A gene pair from the jungle. Discovery, Characterization and Applications of the Enterobacter lignolyticus EIL Efflux system

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The findings presented in this work emerge from the discovery of a multidrug efflux system Enterobacter lignolyticus, a bacterium isolated from Puerto Rican cloud forest soil. This system, which consists of the inner membrane transporter EilA and its cognate repressor EilR, confers tolerance to imidazolium-based ionic liquids. In my research, I characterized these genes using molecular genetics, improved a method of microbial biofuel production using synthetic biology techniques, and demonstrated new biotechnological applications by both decoupling the genes from their natural context and engineering the regulatory elements.
Chapter 1 targets the removal of a bottleneck in the microbial conversion of lignocellulose to biofuels or chemicals. In this process, pretreatment of plant biomass is necessary due to the inherent recalcitrance of lignocellulose. Certain ionic liquids (ILs) are solvents remarkably effective in solubilizing cellulosic biomass. By dissociating lignin from hemicellulose and cellulose in cell walls, enzymatic hydrolysis to fermentable sugars can be achieved. However, ILs are toxic to most microbes, inhibiting growth and subsequent fermentation of sugars to fuels. In searching for a solution to this problem, I discovered a novel molecular system for bacterial resistance to IL toxicity by screening the genome of the IL-tolerant bacterium E. lignolyticus. A single gene was identified that promotes growth in the presence of IL, namely, an multidrug transporter, EilA, which acts to export IL from the cell. In response to changes in external IL levels, expression of the transporter is controlled by a repressor, EilR, providing a self regulating system that maintains cell viability. The gene pair encoding EilA and EilR remains functional when transferred to an Escherichia coli strain that expresses a biosynthetic pathway to produce a terpene-based biofuel. In this host organism, the auto-regulatory efflux system enables growth and biofuel production in a previously toxic environment.
Chapter 2 focuses on the EilR protein and how it performs its task as a transcriptional regulator. Identification of the DNA binding site, the eil-operator,

provided the basis to develop a sensitive EilR-regulated promoter that drives expression of a reporter gene in E. coli. Using this cellular biosensor, I identified a range of cationic dyes with high affinity to the EilR repressor. These anthropogenic ligands are unrelated to ILs, and some of them can induce the biosensor at 2 nanomolar concentrations – up to five orders of magnitude lower than ILs. Activitybased assays with cell extracts indicated a possible way of identifying metabolites as natural inducers. In addition, experiments with homologous repressors and a transporter provided further insights on bacterial regulation of efflux.
The strong binding affinity of EilR to its operator and to the readily available ligands motivated me to use this mechanism to develop an inducible system for gene expression (Chapter 3). The three approaches taken to achieve this goal comprise 1) targeted modifications in the native promoter region, 2) refinements of the biosensor promoter and, 3) insertion of operator sites into early promoters from bacteriophages. Using this approach, I generated a set of tightly repressible promoters that are – upon addition of the characterized effector molecules – inducible over more than four orders of magnitude, reaching expression levels comparable to those of the strongest characterized expression systems. Besides Escherichia coli, these promoters are functional in other distantly related bacteria, such as Pseudomonas putida and Sinorhizobium meliloti. I then introduced the EilRguided regulatory mechanism into the yeast Saccharomyces cerevisiae to show how this bacterial repressor can control the activity of a modified yeast promoter containing EilR-binding sites. The EilR-based molecular switch can therefore serve as a tool for independent gene regulation in prokaryotic and eukaryotic organisms.

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