Homogeneous expression of the P_{BAD} promoter in *Escherichia coli* by constitutive expression of the low-affinity high-capacity AraE transporter

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Genes placed under the control of the arabinose-inducible *araBAD* promoter (P_{BAD}) of *Escherichia coli* are expressed in an all-or-none fashion, in which the percentage of induced cells in the population, rather than the degree of induction in individual cells, varies with the concentration of arabinose in the culture medium. Previous work showed that all-or-none gene expression from P_{BAD} was due to the arabinose-dependent expression of the gene encoding the low-affinity high-capacity transporter (*araE*), and that expression of heterologous genes from P_{BAD} in individual cells could be regulated by placing the *araE* gene under control of an arabinose-independent promoter. Based on these results, two expression systems were developed to allow regulatable control of genes under control of P_{BAD} . In one system, the native *araE* promoter on the chromosome was replaced by constitutive promoters of different strengths. In the second system, the *araE* gene under control of the same constitutive promoters was placed on a medium-copy plasmid. Both systems allow regulatable expression of a plasmid-borne P_{BAD} -controlled heterologous gene and a homogeneous population of cells over a wide range of arabinose concentrations. While the degree of induction varied slightly with the strength of the constitutive promoter, expression was affected most by the arabinose concentration.

Keywords: arabinose transport system, regulatable gene expression, Red recombination, GFP fusions, FACS analysis

INTRODUCTION

Since the first report of its use as an inducible expression system, the arabinose-inducible *araBAD* promoter (P_{BAD}) and regulator (AraC) of *Escherichia coli* have become popular for controlled gene expression in *E. coli* and other bacteria (Guzman *et al.*, 1995). Whether this system is used for control of a single gene or in conjunction with another inducible promoter for control of several genes, the *araC*– P_{BAD} system offers regulatable control of gene expression in the presence of inducer and tight control in the absence of inducer. This weak, yet tightly controlled promoter–regulator system has enabled simultaneous growth and production of both soluble and insoluble heterologous proteins in high-cell-density *E. coli* cultures (Lim *et al.*, 2000).

Recently, the *araC*– P_{BAD} system has been introduced into both Gram-positive and Gram-negative bacterial

hosts (Ben-Samoun *et al.*, 1999; Newman & Fuqua, 1999; Sukchawalita *et al.*, 1999). In *Agrobacterium tumefaciens*, the level of control afforded is significant, although less stringent than that observed in *E. coli* (Newman & Fuqua, 1999). In *Corynebacterium glutamicum*, P_{BAD} requires both arabinose and *araC*, indicating that *E. coli* AraC is capable of interacting with *C. glutamicum* RNA polymerase to induce transcription, but not the CRP protein, as it does in *E. coli* (Ben-Samoun *et al.*, 1999). Given this high degree of flexibility, this broad-host-range promoter is attractive for genetic and metabolic engineering of several different bacteria, since one could make a single genetic construct and use it in several organisms.

Unfortunately, the *araC*– P_{BAD} system and the associated high-capacity, low-affinity L-arabinose transporter AraE display autocatalytic behaviour and suffer from all-or-none expression in *E. coli* (Siegele & Hu, 1997). Rather

than varying the level of gene expression in individual cells of the culture, the concentration of arabinose in the medium changes the fraction of cells that are fully induced. Recently, we showed that expression of *araE* from an arabinose-independent (IPTG-inducible) promoter allows regulatable gene expression control from P_{BAD} in individual cells (Khlebnikov *et al.*, 2000). In this paper we show that expression of *araE* from constitutive promoters of various strengths on medium-copy plasmids or on the chromosome allows homogeneous expression from P_{BAD} and that the level of *araE* expression affects the level of expression from P_{BAD} at a given inducer concentration.

METHODS

General. Bacteria and vectors are listed in Table 1. Each of the three pCP vectors (pCP8, pCP13 and pCP18) contains a constitutive promoter of different strength from *Lactococcus lactis* (Jensen & Hammer, 1998a, b). All DNA manipulations were performed in *E. coli* DH10B using established protocols (Sambrook *et al.*, 1989) or as indicated below. PCR amplification of DNA was done using the Expand High Fidelity PCR System (Roche Molecular Biochemicals) under the conditions recommended by the manufacturer. Oligonucleotides were synthesized by Genemed Synthesis. The restriction digests and ligation reactions were performed as recommended by the restriction enzyme manufacturer (Roche Molecular Biochemicals). The ligated vectors were transformed into electrocompetent cells (*E. coli* DH10B or *E. coli* CW2587) by electroporation (field strength 18 kV cm^{-1}) using a Bio-Rad *E. coli* Pulser.

Plasmid-borne constitutive promoters. To construct the pJAT plasmids, an *XbaI*–*XbaI* fragment (1981 bp for P_{CP8} and P_{CP13} , 1982 bp for P_{CP18}) containing the corresponding constitutive promoter region and the erythromycin-resistance gene was subcloned from the pCP plasmids into the *XbaI* restriction site on pBluescript SK+. The resulting vectors were digested with *ClaI* (a site for which was located between the *XbaI* restriction site and the erythromycin-resistance gene on the constitutive promoter–erythromycin DNA fragment) and *EcoRI* (which cuts in the multi-cloning site of pBluescript SK+ opposite the constitutive promoter–erythromycin DNA fragment), thereby generating smaller fragments that contained the constitutive promoters and erythromycin-resistance gene (1911 bp for P_{CP8} and P_{CP13} , 1912 bp for P_{CP18}) and with appropriate restriction sites at either end for cloning into plasmid pJN105. These *ClaI*–*EcoRI* fragments were ligated to the 4751 bp *ClaI*–*EcoRI* fragment of the broad-host-range, medium-copy-number plasmid pJN105, containing the gentamicin-resistance gene and the pBBR-1 origin of replication. The resulting plasmids were designated pJAT8, pJAT13 and pJAT18.

The *araE* gene was amplified from genomic DNA of *E. coli* W3110 using PCR and the primers for the 5'-end of the gene (5'-CGTGAATTCGTCTTACTCTCTGTCGGCAG-3') and the 3'-end of the gene (5'-CTACGATCGAACGGCC-AAGTGCCCAATCT-3'), and then digested with *EcoRI* and *PvuI*. The medium-copy number vectors pJAT8, pJAT13 and pJAT18 were digested with *EcoRI* and *PvuI*, and ligated with the *EcoRI*–*PvuI* PCR fragment, resulting in plasmids pJAT8*araE*, pJAT13*araE* and pJAT18*araE*.

Construction of strains that express *araE* constitutively from the chromosome. *E. coli* encodes both a high-affinity arabinose transporter (encoded by the *araFGH* operon) and a

low-affinity arabinose transporter (encoded by *araE*) whose synthesis is inducible by arabinose. In order to uncouple the expression of these transporters from this autocatalytic behaviour, new *E. coli* strains were constructed in which the *araFGH* genes are deleted and *araE* is constitutively expressed. Both of these modifications were facilitated by using Red technology (Datsenko & Wanner, 2000) and appropriate PCR products. The DE(*araFGH*) mutation was made by synthesizing a PCR product on pKD83 as template with the primers 5'-TGCACGTTCTCACTGTAATTCTGCGATGTGATA-TTG/CACGTCTTGAGCGATTGTGT-3' and 5'-GAAAA-AACGCTAAATTGTTGCAGAAAAAGCATCAG/ATT-CCGGGGATCCGTCGACC-3', in which bases preceding a slash correspond to homology extensions (H1 or H2) for corresponding priming sites (P1 or P4) as shown in Fig. 1. pKD83 is similar to pKD13 (Datsenko & Wanner, 2000), except pKD83 has the kanamycin resistance gene (*kan903*) from Tn903 (K. A. Datsenko & B. L. Wanner, unpublished results). The DE(*araFGH*) mutation was recombined into the chromosome and verified as described in Fig. 1. Strains carrying this deletion with or without the *kan903* gene are described in Table 1.

pKD85 (P_{CP8} –*araE'*), pKD86 (P_{CP18} –*araE'*) and pKD118 (P_{CP13} –*araE'*) were used as templates with the primers 5'-TTTATCTGCTGTAAAATTAGGTGGTTAATAATAA-TCGTGTAGGCTGGAGCTGCTTC and 5'-ATATTCAT-ACGCCGCTATC-3' to recombine these fusions onto the chromosome. The latter primer corresponds to *araE* sequences in common with these template plasmids and the chromosome (Fig. 2). These plasmids were constructed by synthesizing approximately 850 bp PCR products on the respective pJAT plasmid with the primers 5'-TCAACTGCCTGGCACAAT-3' and 5'-TTCCGCCTCAATATGACG-3'. These PCR products were digested with *XhoI* and *BclI* to release internal promoter-containing fragments of approximately 600 bp, which were then cloned into *XhoI*- and *BamHI*-digested pKD12. The latter plasmid is similar to pKD13 (Datsenko & Wanner, 2000; K. A. Datsenko & B. L. Wanner, unpublished results). The P_{CP8} –, P_{CP13} – and P_{CP18} –*araE'* fusions were recombined into the chromosome of BW25113 by using the Red plasmid pKD46 and selecting Km^R transformants as described elsewhere (Datsenko & Wanner, 2000). The resultant recombinants (BW27270, BW27535 and BW27271) were verified as shown in Fig. 2. Derivatives of the *E. coli* K-12 strain BW25113 carrying both the DE(*araFGH*) mutation and a P_{CP8} –, P_{CP13} – or P_{CP18} –*araE* fusion were constructed by transduction with P1*k*c (Wanner, 1994), resulting in strains BW27749, BW27752 and BW27750. All transductants were similarly verified by PCR before and after FLP-mediated elimination of the kanamycin-resistance genes (BW27783, BW27786 and BW27784). DNA sequence analysis revealed that the CP18 promoter upstream region has two adjacent *BamHI* sites, which were apparently introduced during its original construction (Jensen & Hammer, 1998a). Otherwise, the sequences of the P_{CP8} –, P_{CP13} – and P_{CP18} –*araE* fusions after recombination onto the chromosome and elimination of the resistance marker were as predicted (Fig. 3).

Cell growth and induction studies. Induction studies with arabinose were performed in C medium (Helmstetter, 1968) with 3.4 % (v/v) glycerol as carbon source. Antibiotics were added to the following concentrations: ampicillin, $100 \mu\text{g ml}^{-1}$; chloramphenicol, $34 \mu\text{g ml}^{-1}$; erythromycin and gentamicin, $20 \mu\text{g ml}^{-1}$. *E. coli* CW2587 was grown overnight at 37°C in an air shaker without arabinose to an optical

Table 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype and characteristics*	Reference or source
<i>E. coli</i>		
W3110	λ^- , IN(<i>rrnD</i> – <i>rrnE</i>)1 <i>rph</i> -1	<i>E. coli</i> Genetic Stock Center (Yale University, New Haven, CT)
DH10B	F ⁻ <i>mcrA</i> Δ (<i>mrr</i> – <i>hsdRMS</i> – <i>mcrBC</i>) ϕ 80d <i>lacZ</i> Δ M15 <i>AlacX74 deoR</i> <i>recA1 endA1 araD139</i> Δ (<i>ara</i> , <i>leu</i>)7697 <i>galU galK1 rpsL nupG</i>	Life Technologies
CW2587	<i>araE201</i> Δ <i>araFGH::kan</i> , <i>srl::Tn10 recA59</i>	Horazdovsky & Hogg (1989)
BW25113	<i>lacI^a rrnB3</i> Δ <i>lacZ4787</i> <i>hsdR514</i> DE(<i>araBAD</i>)567 DE(<i>rhaBAD</i>)568	Datsenko & Wanner (2000)
BW25141	<i>lacI^a rrnB3</i> Δ <i>lacZ4787</i> Δ <i>phoBR580</i> <i>hsdR514</i> DE(<i>araBAD</i>)567 DE(<i>rhaBAD</i>)568 <i>galU95</i> Δ <i>endA9 uidA</i> (Δ <i>MluI</i>):: <i>pir</i> (wt) <i>recA1</i>	Datsenko & Wanner (2000)
BW27269	BW25113 DE(<i>araFGH</i>):: <i>kan903</i>	Km ^R with pKD46 and pKD81 PCR product
BW27270	BW25113 Φ (Δ <i>araEp kan P</i> _{CP8} – <i>araE</i>)	Km ^R with pKD46 and pKD85 PCR product
BW27271	BW25113 Φ (Δ <i>araEp kan P</i> _{CP18} – <i>araE</i>)	Km ^R with pKD46 and pKD86 PCR product
BW27378	BW25113 DE(<i>araFGH</i>)	BW27269 Km ^S with pCP20
BW27379	BW25113 Φ (Δ <i>araEp P</i> _{CP8} – <i>araE</i>)	BW27270 Km ^S with pCP20
BW27380	BW25113 Φ (Δ <i>araEp P</i> _{CP18} – <i>araE</i>)	BW27271 Km ^S with pCP20
BW27535	BW25113 Φ (Δ <i>araEp kan P</i> _{CP13} – <i>araE</i>)	Km ^R with pKD46 and pKD118 PCR product
BW27536	BW25113 Φ (Δ <i>araEp P</i> _{CP13} – <i>araE</i>)	BW27535 Km ^S with pCP20
BW27749	BW25113 DE(<i>araFGH</i>) Φ (Δ <i>araEp kan P</i> _{CP8} – <i>araE</i>)	BW27378 Km ^R with P1kc on BW27270
BW27750	BW25113 DE(<i>araFGH</i>) Φ (Δ <i>araEp kan P</i> _{CP18} – <i>araE</i>)	BW27378 Km ^R with P1kc on BW27271
BW27752	BW25113 DE(<i>araFGH</i>) Φ (Δ <i>araEp kan P</i> _{CP13} – <i>araE</i>)	BW27378 Km ^R with P1kc on BW27535
BW27783	BW25113 DE(<i>araFGH</i>) Φ (Δ <i>araEp P</i> _{CP8} – <i>araE</i>)	BW27749 Km ^S with pCP20
BW27784	BW25113 DE(<i>araFGH</i>) Φ (Δ <i>araEp P</i> _{CP18} – <i>araE</i>)	BW27750 Km ^S with pCP20
BW27786	BW25113 DE(<i>araFGH</i>) Φ (Δ <i>araEp P</i> _{CP13} – <i>araE</i>)	BW27752 Km ^S with pCP20
Plasmids		
pBluescriptSK + pCSAK50	ColE1, <i>lacZ</i> , Ap ^r	Stratagene
pCP8	pTC40, P _{BAD} – <i>gfpuv</i> , Ap ^r	Khlebnikov <i>et al.</i> (2000)
pCP13	pAK80, P _{CP8} , Em ^r	Jensen & Hammer (1998a)
pCP18	pAK80, P _{CP13} , Em ^r	Jensen & Hammer, (1998a)
pCP20	pAK80, P _{CP18} , Em ^r	Jensen & Hammer, (1998a)
pJN105	pSC101(Ts ⁻), Ap ^r Cm ^r , cl857 P _r –FLP	Cherepanov & Wackernagel (1995)
pJAT8	pBRR-1, <i>araC</i> –P _{BAD} , Gm ^r	Newman & Fuqua (1999)
pJAT13	pJN105, P _{CP8} , Gm ^r Em ^r	This study
pJAT18	pJN105, P _{CP13} , Gm ^r Em ^r	This study
pJAT18 <i>araE</i>	pJN105, P _{CP18} , Gm ^r Em ^r	This study
pJAT8 <i>araE</i>	pJAT8, P _{CP8} – <i>araE</i> , Gm ^r Em ^r	This study
pJAT13 <i>araE</i>	pJAT13, P _{CP13} – <i>araE</i> , Gm ^r Em ^r	This study
pJAT18 <i>araE</i>	pJAT18, P _{CP18} – <i>araE</i> , Gm ^r Em ^r	This study
pKD12	<i>oriRγ</i> Ap ^r Km ^r	K. A. Datsenko & B. L. Wanner, unpublished
pKD13	<i>oriRγ</i> Ap ^r Km ^r	Datsenko & Wanner (2000)
pKD46	pSC101(Ts ⁻) Ap ^r <i>araC</i> ⁺ P _{BAD} –Red	Datsenko & Wanner (2000)
pKD81	<i>oriRγ</i> Ap ^r Km ^r	K. A. Datsenko & B. L. Wanner, unpublished
pKD85	<i>oriRγ</i> Ap ^r Km ^r P _{CP8} – <i>araE</i> '	This study
pKD86	<i>oriRγ</i> Ap ^r Km ^r P _{CP18} – <i>araE</i> '	This study
pKD118	<i>oriRγ</i> Ap ^r Km ^r P _{CP13} – <i>araE</i> '	This study

* Ap^r, ampicillin resistance; Cm^r, chloramphenicol resistance; Em^r, erythromycin resistance; Gm^r, gentamicin resistance; Km^r, kanamycin resistance.

Fig. 1. Construction of the DE(*araFGH*) mutation. An 1163 bp PCR product containing 36 bp homology extensions (H1 and H2) to the chromosomal *araFGH* region (Blattner *et al.*, 1997) was introduced into BW25113 carrying pKD46 as described elsewhere (Datsenko & Wanner, 2000). pKD46 encodes the Red recombinase from phage λ which promotes highly efficient homologous recombination. In these crosses, recombination occurs between the homologous sequences on the ends of the PCR products and the bacterial chromosome (Datsenko & Wanner, 2000). Km^R transformants were PCR-verified by testing for presence of novel junction fragments of expected sizes when using priming (p) sites that flank the homology regions (BW27269). The *kan903* gene was then eliminated by site-specific recombination by using the Flp plasmid pCP20 (Cherepanov & Wackernagel, 1995), as described previously (Datsenko & Wanner, 2000). The resultant Km^S recombinant (BW27378) was similarly verified by PCR. *kan903* primers: k4 (5'-ACCAGGATC-TTGCCATCCTA-3') and k3 (5'-TAATCGCGCC-TCGAGCAAG-3'). *araF* upstream primer: 5'-GCATTCCAGCGTTGCCATA-3'. *araH* downstream primer: 5'-CGGCAGATGAACGATATG-TG-3'. Priming sites 1 and 4 are as described elsewhere (Datsenko & Wanner, 2000).

Fig. 2. Construction of chromosomal P_{CP8}-, P_{CP13}- and P_{CP18}-*araE* fusions. The construction of the chromosomal P_{CP8}-*araE* fusion strain is illustrated. The P_{CP13}- and P_{CP18}-*araE* fusions on pKD86 and pKD118 were similarly recombined onto the chromosome. The resulting strains are BW27270, BW27535 and BW27271. See Methods for details. These Km^R recombinants and the corresponding Km^S recombinants (BW27379, BW27536 and BW27380) were verified by testing for the presence of novel junction fragments of expected sizes before and after elimination of the kanamycin-resistance gene as described elsewhere. *araE* upstream primer (*araE* up): 5'-CATGGCGAC-CAACAATACTC-3'. *araE* downstream primer: 5'-TTCCGCCTCAATATGACG-3'. Primers k1 and k2 and priming site 1 are as described previously (Datsenko & Wanner, 2000).

density at 600 nm (OD₆₀₀) of 0.6–0.8. The cells were collected by centrifugation (5 min, 15 000 g) and resuspended in fresh C medium with antibiotics to an OD₆₀₀ of 0.1–0.2. Arabinose was added (at time 0 in all plots) to different concentrations, and 1 ml samples were taken at 2 h intervals for analysis.

Culture OD₆₀₀ was measured in a Beckman DU 640 spectrophotometer (Beckman Instruments) and fluorescence was measured in a Versafluor Fluorimeter (Bio-Rad) with

360/40 nm excitation and 510/10 nm emission filters. Flow cytometry was performed on a Beckman-Coulter EPICS XL flow cytometer (Beckman Instruments) equipped with an argon laser (emission at 488 nm/15 mW) and a 525 nm band pass filter. The sampled cells were diluted to an OD₆₀₀ of 0.05–0.1 and kept on ice prior to analysis. For each sample, 30 000 events were collected at a rate between 500 and 1000 events per second.

CP8-*araE* fusion

ttaggtggttaataataatcgtgtaggctggagctgcttcgaagttcctatactttctag
 agaatagggaacttcgaactgcagaaattgtgagcgctcacaattggctagctcgagatct
 gcaggatcccatatgcatctttagtttattcttgacaaacgtattgaggactgatata
 ataggtagtactgttgggtctagaactagtgatccccgggctgcaggaattcgtctt
 actctctgctggcaggaataatg

CP13-*araE* fusion

ttaggtggttaataataatcgtgtaggctggagctgcttcgaagttcctatactttctag
 agaatagggaacttcgaactgcagaaattgtgagcgctcacaattggctagctcgagatct
 gcaggatcccatatgcatctttagtttattcttgacaaacccaccagcttttgggtata
 atacgtgagaactgttgggtctagaactagtgatccccgggctgcaggaattcgtctt
 actctctgctggcaggaataatg

CP18-*araE* fusion

ttaggtggttaataataatcgtgtaggctggagctgcttcgaagttcctatactttctag
 agaatagggaacttcgaactgcagaaattgtgagcgctcacaattggctagctcgagatct
 gcaggatcccgatccttagcattttgagtttattcttgacattgtgtgcttcgggtgt
 ataatactaaagtactgttgggtctagaactagtgatccccgggctgcaggaattcgtc
 ttagctctgctggcaggaataatg

Fig. 3. Sequences of the P_{CP8} -, P_{CP13} - and P_{CP18} -*araE* fusions. The respective promoter regions were PCR-amplified from the chromosomes of BW27379, BW27380 and BW27536 and analysed by automated DNA sequencing with the flanking primers (*araE* up, Fig. 2; and 5'-TATTCATACGCCGCGTATCC-3') in the Microbiology and Molecular Genetics Core Facility at Harvard Medical School. The entire region recombined onto the chromosome in the original PCR (Fig. 2), along with approximately 200 bp of flanking DNA, was analysed. Only the relevant segments are shown. No mutation was detected in regions not shown. Priming site 1 (Datsenko & Wanner, 2000) sequences are in bold and overlined; FRT sites are in italics; the CP8, CP13 and CP18 promoter sequences are underlined. The first 20 and last three nucleotides correspond to the *kdsD-araE* intergenic region and the start codon of *araE*, respectively.

RESULTS

Previously, we showed that independent expression of *araE* in arabinose-transport-deficient strains led to homogeneous *gfpuv* expression from the P_{BAD} promoter (Khlebnikov *et al.*, 2000). For induction of the P_{BAD} promoter a threshold internal arabinose concentration is necessary, and that intracellular arabinose concentration is related to the extracellular arabinose concentration and the arabinose transport capacity of the cell. In order to examine the influence of arabinose concentration and amount of arabinose permease on expression from P_{BAD} we constructed a series of plasmids with constitutive promoters of different strengths, allowing us to vary the amount of permease.

Expression of *araE* from plasmid-borne P_{CP} promoters

Given the results from the previous experiments (the strength of the promoter controlling *araE* appears to affect culture homogeneity) flow cytometry experiments were conducted to examine the effect of *araE* expression from various constitutive promoters on gene expression from the arabinose-dependent P_{BAD} promoter. These experiments were performed in the arabinose-transport-deficient strain *E. coli* CW2587 containing the arabinose-transport gene *araE* on the pJAT vectors (P_{CP} -*araE*) and *gfp* under control of P_{BAD} on the high-copy plasmid pCSAK50 (P_{BAD} -*gfpuv*).

All cultures containing the pJAT*araE* plasmids were

Plasmid-borne *araE*Chromosomal *araE*

Fig. 4. Fluorescence intensity of *E. coli* harbouring pCSAK50 (*araC-P_{BAD}-gfpuv*) and the plasmid-borne (left column) or the chromosomally integrated (right column) constitutive promoters controlling *araE*. The top plot in the right column is the fluorescence intensity for *E. coli* BW27378 (*araE* under control of its native promoter on the chromosome) harbouring pCSAK50. In the second row, the first plot is that for *E. coli* CW2587 harbouring pCSAK50 and pJAT18*araE* and the second plot is that for *E. coli* BW27784 harbouring pCSAK50; row 3 is CW2587 pCSAK50 pJAT13*araE* and BW27786 pCSAK50; and row 4 is CW2587 pCSAK50 pJAT8*araE* and BW27783 pCSAK50. The fluorescence intensity of individual cells was measured by flow cytometry 6 h after addition of arabinose at the indicated concentrations: grey-shaded curve, 0%; orange curve, 2×10^{-5} %; green curve, 2×10^{-4} %; red curve, 2×10^{-3} %; blue curve, 2×10^{-2} %; brown curve, 2×10^{-1} %; purple curve, 2%.

induced homogeneously (Fig. 4). The culture-averaged fluorescence (fluorescence/ OD_{600}) was highest with P_{CP18} but lower and approximately equal for P_{CP8} and P_{CP13} at all inducer concentrations (Fig. 5a). All cultures except CW2587 harbouring pJAT18*araE* grew at approximately the same rate. Because CW2587 harbouring pJAT18*araE* grew much more slowly and reached a lower final density than all other CW2587 cultures the culture-averaged fluorescence was higher for that culture. At the highest arabinose concentration (2%) a decline in the culture-averaged fluorescence was

Fig. 5. Culture-averaged fluorescence (fluorescence/OD₆₀₀) of *E. coli* harbouring the plasmid-borne (a) or the chromosomally integrated (b) constitutive promoters as a function of the inducer concentration. All cultures harboured pCSAK50 (*araC*-P_{BAD}-*gfpuv*). Six hours after addition of arabinose, the culture-averaged fluorescence was determined. Data were corrected for the background fluorescence displayed by control cultures. (a) *E. coli* CW2587 harbouring pCSAK50 and pJAT8araE (black), pJAT18araE (vertical lines) and pJAT13araE (grey). (b) *E. coli* BW27783 (P_{CP8}-araE) harbouring pCSAK50 (black), BW27784 (P_{CP18}-araE) harbouring pCSAK50 (vertical lines), BW27786 (P_{CP13}-araE) harbouring pCSAK50 (grey) and BW27378 (P_E-araE) harbouring pCSAK50 (white).

observed, suggesting that the P_{BAD} promoter was saturated. All control cultures without a functional arabinose transport system displayed a single non-fluorescent population and were not able to grow on arabinose as a carbon source.

Expression of *araE* from P_{CP} promoters in single copy on the chromosome

The constitutive promoters contained on the pJAT plasmids (pCP8, pCP13 and pCP18) were recombined onto the chromosome of the *araFGH* strain BW27378 replacing the arabinose-responsive *araE* promoter. The resulting strains, BW27783 (P_{CP8}-araE), BW27784 (P_{CP18}-araE) and BW27786 (P_{CP13}-araE), as well as the parental strain were transformed with pCSAK50 and induced with various concentrations of arabinose. The homogeneity of induction was measured using flow cytometry.

All cultures containing the chromosomally integrated P_{CP}-araE were homogeneously induced (Fig. 4, bottom

three plots of right column), whereas the parental strain displayed a double population (Fig. 4, top plot of right column). The culture-averaged fluorescence increased with inducer concentration (Fig. 5b), although the difference between the induction at high and low inducer concentrations was less than with the plasmid-borne, constitutively expressed transport genes. In contrast to the strains bearing the pJATaraE plasmids, the culture-averaged fluorescence was highest in cells carrying the chromosomal P_{CP8}, was lower for P_{CP18}, and was lowest for P_{CP13}. Since these experiments were carried out using different strains than those above, differences are probably attributable to strain background or increased stability of the chromosomal constructs.

DISCUSSION

Previously, we have shown that providing arabinose-transport-deficient cells with a plasmid-borne *araE* gene under control of an IPTG-inducible promoter resulted in a homogeneous population of cells expressing the P_{BAD} promoter at all arabinose concentrations (Khlebnikov *et al.*, 2000). As there are many applications for which one may want more than one inducible promoter to control expression of multiple genes, using P_{tac} to control expression of *araE* prevented its use to control expression of another gene. The arabinose-inducible P_{BAD} and the IPTG-inducible P_{lac} (or P_{tac} or P_{trc}) promoters are convenient because they are readily controllable and well characterized. The expression vectors and hosts described here eliminate the all-or-none induction behaviour of P_{BAD} while freeing the *lac* promoter for use with another gene of interest.

Expression of the gene encoding the low-affinity, high-capacity arabinose permease from constitutive promoters eliminated all-or-none induction of P_{BAD}. In general, the level of induction from the P_{BAD} promoter varied most with the concentration of inducer in the medium and slightly with the constitutive promoter strength controlling the arabinose transport gene, whether expressed from the medium-copy plasmids or from the single-copy chromosome. A relatively linear response in P_{BAD} induction was observed over a 1000-fold range of inducer concentration.

These constructs and strains should prove useful for controlled production of regulatory proteins, where a consistent and regulatable response from all cells in a culture is desired, or for expression of genes involved in the synthesis of a secondary metabolite, where under- or overexpression of a given pathway could lead to inefficient production of the desired metabolite.

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