A PLATFORM TECHNOLOGY FOR DYNAMIC CONTROL OF CELL BEHAVIOR
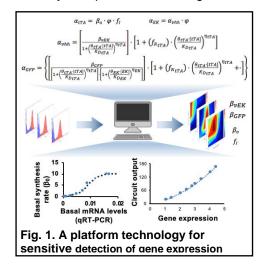
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Mammalian cells rely on complex and highly dynamic gene networks to maintain cellular homeostasis in response to environmental stimuli and intracellular signals. Efficient cellular reprogramming thus requires integration of exogenous components for cell engineering with endogenous cellular networks through feedback control systems. We explored the use of post-translational tools for superior feedback regulation of dynamic behaviors. Specifically, we demonstrated efficient detection and manipulation of the main cellular stress response system – the Unfolded Protein Response (UPR) – for the design of high producing cell lines for protein manufacturing and for the development of cell therapies for sustained protein production.

Our approach is based on the use of the NanoDeg[1] – a bifunctional system that mediates proteasomal degradation of a cellular target with high specificity and exquisite control over rate of decay. To achieve input-dependent post-translational control of the output through the NanoDeg, feedforward loop topologies were explored and compared to conventional strategies for circuit design. We defined the ideal circuit architecture for placing both the reporter output and a reporter-specific NanoDeg under control of a common input and regulate the reporter levels through input-dependent transcriptional and post-translational tools. Transcriptional and post-translational modulation of the output results in lowered basal expression *and* rapid decay of the output upon removal of the input, which, in turn, leads to *enhanced output dynamic range and resolution of the input dynamics*. We deployed this approach and, through iterations of mathematical modeling and experimental tests, built a reporter system for sensitive detection of gene expression. A master cell line containing the components for transcriptional and post-translational control was generated and used to build a comprehensive set of derivative cell lines in which these control elements are linked to the expression of different target genes. Experimental measurements were used to refine the model and generate a predictive tool for establishing experimental conditions for maximal signal amplification and a transfer function correlating the measured signal output with expression of the corresponding target gene (Fig. 1).

This work generated a platform technology for quantitative, multiplexed profiling of gene expression signatures of the UPR with high sensitivity and dynamic resolution of the stimulus causing proteotoxic stress. The relative kinetics of activation of the UPR branches determines cellular fate upon UPR induction (i.e., stress attenuation or apoptosis). This technology will be thus useful for monitoring and manipulating the temporal pattern of activation of the different UPR signaling responses, linking the nature of stimulus causing proteotoxic stress with the outcome of UPR induction, and controlling cellular fate.

This study also provides the design rules of a novel cell engineering technology for building complex genetic networks that govern highly dynamic cellular behaviors. Current work is focused on deploying this technology for manipulating the UPR in response to feedback signals generated at different stages of production of recombinant proteins, thereby enhancing the stress attenuation response and delaying induction of apoptosis. Specifically, we will discuss methods to tune recombinant protein expression with the UPR capacity to cope with proteotoxic stress with the ultimate goal to enhance protein production.

Fig. 1. A platform technology for sensitive detection of gene expression

1. ACS Synth Biol. 2018 Feb 16;7(2):540-552.