

## SYNTHETIC BIOLOGY

## Mammalian signaling circuits from bacterial parts

The ability to engineer synthetic signaling networks has proven challenging for synthetic biology. A breakthrough design strategy shows that bacterial two-component-system-derived parts can be grafted into mammalian cells to create programmable phosphorylation circuitry.

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A cell's ability to sense its external environment and respond in an appropriate fashion is central to its survival and functional identity. Using artificial regulatory networks to reprogram cellular sense and response has thus been a longstanding focus for synthetic biology, with potential applications that range from biosensing to cell-based therapy and regenerative medicine. In natural systems, external signals are universally sensed through networks of transiently interacting intracellular proteins that transmit information post-translationally via reversible chemical marks, typically phosphorylation.

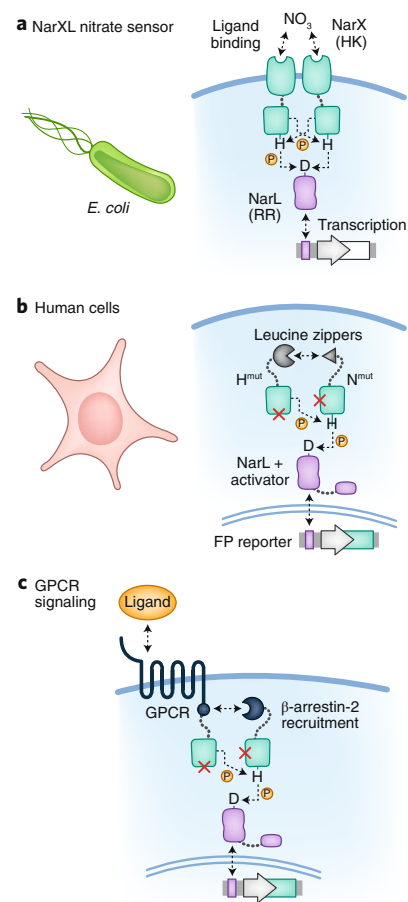
Unfortunately, although a large and ever-growing variety of synthetic transcriptional circuits have been reported over the past two decades<sup>1</sup>, engineered phosphorylation-mediated signaling networks, especially in eukaryotes, have been limited to a handful of examples<sup>2</sup>. Progress has been hampered by a scarcity of biomolecular parts and a lack of design strategies for both establishing post-translational circuit connections and insulating them from unwanted crosstalk with native networks. In this issue of *Nature Chemical Biology*, Mazé and Benenson<sup>3</sup> establish a new, potentially generalizable approach that addresses these challenges; by harnessing the modularity inherent in bacterial two-component systems (TCS), they demonstrate engineering of phosphorylation circuits capable of sensing intra- and extracellular signals in human cells and robustly converting them into transcriptional outputs<sup>4</sup>.

In bacteria, TCSs play a central role in detection and transmission of a broad range of environmental signals, from pH and temperature to small molecules, toxins and antibiotics. Canonical systems typically consist of two proteins: a ligand-sensing transmembrane histidine kinase (HK)—usually a homodimer—that binds to ATP and undergoes histidine autophosphorylation upon ligand binding, and a cognate response regulator (RR) protein that subsequently receives the

phosphoryl group from the HK, resulting in binding to target promoters and transcriptional activation. For their work, Mazé and Benenson<sup>3</sup> focused their engineering efforts on the NarX HK (Fig. 1a), a nitrate sensor from *Escherichia coli* that they previously demonstrated is constitutively phosphorylated when expressed in the cytoplasm of human cells and, further, is capable of inducing a transcriptional reporter via co-expression of an RR (NarL) appended with a eukaryotic transcriptional activator domain<sup>4</sup>.

In the current study, the authors engineered the HK to create a device in which heterospecific protein–protein interactions could be converted into transcriptional activation in HEK293 cells. To accomplish this, they first identified a C-terminal region of the HK (NarX<sup>379–598</sup>) that abolished dimerization-dependent RR phosphorylation and for which activity could be restored synthetically by leucine-zipper-mediated dimerization (Fig. 1b). The authors then created a pair of HK mutants, one lacking the catalytic histidine (H<sup>mut</sup>) and the other incapable of binding ATP (N<sup>mut</sup>). Though neither alone could activate RR through homodimerization, zipper-mediated heterodimerization of the mutants resulted in reporter activation—an effect that could be recapitulated through chemically inducible dimerization by substituting the leucine zipper with the rapamycin-dependent dimerization pair FK506-binding protein (FKBP) and FKBP12-rapamycin binding domain (FRB).

The goal of engineering information flow across the cell membrane in eukaryotes has been a challenge for synthetic biology, and there have been numerous efforts aimed at developing systems for programmable receptor-mediated signaling circuitry<sup>5–7</sup>. As a final demonstration, the authors used H<sup>mut</sup> and N<sup>mut</sup> heterodimerization to couple transcriptional output to ligand binding by a G-protein-coupled receptor (GPCR). By fusing H<sup>mut</sup> to a small-molecule-sensitive GPCR, and N<sup>mut</sup> to  $\beta$ -arrestin-2, which



**Fig. 1 | Harnessing molecular parts from a bacterial two-component system to engineer signaling pathways in mammalian cells. a**, The NarX–NarL (NarXL) system from *E. coli* is shown. **b**, Using a truncated form of the NarX histidine kinase, the authors demonstrate dimerization- (via leucine zipper) dependent reporter activation. By introducing complementing mutations to the shortened HK, they create a heterospecific, interaction-dependent phosphorylation. **c**, These components are used to create a circuit that connects GPCR ligand binding to transcriptional output. P, phosphate group; H, histidine residue on the sensor kinase; D, aspartic acid residue on the response regulator; FP, fluorescent protein.

is recruited to the receptor upon ligand binding, they demonstrated robust dose-sensitive, ligand-dependent transcriptional activation (Fig. 1c).

In principle, phosphorylation circuits offer key performance advantages compared to other classes of synthetic circuitry. Gene network response timescales are constrained by transcriptional and translational delays, and restriction to the nucleus ultimately makes them poorly suited to sense and dynamically respond to rapid, often diffusion-driven changes in a cell's external chemical environment. Although several programmable protease- and intein-based sense-and-respond systems have been reported in mammalian cells<sup>5,8–11</sup>, reliance on irreversible cleavage events limits their activation speed and reversibility compared to phosphorylation networks. To demonstrate the reversibility of their system (HK has intrinsic phosphatase activity that drives histidine dephosphorylation in the absence of ligand), the authors do a careful comparison with a protease-based system<sup>11</sup> that relies on arrestin-mediated recruitment of a protease to liberate GPCR-fused

transcription factors from the membrane. Analysis of the dynamics of the two systems demonstrated that the phosphorylation-based system was more quickly reversed, and thus better suited for recording sequential events.

The engineering strategy employed by the authors offers a potentially far-reaching solution for engineering mammalian cell signaling circuitry. As histidine phosphotransfer is largely unutilized in mammalian cells, the TCS-derived parts enable construction of highly sensitive information channels that can operate orthogonally to native Y- and S/T-phosphorylation machinery. Additionally, because activation of many metazoan signaling pathways is initiated by membrane recruitment events, it may be feasible to use the scheme to couple activation of other receptor classes to circuit output. Scaling of the platform to allow multiplex sensing could enable integration of multiple environmental features, potentially allowing cells deployed in a therapeutic setting to precisely distinguish diseased and healthy tissue.

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#### Competing interests

The authors declare no competing interests.

