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Current Opinion in Microbiology

Review

Clocks and switches: bacterial gene regulation by DNA adenine methylation

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N^6 methylation in adenosine moieties causes changes in DNA structure and can modulate DNA–protein interactions. In both α-Proteobacteria and γ-Proteobacteria, postreplicative formation of N^6-methyl-adenine regulates transcription of specific genes and provides two general types of controls: (i) clock-like controls that permit transient gene transcription during a specific stage of DNA replication; (ii) switch-like controls in which transcription is regulated by a DNA methylation pattern. DNA adenine methylation may also regulate gene expression by affecting nucleoid topology. Recent transcriptomic studies have unveiled novel cases of genes regulated by DNA adenine methylation, including virulence genes of bacterial pathogens.

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Current Opinion in Microbiology 2008, **11**:106–112

This review comes from a themed issue on
Cell regulation
Edited by Claude Parsot and Lucia B. Rothman-Denes

Available online 8th April 2008

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2008.02.012

Introduction

CcrM and Dam are DNA adenine methyltransferases that catalyze postreplicative formation of N^6-methyl-adenine using *S*-adenosyl-methionine as methyl donor [1,2,3•,4•]. In α-Proteobacteria, 5′GANTC3′ motifs serve as targets for CcrM [1]. Dam is found in γ-Proteobacteria, and its target is 5′GATC3′ [1]. Dam and CcrM thus catalyze an identical reaction at similar DNA targets; however, each enzyme has a distinct structure, indicative of independent evolutionary origin [5]. Unlike CcrM [6], Dam is present at all stages in the cell cycle [7]. Another difference is that CcrM is essential while Dam is dispensable in certain genera (e.g., *Escherichia* and *Salmonella*) and essential in others (e.g., *Vibrio*) [1]. A non-essential CcrM homolog with unknown function is found in *E. coli* and *Salmonella* [8]. CcrM methylation is involved in *Brucella abortus* infection [9] and may regulate *Sinorhizobium* differentiation upon infection of legumes [10]. In turn, Dam methylation plays roles in the virulence of *E. coli* [11,12••], *Salmonella* [13,14,15•,16,17], *Yersinia* [18–22], *Vibrio* [23], *Haemophilus* [24], *Pasteurella* [25], *Aeromonas* [26], *Actinobacillus* [27], and *Klebsiella* [28].

Formation of N^6-methyl-adenine lowers the thermodynamical stability of DNA and alters DNA curvature [1,2]. These structural effects can influence DNA–protein interactions, and the methylation state of crucial GATC or GANTC sites can be used as a signal to decide when and where a given protein can interact with DNA [29]. For instance, RNA polymerase and transcription factors can discriminate methylated DNA from hemimethylated DNA at certain promoter regions [1,2]. This discrimination can couple gene transcription to a specific stage of the cell cycle. Furthermore, methylation of specific *E. coli* GATCs can be regulated, giving rise to GATC sites which remain stably hemimethylated or nonmethylated. Some such methylation states regulate gene expression and can be inherited in a manner reminiscent of the DNA methylation patterns occurring in eukaryotic cells [1,2,5]. Epigenetic inheritance of DNA methylation patterns thus permits regulation of gene expression beyond cell cycle-associated control.

DNA replication clocks: transcriptional activation by hemimethylation

In α-Proteobacteria, DNA hemimethylation is used as a timer to couple transcription of cell cycle regulators to replication fork progression. In γ-Proteobacteria, where DNA hemimethylation is transient, transcriptional activation by hemimethylation restricts transcription of certain genes to a brief lapse of the DNA replication cycle.

Cell-cycle-regulated genes in *Caulobacter crescentus*

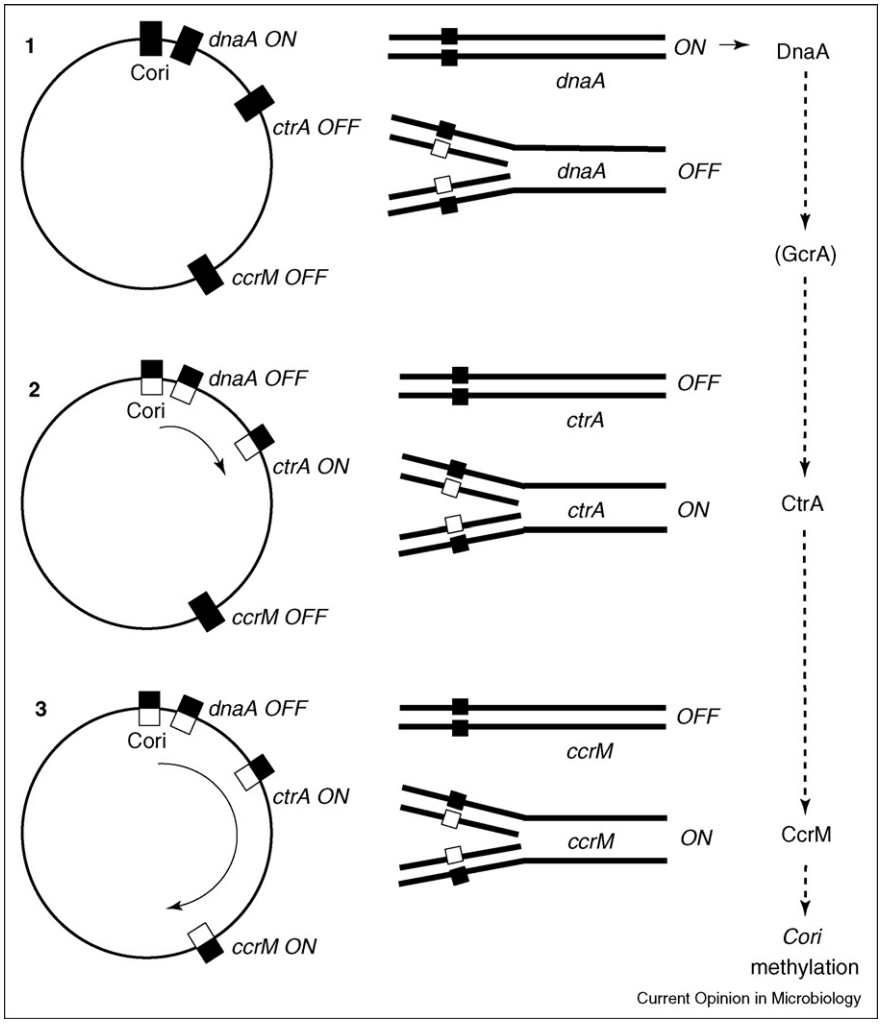
The dimorphic bacterium *Caulobacter crescentus* divides asymmetrically, yielding a stalked cell and a swarmer cell [30]. Chromosome replication occurs only in the stalked cell and starts upon binding of DnaA, the replication initiation protein, to the methylated origin of replication (*Cori*) [30]. At the onset of chromosome replication, the stalked cell does not contain CcrM methylase but the chromosome is fully methylated; hence, replication generates hemimethylated daughter chromosomes [30]. Replication fork progression acts as a timer for co-ordination of the *Caulobacter* cell cycle and involves a cascade of

transient regulators. Transcription of *dnaA* is inhibited shortly after initiation of chromosome replication, since the *dnaA* promoter is inactive in the hemimethylated state [31••] (see below). DnaA activates transcription of *gcrA*, whose product is required for transcription of *ctrA* [32•]. GcrA-mediated activation of *ctrA* transcription occurs upon the passage of the replication fork, when a GANTC site near the −35 module of the *ctrA* p_1 promoter becomes hemimethylated [33]. CtrA is in turn necessary to activate transcription of *ccrM*, which lies near the replication terminus. However, CtrA-mediated activation of *ccrM* transcription requires hemimethylation of two GANTC sites in the *ccrM* leader [34]. Thus *ccrM* transcription is delayed until the replication fork passes through *ccrM* late in the cell cycle. Synthesis of CcrM causes methylation of *Cori* and initiation of a new round of DNA replication [30]. This orderly sequence of events relies on the fact that *ccrM* is transcribed last in the regulatory cascade (Figure 1). Not surprisingly, expression of *ccrM* from a constitutive promoter seriously perturbs the *Caulobacter* cell cycle [34].

Regulation of IS*10* transposition

In the transposase gene of insertion element IS*10*, methylation of a GATC that overlaps the −10 module prevents RNA polymerase binding and keeps the promoter repressed. Hemimethylation caused by replication fork passage permits RNA polymerase binding and *tnp* transcription [35•]. Methylation of the hemimethylated promoter, however, quickly restores repression. Replication-coupled transcription may limit transposition to the moment in which the cell has more than one

Figure 1



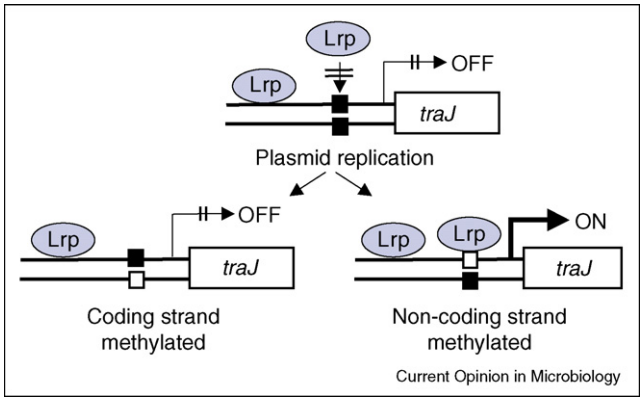
Cell cycle regulation in *Caulobacter crescentus.* In stage 1, the chromosome is methylated (black squares depict methylated GATC sites; white squares depict nonmethylated GATCs), permitting *Cori* activation and *dnaA* transcription. Passage of the replication fork renders both hemimethylated *Cori* and *dnaA*, thereby inhibiting replication initiation (stage 2). DnaA-mediated activation of GcrA will permit *ctrA* transcription as soon as DNA replication renders the hemimethylated *ctrA* promoter (stage 2). In turn, CtrA activates *ccrM* transcription when the replication fork reaches the *ccrM* promoter (stage 3). Synthesis of CcrM will permit methylation of GANTC sites, returning to stage 1.

chromosome, thereby reducing the consequences of potentially lethal 'hops'. Transcription of *tnp* is further reduced by the fact that one of the hemimethylated species of the IS*10 tnp* promoter is transcriptionally more active than the other [35•]. This asymmetric activation of the IS*10 tnp* promoter may further restrain transposase synthesis, avoiding massive DNA strand breaks and/or multiple transposition events.

Regulation of *traJ* transcription in the *Salmonella* virulence plasmid

Transcription of the *tra* operon of the *Salmonella enterica* virulence plasmid, which encodes conjugative pili and products necessary for DNA transfer, requires activation by the TraJ transcription factor [36]. Transcription of *traJ* is in turn controlled by the leucine-responsive regulatory protein (Lrp), a global regulator of transcription [36]. Lrp activates *traJ* transcription by binding to two sites in the *traJ* UAS. One such site contains a GATC and its methylation prevents Lrp binding; hence, in the absence of plasmid replication the *traJ* promoter is repressed [37]. Passage of the replication fork generates hemimethylated GATCs that permit Lrp binding in a strand-specific manner; GATC methylation in the non-coding strand of *traJ* permits Lrp binding, while GATC methylation in the coding *traJ* strand does not [37]. As a consequence, Lrp activates *traJ* expression in only one of the newly replicated plasmids (Figure 2). This design, which is reminiscent of the IS*10* transposase promoter [35], limits TraJ synthesis and thus restrains *tra* operon derepression. Higher expression levels might be perhaps superfluous, if not an energetic waste. As in IS*10*, it is remarkable that a single methyl group can create distinct epigenetic states in DNA molecules that are otherwise identical.

Figure 2



Model for regulation of *traJ* transcription by strand-specific Dam hemimethylation in the *Salmonella* virulence plasmid. The *traJ* UAS contains two binding sites for the transcriptional activator Lrp. GATC methylation inhibits Lrp binding to the downstream site, thus preventing *traJ* transcription in a non-replicating plasmid. Passage of the replication fork renders the hemimethylated *traJ* UAS and permits Lrp binding to one daughter DNA molecule only.

Adjustment of DNA replication clocks: transcriptional repression by DNA hemimethylation

In both *E. coli* and *Caulobacter*, methylation of the chromosome replication origin provides the signal for replication start and also activates synthesis of the DNA replication initiation protein. It is noteworthy that distant evolutionary relatives make use of an analogous signal (GATC or GANTC methylation) to reset their DNA replication clocks.

dnaA (*C. crescentus*)

The *Caulobacter dnaA* gene is located only 2 kb away from the replication origin (*Cori*) and thus becomes hemimethylated shortly after replication initiation [30]. The *dnaA* promoter contains two GATNCs, and its activity is inhibited by hemimethylation; hence, passage of the replication fork renders the promoter inactive [31••]. Because *Caulobacter* stalked cells do not contain CcrM methylase until chromosome replication approaches completion [6], the *dnaA* promoter remains hemimethylated and therefore inactive during most of the cell cycle. When CcrM synthesis occurs, methylation of GANTCs in the *Cori–dnaA* region provides the signal for initiation of the following replication round and permits *dnaA* transcription. Synthesis of DnaA is, however, transient, since its promoter becomes hemimethylated shortly after replication initiation [31••].

dnaA (*E. coli*)

As in *Caulobacter*, the *E. coli dnaA* gene lies near the replication origin (*oriC*) and encodes the main activator of DNA replication [38]. One of the *dnaA* promoters (*dnaA2*) contains three GATC sites and is only active if they are methylated [39,40]. Unlike CcrM, however, synthesis of Dam methylase is not cell cycle regulated [1,3•]. Hence, control of *dnaA* transcription requires an additional element. After DNA replication, the hemimethylated, GATC-rich *oriC-dnaA* region is sequestered by the GATC-binding protein SeqA [41]. Dam methylase activity is locally prevented and the region remains hemimethylated [42]. Initiation of the following chromosome replication round will require SeqA release from both *oriC* and *dnaA* and subsequent GATC methylation by the Dam methylase [1,3•,38]. The switch that resets the *E. coli* replication clock is thus more complex than that of *Caulobacter*, and the need for SeqA action may be seen as a burden imposed by Dam-dependent mismatch repair [1]. In γ-Proteobacteria, Dam methylase is necessary throughout the cell cycle; otherwise, MutHLS-induced DNA strand breaks could become lethal [1].

Phase variation switches: formation of DNA methylation patterns

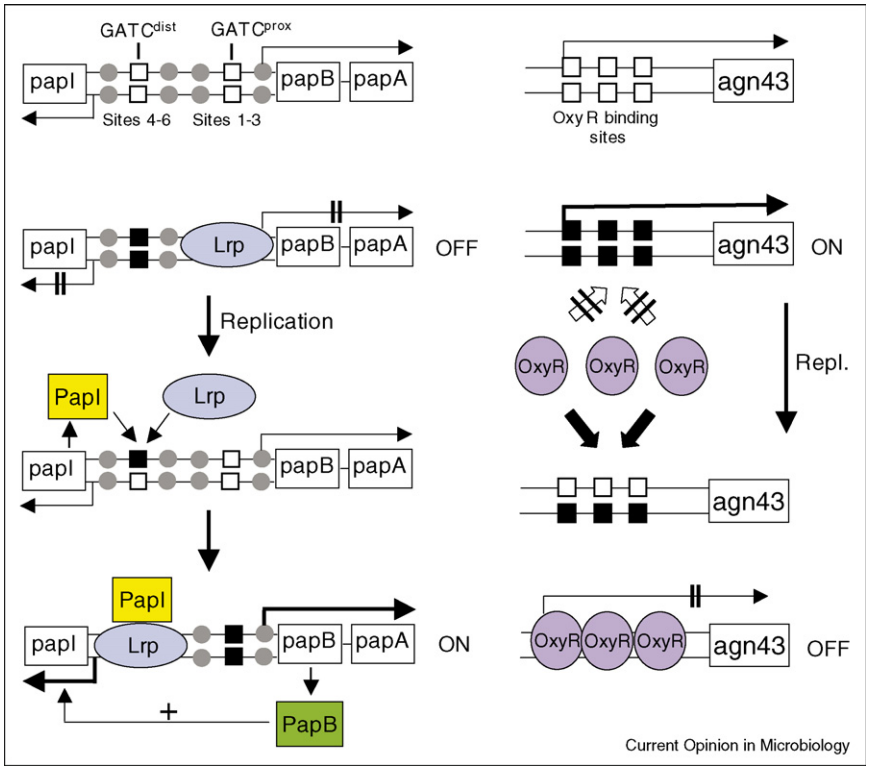
Methylation and transient hemimethylation are not the only possible states of GATC sites. In *E. coli*, protein-mediated exclusion of Dam methylase can

prevent methylation of specific GATCs, and methylation hindrance during two consecutive replication rounds yields nonmethylated DNA [5]. DNA sequences that flank specific GATCs may play a role in this phenomenon by decreasing processivity of the Dam methylase [43••]. Inheritance of nonmethylated GATCs plays diverse roles in phase variation, the reversible generation of surface antigen variants [11].

pap and its relatives

P pili ('pyelonephritis-associated') encoded by the *pap* operon mediate adhesion of uropathogenic *E. coli* to the urinary mucosa [44••]. Synthesis of P pili is subjected to phase variation and switching between ON and OFF states is controlled by Dam methylation [11,44••] (Figure 3). The upstream regulatory sequence of the *papBA* operon contains six binding sites for the leucine-responsive regulatory protein, Lrp. Two of these sites contain GATC motifs: $GATC^{dist}$ in site 5 and $GATC^{prox}$ in site 2. In the OFF state, Lrp binds cooperatively and with high affinity to sites 1–3 and prevents RNA polymerase binding and transcription of *pap* [2,11,44••]. Lrp binding at sites 1–3 has two consequences: (i) it reduces the affinity of Lrp for sites 4-6; (ii) it prevents methylation of daughter DNA molecules after passage of the replication fork, rendering $GATC^{prox}$ nonmethylated after two rounds of replication [2,11]. By contrast, the $GATC^{dist}$ located in the unbound site 5 can undergo a normal cycle of hemimethylation/methylation. The high affinity of Lrp for the nonmethylated $GATC^{prox}$ and its inability to bind a methylated $GATC^{dist}$ create a feedback loop that propagates the OFF state [11,44••]. Switching to the ON state requires that Lrp is translocated to sites 4–6, one of which contains $GATC^{dist}$ [11,44••]. Translocation requires the ancillary protein PapI [45]. The affinity of PapI/Lrp for sites 4–6 is much higher than for sites 1–3, tending to move PapI/Lrp to sites 4–6. Methylation of $GATC^{prox}$ inhibits binding of PapI/Lrp, thus facilitating movement of PapI/Lrp to sites 4–6 [45]. Hence, nonmethylation of $GATC^{dist}$ and methylation of $GATC^{prox}$ define the ON state [44••]. One of the *pap* products, PapB, activates *papI* transcription, thereby propagating the ON state by a positive feedback loop [11,44••]. Although the Pap switch has stochastic elements, it is also regulated by environmental factors via H-NS, RimJ, Crp, and the two-component system

Figure 3



Regulation of *pap* and *agn43*. At the *papBA* regulatory region, Lrp binding at sites 1–3 blocks transcription and methylation of the $GATC^{prox}$ site near the pilin promoter. Transition to phase ON occurs after DNA replication generates a hemimethylated $GATC^{dist}$ (only one of the hemimethylated intermediates is shown) allowing PapI-dependent binding of Lrp to sites 4–6 and formation of the phase ON state. PapB acts in a positive feedback loop. At the *agn43* operator, Dam methylation inhibits OxyR binding in the ON state. Transition to OFF occurs when hemimethylated GATC sites are generated by replication (only one of the hemimethylated intermediates is shown) allowing OxyR binding, which represses transcription and blocks methylation of the *agn43* GATC sites. Detailed descriptions of *pap* and *agn43* regulation can be found in reference [2].

CpxAR [2,11,44••,45]. Lessons learned from the Pap system are directly applicable to understanding the mechanisms of regulation of additional pili regulatory systems including Pef fimbriae of *S. enterica* and Prf, S, Afa, K88, and CS31a pili of *E.coli* [2].

agn43

The *E. coli agn43* gene encodes an autotransporter protein whose synthesis is subjected to phase variation. Switching between ON and OFF states is transcriptional and involves Dam-mediated control of OxyR binding [46]. The *agn43* UAS contains three OxyR binding sites and each has a GATC. Binding of OxyR to the *agn43* UAS represses *agn43* transcription [47]. However, methylation of any two of the three *agn43* GATCs inhibits OxyR binding and permits transcription [48]. Albeit with lower affinity, OxyR also binds a hemimethylated *agn43* UAS [47,48]. When bound, OxyR inhibits DNA methylation, rendering a nonmethylated UAS after two consecutive replication rounds [47]. These observations suggest that in an ON phase cell, DNA replication offers an opportunity to switch from ON to OFF. Hemimethylation of the *agn43* UAS permits competition between OxyR and the Dam methylase, and OxyR binding turns transcription off (Figure 3). In an OFF phase cell, OxyR presumably dissociates from *agn43* DNA, giving a window of opportunity for transition to ON. Immediate methylation of the *agn43* GATC in both DNA strands may prevent OxyR binding and repression. Besides hindrance of repressor binding, Dam methylation plays a second role in *agn43*: methylation of the upstream GATC site increases transcription initiation, which occurs precisely at the G nucleotide of the GATC [49].

DNA adenine methylation as an H-NS antirepressor: regulation of the *finP* gene

The *finP* gene of F-like plasmids encodes an antisense RNA involved in regulation of conjugal transfer. In the *Salmonella* virulence plasmid, and possibly in F and R100 as well, *finP* transcription is activated by Dam methylation [50] and the underlying mechanism is unusual: Dam methylation prevents repression of the *finP* promoter by the nucleoid protein H-NS [50]. However, H-NS binding to DNA fragments containing the *finP* promoter is not altered by their methylation state [50]. Hence, the effect of Dam methylation on *finP* transcription is not local and might reflect differences in nucleoid topology and/or organization in Dam$^-$ mutants. As a consequence, certain regions in the nucleoid of Dam$^-$ mutants might be more efficiently targeted by H-NS [50].

Postranscriptional regulation by DNA methylation: unsolved enigmas

In several genes showing different expression in Dam$^+$ and Dam$^-$ hosts, Dam methylation seems to control synthesis of a protein without affecting the mRNA level (Table 1). The first example of this kind was described in the *E. coli vsr* gene [51], and additional cases have been recently found in bacterial pathogens. In enterohaemorragic *E. coli*, formation of actin pedestals is exacerbated in Dam$^-$ mutants [12••]. Pedestal formation requires delivery of bacterial effectors into epithelial cells via type III secretion. Intimin, the translocated intimin effector (Tir), and the additional effector $EspF_u$ are synthesized at higher levels in Dam$^-$ EHEC indicating that Dam methylation represses their expression [12••]. However, similar amounts of *tir* mRNA are found in Dam$^+$ and Dam$^-$ hosts suggesting that Dam methylation does not regulate *tir* gene transcription nor *tir* mRNA stability [12••]. Another example is found in *Salmonella* pathogenicity island 1 (SPI-1) whose expression is activated by Dam methylation [15•] via postranscriptional control of HilD, the main SPI-1 transcriptional activator (J López-Garrido and J Casadesús, unpublished). Lastly, Dam overproduction has been shown to cause both transcriptional and postranscriptional alterations in the synthesis of *Yersinia enterocolitica* virulence factors [21].

Table 1

Examples of genes regulated by DNA adenine methylation

Species	Gene or operon	Mechanism of regulation	Reference(s)
Caulobacter crescentus	*dnaA*	Transcriptional inhibition by hemimethylation	[31••,32•]
	ccrM	Transcriptional activation by hemimethylation	[34]
	ctrA	Transcriptional activation by hemimethylation	[33]
Escherichia coli	*agn43*	Formation of alternative methylation states at the *agn43* operator	[47,48]
	dnaA	Transcriptional inhibition by hemimethylation	[39,40]
	pap	Formation of alternative methylation states at the *papBA* UAS	[11,43,44••,45]
	tir	Unknown postranscriptional mechanism	[12••]
	tnp (IS*10*)	Activation of RNA polymerase binding by strand-specific hemimethylation	[35•]
	vsr	Unknown postranscriptional mechanism	[51]
Salmonella enterica	*finP* (pSLT)	Dam methylation prevents repression by H-NS	[50]
	traJ (pSLT)	Activation of Lrp binding to the *traJ* UAS by strand-specific hemimethylation	[37]
Yersinia pseudotuberculosis	*rosA*	Unknown postranscriptional mechanism	[21]

The molecular mechanisms involved in these enigmatic cases remain to be identified. Because DNA modification cannot be expected to act at the postranscriptional level, a tentative explanation is that Dam methylation might control transcription of genes whose products are involved in RNA stability, RNA processing, or other postranscriptional events.

Conclusions

DNA–protein interactions sensitive to DNA adenine hemimethylation permit the adjustment of bacterial gene transcription to particular stages of the DNA replication cycle (Table 1). Transcriptional activation by hemimethylation is found in *tnp*(IS*10*) [35•], *traJ* [37], *ctrA* [33], and *ccrM* [34], while transcriptional repression by hemimethylation occurs in *dnaA* [31••,39,40]. A second, more complex type of transcriptional regulation is found in loci regulated by DNA methylation patterns (Table 1). For instance, the ON and OFF states that govern phase variation in *pap* and *agn43* involve nonmethylated GATC sites created by DNA adenine methylation hindrance [11,44••,47]. DNA adenine methylation may also regulate transcription by influencing nucleoid topology as proposed for the *finP* gene [50]. Of special interest for future studies are (i) the growing list of virulence traits governed by DNA adenine methylation in bacterial pathogens [4•], (ii) the finding of novel genes regulated by Dam methylation using high throughput analysis [15•,52], and (iii) the evidence that DNA adenine methylation may regulate hitherto unidentified functions involved in postranscriptional control [12••,21,51].

Acknowledgements

Work in our laboratories is supported by National Institutes of Health grant AI23348 (to DL) and grant BIO2007-67457-CO2-02 from the Spanish Ministry of Education and Science and the European Regional fund (to JC). We are grateful to Javier López-Garrido and Bruce Braaten for critical reading of the manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wion D, Casadesús J: **N6-methyl-adenine: an epigenetic signal for DNA–protein interactions**. *Nat Rev Microbiol* 2006, **4**:183-192.

2. Casadesús J, Low D: **Epigenetic gene regulation in the bacterial world**. *Microbiol Mol Biol Rev* 2006, **70**:830-856.

3. • Løbner-Olesen A, Skovgaard O, Marinus MG: **Dam methylation: coordinating cellular processes**. *Curr Op Microbiol* 2005, **8**:154-160.
A review of the biological functions of Dam methylation with emphasis on evolutionary aspects. A phylogenetic study of the Dam methylase clade is included.

4. • Heusipp G, Fälker S, Schmidt MA: **DNA adenine methylation and bacterial pathogenesis**. *Int J Med Microbiol* 2007, **297**:1-7.
A recent review of the roles of DNA adenine methylation in bacterial pathogens, including a description of the underlying mechanisms if known. An interesting observation is the frequent involvement of DNA adenine methylation in the regulation of adhesion and invasion factors.

5. Low DA, Weyand NJ, Mahan MJ: **Roles of DNA adenine methylation in regulating bacterial gene expression and virulence**. *Infect Immun* 2001, **69**:7197-7204.

6. Stephens C, Reisenauer A, Wright R, Shapiro L: **A cell cycle-regulated bacterial DNA methyltransferase is essential for viability**. *Proc Natl Acad Sci U S A* 1996, **93**:1210-1214.

7. Zhou P, Bogan JA, Welch K, Pickett SR, Wang HJ, Zaritsky A, Helmstetter CE: **Gene transcription and chromosome replication in *Escherichia coli***. *J Bacteriol* 1997, **179**:163-169.

8. Broadbent SE, Balbontin R, Casadesús J, Marinus MG, van der Woude M: **YhdJ, a nonessential CcrM-like DNA methyltransferase of *Escherichia coli* and *Salmonella enterica***. *J Bacteriol* 2007, **189**:4325-4327.

9. Robertson GT, Reisenauer A, Wright R, Jensen RB, Jensen A, Shapiro L, Roop RM 2nd: **The *Brucella abortus* CcrM DNA methyltransferase is essential for viability, and its overexpression attenuates intracellular replication in murine macrophages**. *J Bacteriol* 2000, **182**:3482-3489.

10. Ichida H, Matsuyama T, Abe T, Koba T: **DNA adenine methylation changes dramatically during establishment of symbiosis**. *FEBS J* 2007, **274**:951-962.

11. Hernday A, Krabbe M, Braaten B, Low D: **Self-perpetuating epigenetic pili switches in bacteria**. *Proc Natl Acad Sci U S A* 2002, **99**:16470-16476.

12. •• Campellone KG, Roe AJ, Løbner-Olesen A, Murphy KC, Magoun L, Brady MJ, Donohue-Rolfe A, Tzipori S, Gally DL, Leong JM *et al.*: **Increased adherence and actin pedestal formation by dam-deficient enterohaemorrhagic *Escherichia coli* O157:H7**. *Mol Microbiol* 2007, **63**:1468-1481.
A description of virulence alterations in Dam$^-$ mutants of enterohaemorrhagic *E. coli*, ending with the intriguing conclusion that Dam-mediated regulation is postranscriptional.

13. Heithoff DM, Sinsheimer RL, Low DA, Mahan MJ: **An essential role for DNA adenine methylation in bacterial virulence**. *Science* 1999, **284**:967-970.

14. García-del Portillo F, Pucciarelli MG, Casadesús J: **DNA adenine methylase mutants of *Salmonella typhimurium* show defects in protein secretion, cell invasion, and M cell cytotoxicity**. *Proc Natl Acad Sci U S A* 1999, **96**:11578-11583.

15. • Balbontin R, Rowley G, Pucciarelli MG, Lopez-Garrido J, Wormstone Y, Lucchini S, García-del Portillo F, Hinton JC, Casadesús J: **DNA adenine methylation regulates virulence gene expression in *Salmonella enterica* serovar Typhimurium**. *J Bacteriol* 2006, **188**:8160-8168.
A transcriptomic search for Dam-regulated genes in *Salmonella enterica* carried on under conditions that mimic the intestinal stage of infection. *Salmonella* pathogenicity island I, the fimbrial operon *std*, flagellar and chemotaxis genes, and the Braun lipoprotein gene were found to be under Dam methylation control.

16. Badie G, Heithoff DM, Sinsheimer RL, Mahan MJ: **Altered levels of *Salmonella* DNA adenine methylase are associated with defects in gene expression, motility, flagellar synthesis, and bile resistance in the pathogenic strain 14028 but not in the laboratory strain LT2**. *J Bacteriol* 2007, **189**:1556-1564.

17. Giacomodonato MN, Sarnacki SH, Caccuri RL, Sordelli DO, Cerquetti MC: **Host response to a dam mutant of *Salmonella enterica* serovar enteritidis with a temperature-sensitive phenotype**. *Infect Immun* 2004, **72**:5498-5501.

18. Julio SM, Heithoff DM, Sinsheimer RL, Low DA, Mahan MJ: **DNA adenine methylase overproduction in *Yersinia pseudotuberculosis* alters YopE expression and secretion and host immune responses to infection**. *Infect Immun* 2002, **70**:1006-1009.

19. Fälker S, Schmidt MA, Heusipp G: **Altered Ca(2+) regulation of Yop secretion in *Yersinia enterocolitica* after DNA adenine methyltransferase overproduction is mediated by Clp-dependent degradation of LcrG**. *J Bacteriol* 2006, **188**:7072-7081.

20. Fälker S, Schmidt MA, Heusipp G: **DNA methylation in *Yersinia enterocolitica*: role of the DNA adenine methyltransferase in**

mismatch repair and regulation of virulence factors. *Microbiology* 2005, **151**:2291-2299.

21. Fälker S, Schilling J, Schmidt MA, Heusipp G: **Overproduction of DNA adenine methyltransferase alters motility, invasion, and the lipopolysaccharide O-antigen composition of *Yersinia enterocolitica***. *Infect Immun* 2007, **75**:4990-4997.

22. Taylor VL, Titball RW, Oyston PC: **Oral immunization with a dam mutant of *Yersinia pseudotuberculosis* protects against plague**. *Microbiology* 2005, **151**:1919-1926.

23. Julio SM, Heithoff DM, Provenzano D, Klose KE, Sinsheimer RL, Low DA, Mahan MJ: **DNA adenine methylase is essential for viability and plays a role in the pathogenesis of *Yersinia pseudotuberculosis* and *Vibrio cholerae***. *Infect Immun* 2001, **69**:7610-7615.

24. Watson ME Jr, Jarisch J, Smith AL: **Inactivation of deoxyadenosine methyltransferase (dam) attenuates *Haemophilus influenzae* virulence**. *Mol Microbiol* 2004, **53**:651-664.

25. Chen L, Paulsen DB, Scruggs DW, Banes MM, Reeks BY, Lawrence ML: **Alteration of DNA adenine methylase (Dam) activity in *Pasteurella multocida* causes increased spontaneous mutation frequency and attenuation in mice**. *Microbiology* 2003, **149**:2283-2290.

26. Erova TE, Pillai L, Fadl AA, Sha J, Wang S, Galindo CL, Chopra AK: **DNA adenine methyltransferase influences the virulence of *Aeromonas hydrophila***. *Infect Immun* 2006, **74**:410-424.

27. Wu H, Lippmann JE, Oza JP, Zeng M, Fives-Taylor P, Reich NO: **Inactivation of DNA adenine methyltransferase alters virulence factors in *Actinobacillus actinomycetemcomitans***. *Oral Microbiol Immunol* 2006, **21**:238-244.

28. Mehling JS, Lavender H, Clegg S: **A Dam methylation mutant of *Klebsiella pneumoniae* is partially attenuated**. *FEMS Microbiol Lett* 2007, **268**:187-193.

29. Messer W, Noyer-Weidner M: **Timing and targeting: the biological functions of Dam methylation in *E. coli***. *Cell* 1988, **54**:735-737.

30. Marczynski GT, Shapiro L: **Control of chromosome replication in *Caulobacter crescentus***. *Annu Rev Microbiol* 2002, **56**:625-656.

31. •• Collier J, McAdams HH, Shapiro L: **A DNA methylation ratchet governs progression through a bacterial cell cycle**. *Proc Natl Acad Sci U S A* 2007, **104**:17111-17116.
An elegant study describing the cascade of transient regulators that coordinates the *Caulobacter crescentus* cell cycle.

32. • Collier J, Murray SR, Shapiro L: **DnaA couples DNA replication and the expression of two cell cycle master regulators**. *EMBO J* 2006, **25**:346-356.
CcrM methylation of the *Caulobacter* replication origin provides the start signal for chromosome replication and also activates transcription of the *dnaA* gene. Use of analogous signals for replication control in α-Proteobacteria and γ-Proteobacteria is a remarkable case of evolutionary convergence.

33. Reisenauer A, Shapiro L: **DNA methylation affects the cell cycle transcription of the CtrA global regulator in *Caulobacter***. *EMBO J* 2002, **21**:4969-4977.

34. Reisenauer A, Kahng LS, McCollum S, Shapiro L: **Bacterial DNA methylation: a cell cycle regulator?** *J Bacteriol* 1999, **181**:5135-5139.

35. • Roberts D, Hoopes BC, McClure WR, Kleckner N: **IS*10* transposition is regulated by DNA adenine methylation**. *Cell* 1985, **43**:117-130.
A classical study on the use of hemimethylation as a signal for DNA–protein interactions, which remains a paradigm after two decades.

36. Camacho EM, Casadesús J: **Conjugal transfer of the virulence plasmid of *Salmonella enterica* is regulated by the leucine-responsive regulatory protein and DNA adenine methylation**. *Mol Microbiol* 2002, **44**:1589-1598.

37. Camacho EM, Casadesús J: **Regulation of *traJ* transcription in the *Salmonella* virulence plasmid by strand-specific DNA adenine hemimethylation**. *Mol Microbiol* 2005, **57**:1700-1718.

38. Kaguni JM: **DnaA: controlling the initiation of bacterial DNA replication and more**. *Annu Rev Microbiol* 2006, **60**:351-375.

39. Braun RW, Wright A: **DNA methylation differentially enhances the expression of one of the two *dnaA* promoters *in vivo* and *in vitro***. *Mol Gen Genet* 1986, **202**:246-250.

40. Kücherer C, Lother H, Kölling K, Schauzu MA, Messer W: **Regulation of transcription of the chromosomal *dnaA* gene of *Escherichia coli***. *Mol Gen Genet* 1986, **205**:115-121.

41. Lu M, Campbell JL, Boye E, Kleckner N: **SeqA: a negative modulator of replication initiation in *E. coli***. *Cell* 1994, **77**:413-426.

42. Riber L, Løbner-Olesen A: **Coordinated replication and sequestration of *oriC* and *dnaA* are required for maintaining controlled once-per-cell-cycle initiation in *Escherichia coli***. *J Bacteriol* 2005, **187**:5605-5613.

43. •• Peterson SN, Reich NO: **GATC flanking sequences regulate Dam activity: evidence for how Dam specificity may influence *pap* expression**. *J Mol Biol* 2006, **355**:459-472.
Dam methylase, a highly processive enzyme, may become less processive at GATC sites flanked by specific DNA sequences. Reduced processivity may permit competition between Dam and specific DNA binding proteins, thus permitting the formation of nonmethylated GATCs.

44. •• Hernday A, Braaten B, Low D: **The intricate workings of a bacterial epigenetic switch**. *Adv Exp Med Biol* 2004, **547**:83-89.
A review on phase variation of *E. coli* pyelonephritis-associated pili. Transcription of the *papBA* operon is controlled by Dam methylation, Lrp, and the OFF to ON switching factor, PapI. Additional, host encoded regulators can skew switching frequencies in response to environmental cues.

45. Hernday AD, Braaten BA, Low DA: **The mechanism by which DNA adenine methylase and PapI activate the pap epigenetic switch**. *Mol Cell* 2003, **12**:947-957.

46. Henderson LR, Owen P: **The major phase-variable outer membrane protein of *Escherichia coli* structurally resembles the immunoglobulin A1 protease class of exported protein and is regulated by a novel mechanism involving Dam and OxyR**. *J Bacteriol* 1999, **181**:2132-2141.

47. Haagmans W, van der Woude M: **Phase variation of Ag43 in *Escherichia coli*: Dam-dependent methylation abrogates OxyR binding and OxyR-mediated repression of transcription**. *Mol Microbiol* 2000, **35**:877-887.

48. Waldron DE, Owen P, Dorman CJ: **Competitive interaction of the OxyR DNA-binding protein and the Dam methylase at the antigen 43 gene regulatory region in *Escherichia coli***. *Mol Microbiol* 2002, **44**:509-520.

49. Wallecha A, Munster V, Correnti J, Chan T, van der Woude M: **Dam- and OxyR-dependent phase variation of *agn43*: essential elements and evidence for a new role of DNA methylation**. *J Bacteriol* 2002, **184**:3338-3347.

50. Camacho EM, Serna A, Madrid C, Marqués S, Fernández R, de la Cruz F, Juárez A, Casadesús J: **Regulation of *finP* transcription by DNA adenine methylation in the virulence plasmid of *Salmonella enterica***. *J Bacteriol* 2005, **187**:5691-5699.

51. Bell DC, Cupples CG: **Very-short-patch repair in *Escherichia coli* requires the dam adenine methylase**. *J Bacteriol* 2001, **183**:3631-3635.

52. Oshima T, Wada C, Kawagoe Y, Ara T, Maeda M, Masuda Y, Hiraga S, Mori H: **Genome-wide analysis of deoxyadenosine methyltransferase-mediated control of gene expression in *Escherichia coli***. *Mol Microbiol* 2002, **45**:673-695.