Random switching of the ModA11 type III DNA methyltransferase of *Neisseria meningitidis* regulates Entner-Doudoroff aldolase expression by a methylation change in the *eda* promoter region.

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Abbreviations:

Mod, DNA methyltransferase; SMRT, Single molecule real-time sequencing;

Abstract

Phase variable DNA methyltransferases (Mods) mediate epigenetic regulation of gene expression. These phase variable regulons, called phasevarions, have been shown to regulate virulence and immunoevasion in multiple bacterial pathogens. How genome methylation switching mediates gene regulation is unresolved. *Neisseria meningitidis* remains a major cause of sepsis and meningitis worldwide. Previously we reported that phase variation (rapid on/off switching) of the meningococcal ModA11 methyltransferase regulates 285 genes. Here we show a bioinformatic analysis that reveals only 26 of the regulated genes have a methylation site located upstream of the gene with potential for direct effect of methylation on transcription. To investigate how methylation changes are “read” to alter gene expression, we used a *lacZ* gene fusion approach. We showed a 182 nucleotide region upstream of the *eda* gene (Entner-Doudoroff aldolase) is sufficient to impart methylation-dependent regulation of *eda*. Site directed mutagenesis of the 5'-ACGT^{m6}AGG-3' ModA11 site upstream of the *eda* gene showed that methylation of this site modulates *eda* expression. We show that *eda* is regulated by the PhoB homologue MisR, and that a MisR binding motif overlaps with the ModA11 methylation site. In a MisR mutant, regulation of *eda* is uncoupled from regulation by ModA11 phasevarion switching. The on/off switching of ModA11 leads to the presence or absence of a N6-methyladenine modification at thousands of sites in the genome. Most of these modifications have no impact on gene regulation. Moreover, the majority of the 285 gene regulon that is controlled by ModA11 phasevarion switching (259/285) are not directly controlled by methylation changes in the promoter region of the regulated genes. Our data are consistent with direct control via methylation of a subset of the regulon, like *Eda*, whose regulation will trigger secondary effects in expression of many genes.

Graphical abstract

Keywords: Phasevarion; epigenetic regulation; adenine methylation

Phase variation is the high-frequency, reversible, on/off switching of gene expression, which provides a selective advantage during bacterial adaption to host microenvironments and enables their evasion of host immune defences. One mode of phase variation occurs due to the hypermutation of simple DNA repeats within the open reading frame of a gene, which leads to the reversible loss/gain of repeat units that in turn leads to frameshift mutations and on/off switching of expression [1]. Phase variable DNA methyltransferases have been characterised in many bacterial pathogens, including the pathogenic *Neisseria* species [2-4], *Haemophilus influenzae* [5, 6], *Helicobacter pylori* [7], *Moraxella catarrhalis* [8, 9], *Streptococcus pneumoniae* [10] and *Kingella kingae* [11]. In all of these organisms, the on/off switching of the DNA methyltransferase generates subpopulations with distinct DNA methylation patterns, and this epigenetic heterogeneity results in differential expression of a set of genes within a regulon, called a ‘phasevarion’ (phase variable regulon) [12, 13]. These phasevarions often regulate virulence factors and candidate vaccine antigens, generate altered virulence phenotypes and mediate immunoevasion in *in vitro* and *in vivo* models of disease.

The host adapted pathogen *Neisseria meningitidis* contains three phase variable DNA methyltransferase (Mod) genes associated with Type III Restriction-modification (R-M) systems, ModA, ModB and ModD [2-4]. For each of the *modA*, *modB*, and *modD* genes, different alleles have been identified that are conserved in their N- and C-terminal domains with >90% amino acid identity, but that vary in their central DNA recognition domain (DRD; also known as a Target Recognition Domain or TRD) (> 95% identity within alleles, and typically < 40% identity between alleles). A survey of 1,689 meningococcal isolates indicated that *modA* was present in all isolates, and that the *modA11* allele was one of the most common alleles (present in 27.5% of isolates) [14]. DNA microarray analysis revealed that 285 genes are differentially regulated under iron limitation between ModA11 ON versus ModA11 OFF (*modA11::kan* mutant) states [2]. These genes include vaccine candidates and virulence factors [2], and ModA11 ON has 4-fold higher resistance to the antibiotics ceftazidime and ciprofloxacin than ModA11 OFF [15]. The ModA11 (M.NmeMC58I) recognition site was determined by single molecule, real-time (SMRT) sequencing and methylome analysis to be 5'-nCGY^{m6}AGn-3' [3]. Unlike other phase-variable DNA methyltransferase characterised to date, the ModA11 recognition site is flexible in the nucleotide base before and after the recognition site and the percentage of methylation varies from 4.6%-100% depending on the central Y (C or T) residue present in combination with flanking bases (A, G, T or C) [3]. Epigenetic regulation in bacteria has been studied in detail

for the N6-methyladenine DNA methyltransferase (Dam) in *Escherichia coli* [16], and a previous study in *H. pylori* has shown that ModH5 modulates expression of the flagellin subunit A (*flaA*) gene in a methylation dependent manner [7]. In general, however, the molecular mechanisms of phasevarion-mediated epigenetic regulation are not understood. The ubiquitous nature of phasevarions in pathogenic bacteria suggests that they are key regulators of virulence and immunoevasion [17, 18] and highlights the need for further investigation to understand and predict this mode of gene regulation.

To study the mechanism of how the DNA methyltransferase ModA11 mediates gene expression, bioinformatic analysis was performed to determine if there are ModA11 methylation sites within the promoter region of the 285 ModA11 regulated genes [2]. Our hypothesis is that ModA11 can mediate gene expression either directly at the promoter region of the regulated gene within the phasevarion, or by modulating the expression of regulatory proteins, or indirectly via direct regulation other proteins whose altered expression will have pleiotropic effects on global gene expression by perturbing key biological processes. Our analysis indicated that only 26 of the 285 genes in the phasevarion have ModA11 recognition sites within the upstream intergenic region of the regulated gene (i.e., the complete sequence between the start codon of the regulated gene and the start or stop codon of its upstream gene) (Table 1, Supp Fig 1). This data suggested that most of the genes regulated by the ModA11 phasevarion are regulated indirectly. Of the 26 regulated genes in Table 1, only *nmb1394* (*eda*) contains a ModA11 recognition sequence within the predicted promoter region that is 100% methylated (i.e., this site is 100% methylated in all genomes in the sample, under the growth conditions used). Therefore, *eda* was selected for further analysis. The ModA11 recognition sequence 5'-ACGT^{m6}AGG-3' is located 107 bases upstream of the *eda* ATG start codon, and *eda* has 2.22-fold higher expression in the ModA11 ON vs ModA11 OFF states (Table 1; Fig. 1A).

To investigate how methylation upstream of the regulated *eda* gene alters gene expression, an *eda* promoter-*lacZ* fusion was made. As shown in Fig. 1B, a promoter-less *lacZ* gene, amplified from pBluescriptlacZ::kan [5] using primers lacZ1F 5'-GTTTGTAGAGGCCCCAAGGGGTTATGCTACATCAGAGATTTTGAGACACAACGTG GC-3' and lacZ1R 5'-ACATTAGGCCTTGAAGGAGAGCTACGTTATGGATCCCGTCGTTTTACAACGTTCGT G-3', was cloned into a *N. meningitidis* complementation construct pComPind [19] to

generate pCom*lacZ*. Next the 182bp intergenic region upstream of the *eda* gene was amplified by PCR from *N. meningitidis* strain MC58, using primers SphI_1394_For 5'-GTGGTAGCTAGCTTCTCCGGGTGGAATGGGGT-3' and NheI_1394_Rev 5'-GAAGATGCATGCCTTTTCAGACCGAAGGCCGTC-3', and cloned upstream of *lacZ* in pCom*lacZ* to generate an *eda* promoter-*lacZ* fusion construct (pComPeda-*lacZ*) as shown in Fig. 1B. The gene fusion was initially constructed in *E. coli* and the plasmid was subsequently purified, linearized and transformed into *N. meningitidis* MC58 ModA11 ON (i.e., the *modA11_1R* strain constructed to contain one repeat in *modA11*, which is locked ON and unable to phase vary [3]) and MC58 ModA11 OFF (i.e., the *modA11::kan* strain constructed to generate a *modA11* knockout by insertion of a kanamycin cassette within its open reading frame [2]) (Fig. 1C; Fig. 2A). A promoterless construct was also transformed into MC58 ModA11 ON to act as a control. MC58 ModA11 ON and OFF strains containing the *eda* promoter-*lacZ* gene were streaked on BHI plates containing Levinthal supplement and X-gal (5-bromo-4-chloro-3-indolyl-D-galactopyranoside; 40 µg/ml) to examine LacZ expression. As shown in Fig 2B, the expression of LacZ was high in ModA11 ON when the promoter region is methylated, but low/absent in ModA11 OFF when the promoter region is not methylated. To quantify LacZ expression, a β-galactosidase assay was performed on triplicate samples. To achieve this, overnight bacterial growth was harvested into PBS, cells lysed by repeated freeze–thaw cycles, the supernatant collected after centrifugation, the amount of protein measured by BCA assay (Pierce), and samples normalized so that equal amounts of protein were used in each assay. The amount of β-galactosidase in the cell extracts was measured via ONPG hydrolysis and expressed in Miller units, as described previously [20]. As shown in Fig 2B, 2C, ModA11 ON containing the methylated *eda* promoter region had significantly higher LacZ expression compared to the ModA11 OFF non-methylated *eda* promoter region. No LacZ expression was detected in the MC58 ModA11 ON promoter-less negative control (Fig. 2B, 2C). These results indicate that the 182bp region was sufficient to impart methylation switching dependent expression.

To further investigate ModA11 dependent expression of *eda* and the specific role of the 5'-ACGT^{m6}AGG-3' methylation site in the *eda* promoter region, we used a site directed mutagenesis approach. Two constructs were engineered each with a single point mutation in the methylation site, which would alter methylation from the wild type sequence with 100% methylation (5'-ACGT^{m6}AGG-3') to 0% methylation (5'-ACAT^{m6}AGG-3' or 5'-ACGA^{m6}AGG-3'). Methylation at these sites was not detected in SMRT methylome data [3].

Point mutation constructs were made by inverse PCR using the plasmid pComPeda_{lacZ} as the template and DNA fragments were amplified using reverse primer NMB1394_Rev 5'-CTATCTTAAAACGCTTGAA-3' and forward primers NMB1394_For#3 5'-ACATAGGTTGGATTCTCGAATCCG-3' and NMB1394_For#4 5'-ACGAAGGTTGGATTCTCGAATCCG-3' to introduce the G to A and T to A point mutations, respectively. Amplified PCR products were self-ligated and transformed into *E. coli*. The sequences were confirmed, then the constructs were purified, linearized and transformed into the MC58 ModA11 ON strain. As shown in Fig. 3, the point mutations at the ModA11 methylation recognition site reduces the expression of the *eda::LacZ* fusion, confirming that methylation at 5'-ACGT^{m6}AGG-3' directly affects *eda* gene expression.

The *N. meningitidis* gene *eda* that we investigated encodes a 4-hydroxy-2-oxoglutarate aldolase-2-dehydro-3 deoxyphosphogluconate aldolase, which is also called an Entner-Doudoroff Aldolase or Eda. Eda has an important role in glucose degradation via the Entner-Doudoroff pathway to produce a precursor for cellular building blocks [21]. In *E. coli*, the gene *eda* is regulated by PhoB, which can bind to the Pho box located upstream of *eda* and suppress expression of Eda [22]. A BLAST search of the *N. meningitidis* MC58 genome with the *E. coli* PhoB sequence revealed that the meningococcal gene NMB0595 shares 35% identity with *E. coli* PhoB. In *N. meningitidis*, *in silico* analysis has also indicated that NMB0595 exhibits structural homology with the *E. coli* Pho regulator [23]. NMB0595 has been called MisR in *N. meningitidis* by others [24, 25]. A 15bp MisR binding consensus sequence has been predicted by Tzeng et al [26]; however it has been noted that the consensus may be more degenerate than initially defined [27]. We identified two putative MisR binding motifs within the promoter region of the *eda* gene in MC58, which have 11/15 and 10/15 matches to the MisR consensus sequence predicted by Tzeng et al [26] (see Fig. 4). The second MisR binding motif overlaps the ModA11 site and ModA11 methylation in the *eda* promoter region. The presence of this overlap between the ModA11 methylation target site and MisR repressor binding site suggests a molecular mechanism by which the changes in DNA methylation may be converted into changes in *eda* expression; methylation of this ModA11 site may block binding of the MisR repressor to the *eda* promoter and thereby increase expression, while MisR binding in the ModA11 OFF state, unimpaired by such methylation, may permit MisR repressor binding and thereby lead to lower *eda* expression. The MisR binding site is positioned 50 bp upstream of the predicted -35 and -10 sequences which is not typical for a repressor binding site. There may be other regulatory elements

involved as previously seen for NadA expression in *N. meningitidis* where IHF may permit looping of DNA, allowing interaction of the regulator NadR at operators located distally from the promoter [28]. To investigate whether MisR regulates *eda* expression in *N. meningitidis* MisR mutants were constructed. Briefly, the *misR* gene was amplified from MC58 using primers misRFor 5'- GCCGTCTGAAGACGAAATCCGCAAACCTGCC -3' and misRRev 5'- CAGAATCTTAGGCAACGGCAGCCGTCTGAA -3', was cloned into pGem-Teasy (Promega) to generate pGemT*misR*. Next a *tetM* gene from pGem*tetM* [29] was inserted at nucleotide position 320 of the *misR* open reading frame in pGemT*misR* to generate pGemT*misR::tet* and then transformed into MC58 pComPeda_lacZ ModA11ON and ModA11OFF strains to replace *misR* in the chromosome with the *misR::tet* knockout allele by homologous recombination. As shown in Fig 2B, 2C, LacZ expression is higher in ModA11OFF in the MisR knockout mutant strain (ModA11OFF vs ModA11OFF *misR::tet*). Quantitative RT-PCR of the native *eda* gene also demonstrated that *eda* expression was higher (~ 4-fold) in ModA11OFF *misR::tet* vs ModA11OFF. These data indicate that expression of *eda* is repressed by MisR in the absence of ModA11 methylation and that the cloned 182 bp *eda* promoter region that is fused to the LacZ promoter probe reporter construct is sufficient to mediate this MisR dependent regulation of *eda* at the transcriptional level. In the *misR::tet* mutant strains *eda* is uncoupled from regulation by ModA11 phasevarion switching, confirming that modA11 dependent regulation of *eda* is dependent on MisR, presumably by alternately blocking and permitting the repressor to bind the *eda* promoter in the ON and OFF phases of ModA11 switching of DNA methylation of the MisR binding site. *Eda* is proceeded immediately by the upstream gene in the same orientation. However, terminator sequences were predicted after the upstream gene of *eda* using iTerm-PseKNC [30] suggesting *eda* can be transcribed independently.

In summary, we observed that phase variation of ModA11 in *N. meningitidis* directly mediates regulation of *eda* via methylation of the 5'-ACGT^{m6}AGG-3' sequence within the promoter region of *eda*. Furthermore, DNA methylation may affect binding of the MisR regulator to alter *Eda* expression. *Eda* is a keto-deoxy-phosphogluconate (KDPG) aldolase of the Entner-Doudoroff (ED) pathway, which is involved in catabolizing glucose to pyruvate and glyceraldehyde-3-phosphate. Glucose is the main carbon source available to *N. meningitidis* in human blood and cerebrospinal fluid, and glucose degradation pathways are central for energy generation, with 69% of pyruvate being synthesized via the ED pathway in *N. meningitidis* [27]. Glucose levels regulate energy metabolism and nutrient transport, as

well as expression of virulence factors [31]. The type of available carbon sources has also been found to affect meningococcal complement resistance [32]. As such, altered expression of Eda could impact the expression of various genes and affect bacterial fitness during infection.

The on/off switching of ModA11 leads to the presence or absence of a N6-methyladenine modification at thousands of sites in the genome. Most of these modifications probably have no impact on gene regulation. Moreover, the majority of the 285 gene regulon that is controlled by ModA11 phasevarion switching (259/285) are not directly controlled by methylation changes in the promoter region of the regulated genes. Our data are consistent with direct control via methylation of a subset of the regulon, like Eda, whose regulation will trigger secondary effects in expression of many genes.

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Figure Legends

Figure 1: Schematic of the *eda* gene locus, and the *lacZ* fusion constructs used to study ModA11 methylation.

(A) The gene locus of *nmb1394* (*eda*), which contains a ModA11 recognition sequence (5'-ACGT^{m6}AGG-3') within the predicted promoter region (yellow box), and its flanking genes (*edd* - phosphogluconate dehydratase, *adh* - alcohol dehydrogenase) are shown. The base pair (bp) location of this region in the *Neisseria meningitidis* strain MC58 genome sequence (GenBank Accession NC_003112) is indicated below the line, and dashes represent 200 bp intervals. (B) A promoter-less *lacZ* gene was cloned into a *N. meningitidis* complementation construct pComPtac to generate pCom*lacZ*. This construct contains a chloramphenicol-resistance gene (Cm^R) and is flanked upstream and downstream by regions for integration between the NMB1428 and NMB1429 open reading frames, respectively, of *N. meningitidis* MC58. Following this, 182bp upstream of the *eda* gene was cloned upstream of *lacZ* to generate pComPeda_*lacZ*. (C) The pComPeda_*lacZ* construct was transformed into *N. meningitidis* MC58 and integrated into the genome by homologous recombination.

Figure 2: Phenotypic analysis of *eda* promoter-*lacZ* fusion constructs in *N. meningitidis* MC58 ModA11 ON and ModA11 OFF strains.

(A) The pComPeda_*lacZ* construct was transformed into *N. meningitidis* MC58 ModA11 ON (*modA11* 1R) and ModA11 OFF (*modA11::kan*) strains to enable investigation of the impact of ModA11 methylation in the *eda* promoter region. (B) LacZ expression was investigated in MC58 ModA11 ON, OFF and *misR::tet* strains containing pComPeda_*lacZ* that were grown on agar plates containing and X-gal. Blue colonies indicate expression of LacZ in MC58 ModA11 ON, facilitated by methylation in the *eda* promoter region. White colonies indicate no/low *lacZ* expression in MC58 ModA11 OFF, in the absence of methylation in the *eda* promoter region. Blue colonies of *misR::tet* ModA11 ON and *misR::tet* ModA11 OFF indicate that LacZ expression was restored in the absence of MisR. MC58 ModA11 ON promoter less-*lacZ* is a negative control. (C) *eda* expression was quantified by β -galactosidase assays or with qPCR with the same strains shown in (B). [#] qPCR value is shown for each strain relative to the ModA11 ON strain. Student's *t*-test *p* value as used for comparisons.

Figure 3: Phenotypic analysis of point mutations in ModA11 recognition site demonstrates *lacZ* expression is dependent on the methylation site.

The role of the 5'-ACGT^{m6}AGG-3' methylation site in the *eda* promoter region was investigated using the pComPeda_*lacZ* construct and two constructs with single point mutations in the methylation site that are not methylated by ModA11 (5'-ACAT^{m6}AGG-3' or 5'-ACGA^{m6}AGG-3'). LacZ expression was only detected in MC58 ModA11 ON containing the wild type pComPeda_*lacZ*. No LacZ expression was seen in ModA11 ON containing either mutated construct, or ModA11 OFF containing the wild type pComPeda_*lacZ* that was used as a negative control.

Figure 4: Sequence of the *eda* promoter region.

A region of 182 nucleotides upstream of the ATG site of *eda* is shown, including the ModA11 recognition site (boxed). The predicted transcriptional start site (+1) and the -10 and -35 elements upstream of *eda* were identified using BPRM, Softberry [31] and are underlined and in bold. We also note another candidate -10 sequence, TAAAAT, that is consistent with use in *Neisseria* promoters [32]. The putative MisR binding motifs, based on the MisR consensus sequence (KWWWTGTAARGNNWH) predicted by Tzeng et al [26],

are underlined and in italics. Mismatches to the MisR consensus sequence are shown in lower case. The MisR consensus motif overlaps with the ModA11 site, which is highlighted in grey.

Figure S1: The position of ModA11 recognition sites with respect to regulated genes in the ModA11 phasevarion in *N. meningitidis* MC58. * marks indicate the ModA11 recognition sites at the promoter regions. The coordinates in Genbank Accession NC_003112.2 for each recognition site are shown next to the * marks.

Table 1: Bioinformatic analysis of ModA11 recognition sites within the promoter regions of ModA11 regulated genes.

Gene ID	Gene	Ratio OFF/ON *	ModA11 recognition site^ (5'-nCGY ^{m6} AGn-3')	% methylated^
Increased expression in <i>N. meningitidis</i> modA11::kan mutant				
NMB0021	pilS cassette	1.97	<u>G</u> CGCAGG x 2 sites (20314-20320) (20638-20644)	9.1
NMB0334	glucose-6-phosphate isomerase	2.38	CCGCAGG (342257-342263)	37.4
NMB0740	DNA repair protein RecN	3.51	CCGTAGG (771056-771062)	64.1
NMB0921	hypothetical protein	2.57	CCGCAGA (934642-934649)	44
NMB1500	conserved hypothetical protein	3.25	<u>G</u> CGCAGG (1550782-1550788)	4.6
NMB1540	lactoferrin-binding protein A	2.33	CCGCAGA (1599631- 1599637)	44
NMB1541	lactoferrin-binding protein B	2.22	<u>G</u> CGCAGG (1599921-1599927)	9.1
			<u>G</u> CGCAGG (1600113-1600120)	9.1
NMB1839	formate--tetrahydrofolate	2.22	CCGCAGT (1941555-1941561)	86
NMB1840	ligase conserved hypothetical protein	2.58	ACGCAGC (1941572-1941578)	86.9
NMB2043	IS1106 transposase, putative, degenerate	2.46	<u>G</u> CGCAGG x 5 sites (2165832- 2165839) (2166027-2166033) (2166109-2166115) (2166304-2166310) (2166386-2166392)	9.1
Reduced expression in <i>N. meningitidis</i> modA11::kan mutant				
NMB0143	50S ribosomal protein L4	0.49	TCGTAGC	52.1
NMB0144	50S ribosomal protein L23	0.38	(151747-151753)	
NMB0146	30S ribosomal protein S19	0.51		
NMB0147	50S ribosomal protein L22	0.46		
NMB0148	30S ribosomal protein S3	0.40		
NMB0346	hypothetical protein	0.53	<u>G</u> CGCAGG (354689-354695)	9.1
NMB0535	glucose/galactose transporter	0.53	CCGCAGA (555566-555572)	44
NMB0564	Na(+)-translocating NADH- quinone reductase, subunit F	0.50	CCGCAGA (598174-598180)	44
NMB0568	Na(+)-translocating NADH- quinone reductase, subunit B	0.49		
NMB0623	spermidine/putrescine ABC	0.51	ACGCAGG	76.1

	transporter, periplasmic binding protein		(655581-655587)	
NMB1394	4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate aldolase (Entner-Doudoroff aldolase).	0.45	ACGTAGG (1423415-1423421)	100
NMB1669	iron-starvation protein PigA	0.51	CCGCAGA (1743633-1743639)	44
NMB1729	biopolymer transport protein ExbB	0.49	CCGCAGC (1812843-1812849)	56.5
NMB1937	ATP synthase F1, delta subunit	0.47	<u>G</u> CGCAGC (2037054-2037060)	20.5
NMB2057	50S ribosomal protein L13	0.47	CCGCAGG (2179801-2179807)	37.4
NMB2133	sodium/dicarboxylate symporter family protein	0.44	CCGCAGA (2241556-2241562) ACGCAGC (2241801-2241807)	44 86

* Expression data adapted from Srikhanta et al 2009. The genes listed either have increased or decreased expression in ModA11 OFF (*modA11::kan* mutant strain) relative to ModA11 ON.

^The ModA11 recognition site is 5'-nCGY^{m6}AGn-3', with the percentage of methylation seen at each site dependent on the central base present (Y = C or T) in combination with the flanking bases (n = A, G, T or C) [3]. n = G at the 5' end of the ModA11 recognition site it is underlined to highlight the relatively low % methylation seen for this sequence. The coordinates in Genbank Accession NC_003112.2 for each recognition site are shown below the sequence.

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Highlight

- A bioinformatic analysis that reveals only 9% of the regulated genes have a ModA11 methylation site located upstream with potential for direct effect of methylation on transcription.
- Using a LacZ gene fusion approach, we showed that a single ModA11 methylation site upstream of the *eda* gene (Entner-Doudoroff aldolase) is sufficient to impart methylation-dependent regulation of *eda*.
- Site directed mutagenesis of the ModA11 site upstream of the *eda* gene showed that methylation of this site modulates *eda* expression.
- The on/off switching of ModA11 leads to the presence or absence of a N6-methyladenine modification at thousands of sites in the genome. Most of these modifications have no impact on gene regulation.

Credit Author Statement

FEJ, ALS, AT, KLS and MPJ designed experiments. FEJ, ALS and AT performed experimental protocols. FEJ, KLS and MPJ wrote the manuscript and all authors reviewed the manuscript.

Figure 1

Figure 2

Strain MC58	Methylation site sequence (% methylated)*	Phenotype
ModA11 ON (<i>modA11_1R</i>)	ACGTAGG (100%)
	AC ^A TAGG (0%)
	ACG ^A AAGG (0%)
ModA11 OFF (<i>modA11::kan</i>)	ACGTAGG (0%)

Figure 3

MisR binding motif

ModA11 site

-35

-10

+1

Figure 4