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Rational design of 'controller cells' to manipulate protein and phenotype expression

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ABSTRACT

Coordination between cell populations via prevailing metabolic cues has been noted as a promising approach to connect synthetic devices and drive phenotypic or product outcomes. However, there has been little progress in developing 'controller cells' to modulate metabolic cues and guide these systems. In this work, we developed 'controller cells' that manipulate the molecular connection between cells by modulating the bacterial signal molecule, autoinducer-2, that is secreted as a quorum sensing (QS) signal by many bacterial species. Specifically, we have engineered *Escherichia coli* to overexpress components responsible for autoinducer uptake (*lsrACDB*), phosphorylation (*lsrK*), and degradation (*lsrFG*), thereby attenuating cell–cell communication among populations. Further, we developed a simple mathematical model that recapitulates experimental data and characterizes the dynamic balance among the various uptake mechanisms. This study revealed two controller 'knobs' that serve to increase AI-2 uptake: overexpression of the AI-2 transporter, LsrACDB, which controls removal of extracellular AI-2, and overexpression of the AI-2 kinase, LsrK, which increases the net uptake rate by limiting secretion of AI-2 back into the extracellular environment. We find that the overexpression of *lsrACDBFG* results in an extraordinarily high AI-2 uptake rate that is capable of completely silencing QS-mediated gene expression among wild-type cells. We demonstrate utility by modulating naturally occurring processes of chemotaxis and biofilm formation. We envision that 'controller cells' that modulate bacterial behavior by manipulating molecular communication, will find use in a variety of applications, particularly those employing natural or synthetic bacterial consortia.

1. Introduction

Metabolic engineering exploits the genetic modification of cellular pathways to improve production of metabolites and proteins (Bailey, 1991; Stephanopoulos and Vallino, 1991). Many noteworthy examples have been demonstrated wherein these cells serve as 'factories' for the environmentally sustainable production of energy, materials, and chemicals (Jarboe et al., 2010). Towards this aim, metabolic engineering has incorporated finely tuned synthetic controllers and cells in the creation of artificial networks (Stephanopoulos and Kelleher, 2001; Kramer et al., 2005; Malphettes and Fussenegger, 2006; Boyle and Silver, 2012; Keasling, 2012). The general structure of these synthetic networks is based on control devices that respond to specific stimuli in a predictable fashion (Weber and Fussenegger, 2011; Purnick and Weiss, 2009). However, the task of coordinating among and between cell populations remains a critical challenge that can limit the production of desired end-products (Nielsen et al., 2014; Cameron et al., 2014). A further challenge is controlling the partitioning of resources that (i) maintain native metabolism and (ii) adequately support product synthesis (Way et al., 2014; Solomon and Prather, 2011). One creative approach to address both of these challenges is through the leveraging of cell–cell communication networks, and these have been the target of a variety of dynamic control systems (Farmer and Liao, 2000; Kobayashi et al., 2004; Tsao et al., 2012). Using the native bacterial signaling network known as quorum sensing (Fuqua et al., 1994; Surette and Bassler, 1998), we have previously shown the ability to reduce the metabolic burden (Bentley et al., 1990; DeLisa et al., 2001) and 'program' cell populations through the metabolic cue, autoinducer-2 (Tsao et al., 2010, 2011). However, while there has been much development in multicellular systems that respond to metabolic cues (Kuipers et al., 1998; Tamsir et al., 2011; Wood et al., 2011; Basu et al., 2004), control of the intensity of these metabolic cues to fulfill the potential of spatiotemporal control has not been realized.

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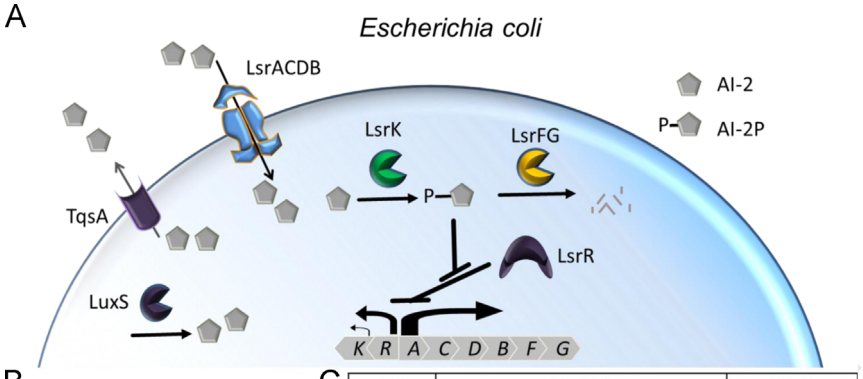

B

	LsrACDB	LsrK	LsrFG
LW12 pTrcHisB	Native	Native	Native
LW12 pLsrFG	Native	Native	Induced
LW12 pLsrK	Native	Induced	Native
LW12 pLsrACDB	Induced	Native	Native
LW12 pLsrACDBFG	Induced	Native	Induced

C

Strain	Genotype	Source
LW12	W3110 ΔluxS::Kan T	(Liang et al., 2005)
Plasmid	**Description**	**Source**
pTrcHisB	Cloning Vector Ap^r	Invitrogen
pLsrFG	pTrcHisB derivative, containing *lsrFG*, Ap^r	This study
pLsrK	pTrcHisB derivative, containing *lsrK*, Ap^r	This study
pLsrACDB	pTrcHisB derivative, containing *lsrACDB*, Ap^r	This study
pLsrACDBFG	pTrcHisB derivative, containing *lsrACDBFG*, Ap^r	This study

Scheme 1. Panel (A) depicts the AI-2 quorum sensing network. LuxS generates AI-2 from metabolic precursors, which is then exported out of the cell by TqsA. AI-2 is primarily taken up through the ABC-type transporter Lsr, and then phosphorylated by LsrK to AI-2P. AI-2P depresses the response regulator LsrR, thereby activating transcription of the lsr operon. AI-2P is degraded by LsrF and LsrG. Panel (B) depicts the 'controller cells' that are engineered through the overexpression of distinct components of the lsr system. 'Native' indicates native production, while 'Induced' indicateso over-expression. Panel (C) illustrates the strains, plasmids, descriptions and sources used for these 'controller cells'.

In this work, we have developed bacterial AI-2 consumers, 'controller cells', which can be deployed to control AI-2 in a predictable fashion using the now well-characterized quorum sensing mechanisms of *Escherichia coli* (Scheme 1A). AI-2 is synthesized and recognized by a wide variety of bacteria (Quan and Bentley, 2012; Pereira et al., 2009); correspondingly its use as a potential target for modulating QS activities among different cell types is of interest. The use of genetically engineered bacteria to 'quench' extracellular AI-2 was first described by Xavier et al. Xavier and Bassler (2005), where genetic deletions of its synthase (*luxS*) and its repressor (*lsrR*) were used to interfere with bacterial communication. However, the interrelated complexity of QS networks renders the elucidation of its mechanisms difficult, and the production of simple, 'modular' networks would enrich the understanding of these actions (Carter et al., 2012). We have addressed this through the model-based design, construction, and characterization of these 'controller cells' to modulate the external AI-2 environment. These cells are designed via the compartmentalization of different aspects of AI-2 processing: uptake (*lsrACDB*), phosphorylation (*lsrK*), and degradation (*lsrFG*) (Scheme 1B and C).

These 'controller cells' provide the ability to regulate extracellular AI-2 and modulate synthetic circuits. Further, we show that the ability to quench extracellular AI-2 through 'controller cells' can attenuate the native cell–cell behaviors of chemotaxis and biofilm formation. By teasing apart the regulatory network for AI-2, we have enhanced our understanding of the collective population-scale response to AI-2. In this way, systems can be designed wherein we decouple the consumption of AI-2 from bacterial population density and its emergent behavior. With the addition of 'controller cells', we provide an orthogonal means to modulate QS activity, demonstrate their use as mediators of heterologous protein and phenotype expression, and provide a modeling foundation to guide QS-communication.

2. Materials and methods

2.1. Plasmid construction

The bacterial strains and plasmids used in this study are listed in Table S1, and were constructed according to standard procedures (Sambrook et al., 1989). Briefly, plasmid pTrcHisB (Invitrogen) was used as the backbone to construct plasmids pLsrFG, pLsrK, pLsrACDB, and pLsrACDBFG. The sequences for *lsrFG*, *lsrK*, *lsrACDB*, and *lsrACDBFG* were amplified by PCR using Q5 polymerase (New England Biolabs) from *E. coli* K-12 strain W3110. These PCR inserts were ligated into XhoI-digested pTrcHisB using Gibson assembly (Gibson et al., 2009) and then transformed into LW12 (W3110 Δ*luxS*) (Wang et al., 2005). Oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA) and are listed in Table S2. Cloning was verified with sequencing and Western Blot.

2.2. AI-2 assay

Cultured media was tested for the presence of AI-2 by inducing luminescence in *Vibrio harveyi* reporter strain BB170 (Bassler et al., 1997). Briefly, BB170 was grown for 16 h with shaking at 30 °C in AB (AI-2 Bioassay) media. AB media is made by adjusting 400 mL of distilled (DI) water to pH 7.5, and adding 7 g of NaCl, 2.4 g of $MgSO_4$, 0.8 g casamino acid, and 8 mL of glycerol. AB media is supplmented with 400 μL of potassium phosphate buffer (K_2HPO_4 10.71 g and 5.24 g KH_2PO_4 in 100 mL of DI water), 400 μL of 0.1 M L-arginine (0.1742 g in 10 mL of DI water), 40 μL of riboflavin (10 μg/mL), 40 μL of thiamine (1 mg/mL) and 40 μL kanamycin (50 mg/mL).

Overnight cultures were diluted 1:5000 in fresh AB media with kanamycin, and aliquoted into sterile 12 × 75-mm tubes (Fisher Scientific). Test samples were added to BB170 cultures at a final concentration of 10% (vol/vol). Luminescence was measured by quantifying light production with a luminometer (EG&G Berthold LB 9509 Jr) and assays were adjusted, if needed, so that values were in the linear range. Data are presented as 'fold change' compared to negative controls. All conditions were tested in triplicate. In experiments with supplemented chemically-synthesized AI-2, we report AI-2 activity normalized to the initial concentration, as our previous study showed a linear correlation between AI-2 concentration and resultant bioluminescent AI-2 activity (Zargar et al., 2015).

2.3. AI-2 uptake profiles of 'controller cells'

Chemically synthesized AI-2 (Smith et al., 2009) was generously provided by the Sintim research group. Each strain was reinoculated by diluting an overnight culture to 3% volume in