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Modulation of gene expression from the arabinose-inducible *araBAD* promoter

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The arabinose-inducible P_{BAD} promoter suffers from all-or-none gene expression in which cells harboring the natively controlled arabinose transport gene (*araE*) are either induced or uninduced, the relative fraction of which is controlled by the concentration of arabinose. The population-averaged variation in expression from P_{BAD} as a function of inducer concentration is proportional to the percentage of cells that are fully induced (vs. uninduced) rather than the level of expression in individual cells. Because of its undesirable effects on the expression of heterologous genes, the all-or-none phenomenon was eliminated in *Escherichia coli* by expression of *araE* from arabinose-independent (either the *Lactococcus lactis* constitutive or IPTG-inducible *lac*) promoters. In these arabinose-transport engineered cells, variation in P_{BAD} expression with arabinose concentration was a result of variation of the expression level in individual cells with all cells in the population having approximately the same induction level.
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Keywords: arabinose; constitutive promoter; *Escherichia coli*; regulatable control; arabinose transport

Introduction

The arabinose-inducible *araBAD* promoter (P_{BAD}) has been used extensively for expression of heterologous genes in *Escherichia coli* and other hosts [1,4,12,15,16,18-20]. In its simplest form, P_{BAD} and the divergently transcribed *araC*, which encodes the AraC regulatory protein, are placed in an expression vector and transformed into a host capable of transporting arabinose into the cell [2]. When arabinose is added to the medium and transported into the cell, it binds to AraC, which in turn binds to the $araI_1$ and $araI_2$ sites between P_{BAD} and P_C [17]. In the absence of arabinose, the AraC represses transcription from P_{BAD} and P_C. This mechanism allows the *araC*-P_{BAD} expression systems to provide: (i) a relatively low level of background expression in the absence of arabinose and (ii) modulation of the expression level by addition of L-arabinose. In a similar manner, AraC regulates transcription from the *araE* promoter (P_E) and production of the low-affinity, high-capacity AraE arabinose transporter.

The tight control offered by P_{BAD} and P_E results in a phenomenon (all-or-none gene expression) that may not be desirable for the expression of heterologous genes. When concentrations of arabinose intermediate between zero and that necessary for maximal induction are used to induce expression, a fraction of the cells become fully induced and the remainder are uninduced. One hypothesis for why this phenomenon occurs is that those cells that encounter inducer and are capable of transporting the inducer into the cell are able to induce expression from P_{BAD} and P_E, resulting in more AraE, more arabinose inside the cell, and even higher expression from P_{BAD} and P_E; those cells that are not able to transport enough inducer to initiate expression from P_{BAD} and P_E remain uninduced. Recently, we demonstrated that expression of *araE* from an arabinose-independent promoter resulted in a homogeneous population of cells at all inducer concentrations and regulatable promoter control in each cell of the population [10].

In this paper we report on the development of several host/transporter systems that allow homogeneous expression from P_{BAD} and tight control over gene expression.

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Materials and methods

The bacteria and vectors used are summarized in Table 1. All DNA manipulations were performed in *E. coli* DH10B using established protocols [13] or as indicated below. Polymerase chain reaction (PCR) amplification of DNA was performed by using the Expand™ high-fidelity PCR system (Roche Molecular Biochemicals, Indianapolis, IN) under conditions recommended by the manufacturer. Sequencing and DNA oligonucleotide primer synthesis were preformed by Genemed Synthesis (South San Francisco, CA). 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, *E. coli* CW2513 or *E. coli* CW2587) by electroporation (field strength 18 kV/cm) using a Bio-Rad *E. coli* Pulser™ (Bio-Rad, Hercules, CA).

Constitutive promoters of different strengths from *Lactococcus lactis* [7,8] were subcloned from the original vectors (pCP8, pCP13, and pCP18) onto the broad-host-range, low-copy-number plasmid pJN105, containing the gentamycin resistance gene and the pBBR-1 origin of replication [9]. The resulting plasmids were designated pJAT8, pJAT13, and pJAT18. The *araE* gene was amplified from genomic DNA of *E. coli* W3110 using PCR and placed on pJAT8, pJAT13, and pJAT18 resulting in plasmids pJAT8*araE*, pJAT13*araE*, and pJAT18*araE*, respectively [9] or on the medium-copy number vectors pMMB206 and

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

Strain or plasmid	Genotype and characteristics	Reference or source
E. coli		
DH10B	F$^-$-*mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) ϕ80d*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *end*A1 *ara*D139 Δ*(ara, leu) 7697 gal*U *gal*K1 *rps*L *nup*G	Life Technologies (Gaithersburg, MD)
CW2513	Wild type	[6]
CW2587	CW2513 *araE*201, Δ*araFGH:kan*, *srl*Tn10 *rec*A59	[6]
Plasmids		
pMMB206	RSF1010, $lacI^q/P_{taclacUV5}$, Cm^r, broad-host-range	[11]
pMMB207	RSF1010, $lacI^q/P_{tac}$, Cm^r, broad-host-range	[11]
pAK01	pMMB206, $P_{taclacUV5}$*araE,* Cm^r	[10]
pAK02	pMMB207, P_{tac}*araE,* Cm^r	[10]
pCSAK50	pTC40, P_{BAD}*gfpuv,* Ap^r	[10]
pJAT8	pJN105, P_{CP8}, Gm^r, Erm^r	[9]
pJAT13	pJN105, P_{CP13}, Gm^r, Erm^r	[9]
pJAT18	pJN105, P_{CP18}, Gm^r, Erm^r	[9]
pJAT8*araE*	pJAT8, P_{CP8}*araE*, Gm^r, Erm^r	[9]
pJAT13*araE*	pJAT13, P_{CP13}*araE*, Gm^r, Erm^r	[9]
pJAT18*araE*	pJAT18, P_{CP18}*araE*, Gm^r, Erm^r	[9]

Ap^r, ampicillin resistance; Cm^r, chloramphenicol resistance; Erm^r, erythromycin resistance, Gm^r, gentamycin resistance.

pMMB207 resulting in plasmids pAK01 and pAK02, respectively [10].

Induction studies were performed in C medium (3 g/l KH_2PO_4, 6 g/l Na_2HPO_4, 3 g/l NaCl, 2 g NH_4Cl, 0.25 g/l $MgSO_4$) [5] with 3.4% glycerol as carbon source. Antibiotics were added to yield the following concentrations: ampicillin, 100 μg/ml; chloramphenicol, 34 μg/ml; erythromycin and gentamycin, 20 μg/ml. *E. coli* CW2513, CW2587, or DH10B was grown overnight at 37°C in an air shaker without arabinose to an optical density at a wavelength of 600 nm (OD_{600}) of 0.6–0.8. Cells were collected by centrifugation (5 min, 15,000×*g*) and resuspended in fresh C medium with antibiotics to an OD_{600} of 0.1–0.2. Arabinose was added to different concentrations (time=0 in all time-course induction studies), and 1-ml samples were taken at 2-h intervals for analysis.

Culture density (OD_{600}) was measured, and culture-averaged fluorescence was measured on a Versafluor™ fluorimeter (Bio-Rad) with 360/40 nm excitation and 510/10 nm emission filters. Single-cell fluorescence was determined using a Beckman-Coulter EPICS XL flow cytometer (Beckman Instruments, Palo Alto, CA) 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 OD_{600} 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/s.

Results

Previously, we showed that independent expression of *araE* in arabinose transport-deficient strains led to homogeneous expression of *gfpuv*, the gene encoding the UV-excitable green fluorescent protein, from the P_{BAD} promoter [10]. 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 different strength constitutive and IPTG-inducible promoters allowing us to vary the amount of arabinose permease and subsequently the level of arabinose inside the cells.

Expression of araE from P_{tac}/$P_{taclacUV5}$ promoters

To demonstrate that arabinose-independent control of *araE* would allow homogeneous induction of expression from P_{BAD}, *araE* was placed under control of P_{tac} and $P_{taclacUV5}$ on the medium copy, broad-host-range pMMB plasmids and cotransformed with pCSAK50 (P_{BAD}*gfpuv*). In general, those cells that were induced reached their highest level of induction after 6 h of incubation, and the percentage of cells that became fully induced

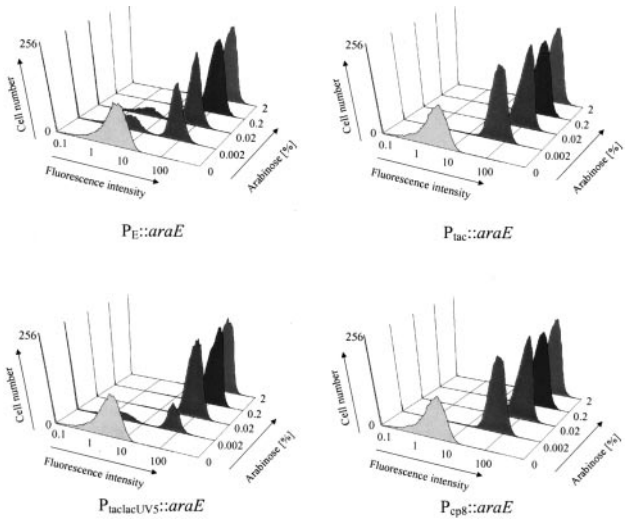

Figure 1 Histograms showing the number of cells with a given fluorescence as a function of the arabinose concentration in cultures with *araE* under control of different promoters. All cultures harbored pCSAK50 (*araC*-P_{BAD}*gfpuv*). Wild-type *E. coli* CW2513 with the natively controlled *araE* gene (P_E*araE*) harboring pMMB207 was used as a control strain. Six hours after addition of arabinose, the fluorescence in single cells was determined. The population distribution observed from cells harboring pJAT13*araE* or pJAT18*araE* was identical to that from cells harboring pJAT8*araE* (data not shown).

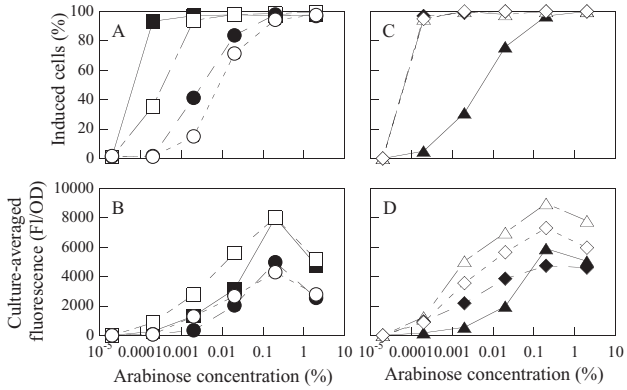

Figure 2 The percentage of the culture that was induced (A, C) and the population-averaged fluorescence (B, D) as a function of the arabinose concentration 6 h after induction. (A, B) Circles, *E. coli* CW2587 pCSAK50 (*araC*-P_{BAD}*gfpuv*) pAK01 ($P_{taclacUV5}$*araE*). Squares, *E. coli* CW2587 pCSAK50 pAK02 (P_{tac}*araE*) (data taken from Ref. [10]). Filled symbols, induced with 32 μg/ml IPTG. Open symbols, no ITPG present. (C, D) Filled diamonds, *E. coli* CW2587 pCSAK50 pJAT18*araE* (P_{cp18}*araE*). Open diamonds, *E. coli* CW2587 pCSAK50 pJAT8*araE* (P_{cp8}*araE*). Filled triangles, *E. coli* CW2587 pCSAK50 pJAT13*araE* (P_{cp13}*araE*). Open triangles, *E. coli* CW2513 with the native-controlled *araE* gene (P_E*araE*).

at 6 h varied with arabinose concentration and the type (or strength) of the promoter controlling *araE*. For expression of *araE* under control of P_{tac}, the culture-average fluorescence varied with the arabinose concentration both with and without IPTG induction of *araE* (Figures 1 and 2). In the absence of IPTG, the percentage of induced cells in the population varied only with the lowest arabinose concentration (less than 0.002%); at 0.0002% arabinose, only 40% of cells displayed fluorescence above background 6 h after addition of arabinose. A different trend was observed with the weaker $P_{taclacUV5}$. At low arabinose concentrations, only a small fraction of the cells was induced. At all arabinose concentrations, the proportion of induced cells in the population was slightly higher for cultures containing IPTG than for cultures without IPTG.

Expression of araE from P_{cp} promoters

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

All cultures containing the pJAT*araE* plasmids were homogeneously induced (Figures 1 and 3). The culture-averaged fluorescence (FL/OD) varied with the constitutive promoter strength $P_{CP13} < P_{CP8} < P_{CP18}$. All control cultures without a functional arabinose transport system displayed a single non-fluorescent population and were not able to grow on arabinose at any concentration as the sole carbon source. In the control experiment with *araE* expressed from its native promoter on the chromosome, two subpopulations were observed at low to intermediate arabinose concentration and a single, homogeneous population was observed only when using high inducer concentrations.

Effect of promoter strength

Two factors affected reporter gene expression from P_{BAD}: (i) the external arabinose concentration and (ii) the level of *araE* expression. When *araE* was expressed from the relatively weak IPTG-inducible $P_{taclacUV5}$ or constitutive P_{cp13} promoter, there was little or no expression from P_{BAD} except at the highest arabinose concentrations (Figure 2). Interestingly, expression of *araE* from $P_{taclacUV5}$ gave rise to two populations at low to intermediate arabinose concentrations, whereas expression of *araE* from P_{cp13} (also a relatively weak promoter) gave rise to a homogeneous population at all arabinose concentrations. When *araE* was expressed from the strong promoters $P_{cp8,}$ P_{tac}, and P_{cp18}, the cells were homogeneously induced at all arabinose concentrations.

A higher culture-averaged fluorescence was observed when P_{CP8}, P_{CP18}, and P_{tac} promoters drove expression of *araE*, whereas a lower level of induction was observed in cultures harboring pJAT13*araE* (P_{CP13}*araE*) and pAK01 ($P_{taclacUV5}$*araE*) (Figure 3). In general, the stronger the promoter driving expression of *araE*, the higher the culture-averaged fluorescence.

Discussion

The arabinose-inducible *araBAD* promoter (P_{BAD}) has been used extensively for modulation of gene expression [1,4,12,15,16, 18-20]. Its primary merits are tight control of gene expression, particularly in the absence of inducer, and regulatable control with varying concentrations of inducer [4]. These qualities are particularly important for the expression of genes whose product may be toxic or for the modulation of fluxes through metabolic pathways. Unfortunately, it was shown recently that the promoter is not regulatable in individual cells but suffers from all-or-none gene expression [14]. Cells are either fully induced or uninduced,

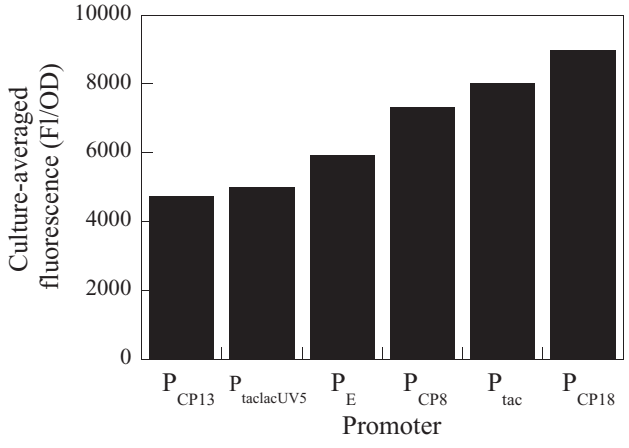

Figure 3 Comparison of culture-averaged fluorescence of *E. coli* with *araE* under the control of various arabinose-independent promoters (CW2587) and under the control of the arabinose-inducible P_E promoter (CW2513). All cultures harbored pCSAK50 (*araC*-P_{BAD}*gfpuv*) and were induced with 0.2% of arabinose. Six hours after addition of arabinose, the population-averaged fluorescence (FL/OD) was determined. Cultures harboring pAK01 ($P_{taclacUV5}$*araE*) and pAK02 (P_{tac}*araE*) were also induced with 32 μg/ml of IPTG. Data were corrected for the background fluorescence displayed by control cultures harboring pCSAK50 and the corresponding pJAT plasmid without the *araE* gene or pMMB206/pMMB207, which were induced with 32 μg/ml of IPTG. Wild-type *E. coli* CW2513 with native *araE* gene (P_E*araE*) was used as a control.

and the fraction of cells that are induced changes as the amount of inducer in the growth medium is varied.

Recently, we showed with simulations [3] and then experimentally [10] that all-or-none gene expression occurred because the promoters for the genes encoding the arabinose transporters (*araE* and *araFGH*) were under arabinose-inducible control. Here, we showed that expression of the gene encoding the low-affinity high-capacity arabinose permease (*araE*) from arabinose-independent — constitutive as well as strong inducible P_{tac} — promoters eliminated all-or-none induction of P_{BAD}. A linear response in P_{BAD} induction to arabinose concentration was observed over three orders of magnitude of inducer concentration. The use of constitutive promoters of different strengths would allow one to pick a particular desired range of gene expression and fine tune the expression level with the arabinose concentration. This approach might be particularly useful in metabolic engineering for balancing pathways [15,16] or for controlled expression of toxic proteins. Further, the use of constitutive promoters for expression of *araE* frees the IPTG-inducible lac promoters for other uses.

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References

1 Ben-Samoun K, G Leblon and O Reyes. 1999. Positively regulated expression of the *Escherichia coli* araBAD promoter in *Corynebacterium glutamicum*. *FEMS Microbiol Lett* 174: 125–130.
2 Carra JH and RF Schleif. 1993. Variation of half-site organization and DNA looping by AraC protein. *EMBO J* 12: 35–44.
3 Carrier TA and JD Keasling. 1999. Investigating autocatalytic gene expression systems through mechanistic modeling. *J Theor Biol* 201: 25–36.
4 Guzman LM, D Belin, MJ Carson and J Beckwith. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* 177: 4121–4130.
5 Helmstetter CE. 1968. DNA synthesis during the division cycle of rapidly growing *Escherichia coli* B/r. *J Mol Biol* 31: 507–518.
6 Horazdovsky BF and RW Hogg. 1987. High-affinity L-arabinose transport operon. Gene product expression and mRNAs. *J Mol Biol* 197: 27–35.
7 Jensen PR and K Hammer. 1998. Artificial promoters for metabolic optimization. *Biotechnol Bioeng* 58: 191–195.
8 Jensen PR and K Hammer. 1998. The sequence of spacers between the consensus sequences modulates the strength of prokaryotic promoters. *Appl Environ Microbiol* 64: 82–87.
9 Khlebnikov A, KA Datsenko, T Skaug, BL Wanner and JD Keasling. 2001. Homogeneous expression of the PBAD promoter in *Escherichia coli* by constitutive expression of the low-affinity, high-capacity AraE transporter. *Microbiology* 147: 3241–3247.
10 Khlebnikov A, O Risa, T Skaug, TA Carrier and JD Keasling. 2000. A regulatable arabinose-inducible gene expression system with consistent control in all cells of a culture. *J Bacteriol* 182: 7029–7034.
11 Morales VM, A Bäckman and M Bagdasarian. 1991. A series of wide-host-range low-copy-number vectors that allow direct screening for recombinants. *Gene* 97: 39–47.
12 Newman JR and C Fuqua. 1999. Broad-host-range expression vectors that carry the L-arabinose-inducible *Escherichia coli* araBAD promoter and the araC regulator. *Gene* 227: 197–203.
13 Sambrook J, T Maniatis and EF Fritsch. 1989. Molecular Cloning : A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
14 Siegele DA and JC Hu. 1997. Gene expression from plasmids containing the araBAD promoter at subsaturating inducer concentrations represents mixed populations. *Proc Natl Acad Sci USA* 94: 8168–8172.
15 Smolke CD, TA Carrier and JD Keasling. 2000. Coordinated, differential expression of two genes through directed mRNA cleavage and stabilization by secondary structures. *Appl Env Microbiol* 66: 5399–5405.
16 Smolke CD, VJJ Martin and JD Keasling. 2001. Controlling the metabolic flux through the carotenoid pathway using directed mRNA processing and stabilization. *Met Eng* 3: 313–321.
17 Soisson SM, B Macdougall-Shackleton, R Schleif and C Wolberger. 1997. Structural basis for ligand-regulated oligomerization of AraC. *Science* 276: 421–425. (Washington, D. C.).
18 Sukchawalita R, P Vattanaviboona, R Sallabhana and S Mongkolsuk. 1999. Construction and characterization of regulated L-arabinose-inducible broad host range expression vectors in *Xanthomonas*. *FEMS Microbiol Lett* 181: 217–223.
19 Van Dien SJ and JD Keasling. 1998. Optimization of polyphosphate degradation and phosphate secretion using hybrid metabolic pathways and engineered host strains. *Biotechnol Bioeng* 59: 754–761.
20 Van Dien SJ, S Keyhani, C Yang and JD Keasling. 1997. Manipulation of independent synthesis and degradation of polyphosphate in *Escherichia coli* for investigation of phosphate secretion from the cell. *Appl Environ Microbiol* 63: 1689–1695.