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Increasing the dynamic control space of mammalian transcription devices by combinatorial assembly of homologous regulatory elements from different bacterial species

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ABSTRACT

Prokaryotic transcriptional regulatory elements are widely utilized building blocks for constructing regulatory genetic circuits adapted for mammalian cells and have found their way into a broad range of biotechnological applications. Prokaryotic transcriptional repressors, fused to eukaryotic transactivation or repression domains, compose the transcription factor, which binds and adjusts transcription from chimeric promoters containing the repressor-specific operator sequence. *Escherichia coli* and *Chlamydia trachomatis* share common features in the regulatory mechanism of the biosynthesis of L-tryptophan. The repressor protein TrpR of *C. trachomatis* regulates the trpRBA operon and the TrpR of *E. coli* regulates the trpEDCBA operon, both requiring L-tryptophan as a co-repressor. Fusion of these bacterial repressors to the VP16 transactivation domain of *Herpes simplex* virus creates synthetic transactivators that could bind and activate chimeric promoters, assembled by placing repressor-specific operator modules adjacent to a minimal promoter, in an L-tryptophan-adjustable manner. Combinations of different transactivator and promoter variants from the same or different bacterial species resulted in a multitude of regulatory systems where L-tryptophan regulation properties, background noise, and maximal gene expression levels were significantly diverse. Different L-tryptophan analogues showed diverse regulatory capacity depending on the promoter/transactivator combination. We believe the systems approach to rationally choose promoters, transactivators and inducer molecules, to obtain desired and predefined genetic expression dynamics and control profiles, will significantly advance the design of new regulatory circuits as well as improving already existing ones.

1. Introduction

Inducible activation and repression of transgene expression in mammalian cells has become a fundamental technology widely utilized in the field of synthetic biology (Khalil and Collins, 2010; Weber and Fussenegger, 2010). In recent years, the construction of regulated gene expression systems have been implemented in areas such as biopharmaceutical manufacturing (Ulmer, 2006; Weber and Fussenegger, 2007), functional genomics (Kawaguchi et al., 2002; Malleret et al., 2001), drug discovery (Sharpless and Depinho, 2006; Weber et al., 2008), and in prototype gene- and cell-based therapies (Kemmer et al., 2010; Weber and Fussenegger, 2012; Ye et al., 2011; Yung et al., 2006). Genetic circuits such as switches (Deans et al., 2007; Gardner et al., 2000; Greber et al., 2008; Kramer et al., 2004b), logic formulas (Auslander et al., 2012; Kramer et al., 2004a; Rinaudo et al., 2007), (semi-) synthetic regulatory cascades (Kramer et al., 2003), hysteretic networks (Kramer and Fussenegger, 2005) time-delay circuits (Weber et al., 2007) and oscillators (Danino et al., 2010; Stricker et al., 2008; Tigges et al., 2009) have been constructed enabling cells to perform advanced regulatory tasks. Many inducible mammalian gene expression systems utilize heterologous transcription factors designed by fusing a prokaryotic repressor with a eukaryotic activation domain (Gitzinger et al., 2009; Urlinger et al., 2000; Weber et al., 2002). Chimeric promoters are also used and are constructed by placing the repressor-specific operator sequence upstream of a minimal eukaryotic promoter. External inducer molecules are used to regulate the affinity of the transcription factor to its cognate operator site, thereby regulating the transgene expression. Traditionally, the repressors and their specific operators, of same prokaryotic origin, are used in mammalian gene expression systems (Gossen and Bujard, 1992; Tigges and Fussenegger, 2009; Weber and Fussenegger, 2009).

In biological systems, the processes of transcription, translation and protein degradation have an ever present problem with noise and

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background signals (Ozbudak et al., 2002). A major goal of any biotechnological development is the reduction and control of these variables. Heterologous inducible mammalian gene regulation systems are no exception to this rule and many attempts have been tried to improve the robustness of these systems and reduce their leakiness. These strategies include modification of the transactivation domain, regulating the number of operator repeats and varying the length of the base pair linker between the operator sequence and the minimal promoter, to optimize the binding of the transcription regulator to the operator DNA (Fussenegger et al., 2000; Gitzinger et al., 2009). More sophisticated attempts to control leaky expression and noise include the construction of complex gene networks containing feedback motifs (Hooshangi and Weiss, 2006). To control these factors we utilized a systems approach based on changes to the DNA sequences of the operators and repressor proteins of homologous regulons from different bacteria.

The biosynthesis of L-tryptophan in both *Escherichia coli* and *Chlamydia trachomatis* is regulated by the aporepressor TrpR and L-tryptophan is required as a corepressor. TrpR binds in the presence of L-tryptophan to its cognate operator upstream of the trpRBA operon in *C. trachomatis* and the trpEDCBA operon in *E. coli*. Thereby, TrpR represses the transcription of genes responsible for L-tryptophan synthesis (Akers and Tan, 2006; Gunsalus and Yanofsky, 1980; Joachimiak et al., 1983). Since the molecular mechanism of L-tryptophan-responsive transcriptional regulation is similar in both organisms we wanted to investigate whether the regulatory genetic elements of *E. coli* are able to recognize and interact with the complementary elements from *C. trachomatis* to regulate gene expression in a L-tryptophan-inducible manner.

We demonstrate using human embryonic kidney cells (HEK-293) that the trans-species approach results in L-tryptophan regulation systems with varying characteristics dependent on the combination of the genetic elements, e.g., the transactivators and operators used. We believe this approach could improve the design of new regulatory systems as well as improving existing ones.

2. Materials and methods

2.1. Vector constructions

All vectors and oligonucleotides used in this study are described in Table 1.

2.2. Cell culture

Human embryonic kidney cells (HEK-293, ATCC: CRL-1573) were cultivated in Dulbecco's modified Eagle's medium without L-tryptophan (L-tryptophan-free, L-phenylalanine-free, L-tyrosine-free DMEM; [Cell culture technologies, Gravesano, Switzerland])

Table 1
Plasmids used and designed in this study.

Plasmid	Description and cloning strategy	Reference or source
pMF111	Vector for tetracycline-responsive SEAP expression ($P_{hCMV*-1}$-*seap*-pA).	(Fussenegger et al., 1997a)
pSAM200	Vector for constitutive expression of tTA.	(Fussenegger et al., 1997b)
pSEAP2-control	Vector for constitutive expression of SEAP (P_{SV40}-*seap*-pA).	Clontech, Carlsbad, CA, USA
pWB22	Vector for L-tryptophan-inducible SEAP expression (P_{TRTC}-*seap*-pA).	(Bacchus et al., 2012)
pWB24	Vector for constitutive expression of the *C. trachomatis*-derived L-tryptophan-dependent transactivator TRT_C (P_{SV40}-trt_C-pA).	(Bacchus et al., 2012)
pWB57	Vector for constitutive expression of the *E. coli*-derived L-tryptophan-dependent transactivator TRT_E (P_{SV40}-trt_E-pA). $TrpR_E$ was PCR amplified from *E. coli* using oligonucleotides OWB79 (5′-catgcggccgcgaattccaccATGGCCCAACAATCACCCTATTCAGCAG-′3) and OWB80 (5′-gtcgatgcgcgctATCGCTTTTCAGCAACACCTCTTCCAGC-′3), restricted with *Eco*RI/*Bss*HII and cloned into pSAM200 (*Eco*RI/*Bss*HII).	This work
pWB58	Vector for L-tryptophan-inducible SEAP expression ($P_{TRTC-P1}$-*seap*-pA). Oligonucleotides OWB83 (5′-gcatctcgagatct*agtaatattataatattacaattgtaatattatataattaca*aggatcctgcagGTCGA GCTCGGTACCCGGGTCG-′3, tandem C-P1 operator sites in italics) and OWB88 (5′-GCTTCTGCAGCTCGAGGCCACTGG-′3) were used to PCR amplify SEAP from pMF111. The fragment was restricted with *Bgl*II/*Bss*HII and inserted into pSEAP2control (*Bgl*II/*Bss*HII).	This work
pWB59	Vector for L-tryptophan-inducible SEAP expression ($P_{TRTC-P2}$-*seap*-pA). $P_{TRTC-P2}$ was synthesized by GenScript Corporation (Piscataway, NJ, USA) and restricted with *Bgl*II/*Eco*RI and inserted into pWB22 (*Bgl*II/*Eco*RI).	This work
pWB61	Vector for L-tryptophan-reponsive SEAP expression ($P_{TRTC-TRTE}$-*seap*-pA). $P_{TRTC-TRTE}$ was synthesized by GenScript Corporation (Piscataway, NJ, USA) and restricted with *Bgl*II/*Eco*RI and inserted into pWB22 (*Bgl*II/*Eco*RI).	This work
pWB62	Vector for L-tryptophan-inducible SEAP expression ($P_{TRTE-TRTC}$-*seap*-pA). Oligonucleotides OWB90 (5′- gcatgagatct*aagcttcgtactagttaactagtacgcgtactagttaactagtacg*TTGTAATATTATAG CATTACAATTGTAATATTATAG-′3, $TrpR_E$-specific tandem operator sites in italics) and OWB87 (5′-GTGCGCGGCGTCGGTGG-′3) were used to PCR amplify SEAP from pWB22. The fragment were restricted with *Bgl*II/*Bss*HII and inserted into pSEAP2control (*Bgl*II/*Bss*HII).	This work
pWB95	Vector for L-tryptophan-responsive SEAP expression (P_{TRTE}-*seap*-pA). P_{TRTE} was synthesized by GenScript Corporation (Piscataway, NJ, USA) and restricted with *Bgl*II/*Eco*RI and inserted into pWB22 (*Bgl*II/*Eco*RI).	This work
pWW35	Vector for constitutive expression of ET1	(Weber et al., 2002)
pWW37	Vector for erythromycin-responsive SEAP expression (P_{ETR}-*seap*-pA).	(Weber et al., 2002)

Restriction endonuclease-specific sites are underlined in oligonucleotide sequences. Annealing base pairs contained in oligonucelotide sequences are shown in capital letters.

Abbreviations: ET1, Erythromycin-dependent transactivator; pA, SV40-derived polyadenylation site; P_{ETR}, Erythromycin-responsive promoter; P_{hCMV}, Human cytomegalovirus immediate early promoter; $P_{hCMV*-1}$, Tetracycline-responsive promoter; P_{SV40}, Simian virus 40 promoter; P_{TRTC}, *Chlamydia trachomatis*-derived L-tryptophan-responsive promoter; P_{TRTE}, *Escherichia coli*-derived L-tryptophan-responsive promoter; $P_{TRTC-P1}$, L-tryptophan-responsive promoter with perfect *Chlamydia trachomatis* palindrome operator sequence 1; $P_{TRTC-P2}$, L-tryptophan-responsive promoter with perfect *Chlamydia trachomatis* palindrome operator sequence 2; $P_{TRTC-TRTE}$, L-tryptophan-responsive promoter with O_C–O_E orientation; $P_{TRTE-TRTC}$, L-tryptophan-responsive promoter with O_E–O_C orientation; SEAP, Human placental secreted alkaline phosphatase; $TrpR_C$, *Chlamydia trachomatis*-derived L-tryptophan repressor; $TrpR_E$, *Escherichia coli*-derived L-tryptophan repressor; TRT_C, *Chlamydia trachomatis*-derived L-tryptophan-dependent transactivator; TRT_E, *Escherichia coli*-derived L-tryptophan-dependent transactivator; tTA, Tetracycline-dependent transactivator; VP16, *Herpes simplex*-derived transactivation domain.