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Regulated Expression of the *Escherichia coli dam* Gene

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Regulated expression of the *Escherichia coli dam* gene has been achieved with the *araBAD* promoter lacking a ribosome binding site. Cultures of *dam* mutants containing plasmid pMQ430 show no detectable methylation in the absence of arabinose and complete methylation in its presence. Dam methyltransferase is a substrate for the Lon protease.

In *Escherichia coli* K-12, about 2% of adenines are modified to *N*6-methyladenine by the action of the Dam methyltransferase (8). This modification occurs at GATC sequences in transiently hemimethylated double-stranded DNA trailing the replication fork. Hemimethylated DNA has one parental chain which is methylated, but its newly synthesized complementary strand is not methylated. Methylation of hemimethylated DNA is delayed due to the low concentration of Dam methyltransferase, which is present at about 130 molecules per cell (2). Increasing Dam methyltransferase levels reduce the amount of hemimethylated DNA (3).

The large number of phenotypic differences associated with Dam deficiency in *E. coli* is consistent with multiple roles for *dam* methylation (8, 11). These roles can broadly be defined as affecting (i) DNA mismatch repair (12), (ii) alterations in gene expression (15), (iii) the initiation of chromosome replication (1), and (iv) the chromosome structure (6). The phenotypes of *dam* mutants have been explored using presumed missense (e.g., *dam-3*) and insertion and deletion (e.g., *dam-13*::Tn*9* and *dam-16*::Kan, respectively) mutations. In spite of numerous attempts, no temperature-sensitive *dam* alleles have been isolated from *E. coli*, although such alleles would be very useful in functional studies to turn Dam methylation on and off. These alleles would also allow testing of the effects of partially methylated or hemimethylated DNA on cell functions.

In an alternative approach, we placed the *dam* gene borne on a chromosomal *Xba*I-*Hin*dIII fragment from pYIN2 (13) under the control of the *araBAD* promoter in vector pBAD18 (5), creating pMQ400. In the absence of inducer, however, the cells still showed a Dam$^+$ phenotype, as was determined by the action of *Dpn*I (digests methylated *dam* DNA), *Dpn*II (digests unmethylated *dam* DNA), and *Sau*3AI (digests both methylated and unmethylated *dam* DNA) on total DNA extracted from cultures using a MasterPure DNA purification kit (Epicentre). To reduce the amount of Dam produced from the plasmid, we removed the ribosome binding site downstream of the *araBAD* promoter in pMQ400. This was achieved by removing the native DNA upstream of the ATG of the *dam* gene and fusing the translation initiation codon (an *Nla*III site, ↓CATG) directly to the *Sph*I site (G↓CATG) in the polylinker sequence of pBAD18. The resulting construct was designated pMQ430.

Log-phase cultures of strain GM3819 (*dam-16*::Kan [16]) (Table 1) bearing pMQ430 growing in L broth show no detectable methylation of chromosomal DNA in the absence of inducer (Fig. 1). Upon exposure to 0.2% arabinose, however, chromosomal DNA was completely methylated within one generation (40 min), as was determined with the restriction enzymes *Dpn*I, *Dpn*II, and *Sau*3AI (Fig. 1). Removal of the inducer after a 40-min exposure and its replacement with 0.2% glucose led to a slow reduction in chromosomal methylation requiring 4.5 h or nine generations to effect complete loss of detectable methylation (Fig. 2). It should be noted that neither *Dpn*I nor *Dpn*II digests hemimethylated DNA. The cells showed no decrease in growth rate (30-min generation time) or cell number during this period. The time required to demethylate chromosomal DNA was longer than expected, and we suspect that this may be due to the slow degradation of the inducer. Consequently, we have not quantitated it further by the use of Southern or Western blots.

We have also tested the effect of sequentially turning Dam production on and off during cell growth. Cultures were exposed to 0.2% arabinose for 60 min, diluted, and then allowed to grow logarithmically in L broth with 0.2% glucose for 14 generations. The culture was again exposed to inducer, and the

TABLE 1. Description of *E. coli* K-12 strains[a]

Strain	Relevant characteristics	Source
GM2807	Hfr *dam-16*::Kan (PO68) *thi-1 relA1*	Lab stock
AB1157	F$^-$ *thr-1 leuB6 thi-1 argE3 hisG4 proA2 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL31 tsx-33 supE44 rfbD1 kdgK51*	Lab stock
GM3819	Like AB1157 but *dam-16*::Kan	Lab stock
GM7428	pMQ430/N4454	Lab Stock
GM7481	pMQ430/EC18 *lon*::Tn*10 dam-13*::Tn*9*	R. Woodgate
GM7482	pMQ430/EC22 *clpP*::Kan *dam-13*::Tn*9*	R. Woodgate
GM7483	pMQ430/EC28 *clpX*::Kan *dam-13*::Tn*9*	R. Woodgate
GM7484	pMQ430/EC210 *rcsA166*::Kan *dam-13*::Tn*9*	R. Woodgate
GM7485	pMQ430/SG22099 *clpA319*::Kan *dam-13*::Tn*9*	S. Gottesman
GM7486	pMQ430/SG2210 *clpB*::Kan *dam-13*::Tn*9*	S. Gottesman
N4454	Like AB1157 but DE(*ruvABC*)::Cam	R. Lloyd

[a] The EC derivatives also carry the following markers: DE(*umuDC*)::Erm *sulA211 thi-1* DE(*lac-gpt*)35 *ilv*(Ts) *mtl-1 rpsL31.* Full descriptions of *dam* mutant strains can be found at http://users.umassmed.edu/martin.marinus/dstrains.html.

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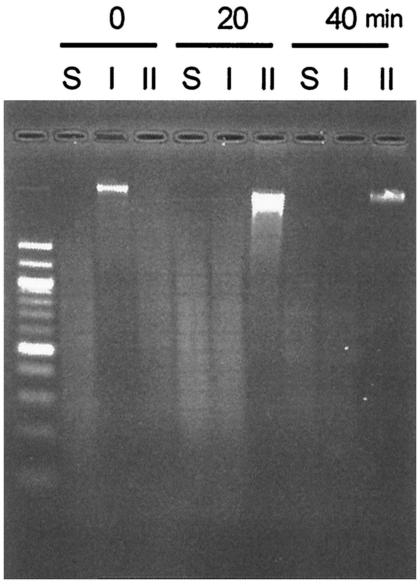

FIG. 1. Effect of adding inducer to log-phase cultures. Arabinose was added to a culture of pMQ430/GM3819 growing in L broth to a final concentration of 0.2% for 20 and 40 min. Cells were harvested, and total DNA was extracted and digested with *Sau*3A (lanes S) (cuts independently of methylation status), *Dpn*I (lanes I) (cuts methylated DNA), and *Dpn*II (lanes II) (cuts unmethylated DNA). First lane, 100-bp ladder.

cycle was repeated two more times. We found that there was no change in growth rate or numbers of CFU during these cycling experiments. We conclude that alternating cycles of methylation and demethylation have no deleterious effects on cell growth.

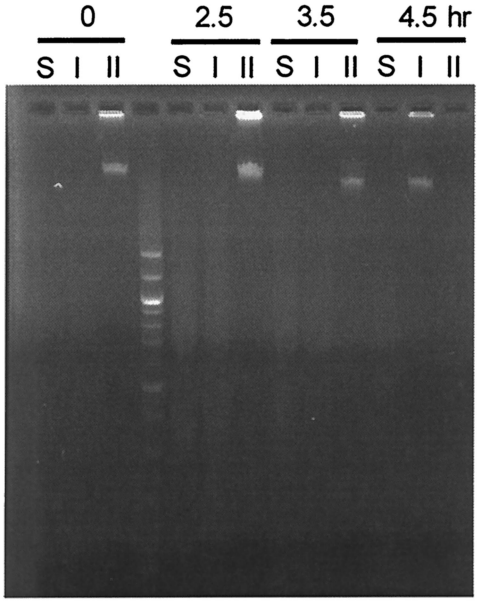

FIG. 2. Loss of methylation after removal of inducer. Log-phase cells of pMQ430/GM3819 were exposed to arabinose, harvested, and resuspended in L medium with glucose. The cells were kept in the logarithmic phase of growth by dilution into fresh medium. Samples were removed at 0, 2.5, 3.5, and 4.5 h. For each time point the order of digestion was *Sau*3A (lanes S), *Dpn*I (lanes I), and *Dpn*II (lanes II). Fourth lane, 100-bp ladder.

TABLE 2. Dam methylation in protease-deficient strains[a]

Genotype	Dam methylation
Wild type	−
lon::Tn*10*	+
clpA319::Kan	−
clpB::Kan	−
clpP::Kan	−
clpX::Kan	−
rcsA166::Kan	−

[a] Log-phase *dam-13*::Tn*9* bacteria containing pMQ430, with or without a protease defect, were exposed to arabinose and after growth in the absence of inducer were assayed for Dam methylation in total chromosomal DNA.

Several proteases have been described for *E. coli* (4), and we wanted to determine if any were active in degrading Dam methyltransferase. We constructed pMQ430/*dam-13*::Tn*9* derivatives of a series of protease-deficient strains and determined the state of DNA methylation 14 generations after a 60-min exposure to inducer. The results in Table 2 show that under these conditions, methylated DNA was found only in a Lon-deficient strain, thus implicating it in Dam methyltransferase stability. This is the first direct evidence for proteolytic degradation of Dam and confirms previous indirect data to this effect (7).

We tested the utility of pMQ430 to produce Dam^+ or Dam^- conditions in conjugal crosses between a *dam* Hfr strain (GM2807 Kan^r Str^s [Table 1]) and a recipient strain (GM7428 Str^r [Table 1]) bearing a *ruvABC*::Cam deletion. Logarithmic-phase cells were mixed in a ratio of 1 donor to 10 recipients (at about 10^8 per ml), mated for 60 min, and plated on selective media with or without arabinose. As shown in Table 3, no *dam ruvABC* recombinants (Kan^r Str^r) were recovered in the absence of arabinose, confirming previous results indicating that this combination of mutations is lethal (10). A few recombinant colonies did appear on the selective plates, but although these were *dam* by their kanamycin-resistant phenotype, they were also chloramphenicol sensitive, indicating the loss of the *ruvABC* allele and its replacement by the wild-type genes. The same low frequency of colonies was obtained when the mating mixture was plated in the presence of 0.002 and 0.02% arabinose. At a concentration of 0.2% arabinose, however, we observed a high frequency of recombinants which were sensitive to UV light and chloramphenicol resistant, indicating a *ruvABC* defect. These recombinants were resistant to ampicillin, indicating retention of pMQ430. They were unable to grow when they were patched onto the same selective medium with-

TABLE 3. Recombinant formation in crosses between Hfr GM2807 (*dam-16*::Kan) and pMQ430/GM7323 (Δ*ruvABC*::Cam Str^r)[a]

% Arabinose	Recombination frequency
None	5 (Cam^s UV^r)
0.2	1,250 (Cam^r UV^s)
0.02	4 (Cam^s UV^r)
0.002	2 (Cam^s UV^r)

[a] Bacteria were mated for 60 min and then plated for Kan^r Str^r recombinants on plates with various concentrations of arabinose. The numbers of recombinants in 50 μl of mating mixture are shown. The Cam^s UV^r recombinants arise from the transfer of the wild-type *ruvA*, *-B*, and *-C* genes from the donor.

out arabinose, thereby confirming that the *dam ruvABC* combination is inviable.

The viability of *E. coli* strains carrying the *dam-16*::Kan deletion mutation indicated that the loss of Dam methyltransferase is not a lethal event provided that recombination proficiency is not impaired (9). The present study with pMQ430 indicates that cells can tolerate the progressive loss of Dam methylation (and the formation of partially methylated DNA) and subsequent remethylation without any effect on growth. These findings imply that it is highly unlikely that any essential genes have their transcription coupled to hemimethylated DNA in a fashion similar to that of the transposase gene of Tn*10* (17). Our results also make it highly unlikely that hemi- or partial methylation of the overabundant GATC sites in the *oriC* region (14) is essential for the initiation of chromosome replication. Finally, regulating transcription of the *dam* gene on plasmid pMQ430 is a simple way to manipulate the level of Dam methyltransferase in the cell in the absence of any temperature-sensitive mutations in the gene.

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