

Supplementary Methods

Mathematical modeling and simulations

Kinetics of epigenetic switching

There are five states for the model; fully methylated (M_F), hemi-methylated (M_H), unmethylated unbound (U_N), unmethylated + OxyR (U_O) and “repressed” unmethylated + OxyR (OFF). The switching reactions for the M_F , M_H , U_N , U_O and OFF states are the respective first order reactions:

$$\frac{dM_F}{dt} = k_M M_H; \quad [1]$$

$$\frac{dM_H}{dt} = k_H U_N - k_M M_H; \quad [2]$$

$$\frac{dU_N}{dt} = k_{.O} U_O - (k_O + k_H) U_N; \quad [3]$$

$$\frac{dU_O}{dt} = k_{.R} U_{OFF} + k_O U_N - (k_{.O} + k_R) U_O; \quad [4]$$

$$\frac{dOFF}{dt} = -k_{.R} U_{OFF} + k_R U_O. \quad [5]$$

k_M , k_H , k_O , $k_{.O}$, k_R , $k_{.R}$ are rate constants which are defined as the number of cell transitions per generation. The M_F , M_H , U_N , U_O and OFF states also change due to exponential growth which occurs at rates γ_M , γ_H , γ_N , γ_O and γ_R respectively. The combined switching and growth rate changes for each state are consecutive components of $\frac{d}{dt} \mathbf{x}^j(t)$, which describes the changes in each state in generation j , from the end of one round of DNA replication ($t=0$) until the start of the next replication ($t=\tau$) (**Methods**). Integrating $\mathbf{x}^j(t)$ at τ provides the number of cells in each state prior to DNA replication.

At DNA replication each fully methylated site is converted into two hemi-methylated DNA sites and each hemi-methylated site is converted into one hemi-methylated and one unmethylated site. The unmethylated states are otherwise unaffected by DNA replication. The behavior of the system associated with DNA replication changes are in the table below.

		<i>After DNA replication</i>				
		M_F	M_H	U_N	U_O	OFF
<i>Before DNA replication</i>	M_F	0	1	0	0	0
	M_H	0	0.5	0.5	0	0
	U_N	0	0	1	0	0
	U_O	0	0	0	1	0
	OFF	0	0	0	0	1

The diagonal elements of the table are equal to one for the U_N , U_O and OFF states because cells in these states do not transition out of these states at DNA replication. Transforming $\mathbf{x}^j(\tau)$ by \mathbf{D} , a matrix with the DNA replication associated transitions shown in the above table gives the initial conditions for the next generation $j+1$; that is,

$$\mathbf{x}^{j+1}(0) = \mathbf{D}\mathbf{x}^j(\tau), \text{ where } \mathbf{D} = \begin{pmatrix} 0 & 1 & 0 & 0 & 0 \\ 0 & 0.5 & 0.5 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}. \quad [6]$$

Methylated loss

All methylated sites (M_F) become hemi-methylated (M_H) and most are rapidly re-methylated before the next DNA replication. The M_H cells that are derived from the M_F state (rather than from the U_N state) are designated M_{FH} . The fraction of M_{FH} cells remaining in the M_H state after period τ is

$$\int_0^\tau \frac{dM_{FH}(t)}{M_{FH}} = \int_0^\tau -k_M dt = e^{-k_M \tau} \quad [7]$$

Half of this remaining fraction will transition to the U_N state and this is defined as “methylated loss”.

Unmethylated loss

The fraction of cells that are gained and lost from the unmethylated states is relatively small and the reactions are in equilibrium and therefore,

$$\frac{dU_N}{dt} = \frac{dU_O}{dt} = \frac{dOFF}{dt} \sim 0. \quad [8]$$

Consequently, by Eq. 3-5, the U_N , U_O and OFF states have the relationships,

$$U_O = \frac{k_o}{k_{-o}} U_N = K_o U_N \text{ and} \quad [9]$$

$$\text{OFF} = \frac{k_R}{k_{-R}} U_O = K_R U_O. \quad [10]$$

K_O and K_R are the equilibrium constants for the U_N to U_O and the U_O to OFF transitions respectively. Because the U_N state is essentially constant within a generation, the number of cells transitioning to the M_H state is the product of the rate constant (k_H), U_N and the period τ . Therefore the fraction of unmethylated cells that switch (“unmethylated loss”) is

$$\frac{k_H U_N \tau}{U_{\text{Total}}} = k_H F_{\text{UN}} \tau, \quad [11]$$

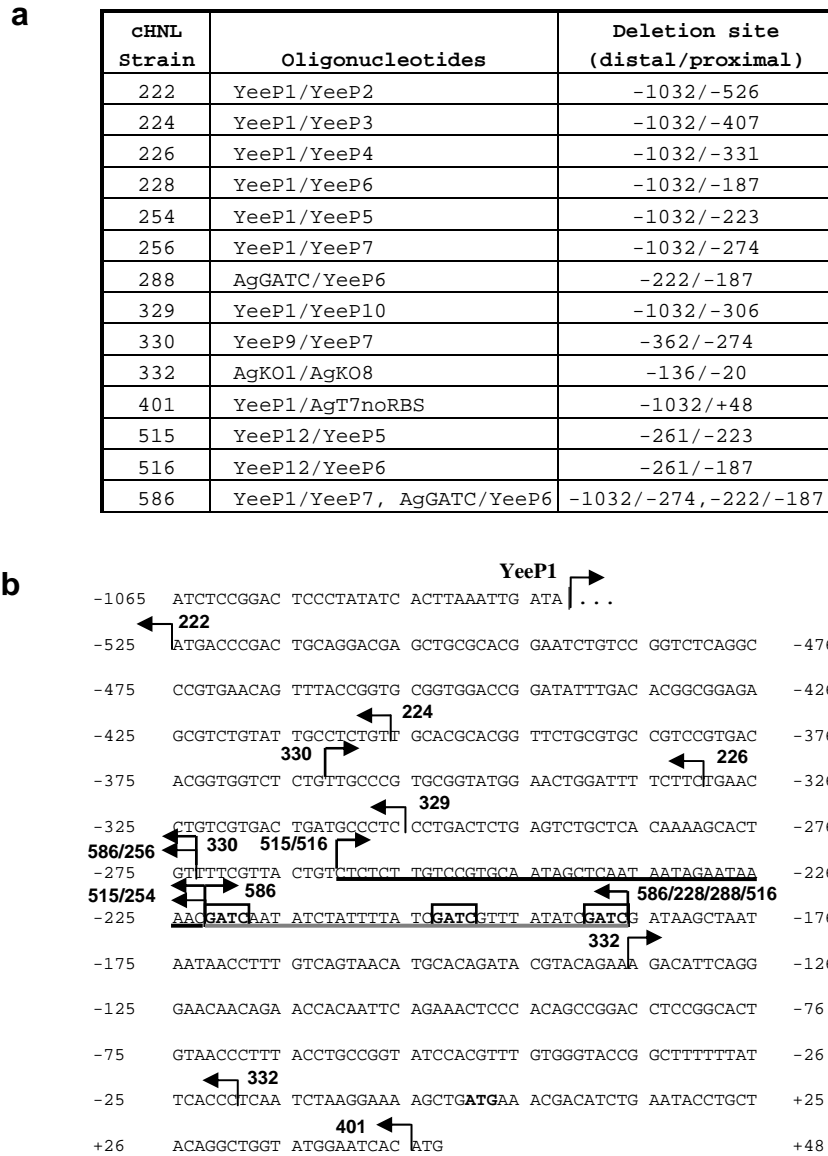
where $F_{\text{UN}} = U_N / U_{\text{Total}} = U_N / (U_N + U_O + \text{OFF})$.

Switching in wild-type and mutant strains

The rate of switching from the methylated to the unmethylated state in wild-type cells is 7×10^{-3} cells/generation¹. Substitution of this methylated loss into Eq. 8 gives a $k_M = 4.3$ /generation. k_M is the conversion rate of hemi-methylated DNA to fully methylated DNA which is estimated to be 30-40 times greater than the rate for the U_N to M_H methylation reaction (k_H)². Therefore $k_H = 0.12$ (that is, $1/35^{\text{th}}$ of k_M). The unmethylated loss is 2×10^{-3} cells/generation¹. Substitution of this value and k_H into Eq. 11 indicates that F_{UN} is 0.016 (1.6%). The fraction of cells in the OFF, intermediate ($U_N + U_O$) and ON ($M_F + M_H$) states is obtained directly from the histogram by measuring the area of each with a three Gaussian distribution fit. The observed wild-type fraction of unmethylated cells in the OFF state and partial states is 92.5% and 7.5% respectively. The subtraction of F_{UN} (1.6%) from the partial state fraction provides the sub fraction of unmethylated cells in the U_O state (5.9 %). The equilibrium constants, K_O and K_R are calculated using the fraction of cells in U_N , U_O and OFF states by Eq. 9 and 10. K_R was determined for each mutant strain assuming the same K_O value since the concentration of OxyR and its binding site were unaltered.

Supplementary References

1. Hasman, H., Schembri, M.A. & Klemm, P. Antigen 43 and type 1 fimbriae determine colony morphology of *Escherichia coli* K-12. *J Bacteriol* **182**, 1089-95 (2000).
2. Urig, S. et al. The *Escherichia coli* dam DNA methyltransferase modifies DNA in a highly processive reaction. *J Mol Biol* **319**, 1085-96 (2002).



Supplementary Figure 1. Cis-regulatory deletion strains. a, Oligonucleotides used to construct the cis-regulatory deletions strains. Nucleotide numbering is relative to the start codon. **b,** The location of the proximal and distal ends of the *agn43* regulatory region deletions. Bold numbers indicate the strain number. The start codon and GATC sites are shown in bold font. Black arrows face downstream and upstream to respectively indicate the distal and proximal extent of the deletions. Grey and black underline indicates the switch and promoter regions.

Supplementary Table 1. Bacterial strains and plasmids.

Strain	Description*	Reference or Source
MC4100	Genetic description is available ¹	M. van der Woude
cHNL13B	MC4100, <i>agn43::agn43(0->+48bp):T7RNApol:CamR</i>	This study
cHNL135	cHNL13B+pHL32	This study
cHNL25	cHNL135, Δdam	This study
cHNL26	cHNL135, $\Delta oxyR$	This study
cHNL140	cHNL135, pTP166	This study
cHNL143	MC4100+pHL32	This study
cHNL149	cHNL135, pHL34	This study
cHNL172	cHNL135, $\Delta dam \Delta oxyR$	This study
cHNL222	cHNL135, $\Delta agn43(-1032/-526)$	This study
cHNL224	cHNL135, $\Delta agn43(-1032/-407)$	This study
cHNL226	cHNL135, $\Delta agn43(-1032/-331)$	This study
cHNL228	cHNL135, $\Delta agn43(-1032/-187)$	This study
cHNL254	cHNL135, $\Delta agn43(-1032/-223)$	This study
cHNL256	cHNL135, $\Delta agn43(-1032/-274)$	This study
cHNL288	cHNL135, $\Delta agn43(-222/-187)$	This study
cHNL329	cHNL135, $\Delta agn43(-1032/-306)$	This study
cHNL330	cHNL135, $\Delta agn43(-362/-274)$	This study
cHNL332	cHNL135, $\Delta agn43(-136/-20)$	This study
cHNL401	cHNL135, $\Delta agn43(-1032/48)$	This study
cHNL515	cHNL135, $\Delta agn43(-261/-223)$	This study
cHNL516	cHNL135, $\Delta agn43(-261/-187)$	This study
cHNL586	cHNL135, $\Delta agn43(-1032/-274)$, $\Delta agn43(-222/-187)$	This study
	* numbering is relative to the start codon (position 0)	
Plasmids		
pZE11/pZE21/pZA21	pZ expression system	2
pTP166	pBR322 with Dam under Tac promoter	3
pHL16	<i>agn43</i> regulatory region:T7RNApol:CamR	This study
pHL32	pT7RNApol:GFP3.1mut, KanR	This study
pHL34	OxyR inserted into pZE21	This study

Supplementary References

1. Peters, J.E., Thate, T.E. & Craig, N.L. Definition of the Escherichia coli MC4100 genome by use of a DNA array. *J Bacteriol* **185**, 2017-21 (2003).
2. Lutz, R. & Bujard, H. Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res* **25**, 1203-10 (1997).
3. Marinus, M.G., Poteete, A. & Arraj, J.A. Correlation of DNA adenine methylase activity with spontaneous mutability in Escherichia coli K-12. *Gene* **28**, 123-5 (1984).

Supplementary Table 2. Oligonucleotide sequences.

Name	Function	Sequence
AgmvwBamHIF	Amplify <i>agn43</i> regulatory region for insertion into pHL16 and used with Ag3rCamR for lambda red integration	CGGGATCCTCCTGCACAGGCACAGAAT
AgmvwSphIR	Amplify <i>agn43</i> regulatory region for insertion into pHL16	TTTACGCATGCTCATGTGATTCCATACCAG
T7SphIF	Amplify T7 RNA polymerase gene for insertion into pHL16	ACATGCATGCACACGATTAACATCGCTAAGAAC
T7ApaIR	Amplify T7 RNA polymerase gene for insertion into pHL16	TTAGGGCCCTTACGCGAACGCGAAGTCCGACTC
CamApaIF	Amplify chloramphenicol gene for insertion into pHL16	GACGGGCCCATATCTGGCGAAAATGAGACGTTG
CamAatIIR	Amplify chloramphenicol gene for insertion into pHL16	CATGACGTCATGGAGTCTGAGGTCATTACTG
Ag3rCamR	Lambda Red integration of pHL16 sequence into cHNL135	CCGGGACCACAGAGAGGCGATGGTCTGTCAGAAAGGTC ACATTCAGTGTGATGGAGTCTGAGGTCATTACTG
T7seqR	Sequencing of <i>agn43</i> promoter in cHNL135	CGTTATCCGCAACCTCACCAGCTT
AgUpmvwF	Sequencing of <i>agn43</i> promoter in cHNL135	CGGAGCCCTGCCATGAATGGGATA
Ag8XmaIF	Sequencing of <i>agn43</i> promoter in cHNL135	TCCACCCGGGTGCTGTTGTGGTGACGCAGGC
YeePUpF	Confirmation and sequencing of <i>agn43</i> promoter deletion. Used with T7seqR as reverse primer	GATGAGTCTG CGTGGAGCG TGTT
pT7AatIIF	Amplify T7 RNA polymerase promoter into pHL32	CATGACGTCTAATACGACTCACTATAGGG
GfpT7TermApaIR	Amplify GFP for insertion into pHL32	ATAGGGCCAAAAAACCCCTCAAGACCCGTTAGAGGCC CCAAGGGGTTATGGTCAGCTAATTAAGCTTATTTG AACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTT T
T7 terminator seq.	Reverse complement used in GfpT7termApaIR	AATTAGTTAGTCAGCATGAAGAAAAATCGCGCTTTTTTG AAGTGGCAGGGTGTAGGCTGGAGCTGCTTC
DampkD1F	Deletion of Dam using pkD13 plasmid	TTATTTTTTCGCGGGTGAACGACTCCTGGTTGTACAAA GCCAGCAGTTATTCCGGGGATCCGTCGACC
DampkD4R	Deletion of Dam using pkD13 plasmid	ATGAATATTCGTGATCTTGAGTACCTGGTGGCATTGGCT GAACACCGCCAGTGTAGGCTGGAGCTGCTTC
OxpkD1F	Deletion of OxyR using pkD13 plasmid	TTAAACCGCCTGTTTAAAACCTTATCGAAATGGCCATCC ATTCTTGCGCATTCGGGGATCCGTCGACC
OxpkD4R	Deletion of Dam using pkD13 plasmid	ATCACCAAAA AGGGTGAATC TCCGGACTCC CTATATCACT TAAATTGATA GTGTAGGCTGGAGCTGCTTC
DamUpF	Confirmation and sequencing of Dam deletion	CTGAAGTAATCAAGGTTATCTCCC
DamInR	Confirm Dam deletion (site lies within Dam)	CGGCCTGTACGTACTCATCAGTAC
DamDR	Confirmation and sequencing of Dam deletion	CACCATTGGCCCAATCGTCAGATT
OxUpF	Confirmation and sequencing of OxyR deletion	GGAGATCCGCAAAAGTTCACGTTG
OxInR	Confirm OxyR deletion (site lies within OxyR)	CAGCATTCCCGCCTGGGTGAACAA
OxDR	Confirmation and sequencing of OxyR deletion	GGAACAGAAAGGTGGCGGCAACAC
YeeP1	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	ATCACCAAAA AGGGTGAATC TCCGGACTCC CTATATCACT TAAATTGATA GTGTAGGCTGGAGCTGCTTC
YeeP2	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GCCTGAGACCGGACAGATTCCGTGCGCAGCTCGTCCTGC AGTCGGGTCATATTCGGGGATCCGTCGACC
YeeP3	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GGGCAACAGAGACCACCGTGTACGGACGGCACGCAGA ACCGTGCCTGCAATTCGGGGATCCGTCGACC
YeeP4	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	TTTTGTGAGCAGACTCAGAGTCAGGGAGGGCATCAGTCA CGACAGGTTCAATTCGGGGATCCGTCGACC
YeeP5	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	ATTATTAGCTTATCGATCGATATAAACGATCGATAAAAAT AGATATTGATCATTCCGGGGATCCGTCGACC
YeeP6	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	TTCTGTACGT ATCTGTGCAT GTTACTGACA AAGGTTATTA TTAGCTTATC ATTCCGGGGATCCGTCGACC
YeeP7	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GTTTTATTCTATTATTGAGCTATTGCACGGACAAGAGAG ACAGTAACGAAAATTCGGGGATCCGTCGACC
YeeP9	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	CTCTGTTGCACGCACGGTCTGCGTGCCGTCCGTGACAC GGTGGTCTCTGGTGTAGGCTGGAGCTGCTTC
YeeP10	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	AGAGAGACAGTAACGAAAACAGTGTCTTTTGTGAGCAGA CTCAGAGTCAGGATTCCGGGGATCCGTCGACC
YeeP12	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GCCCTCCCTGACTCTGAGTCTGCTCAAAAAGCACTGTTT TCGTTACTGTGTAGGCTGGAGCTGCTTC

AgGATC	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	TTCGTTACTGTCTCTCTTGTCCGTGCAATAGCTCAATAAT AGAATAAAAACGTGTAGGCTGGAGCTGCTTC
AgKO1	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GATAAGCTAATAATAACCTTTGTCAGTAACATGCACAGA TACGTACAGAAGTGTAGGCTGGAGCTGCTTC
AgKO8	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GCCTGTAGCAGGTATTCAGATGTCGTTTCATCAGCTTTTC CTTAGATTGAATTCGGGGATCCGTCGACC
AgT7noRBS	Deletion of <i>agn43</i> regulatory region using pkD13 plasmid	GCCAGTTCGATGTCAGAGAAGTCGTTCTTAGCGATGTTA ATCGTGTGCATATTCGGGGATCCGTCGACC
T7seqF1	PCR and sequencing of the T7 RNA polymerase gene	ATGAACACGATTAACATCGCTAAG
T7seqF451	PCR and sequencing of the T7 RNA polymerase gene	TTCGGTCGTATCCGTGACCTTGAA
T7seqF901	PCR and sequencing of the T7 RNA polymerase gene	AGTAAGAAAGCACTGATGCGCTAC
T7seqF1441	PCR and sequencing of the T7 RNA polymerase gene	TTCATTGAGGAAAACCACGAGAAC
T7seqF1891	PCR and sequencing of the T7 RNA polymerase gene	AAGCGTTCAGTCATGACGCTGGCT
T7seqF2341	PCR and sequencing of the T7 RNA polymerase gene	AACTTTGTACACAGCCAAGACGGT
T7seqR541	PCR and sequencing of the T7 RNA polymerase gene	AGCCTCGACAACCTTGCAATAAATGC
T7seqR991	PCR and sequencing of the T7 RNA polymerase gene	GGCGACCGCTAGGACTTTCTTGTT
T7seqR1531	PCR and sequencing of the T7 RNA polymerase gene	GTACTIONAAGCAGAACGCAAGGAA
T7seqR1981	PCR and sequencing of the T7 RNA polymerase gene	AGTGAACATCAGACCCTTGCCGGA
T7seqR2431	PCR and sequencing of the T7 RNA polymerase gene	CGGAATGGTACCGAAGGAGTCGTG
CamSeqR1	PCR and sequencing of the T7 RNA polymerase gene	GCCCGGTAGTGATCTTATTTCATT