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Insertion Mutations in the *dam* Gene of *Escherichia coli* K-12

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Summary. The *dam* gene of *E. coli* can be inactivated by insertion of Tn*9* or Mu*d* phage. Strains bearing these mutations are viable indicating that the *dam* gene product is dispensable.

The product of the *dam* gene is a DNA adenine methylase which methylates -GATC- sequences in double stranded DNA (Marinus 1982). To determine if the enzyme is essential for viability of *E. coli*, we set out to isolate derivatives in which transposons were inserted into the *dam* gene.

Strain GM688 (Δ*lac-pro*$_{X111}$) an Hfr strain which transfers *dam* as an early marker was infected with Mu*c*ts *d*II301 (Ap, *lac*), plated on medium containing ampicillin (Casadaben and Cohen 1979) and replica plated to isolate cells sensitive to 2-aminopurine. Three such strains, none of which produce detectable β-galactosidase, were isolated from about 10,000 survivors. From one of these lysogens a derivative was isolated which is able to grow at 42°, has lost Mu immunity and is ampicillin resistant. This derivative was designated *dam-12*::Mu*d* and the mutation can be easily transferred into other genetic backgrounds by conjugation.

The *dam-12*::Mu*d* mutation should result in a chromosomal rearrangement compared to wild type. Figure 1 shows a restriction map of the *dam* gene region of the *E. coli* chromosome (Arraj and Marinus 1983) and Fig. 2 shows the results of hybridizing p*dam*118 DNA (Fig. 3) to DNA from *dam*$^+$ and *dam-12*::Mu*d* strains. The data for the *dam*$^+$ strain (Fig. 2, lanes e–h) are exactly as predicted from the map in Fig. 1. This indicates that the restriction map of the *dam-trpS* insert in pMQ3 (Arraj and Marinus 1983), from which Fig. 1 is derived, is the same as that for the chromosome. In the *dam-12*::Mu*d* strain, the 2.45 kb *Bam*H1-*Kpn*I fragment is not seen (lane d) and this indicates that the Mu*d* insertion in the *dam* gene must be distal to the *Bam*H1 site. The probe DNA (p*dam*118) will hybridize not only to *dam* DNA but also to the Ap (ampicillin resistance) gene in Mu*d*. Since the Ap gene contains a *Pst*I site, and that the *dam* gene should be disrupted by the insertion, two, three or four hybridization bands could be expected depending on the distribution of restriction enzyme sites in Mu*d*. *Pst*I plus *Eco*RI digestion reveals four bands (Fig. 2b) indicating that the two parts of *dam* and Ap are on separate restriction fragments. *Pst*I plus *Sal*I or *Pst*I plus *Kpn*I digestion yields three bands indicating

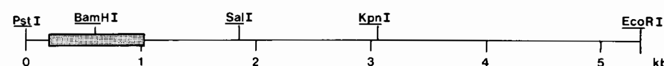

Fig. 1. Restriction map of the *dam-trpS* region of the *E. coli* chromosome. The stipled box represents the *dam* gene. The *Kpn*I site is in the *trpS* gene (Arraj and Marinus 1983)

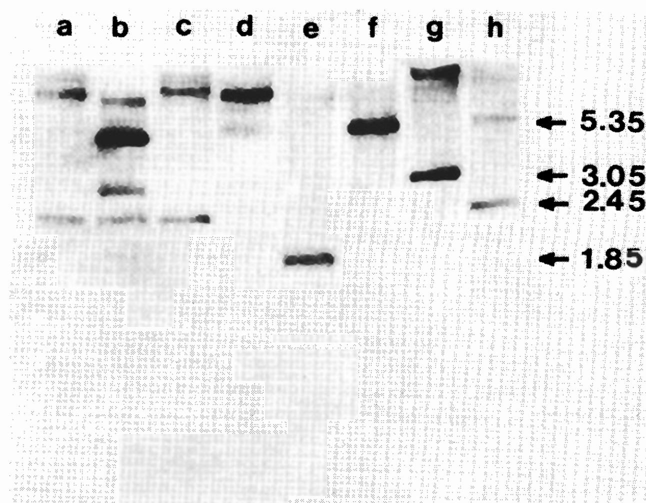

Fig. 2. Hybridization of p*dam*118 DNA to DNA from *dam*$^+$ and *dam-12*::Mu*d* strains. DNA from *dam*$^+$ (lanes e–h) and *dam-12*::Mu*d* (lanes a–d) strains was digested with *Pst*I plus *Sal*I (lanes a, e); *Pst*I plus *Eco*R1 (lanes b, f); *Pst*I plus *Kpn*I (lanes c, g) and *Bam*H1 plus *Kpn*I (lanes d, h) and subjected to agarose gel electrophoresis. The fragments were transferred to diazophenylthioether paper and then exposed to P^{32} labeled p*dam*118 probe under high stringency conditions. The paper was then subjected to autoradiography. The numbers at the right of the Figure indicate kilobase coordinates

that one fragment contains part of *dam* and part of Ap in addition to the two fragments each of which hybridizes to *dam* or Ap. *Kpn*I plus *Bam*HI digestion yields two bands, the upper band containing the Ap gene and part of *dam*. In sum, the results in Fig. 2 are consistent with an interruption of the *dam* gene sequence.

A Tn*9* insertion into the proximal part of the *dam* gene of p*dam*118 (Brooks et al. 1983) was obtained by transposition of the element from phage lambda::Tn*9*. The structure of the resulting plasmid pMQ71 is shown in Fig. 3. To replace the *dam*$^+$ allele on the *E. coli* chromosome with the *dam*::Tn*9* insertion on pMQ71 we used a method developed by Graham Walker (personal communication) which

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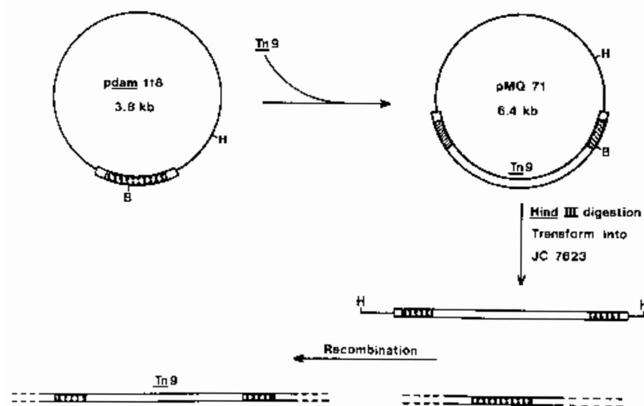

Fig. 3. Replacement of the *dam*$^+$ gene by *dam*::Tn*9*. After insertion of Tn*9* into the *dam* gene on p*dam*118, the plasmid was linearized and transformed into *E. coli* strain JC7623. The stipled box represents the *dam* gene. B and H denote *Bam*HI and *Hin*dIII recognition sites

is shown in Fig. 3. pMQ71 was digested with *Hin*dIII (which cuts the vector), transformed into JC7623 (*recB21 recC22 sbcB15*) and chloramphenicol resistant colonies were isolated. Of these 1/43 was *dam*$^-$ and ampicillin sensitive. The mutation in this strain was designated *dam-13*::Tn*9* and can be moved into different genetic backgrounds by Pl*vir* transduction. Although the *dam-13*::Tn*9* mutation could have arisen by transposition from pMQ71 into the host *dam* gene, the high frequency with which the mutation was isolated leads us to believe that a recombination mechanism is more plausible.

The *dam-12*::Mu*d* and *dam-13*::Tn*9* mutations are 96% (933 transductants analysed) and 92% (899 transductants) respectively co-transducible with *aroB351*. These are the expected approximate linkage values (Marinus 1973). In these crosses, a *dam-12*::Mu*d aroB351* strain was transduced to Aro$^+$ with P1 phage grown on the wild type and an *aroB351* strain was transduced to Aro$^+$ with phage grown on the *dam-13*::Tn*9 aroB*$^+$ mutant. Both mutations are recessive in F-*dam*$^+$ diploids and fail to complement the *dam-3* mutation carried on an F factor.

Neither *dam-12*::Mu*d* or *dam-13*::Tn*9* has any detectable DNA methylase activity in vivo or in crude extracts as determined by the procedure of Marinus and Morris (1973) and Bale et al. (1979). DNA from the mutant strains is degraded by *Mbo*I whereas wild type DNA is resistant (data not shown; Lacks and Greenberg 1977).

The phenotypes of *dam-12*::Mu*d* and *dam-13*::Tn*9* strains which have so far been studied do not differ significantly from those of *dam-3* cells. The data for spontaneous mutability are shown in Table 1.

We have also constructed a strain which contains *dam-12*::Mu*d*, is deficient for the *dcm* (DNA cytosine methylation) methylase and which is *hsdS2* (host specificity). This strain, which has no detectable DNA methylation, is viable.

The data above indicate that the *dam* enzyme is not required for viability of *E. coli*. It shows also that DNA adenine methylation is not required for DNA replication (cf. Lark 1968) or for initiation of DNA replication at the *ori* region which has more -GATC- sequences than expected on a random basis (Sugimoto et al. 1979; Meijer et al. 1979). There can be no essential genes which are regulated solely by methylation in the same manner as the *mom* gene of phage Mu (Hattman 1982; Plasterk et al. 1983). Finally

Table 1. Spontaneous mutation frequencies in *dam* mutants

Genotype	Rif^R	Val^R
dam$^+$	0.3	38
dam-12::Mu*d*	73	1,394
dam-13::*Tn9*	113	1,014
dam-3	69	1,026

The number of rifampicin (Rif) or valine (Val) resistant mutants per 10^8 cells plated is shown. Each number represents the average of five separate determinations (Bale et al. 1979)

that strains bearing the *dam-3* mutation have the same phenotypes as those strains described in this paper, indicates that the *dam-3* mutation completely eliminates *dam* gene activity (Lacks and Greenberg 1976; Bale et al. 1979).

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