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Published paper

Husnain, S., Busby, S.J.W., Thomas, M.S. (2009) *Downregulation of the Escherichia coli guaB promoter by upstream-bound cyclic AMP receptor protein*, Journal of Bacteriology, 191 (19), pp. 6094-6104

<http://dx.doi.org/10.1128/JB.00672-09>

1 **Supplementary Figure 1. Involvement of CRP in GRDC of P_{guaB} .** Data from
2 Figure 4 are replotted to show relative promoter activities. The β -galactosidase
3 activity specified by each promoter was normalized to the β -galactosidase activity
4 corresponding to a growth rate of one doubling per hour. The β -galactosidase activity
5 specified by each promoter (in Miller units) at a growth rate of one doubling per hour
6 was as follows: Δcrp , 2763; $\Delta crp/pLG339CRP$, 1425; $\Delta crp/pLG339CRP159L$, 782;
7 $\Delta crp/pLG339CRP101E$, 1333; P_{guaB} (CRP -106.5), 3091; P_{guaB} (CRP -128.5), 3035.

8
9 **Supplementary Figure 2. Effect of growth rate on the intracellular levels of CRP.**

10 Strain VH1000G-133 was grown at different rates using 'standard media'. Cells were
11 sonically disrupted, and 2.4 μ g of total cell protein was used for SDS-PAGE and
12 western blotting with polyclonal anti-CRP antibody. Samples were loaded in order of
13 increasing growth rate, and are as follows: M9 minimal medium + 5 μ g/ml thiamine
14 and (1) glycerol, (2) succinate + 20 amino acids, (3) glycerol + 20 amino acids, (4)
15 glycerol + 1% (w/v) casamino acids, (5) glycerol + 2% (w/v) casamino acids, (6)
16 glucose + 20 amino acids and (7) glucose + 0.8% (w/v) casamino acids. Lanes 8 was
17 loaded with a lysate isolated from strain VH1000G-133 Δcrp , grown in M9 minimal
18 medium + 5 μ g/ml thiamine and fructose. Carbon sources were included at a final
19 concentration of 0.4% (w/v), and 20 amino acids were each present at 20 μ g/ml. In the
20 plot of CRP band intensity versus growth rate, the band intensity is expressed relative
21 to the intensity of the band corresponding to the highest cell growth rate (i.e., lane 7),
22 which was assigned a value of 1.0. Each data point is the mean (with standard error)
23 of data obtained from two independent experiments.

24

1 **Supplementary Figure 3. Effect of exogenously added cAMP on GRDC of P_{guaB} .**

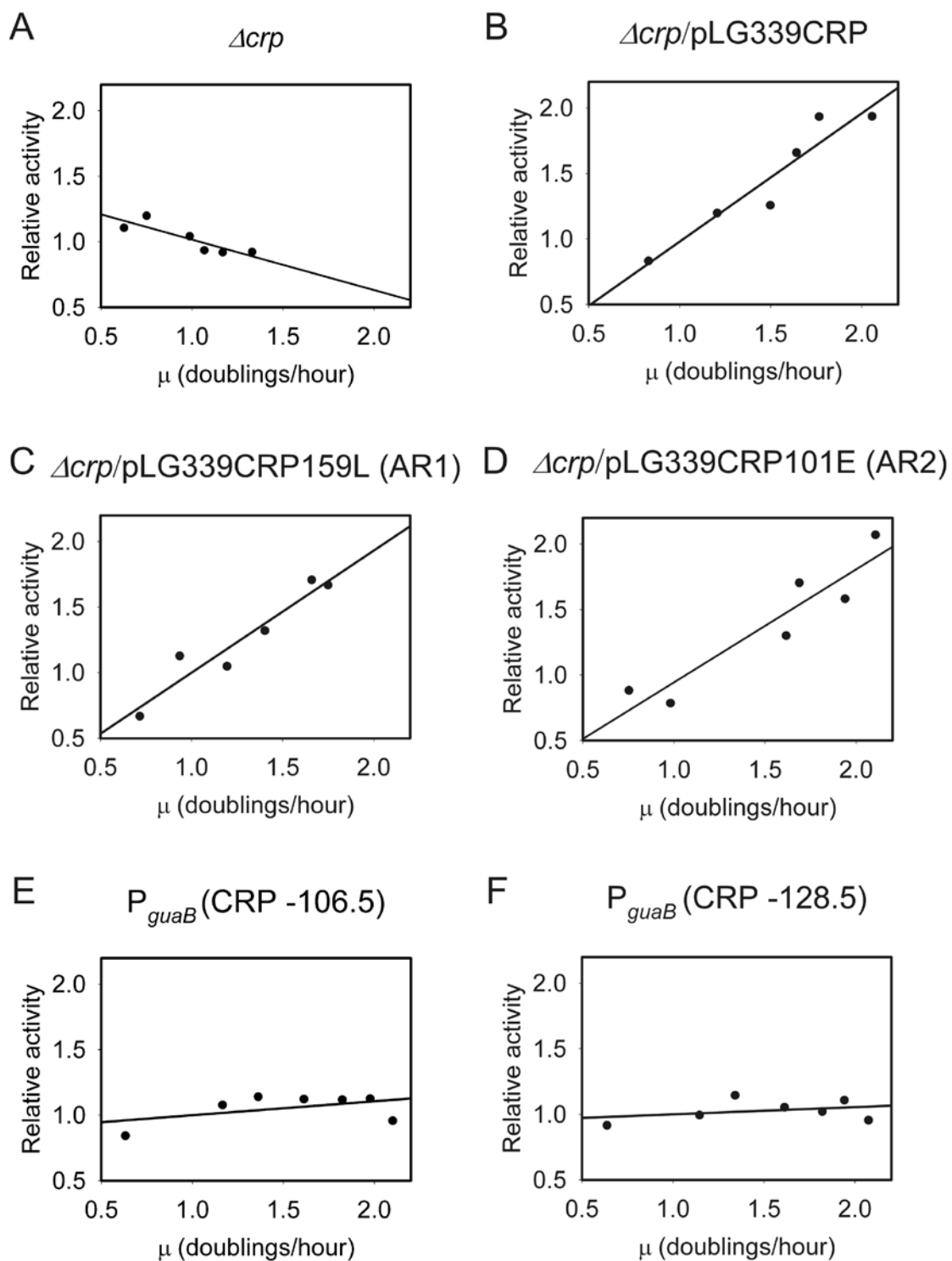
2 Data from Figure 6B and 6D are replotted to show relative promoter activities. The β -
3 galactosidase activity specified by each promoter was normalized to the β -
4 galactosidase activity corresponding to a growth rate of one doubling per hour. The β -
5 galactosidase activity specified by each promoter (in Miller units) at a growth rate of
6 one doubling per hour was as follows: $P_{guaB}(-133 \text{ to } +36)/\Delta cyaA + cAMP$, 1537;
7 $P_{guaB}(-133 \text{ to } +36, G-122C)/\Delta cyaA + cAMP$, 4617.

8

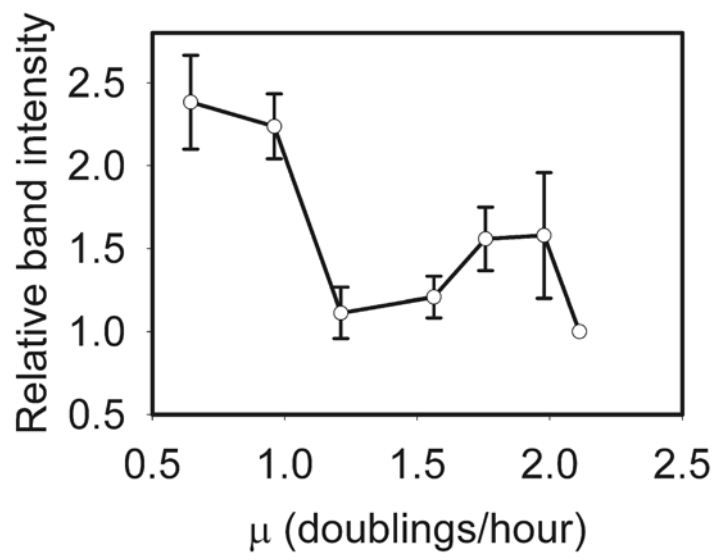
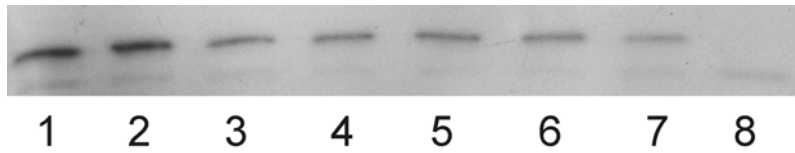
9 **Supplementary Figure 4. Growth rate-dependent control of the $guaB$ promoter.**

10 A strain harbouring a fusion of the wild type $guaB$ promoter ($P_{guaB}(-133 \text{ to } +36)$) to
11 $lacZ$ was grown at different cellular growth rates in M9 minimal salts medium
12 supplemented with the carbon sources listed in the accompanying table, whereupon
13 the β -galactosidase activity was determined (expressed as Miller units). Each data
14 point represents the mean promoter activity and mean growth rate, and was calculated
15 using data obtained from at least three independent experiments. Media highlighted in
16 the same colour supports a similar growth rate and similar P_{guaB} activity.

Supplementary Figure 1

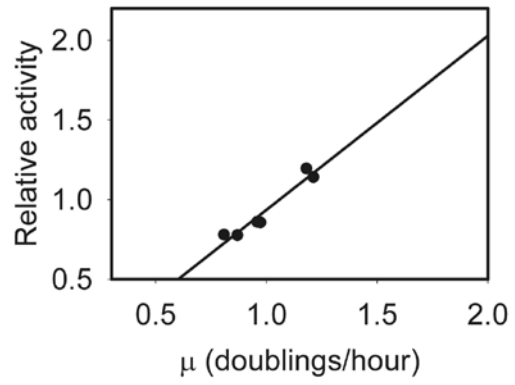


Supplementary Figure 2

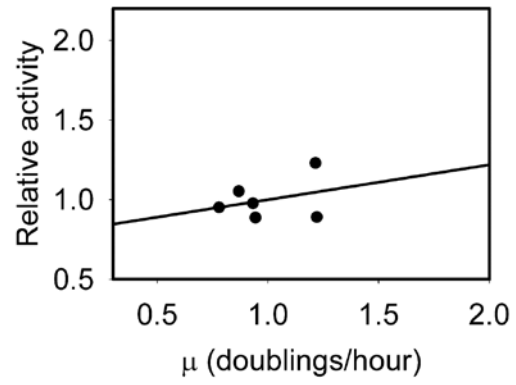


Supplementary Figure 3

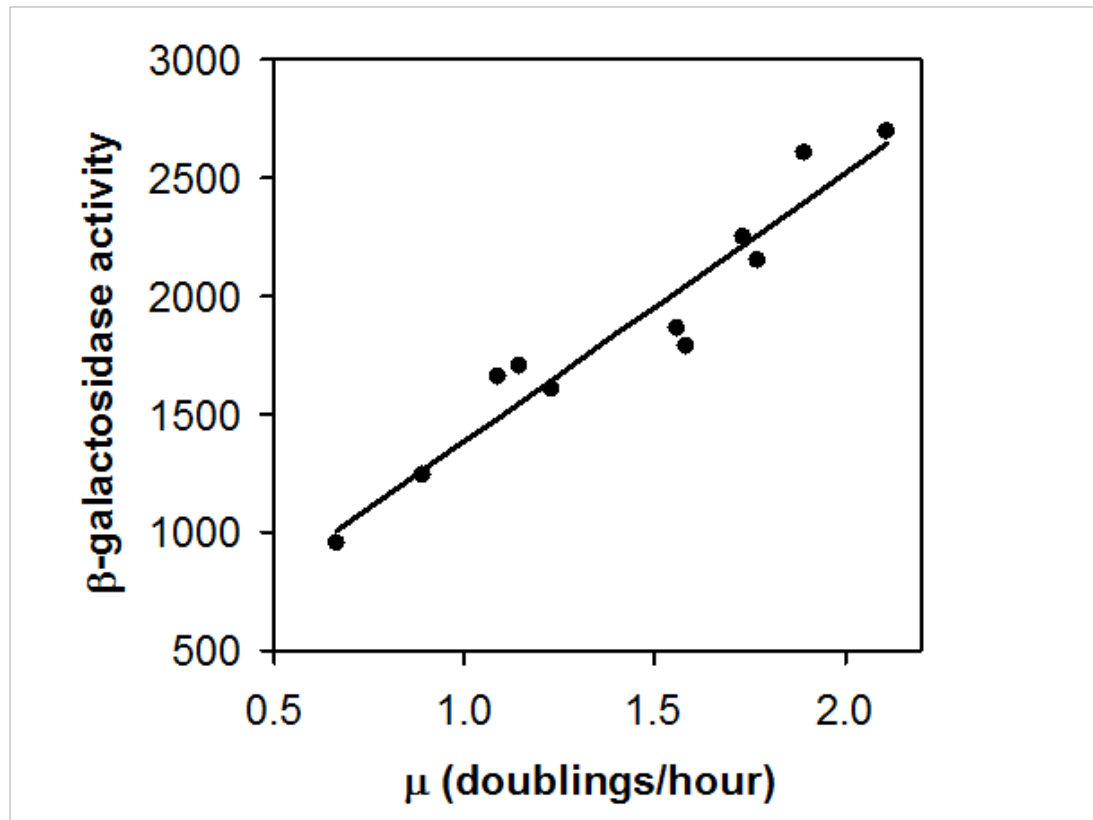
A P_{guaB} (-133 to +36)/ $\Delta cyaA$
+ cAMP



B P_{guaB} (-133 to +36, G-122C)/
 $\Delta cyaA$ + cAMP



Supplementary Figure 4



Medium	doublings/hour	β-gal activity
glycerol	0.66	951.35
fructose	0.89	1242.23
succinate + 20 amino acids	1.09	1661.10
glucose	1.15	1707.78
glycerol + 20 amino acids	1.23	1608.60
fructose + 20 amino acids	1.56	1861.07
glycerol + 1% casamino acids	1.58	1789.46
fructose + 1% casamino acids	1.73	2250.17
glycerol + 2% casamino acids	1.77	2151.64
glucose + 20 amino acids	1.89	2603.39
glucose + 0.8% casamino acids	2.11	2696.15

Supplementary TABLE 1. Oligonucleotide primers used for promoter construction

Primer	Sequence (5' to 3') ^a	Promoter constructed ^b
Forward primer		
P _{guaB} -253	gcgc <u>GAATTC</u> AGCTGGTTGCGTGAAATTAGA	P _{guaB} (-253 to +36)
		P _{guaB} (-253 to +10)
P _{guaB} -133	gcgc <u>GAATTC</u> AGGTAACATGTGAGCGAG	P _{guaB} (-133 to +36)
P _{guaB} -133G7C	gcgc <u>GAATTC</u> AGGTAACATGTCAGCGAGATCAAATTCTAA	P _{guaB} (-133 to +36, G-122C)
P _{guaB} -133A18C	gcgc <u>GAATTC</u> AGGTAACATGTGAGCGAGATCACATTCTAAATCAGCAG	P _{guaB} (-133 to +36, A-111C)
P _{guaB} -117	gcgc <u>GAATTC</u> AGATCAAATTCTAAATCAGCAG	P _{guaB} (-117 to +36)
P _{guaB} -37	gcgc <u>GAATTC</u> GACTGCAGTGGTACCTAGGAATGGTAGATGCAATCGGTTACG	P _{guaB} (-37 to +36)
P _{guaB} -133CRPUP	gcgc <u>GAATTC</u> ACATGTGAGCGAGATCAAATTCAGAGACTGTTCTAAATCAGCAGGTTA	P _{guaB} (CRP -128.5)
P _{guaB} -133CRPDN	gcgc <u>GAATTC</u> AGGTAACAGACTGGCGACATGTGAGCGAGATCAAATTCGTTATTCAGTCGATA GTA	P _{guaB} (CRP -106.5)
Reverse primer		
P _{guaB} +10	gcgc <u>AAGCTT</u> TAAATATTGCCGCGGCATTATA	P _{guaB} (253 to +10)
P _{guaB} +36	gcgc <u>AAGCTT</u> GGCAATATCTCGACCAGAG	All remaining constructs with endpoints at +36.

^aRestriction sites for *Eco*RI and *Hind*III are underlined. Oligonucleotides also contain a GC clamp immediately preceding a restriction site at the 5' end (shown in lower case).

^bRefer to Table 1 for information about promoters used in this work.