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Bile acid-controlled transgene expression in mammalian cells and mice

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ABSTRACT

In recent years, using trigger-inducible mammalian gene switches to design sophisticated transcription-control networks has become standard practice in synthetic biology. These switches provide unprecedented precision, complexity and reliability when programming novel mammalian cell functions. Metabolite-responsive repressors of human-pathogenic bacteria are particularly attractive for use in these orthogonal synthetic mammalian gene switches because the trigger compound sensitivity often matches the human physiological range. We have designed both a bile acid-repressible ($BEAR_{OFF}$) as well as a bile-acid-inducible ($BEAR_{ON}$) gene switch by capitalizing on components that have evolved to manage bile acid resistance in *Campylobacter jejuni*, the leading causative agent of human food-borne enteritis. We have shown that both of these switches enable bile acid-adjustable transgene expression in different mammalian cell lines as well as in mice. For the $BEAR_{OFF}$ device, the *C. jejuni* repressor CmeR was fused to the VP16 transactivation domain to create a synthetic transactivator that activates minimal promoters containing tandem operator modules (O_{cme}) in a bile acid-repressible manner. Fusion of CmeR to a transsilencing domain resulted in an artificial transsilencer that binds and represses a constitutive O_{cme}-containing promoter until it is released by addition of bile acid ($BEAR_{ON}$). A tailored multi-step tuning program for the inducible gene switch, which included the optimization of individual component performance, control of their relative abundances, the choice of the cell line and trigger compound, resulted in a $BEAR_{ON}$ device with significantly improved bile acid-responsive control characteristics. Synthetic metabolite-triggered gene switches that are able to interface with host metabolism may foster advances in future gene and cell-based therapies.

1. Introduction

Innovations in synthetic biology have made the design of trigger-controlled devices that can precisely fine-tune the expression of transgenes possible. These recent developments are significant because they can be used to reprogram mammalian cells to execute desired activities or interface with complex metabolic pathways (Kemmer et al., 2010; Ye et al., 2011, 2013). Synthetic transcription control devices have been instrumental for gene function analysis and functional genomic research (Malleret et al., 2001; Baumgartel et al., 2008), constructing genetic toggle switches and genetic counters (Gardner et al., 2000; Kramer et al., 2004; Friedland et al., 2009), discovering new drugs and manufacturing biopharmaceuticals (Sharpless and Depinho, 2006; Weber et al., 2008; Wurm, 2004; Ulmer et al., 2006; Weber and Fussenegger, 2007; Gitzinger et al., 2009b) as well as designing specialized biomaterials (Greber and Fussenegger, 2007; Weber and Fussenegger, 2006; Ehrbar et al., 2008). Artificial transcription controllers have become essential components of molecular design, allowing construction of highly complex gene networks such as oscillators (Elowitz and Leibler, 2000; Deans et al., 2007; Tigges et al., 2009, 2010), biocomputers (Xie et al., 2011; Ausländer et al., 2012), inter-cellular communication devices (Bacchus et al., 2012), and closed-loop control circuits known as prosthetic networks that correct metabolic disorders (Kemmer et al., 2010). Trigger-adjustable repressors found in prokaryotes have been an especially fruitful source of molecular parts for use in the assembly of orthogonal mammalian transcription-control devices (Ausländer and Fussenegger, 2013; Urlinger et al., 2000; Weber et al., 2002; Gitzinger et al., 2009b, 2012). Repressors derived from human-pathogenic bacteria are particularly attractive components because their trigger sensitivity often matches the human physiological range. This is a result of years of co-evolution during which the pathogen was selected to optimally interface and synchronize with its host's metabolism (Ausländer and Fussenegger, 2013; Bacchus et al., 2013; Hartenbach et al., 2007; Weber et al., 2007; Kemmer et al., 2010).

Campylobacter jejuni is the leading bacterial cause of human food-borne enteritis in industrialized countries and has become increasingly resistant to antimicrobials (Klančnik et al., 2012). Over time, *C. jejuni* has evolved multiple mechanisms to adapt to

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antibiotic treatments as well as the human gastrointestinal environment. As part of this adaptation, *C. jejuni* uses a three-gene operon called *cmeABC* that encodes a multidrug efflux system able to confer resistance to structurally diverse antimicrobial compounds (Lin et al., 2002, 2005b). Expression of *cmeABC* is controlled by the transcriptional repressor CmeR which directly binds to the inverted repeat operator O_{Cme} within the P_{cmeABC} promoter and represses the transcription of *cmeABC* (Lin et al., 2005a; Routh et al., 2009). CmeR is a member of the TetR family of transcriptional regulators and contains typical motifs of this family, namely an N-terminal DNA-binding helix-turn-helix domain as well as a C-terminal region predicted to be involved in recognizing the inducer molecule (Routh et al., 2009; Gu et al., 2007). Bile acids, a key compound family that defines the gastrointestinal microenvironment, have recently been shown to bind and dose-dependently inactivate the CmeR repressor, resulting in induction of the CmeABC multidrug efflux pump (Lei et al., 2011; Kurinčič et al., 2012). Timely induction of the multidrug efflux pump, which contributes to *C. jejuni*'s resistance to a broad spectrum of antimicrobials as well as to bile acids, is essential for the pathogen's colonization of the intestinal tract (Lin et al., 2005b; Young et al., 2007).

Bile acids have long been known to act as emulsifiers that improve digestion and absorption of lipids and fat-soluble vitamins in the small intestine (Insull, 2006; Lefebvre et al., 2009; Hofmann, 2009). Recently, they have been shown to act as hormones involved in the regulation of metabolic processes, including the maintenance of energy homeostasis (Staels and Fonseca, 2009), and therefore to have a much greater impact on human physiology than previously understood. Bile acids are synthesized from cholesterol in the liver (Hofmann, 1999a, 2009). The immediate products of the bile acid synthetic pathways are the primary bile acids, cholic acid and chenodeoxycholic acid, which are conjugated with taurine or glycine for improved hydrophilicity before storage in the gallbladder as mixed micelles. Primary bile acids are also converted to secondary bile acids such as deoxycholic or lithocholic acids by the action of the intestinal bacterial flora (Thomas et al., 2008; Enhsen et al., 1998; Ridlon, 2005). Upon ingesting food, gallbladder contraction releases micellar bile acids into the intestinal lumen to facilitate digestion. These bile acids are subsequently reabsorbed and recycled via enterohepatic circulation (Hofmann, 1999b).

Capitalizing on *C. jejuni*'s bile acid-sensing components, we have engineered a bile acid-responsive transcription-controlling device that is able to reversibly fine-tune transgene expression in different mammalian cell lines as well as in mice. By constructing this device, we have made a gene switch responsive to a critically important metabolic trigger compound available. It will expand the metabolic intervention portfolio and may foster novel advances in gene- and cell-based therapies.

2. Materials and methods

2.1. Bile acid sensor components

Comprehensive design and construction details for all expression vectors are provided in Table 1.

2.2. Cell culture and transfection

Human embryonic kidney cells (HEK-293T, ATCC: CRL-11268), Baby hamster kidney cells (BHK-21, ATCC: CCL-10), African green monkey kidney cells (COS-7, ATCC: CRL-1651), human cervical adenocarcinoma cells (HeLa, ATCC: CCL-2) and human fibrosarcoma cells (HT-1080, ATCC: CCL-121) were cultivated in a Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Basel, Switzerland) supplemented with 10% (v/v) fetal calf serum (FCS, PAN Biotech GmbH, Aidenbach, Germany, Lot no. P251110) or 10% (v/v) charcoal-stripped fetal bovine serum (cFBS, Sigma-Aldrich, Munich, Germany, Cat. no. F6765, Lot. no. 11C151) and 1% (v/v) penicillin/streptomycin solution (Sigma-Aldrich, Munich, Germany). Wild-type Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultured in ChoMaster® HTS (Cell Culture Technologies GmbH, Gravesano, Switzerland) containing 1% penicillin/streptomycin. All cell types were cultivated at 37 °C in a humidified atmosphere containing 5% CO_2. For co-transfection of BHK-21, CHO-K1, COS-7, HEK-293, HeLa and HT-1080 cells, 40,000 cells were seeded per well of a 24-well plate 12 h prior to transfection and were incubated for 6 h with a 4:1 PEI:DNA mixture (Polyethyleneimine; MW 40,000, Polysciences, Inc., Warrington, USA). After transfection, all cells were maintained in their specific medium containing different concentrations of inducer. Reporter protein levels were measured after 48 h unless stated otherwise.

2.3. Colorimetric SEAP assay

Production of the human placental secreted alkaline phosphatase (SEAP) was quantified in culture supernatants according to a p-nitrophenylphosphate-based light absorbance time course (Schlatter et al., 2002). Serum levels of SEAP were profiled using a chemiluminescence-based assay (Roche Diagnostics GmbH, Mannheim, Germany).

2.4. Inducer compounds, cholic acid and its derivatives

Cholic acid (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland; Cat. no. C9282), chenodeoxycholic acid (Sigma-Aldrich Chemie GmbH; Cat. no. C8261), lithocholic acid (Sigma-Aldrich Chemie GmbH; Cat. no. L6250), glycocholic acid (Sigma-Aldrich Chemie GmbH; Cat. no. G7132), taurocholic acid (Calbiochem, La Jolla, USA; Cat. no. 580217), deoxycholic acid (Acros Organics, Geel, Belgium; Cat. no. 21859), and water soluble cholesterol (Sigma-Aldrich Chemie GmbH; Cat. no. C4951) were prepared as 10 mM stock solutions in water and adjusted to pH 7.

2.5. Animal experiments

Intraperitoneal implants were produced by encapsulating pKR69/pKR114-transgenic HEK-293 cells in coherent alginate-poly-(L-lysine)-alginate beads (400 μm; 200 cells/capsule) using an Inotech Encapsulator Research Unit IE-50R (Buchi Labortechnik AG, Flawil, Switzerland) set to the following parameters: 200 μm nozzle with a vibration frequency of 1023 Hz and 900 V for bead dispersion, and 20 mL syringe operated at a flow rate of 403 units. 400 μL of serum-free DMEM containing 2×10^6 microencapsulated pKR69/pKR114-transgenic HEK-293 cells (200 cells/capsule) was injected intraperitoneally into 14-week-old female CD1 mice (Janvier S.A.S., Le Genest-Saint-Isle, France), which received twice-daily injections of cholic acid (200 μl, 0–100 mg/kg), chenodeoxycholic acid or deoxycholic acid (20 μl, 0–30 mg/kg). Blood samples were collected and the serum was isolated using BD Microtainer® SST tubes according to the manufacturer's protocol (Beckton Dickinson, Plymouth, UK) before serum SEAP levels were scored as described above. All experiments involving animals were performed according to the directives of the European Community Council (2010/63/EU), approved by the French Republic (No. 69266309), and carried out by Ghislaine Charpin-El Hamri at the Institut Universitaire de Technology (IUTA, F-69622 Villeurbanne Cedex, France).