unit, *urf74.3*, is unknown (but dispensable).

At present we can only speculate as to the function of the *cde* gene product. A simple explanation would be that it is a regulator which interacts with RNA polymerase at the *dam* P2 promoter to decrease the frequency of transcription initiation at low growth rates. Alternatively, the regulator could interact with the promoter region to alter its affinity for RNA polymerase, or the Cde product could control the synthesis of factors required for transcription initiation. That the *cde* gene product may be an antisense RNA which acts in a differential manner at different growth rates to interfere with transcription initiation cannot be ruled out. It should be possible to test these ideas, *in vitro*, once the gene and its product have been identified.

<i>dam</i> P2	TGGTGATTATTTCAG	TTGCCA	AACCCGCTGGAGTATTGA	GATAAT	TTTCAGT	CT
<i>rrnA</i> P1	GATTTTAAATTTCCCTC	TTGTCA	GGCCGGAATAACTCCC	TATAAT	GCGCGAG	CA
<i>rrnB</i> P1	TATTTTAAATTTCCCTC	TTGTCA	GGCCGGAATAACTCCC	TATAAT	GCGCCAC	CA
<i>rrnC</i> P1	TATTTTAAATTTCCCTC	TTGTCA	GGCCGGAATAACTCCC	TATAAT	GCGCCAC	CA
<i>rrnD</i> P1	AAGATCAAAAAATAC	TTGTGC	AAAAAATGGGATCCC	TATAAT	GCGCCTC	CG
<i>rrnE</i> P1	TCTGCAATTTTCTA	TTGCGG	CCTGCGGAGAACTCCC	TATAAT	GCGCCTC	CA
<i>rrnG</i> P1	TTTTTATATTTTCCG	TTGTCA	GGCCGGAATAACTCCC	TATAAT	GCGCCAC	CA
<i>rrnH</i> P1	AAATGCATTTTCCGC	TTGTCT	TCCTGAGCCGACTCCC	TATAAT	GCGCCTC	CA

Fig. 4. Alignment of various growth rate regulated promoters. The -10 and -35 regions are shown in bold type. The sequences of the *rrn* promoters are from Condon *et al.* (1992).

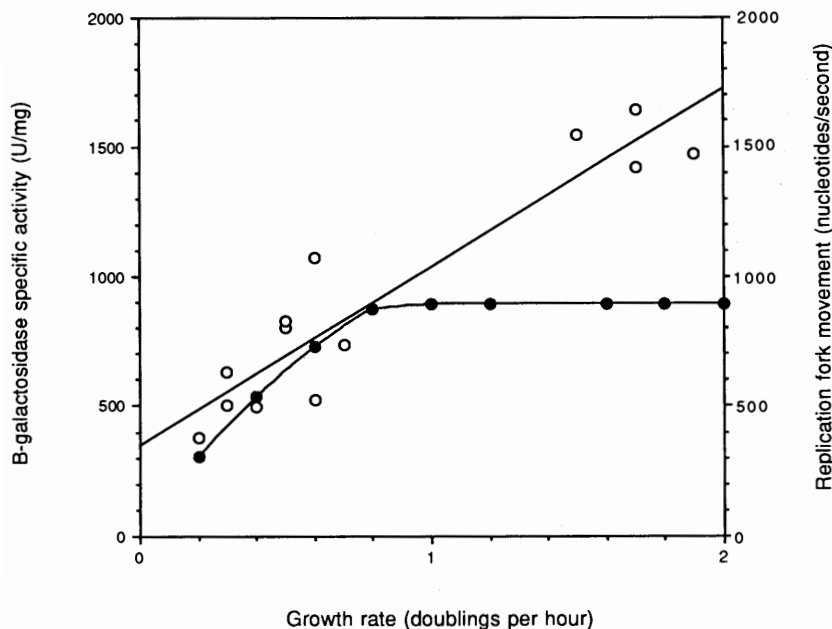


Fig. 5. Relationship between growth rate, *dam* gene expression and fork movement. Beta-galactosidase specific activity from a *dam* P1–P5::*lacZ* transcriptional fusion is expressed as units per mg of protein from cells grown at different growth rates. Replication fork movement data were obtained from Cooper and Helmstetter (1968) and Kubitschek and Newman (1978). Filled symbols indicate replication fork movement and open symbols the β -galactosidase specific activity in strain LJ391.

Experimental procedures

Bacterial strains and plasmids

All the strains used (except KL335) are derivatives of LJ24 (Rasmussen *et al.*, 1991). Strains LJ385, LJ387, LJ391, LJ460 and LJ461 carry *dam*::*lacZ* fusions integrated at *attB* and were isolated as described by Atlung *et al.* (1991) and Diederich *et al.* (1992). Strains LJ391, LJ387 and LJ385 contain the *lac* operon fusion plasmids pALO163, pALO214 and pALO183 respectively (Løbner-Olesen *et al.*, 1992). The *dam* promoter regions in strains LJ460 and LJ461 were initially isolated as 240 and 520 bp polymerase chain reaction products amplified by Vent DNA polymerase (New England Biolabs) in a PTC-100 Programmable Thermal Controller (MJ Research). The oligonucleotide primers MM75 (AGATCTGAAACCTCATAAGATAT and MM76 (AGATC-TTTGAGAGTTGGTGCTCTT) were obtained from Operon Technologies (Alameda) and pALO163 DNA (Løbner-Olesen *et al.*, 1992) served as template. Strains LJ513 and LJ514 carry the *rmB*::*lacZYA* lambda prophages from RLG1100 and RLG1019 respectively (Gaal *et al.*, 1989). Strain ALO838 carries a chromosomal *dam*::*lacZ* fusion (Løbner-Olesen *et al.*, 1992) and RJ1617 (MC1000 *fis*::Tn5; Johnson *et al.*, 1988) was the source of the *fis* mutation. KL335 is an Hfr with *lacI3* and *lacZ608*(Am) mutations and was obtained from K. B. Low (Yale University). Plasmids pBR322 (Bolivar *et al.*, 1977) and pMS2 (Løbner-Olesen and Marinus, 1992) have been described previously. pNO1301 (*rmB*) was obtained from R. L. Gourse and described in Jinks-Roberts *et al.* (1983).

Primer-extension analysis

RNA was isolated from cells by the hot-phenol method described by Gerdes *et al.* (1992). The total amount of RNA in each sample was determined by measuring the optical

density at 260 nm (OD_{260}), and $10 \mu\text{g ml}^{-1}$ of total RNA was used for each primer-extension reaction. Primer extensions were done according to the method of Gerdes *et al.* (1992), using primer MM84 (CCCCTTCGCTCAGAG) and the resulting fragments were separated on 8% denaturing polyacrylamide gels, together with a labelled *MspI* digest of pBR322. For the induction of the stringent response, valine was added to a final concentration of $200 \mu\text{g ml}^{-1}$ and for messenger half-life determination rifampicin was added to a final concentration of $300 \mu\text{g ml}^{-1}$. Samples were taken at intervals and the RNA extracted and processed as described above.

Growth conditions, media and enzyme assay

Cells were grown in either LB medium or AB minimal medium (Clark and Maaloe, 1967) supplemented with $1 \mu\text{g ml}^{-1}$ thiamine and either 0.2% glucose and 1% casamino acids, 0.2% glucose, 0.2% glycerol, 0.4% succinate or 1% acetate. Beta-galactosidase activity was assayed in crude extracts obtained by sonication. Enzyme levels were determined as an average of five samples taken at different optical densities of the culture. Units of activity are expressed as described by Miller (1972) with the modification that instead of correction for absorbance at 550 nm by cell debris, this was removed by high-speed centrifugation. The β -galactosidase specific activities were corrected for relative gene dosage using the formula of Collins and Pritchard (1973). The C period of the cell cycle was calculated using the formula of Kubitschek and Newman (1978). The slopes of the plots for growth rate regulation were obtained as described by Dickson *et al.* (1989) using a scaling procedure which allows comparison of the slopes on a uniform basis for promoters of widely different activities. The β -galactosidase activity per mg of protein of each operon fusion strain was plotted against the growth rate of the culture (doubling per hour) and a linear regression analysis was performed. All activities were scaled to values at the specific

growth rate equal to 1.0. The activities at all growth rates were then divided by the scaling factor and replotted. Linear regression analysis was performed and the resulting slopes are shown in Fig. 1.

Isolation and mapping of *cde* mutants

Lambda NK1098 (Way *et al.*, 1984) was used to introduce the miniTn10 transposon into strain KL335/pALO163 as described by Way *et al.* (1984). Mutants which formed red colonies were selected on MacConkey plates supplemented with 1% lactose. The transposon insertions were moved to strain LJ24 by P1 transduction and selection for tetracycline resistance.

The methods for DNA hybridization (Southern, 1975) have been described elsewhere (Løbner-Olesen and Marinus, 1992). The labelled probe was the 1554 bp *HindIII*-*XbaI* fragment from miniTn10.

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