

NOVEL TRANSCRIPTION FACTOR-BASED BIOSENSOR

RELATED PATENT APPLICATIONS

[0001] The application claims priority to U.S. Provisional Patent Application Ser. No. 61/172,678, filed Apr. 24, 2009, which is herein incorporated by reference in its entirety.

STATEMENT OF GOVERNMENTAL SUPPORT

[0002] The invention described and claimed herein was made in part utilizing funds supplied by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231 and NIH Grant No. GM008352. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] This invention relates generally to a transcription factor-based biosensor.

BACKGROUND OF THE INVENTION

[0004] In vivo biosensors for small molecules remain a valuable means through which one can design regulatory networks, build screens/selections, and quantify in vivo concentrations of metabolites of interest following system perturbations. Transcription activator and repressor proteins—proteins that modulate the activity of the RNA polymerase on a given promoter—serve as perhaps the most straight forward and controllable option for an in vivo biosensor. Further, transcription repressor/activator proteins are numerous in nature and there exist a wide range of low molecular weight ligands to which they respond. The value of an in vivo biosensor is intrinsically founded upon its transcription profile. An ideal in vivo biosensor would possess little-to-no basal level of expression in the absence of an input signal and the output signal would be linear over a large dynamic range.

[0005] Putatively, σ^{54} -dependent promoters and their associated activator proteins are ideally suited for use as in vivo biosensors. The sigma subunits of RNA polymerase specifically bind to DNA sequence elements and are responsible for differential gene expression. The primary, and most well understood, sigma factor is σ^{70} . σ^{70} associates with the core RNA polymerase (E) to transcribe housekeeping genes. The complex E- σ^{70} alone can be sufficient to catalyze the open promoter complex and allow RNA transcription. While activity can be controlled by various repressor proteins, leaky expression persists.

[0006] Absolute control, however, is accomplished through the use of the much less common complex E- σ^{54} . While most bacteria contain several alternative σ factors of the σ^{70} class, usually only one σ^{54} form exists. A σ^{54} -governed promoter is unique in that hydrolysis of the promoter DNA by an activator protein is an absolute requisite for transcription, imparting an intrinsically very low level of basal expression. ATP hydrolysis by activator proteins can be triggered by phosphorylation, binding of low molecular weight ligands, or protein-protein interaction. Further, without the need for a repressor protein, transcription levels can be tightly controlled over a large dynamic range. Lastly, the σ^{54} -based transcription system is a dedicated transcription system with little cross-talk; there exist close to 100 σ^{54} molecules of in *E. coli* (compared to 700 of σ^{70}), while there are only 20 σ^{54} -governed promoters.

[0007] Use of σ^{54} -dependent promoters and their cognate activator proteins as biosensors in *E. coli* has been limited to

date. Most of the 20 native *E. coli* σ^{54} -dependent promoters are induced in response to nitrogen limitation, potentially minimizing their use in biotechnology applications; however, prpBCDE promoters from *Salmonella enteric* serovar Typhimurium and *E. coli* have been explored by Lee, et al. as a propionate-inducible expression system (Lee, S. K., Keasling, J. D. A Propionate-Inducible Expression System for Enteric Bacteria. *Appl. Environ. Microbiol.* 71, 6856-6862 (2005)).

SUMMARY OF THE INVENTION

[0008] The present invention provides for a system comprising a BmoR transcription factor, a σ^{54} -RNA polymerase, and a pBMO promoter operatively linked to a reporter gene, wherein the pBMO promoter is capable of expression of the reporter gene with an activated form of the BmoR and the σ^{54} -RNA polymerase.

[0009] The present invention provides for a system comprising a first nucleic acid encoding a BmoR, and a second nucleic acid encoding a pBMO promoter operatively linked to a reporter gene, wherein the system is capable of expressing a σ^{54} -RNA polymerase and the pBMO promoter is capable of expression of the reporter gene.

[0010] The present invention provides for a modified host cell comprising a first nucleic acid encoding a BmoR, and a second nucleic acid encoding a pBMO promoter operatively linked to a reporter gene, wherein the modified host cell is capable of expressing a σ^{54} -RNA polymerase.

[0011] The present invention provides for an isolated or purified or recombinant BmoR. In some embodiments, the BmoR is bound to a DNA.

[0012] The present invention provides for a method for sensing a C₂-C₈ alcohol, aldehyde, or mixture thereof, comprising: (a) providing a modified host cell of the present invention, and (b) detecting the expression of the reporter gene.

[0013] In some embodiments of the invention, the (b) detecting step comprises detecting the gene product of the reporter gene. In some embodiments of the invention, the gene product of the reporter gene increases or decreases the doubling time of the modified host cell. In some embodiments of the invention, the gene product of the reporter gene causes the modified host cell to become resistant or sensitive to a compound.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The foregoing aspects and others will be readily appreciated by the skilled artisan from the following description of illustrative embodiments when read in conjunction with the accompanying drawings.

[0015] FIG. 1 shows the induction of β -galactosidase activity in a *P. butanovae* lacZ reporter strain (X::lacZ) in which the bmo promoter controls the expression of a lacZ reporter cassette (Panels A and B), and in a mutant R8-X::lacZ reporter strain in which bmoR is insertionally inactivated (Panels C and D). (Panels A and C) Induction of β -galactosidase in X::lacZ (Panel A) and in mutant R8-X::lacZ (Panel C) in response to alcohols (C₂-C₈) following growth on lactate. (Panels B and D) Induction of β -galactosidase in X::lacZ (Panel B) and in mutant R8-X::lacZ (Panel D) in response to alcohols (C₂-C₈) following growth on propionate. (See Kurth, E. G., Doughty, D. M., Bottomley, P. J., Arp, D. J. & Sayavedra-Soto, L. A. Involvement of BmoR and BmoG in