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Bacterial two-component systems as sensors for synthetic biology applications

John T. Lazar¹, Jeffrey J. Tabor^{2,3,4}

¹Department of Chemical and Biomolecular Engineering, Rice University, Houston, TX, USA

²Department of Bioengineering, Rice University, Houston, TX, USA

³PhD Program in Systems, Synthetic, and Physical Biology, Rice University, Houston, TX, USA

⁴Department of Biosciences, Rice University, Houston, TX, USA

Abstract

Two-component systems (TCSs) are a ubiquitous family of signal transduction pathways that enable bacteria to sense and respond to diverse physical, chemical, and biological stimuli outside and inside the cell. Synthetic biologists have begun to repurpose TCSs for applications in optogenetics, materials science, gut microbiome engineering, and soil nutrient biosensing, among others. New engineering methods including genetic refactoring, DNA-binding domain swapping, detection threshold tuning, and phosphorylation cross-talk insulation are being used to increase the reliability of TCS sensor performance and tailor TCS signaling properties to the requirements of specific applications. There is now potential to combine these methods with large-scale gene synthesis and laboratory screening to discover the inputs sensed by many uncharacterized TCSs and develop a large new family of genetically-encoded sensors that respond to an unrivaled breadth of stimuli.

Introduction

Synthetic biologists program cells to sense and respond to extra- and intracellular stimuli for applications in medicine[1], agriculture[2], chemicals synthesis[3] and many other areas. For example, a probiotic strain of *E. coli* that naturally colonizes tumors was recently engineered to lyse and release immune checkpoint-inhibiting nanobodies upon reaching high intra-tumoral density[4]. By enabling local delivery of these potent biologic drugs, this approach could improve the efficacy of cancer immunotherapy while decreasing side effects. In other work, researchers are engineering bacteria to sense stresses imposed by heterologous metabolic and genetic pathways and activate the expression of enzymes or stress-response systems that ameliorate these effects [5]. Such 'host-aware' design strategies

JJT is a founder of PanaBio, a company that aims to commercialize diagnostic and therapeutic bacteria.

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could increase the stabilities of engineered genetic systems and improve the yields of industrial fermentations, among other benefits.

To program cells to sense and respond to stimuli, synthetic biologists utilize genetically-encoded sensors. The canonical synthetic biological sensor is an RNA or protein that binds to a ligand or perceives a biochemical or physical property of the environment and responds by altering gene expression. Riboswitches are a well-studied family of RNA-based sensors. The prototypical riboswitch is encoded on the 5' end of a messenger RNA and comprises an aptamer domain linked to a gene regulatory domain. In the presence of a cognate stimulus (a.k.a. input), the aptamer domain undergoes a conformational rearrangement that is transmitted to the gene regulatory domain, resulting in a change in the level of expression of the gene encoded on the mRNA *in cis* [6]. Riboswitch inputs are often involved in fundamental biological processes and include metal ions, molecules involved in RNA metabolism, and amino acids[7]. The gene regulatory domains of riboswitches most often modulate transcription of the downstream mRNA by exposing or obscuring transcriptional terminators, translation of the downstream open reading frame by exposing or obscuring a ribosome binding site, or mRNA splicing[8]. Synthetic riboswitches that sense inputs not known to be detected by their natural counterparts have been engineered by rational design[9-11], directed evolution[12], and machine learning[13]. One notable recent study utilized a physics-based model of riboswitch structure and function to engineer translation-regulating sensors of theophylline, tetramethylrosamine, fluoride, dopamine, thyroxine, and 2,4 dinitrotoluene with activation ratios up to several hundred-fold [14]. Though good progress is being made on riboswitch design, the repertoire of inputs that these RNA-based sensors can detect remains small. On the other hand, nature has provided a wealth of protein-based sensors that sense diverse inputs relevant to synthetic biology applications.

One-component systems (OCSs) are the largest family of bacterial signal transduction pathways [15,16] and the most frequently-used family of genetically-encoded sensors. The typical OCS comprises an allosteric transcription factor (aTF) and a target (a.k.a. output) promoter. The aTF usually consists of an N-terminal sensor domain linked to a C-terminal DNA-binding domain (DBD). In the presence of the input, the aTF sensor domain allosterically modulates the activity of the DBD, and thus the rate of transcription from the output promoter. A handful of well-characterized OCSs (e.g. LacI, TetR, AraC, LuxR) are often used as model sensors in synthetic biology studies. Recent efforts based on genome mining, directed evolution, protein engineering, and computational protein design have generated several dozen new OCS sensors, including those that detect inputs linked to bacterial or host physiology [17-21]. It is likely that many new OCSs will be characterized and engineered for synthetic biology applications going forward.

Two-component systems (TCSs) are the largest class of multi-step signal transduction pathways in nature[22] and an important family of sensors for synthetic biology. The classical TCS sensor comprises a sensor histidine kinase (SHK), a response regulator (RR), and an output promoter (Fig. 1). A given SHK contains an N-terminal sensor domain linked to C-terminal transmitter domain, often by a transmembrane region[23]. The presence of the input causes the SHK sensor domain to undergo a conformational rearrangement that is relayed to the transmitter domain, typically increasing kinase activity. In the activated

state, the transmitter domain phosphorylates the partner RR on a conserved aspartate residue within an N-terminal receiver (REC) domain. Similar to aTFs, most RRs contain C-terminal DBDs that regulate transcription[24]. Phosphorylation induces a conformational switch that activates the DBD, often via REC-mediated homodimerization[25-27]. Most SHK transmitter domains also dephosphorylate their cognate RRs in the absence of input, thus de-activating the TCS response. A spectrum of signal transduction pathways that elaborate upon the core TCS architecture are present in bacteria, archaea, and non-animal eukaryotes[28-30]. However, the prototypical TCS is predominantly found in bacteria.

TCS performance features

Though OCSs are simpler and more abundant, TCSs offer several advantages for synthetic biology. First, SHKs can be membrane-bound or cytoplasmic while OCSs are almost exclusively cytoplasmic[15]. Accordingly, TCSs can sense extracellular, intra-membrane, or intracellular inputs while OCSs typically sense intracellular inputs. From a sensor design perspective, a transporter must be co-expressed alongside an OCS if the input does not naturally diffuse or is not naturally transported across the membrane [31]. As transporters are not readily available for many compounds (e.g. large molecular weight species), OCSs tend to sense a more restricted range of inputs than TCSs. Second, the bi-functional kinase/phosphatase activity of SHKs makes TCS output signals (phosphorylated RR and thus transcription rate) relatively insensitive to changes in the expression levels of SHKs and RRs[32,33]. This built-in robustness can buffer TCS responses against gene expression noise or fluctuations in SHK and RR expression that arise from changing growth conditions. Third, we recently demonstrated that SHK phosphatase activity acts as a built-in knob for tuning TCS detection threshold[34]. In particular, by introducing transmitter domain mutations that specifically reduce SHK phosphatase activity, TCSs can be made to respond to their inputs at up to two orders of magnitude lower concentrations. Furthermore, we demonstrated that the first variable residue in the transmitter domain GXGXG motif, which is present in 64% of SHKs, can be mutated to different hydrophobic residues to tune the detection thresholds of TCSs even in the absence of well-characterized phosphatase-altering mutations. This phosphatase tuning method is simpler to implement than computational design and directed evolution, which are frequently used to tune OCS sensitivity.

TCSs have evolved to sense a remarkable assortment of inputs. Classes of known TCS inputs include light, temperature, pH, metals, nutrient availability, respiratory electron acceptors, oxidizing agents, small molecule metabolites, inter-bacterial communication signals, antibiotics, antimicrobial peptides, oligosaccharides, proteins, hormones, and other host-derived signals (Table 1). Some SHKs are highly specific for a single input. For example, *E. coli* NarX and *Shewanella halifaxensis* ThsS are activated by the terminal electron acceptors nitrate (NO_3^-) and thiosulfate ($\text{S}_2\text{O}_3^{2-}$), but discriminate against closely-related compounds such as nitrite (NO_2^-) and tetrathionate ($\text{S}_4\text{O}_6^{2-}$), respectively[35,36]. Other SHKs sense multiple inputs characteristic of a specific environment. For example, *Salmonella* Typhimurium PhoQ is activated by low divalent cation concentrations, acidic pH, and antimicrobial peptides – three distinct stimuli that are likely to be encountered by the bacterium during the infection of a host[37]. SHKs can detect multiple inputs via multiple binding sites in a single sensor domain[37], or via multiple sensor domains[38].

Finally, many SHKs sense general phenomena such as membrane disruption that occur in the presence of a wide range of inputs[39].

Repurposing TCSs as sensors for synthetic biology

Researchers have begun to utilize TCSs as sensors for synthetic biology applications. For example, light-responsive TCSs (Table 1) have been used to spatially manipulate gene expression across two-dimensional bacterial lawns[40-42], program bacteria to perform the image processing function of edge detection[43], pattern biofilm deposition on ceramics, polystyrene, and cotton[44], introduce single-base pair edits in genomic DNA[45], characterize the input/output dynamics of transcriptional regulatory circuits[46], dynamically control metabolic pathway flux[47-49], and characterize how gut bacterial metabolite secretion can impact host health and longevity[50]. In another series of studies, TCSs activated by thiosulfate, tetrathionate, and acidic pH (Table 1) have been used to program bacteria to sense and report intestinal inflammation in mouse models of colitis and Crohn's Disease [36,51,52]. Such diagnostic gut bacteria could be advanced to enable long-term monitoring and treatment of inflammatory bowel diseases with less invasiveness and fewer side-effects compared to current standards of care.

It is often necessary to replace the evolved gene regulatory systems that govern TCS function prior to using them as sensors (Fig. 2a). First, *shk* and *rr* genes are generally regulated by multiple interacting pathways[53]. These evolved TCS regulatory networks can cause problems such as silencing[54] or transient input responses[55] that can compromise TCS sensor function. These challenges can often be overcome using genetic refactoring (Fig. 2b). Here, evolved promoters, ribosome binding sites (RBSs), and terminators are replaced with well-characterized alternatives that function reliably in a bacterium of interest. The open reading frames (ORFs) of *shk*, *rr*, and any required auxiliary genes can also be computationally redesigned to eliminate known and unknown regulation while increasing translational efficiency in a heterologous host[56]. Though TCSs are relatively insensitive to the levels of their component proteins, it is still important to optimize *shk* and *rr* expression levels during refactoring. TCSs are generally more sensitive to RR than SHK levels. If total RR abundance is too low, there will be too few phosphorylated RRs to bind to output promoters and activate transcription in response to the input. Conversely, if total RR abundance is too high, output promoters will often exhibit strong activity in the absence of any input. This problem can arise due to promoter binding by non-phosphorylated RRs, which exist in an equilibrium between inactive and active conformations[24,57]. It can also arise due to residual SHK kinase activity in the absence of input, or alternative sources of RR phosphorylation such as small molecule donors[58] or non-cognate SHKs[59], the effects of which become pronounced at high total RR expression. Very low and very high SHK expression often degrade TCS responses[34,36,52,60,61] and should be avoided. SHK and RR abundances can be co-optimized using orthogonal gene expression inducers (e.g. IPTG, aTc) or libraries of constitutive promoters or RBSs of different strengths[34,36,52,54,60-62].

Unfortunately, many evolved TCS output promoters can only be activated to a small extent. Furthermore, TCS output promoters are often cross-regulated by alternative pathways or

silent in heterologous hosts[36,54,61,62]. These challenges can be addressed by introducing mutations or truncations into output promoters to remove unwanted regulatory sites or nested constitutive promoters that generate leaky transcription[36,54,60,61]. TCSs can also be rewired to non-cognate output promoters with superior performance features. The traditional approach to TCS rewiring is to make a chimera between an SHK sensor domain of interest and a non-native transmitter domain. Here, the chimeric SHK controls the phosphorylation of a non-cognate RR and thereby transcription from a non-cognate output promoter. Though sensor domain swapping has been used to engineer numerous TCS sensors[40,63-71], the allosteric mechanisms that enable communication between sensor and transmitter domains are intricate and incompletely understood. As a result, general strategies for TCS sensor domain swapping remain elusive. Approaches based SHK-RR interface swapping[72] and protein scaffolds that redirect SHK phosphorylation to non-cognate RRs[73] have also shown promise for TCS rewiring. However, these approaches tend to yield TCSs whose activity is not dependent on the presence of the input.

Recently, we developed a general method for rewiring TCSs to well-characterized output promoters by modularly swapping RR DBDs[61]. In particular, we identified standard amino acids at which the DBDs of RRs from the OmpR/PhoB or NarL/FixJ sub-families can be removed and replaced with those from structurally-related but functionally unrelated RRs. In addition, we developed standard output modules (e.g. the CcaR DBD and its *P_{cpcG2-172}* output promoter for OmpR/PhoB family RRs, and the YdfI DBD and its *P_{ydfI115}* output promoter for NarL/FixJ RRs) to which TCSs from those families can be rewired with high rates of success. OmpR/PhoB and NarL/FixJ constitute over 70% of all transcription-regulating RRs[24], suggesting that thousands of TCSs could be characterized and potentially deployed as sensors using this approach.

Due to similarities in the sequences and structures of their interaction interfaces, SHKs and RRs from different TCSs may cross-talk with one another in the same cell. Such phospho-signaling cross-talk could compromise the fidelity of a given TCS sensor in the complex cellular environment. However, work by Laub and colleagues has demonstrated that SHKs generally exhibit a high degree of specificity toward their cognate RRs in the cell due to a large and relatively unoccupied interaction sequence space[70]. This result suggests that synthetic biologists can utilize multiple TCS sensors in a single cell without a high risk of phospho-signaling cross-talk. In the event that phospho-signaling cross-talk is a problem, this group also demonstrated that the evolved interaction interfaces can be replaced with insulated versions that have been shown not to cross-talk with other TCSs. The authors used this approach to engineer a system based on a sensor domain-swapped version of PhoQ that responds to the plant cytokinin trans-zeatin and does not cross-talk with any native TCS in *E. coli*[70] (Table 1).

Porting TCSs into eukaryotes

There is substantial interest in using TCSs to endow eukaryotic cells with novel sensing capabilities. In early work, an engineered *E. coli* Trg-PhoR system was used to sense 2,4,6-trinitrotoluene via an interaction with the computationally-designed extracellular accessory protein TNT.R3 in *A. thaliana*[74,75]. To achieve transcription regulation in plants, the

RR PhoB was fused to a VP64 transactivation domain and used to activate a minimal plant promoter engineered to contain multiple *Pho* operator sites. Porting TCSs into plants more broadly remains a challenge. In other work, Benenson and colleagues expressed the *E. coli* TCSs EnvZ-OmpR, NarX-NarL, and DcuS-DcuR in mammalian cells[76]. Similar RR:transactivator domain fusions and synthetic eukaryotic output promoter design strategies were used to enable control of transcription. While these three TCSs were capable of phospho-signaling and transcriptional activation, they did not respond to their cognate inputs. The reasons for sensing failure are unclear and warrant further investigation. One likely source of the problem is an incompatibility between bacterial SHK transmembrane regions and eukaryotic membranes. In an interesting recent follow-up study, this group fused two NarX transmitter domain mutants that must be brought into close physical proximity to phosphorylate NarL to a G-protein coupled receptor- β arrestin pair that heterodimerize in the presence of ligands including procaterol[77]. This engineered pathway generates large transcriptional responses to these ligands in mammalian cells. This approach may be useful for engineering synthetic mammalian signaling pathways that do not cross-talk with endogenous systems.

Harnessing nature's treasure trove of TCS sensors

Despite exciting progress, TCSs remain a largely untapped treasure trove of sensors for synthetic biology. The number of TCSs with well-characterized inputs is on the order of one hundred (Table 1). However, many thousands of TCSs are present in bacterial genomes[34,78] and the inputs of most of these systems cannot currently be predicted. A major impediment to identifying the inputs of these orphan TCSs is that most bacteria are intractable; they cannot be cultured nor genetically-manipulated in the laboratory. Synthetic biology methods are helping to overcome this challenge. If an output promoter of an orphan TCS is known or can be inferred from genomic context, the system can be introduced into a tractable organism such as *E. coli* or *B. subtilis*[36]. If an output promoter is not known or does not function well in laboratory conditions, sensor domain swapping or DBD-swapping can be used to replace it with an alternative that functions reliably. In either case, the orphan TCS can then be screened against targeted input panels designed by analyzing the function of genes residing adjacent to the TCS or the environment in which the native organism lives[36], complex samples representative of those environments, or even large panels of untargeted chemicals. If inputs of interest are known, commercial gene synthesis can be used to apply this process to hundreds or thousands of orphan TCS pathways in order to identify novel sensors. For example, libraries of orphan TCSs from the human gut microbiome could be synthesized and screened against disease biomarkers found in the gut. If a TCS responsive to the biomarker is found, it can be converted into a high-performance sensor using genetic refactoring and phospho-signaling insulation. Finally, the detection threshold of the new sensor can be matched to the needs of applications such as engineering bacteria that diagnose and treat disease[79]. Similar approaches are being taken to engineer bacteria to detect soil nitrate levels [34], which could eventually be coupled with engineered nitrogen fixation pathways[2] to maintain nitrogen homeostasis in soil without fertilizer.

Conclusions

Though they are more complex than alternatives such as riboswitches and OCSs, TCSs offer a number of benefits as sensors for synthetic biology. First, due to the transmembrane architecture of most SHKs, TCSs can sense inputs that are both accessible and inaccessible to cytoplasmic sensors. Additionally, TCS phospho-signaling increases the reliability of sensor function in the face of variable protein expression levels while also providing a built-in knob for tuning TCSs to respond to different input concentrations. It is likely that bacteria have exploited these and other features of TCSs to better adapt to diverse environmental conditions. Synthetic biologists are increasingly taking advantage of these same properties to endow bacteria with artificial sense and respond capabilities for new engineering applications. However, we have only reached the tip of the iceberg. Bacterial genomes host a huge number of uncharacterized TCSs that likely sense inputs of agricultural, biotechnological, environmental, medical, physiological, and scientific relevance for which no biosensors are currently available. Recent methods for porting TCSs into laboratory bacteria are accelerating the pace at which their inputs can be discovered. Recapitulating TCS function in eukaryotes remains an important challenge that will likely require both new biological insights and new engineering approaches to solve. Overall, the breadth of inputs that they sense combined with the robustness and programmability of their performance will make TCSs an important family of sensors for synthetic biology in the future.

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Highlights:

- Two-component systems (TCSs) are a large class of bacterial signaling pathways that can detect an incredibly wide variety of stimuli
- Recent synthetic biology methods can reduce cross-talk, increase dynamic range, and adjust TCS detection thresholds
- Many TCSs can now be reliably rewired to synthetic output promoters, facilitating elucidation of their inputs
- TCSs are being applied to the gut microbiome, metabolic engineering, and biomaterial production among other applications.

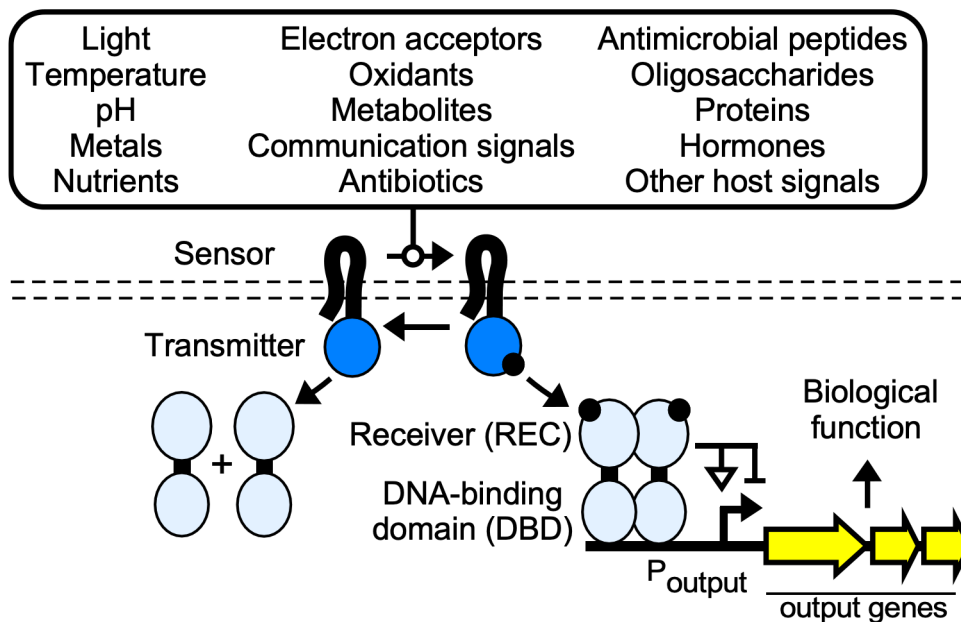
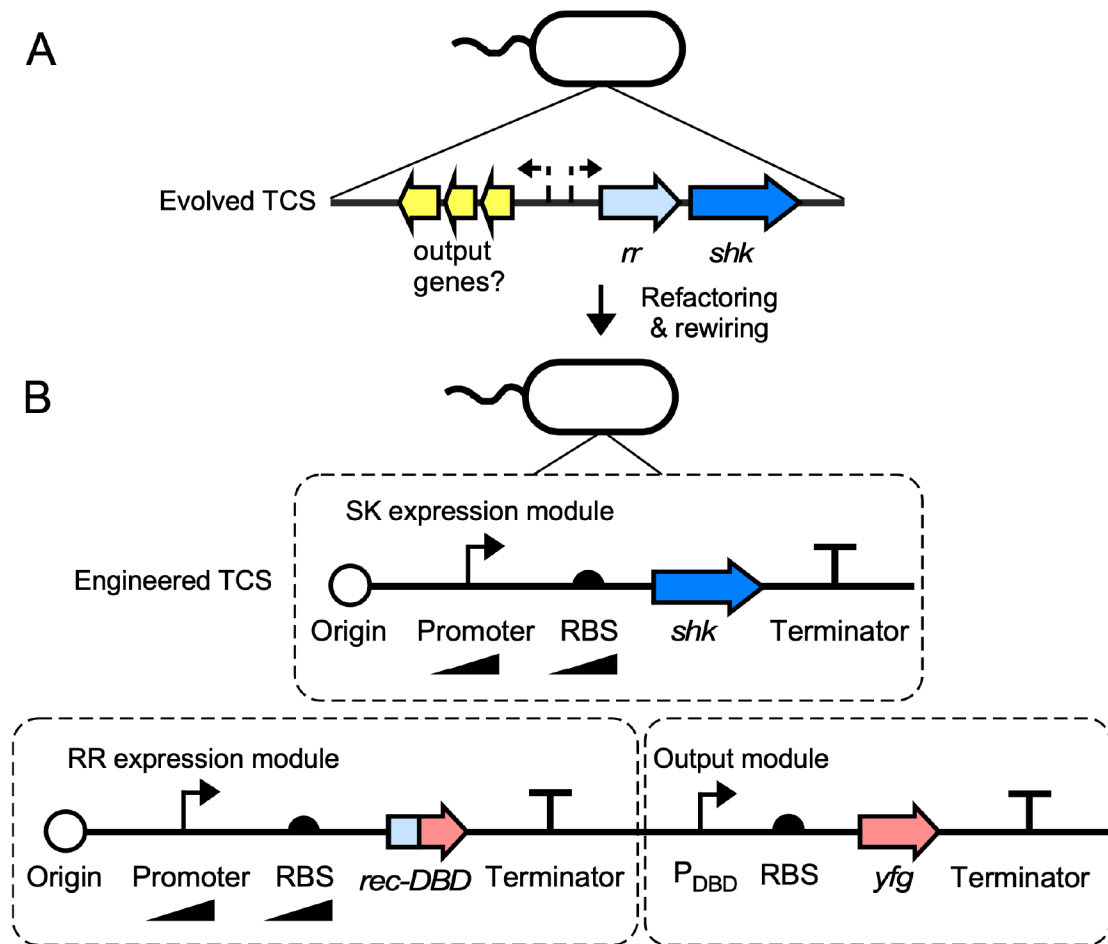


Figure 1. Bacterial two-component systems and their function. Inputs are shown inside of the rounded box. Sensor histidine kinase (SHK): Black/dark blue. Phosphoryl group: black circle. Response regulator (RR): light blue. P_{output} : output promoter.

**Figure 2.**

Refactoring and rewiring TCSs. (A) Evolved TCSs can be computationally identified in bacterial genomes due to the conservation of SHK and RR domain architectures and the fact that the genes encoding interacting SHK-RR pairs often reside adjacent to one another. However, the promoters (dashed bent lines) and RBSs driving the expression of *shk* and *rr* genes can be difficult to predict and characterize from sequence information. This problem is exacerbated for output promoters, which may or may not reside adjacent to *shk* and *rr* genes. (B) TCSs can be engineered to function more reliably by replacing native promoters and RBSs with well-characterized synthetic versions. Promoters and RBSs of different strengths should be screened to achieve optimal SHK and RR expression levels. If an evolved output promoter is unknown or has undesirable features, it can be replaced by DBD swapping, wherein the native *rr* gene is replaced by a chimeric *rec-DBD* gene and the native output promoter is replaced by a well-characterized promoter that responds to the Rec-DBD protein (P_{DBD}). *yfg*: your favorite gene.

Table 1

Available two-component system (TCS) sensors. Often, numerous TCSs sense a given input and a given response regulator regulates numerous output promoters. In these cases, one well-characterized TCS or output promoter is listed for simplicity.

| Input | Native organism(s) | SHK | RR | Output promoter | Performance notes | Reference |
|-----------------------------------------------------|---------------------------------------------------|-------------|----------------------------------------------------|-------------------------|-----------------------------------------------------------------------------------------------------|-----------|
| Light | | | | | | |
| UV-violet light | <i>Synechocystis</i> PCC6803 | UirS | UirR | P_{uirR} | 5-fold activation. Also reported to sense ethylene | [54] |
| Blue light | <i>B. subtilis</i> , <i>B. japonicum</i> | YF1 | FixJ | P_{fixJ} | Chimeric YvA-FixL SHK. 460-fold activation when coupled to transcriptional inverter (pDushk system) | [80] |
| Green light | <i>Synechocystis</i> PCC6803 | CcaSmini#10 | CcaR | $P_{opcG2-172}$ | 600-fold activation | [81] |
| Red light | <i>Synechocystis</i> PCC6803, <i>E. coli</i> | Cph8* | OmpR | $P_{ompR112}$ | 80-fold de-activation | [60] |
| Near infrared light | <i>Rps. palustris</i> , <i>B. japonicum</i> BTA11 | BphP1 | PpsR2 | P_{Brc_crrE} | Signaling based on RR sequestration. 2-fold activation | [62] |
| Temperature | | | | | | |
| 25°C | <i>B. subtilis</i> | DesK | DesR | P_{des} | Fully activated and repressed at 25°C and 37°C, respectively | [82] |
| pH | | | | | | |
| Acidic pH (<6.2) | <i>S. oneidensis</i> , <i>B. subtilis</i> | SO_4387 | SO_4388 ^{REC-} PsdR _{DBD} 137 | $P_{psdA110}$ | Used to sense small intestinal inflammation in mice | [52,61] |
| Acidic pH (extracellular) | <i>H. pylori</i> | ArsS | ArsR | P_{amiF} , P_{amiF} | Activated in the human stomach | [83,84] |
| Metals | | | | | | |
| As ³⁺ (extracellular) | <i>A. tumefaciens</i> | AioS | AioR | P_{aioB} | Requires AioX inner membrane accessory protein | [85] |
| Ca ²⁺ (extracellular) | <i>P. aeruginosa</i> PAO1 | CarS | CarR | P_{carO} | CarS is related to PhoQ but senses the presence not absence of the divalent cation | [86] |
| Cu ⁺ (extracellular) | <i>E. coli</i> | CusS | CusR | P_{cusC} | Also activated by Ag (I) | [87-89] |
| Cu ²⁺ (extra- and intracellular) | <i>Synechocystis</i> PCC 6803 | CopS | CopR | P_{copM} | CopS partially localizes to thylakoid membranes | [90] |
| Cu ²⁺ (extracellular) | <i>M. xanthus</i> | CorS | CorR | P_{aioA} | | [91] |
| Fe ²⁺ , Fe ³⁺ (extracellular) | <i>S. marcescens</i> | RssA | RssB | P_{pvcA} | Activity can be tuned with the natural product 2-isocyano-6,7-dihydroxycoumarin | [92] |
| K ⁺ (extracellular, intracellular) | <i>E. coli</i> | KdpD | KdpE | P_{kdpF} | Inactivated by K ⁺ | [93,94] |
| Ni ²⁺ | <i>Synechocystis</i> PCC6803 | NrsS | NrsR | P_{nrsB} | 10-fold activation. Also weakly activated by Co ²⁺ | [95] |
| U | <i>E. coli</i> | UzcS | UzcR | P_{urcA} | Sensitivity and specificity improved by coupling to UtrRS via AND gate | [96] |

| Input | Native organism(s) | SHK | RR | Output promoter | Performance notes | Reference |
|-----------------------------------------------------|---------------------------------|-----------|----------------------------------------------|-------------------------|--------------------------------------------------------------------------------------------------------------------------------|-----------|
| Zn ²⁺ | <i>E. coli</i> | ZraS | ZraR | P _{zraP} | P _{zraP} 's σ^{54} -dependent | [97] |
| Nutrient availability | | | | | | |
| Nitrogen limitation | <i>E. coli</i> | NtrB | NtrC | P _{ndp} | Requires the accessory protein GlnB _{ogl} . | [98] |
| Respiratory electron acceptors | | | | | | |
| O ₂ | <i>S. meliloti</i> | FixL | FixJ | P _{nifA} | O ₂ inhibits pathway. O ₂ binds FixL via covalently attached heme. | [99] |
| Thiosulfate | <i>S. halifaxensis</i> | ThsS | ThsR | P _{thbA342} | Activated proportional to DSS-induced inflammation in mouse colon | [36] |
| Tetrathionate | <i>S. baltica</i> | TtrS | TtrR | P _{trb185-269} | 100-fold dynamic range | [36] |
| Nitrate | <i>E. coli</i> | NarX | NarL _{REC} -YdfL _{DBD} 131 | P _{ydfL115} | 1300-fold activation in <i>B. subtilis</i> . | [61] |
| Nitrate OR Nitrite | <i>E. coli</i> | NarQ | NarP | P _{nrfA} | NarQ also responds to nitrate. NarX cross-phosphorylates NarP. NarL binds NarP output promoters. | [100] |
| Trimethyl amine N-oxide (TMAO) | <i>E. coli</i> | TorS | TorR _{REC} -PsdR _{DBD} 137 | P _{psdA110} | DBD swapping eliminates O ₂ cross repression at native output promoter. Requires periplasmic TorT accessory protein | [61] |
| Oxidizing agents | | | | | | |
| O ₂ , H ₂ O ₂ , NO | <i>S. aureus</i> | AirS | AirR | P _{airO} | AirS requires a [2Fe-2S] ²⁺ cluster | [101,102] |
| Small molecule metabolites | | | | | | |
| α -ketoglutarate (extracellular) | <i>P. aeruginosa PAO1</i> | MifS | MifR | P _{PA5550} | 10-fold activation. Weak response to glutarate | [103] |
| Butanol | <i>C. acetobutylicum</i> | BtrK | BtrR | P _{btrT} | Involved in butanol tolerance | [104] |
| C4-dicarboxylates (extracellular) | <i>E. coli</i> | DcuS | DcuR | P _{ndcA} | 22-fold activation | [105] |
| Citrate | <i>K. pneumoniae</i> | CitA | CitB | P _{citC} | Requires anaerobic conditions | [106] |
| Fucose | <i>E. coli</i> | Fushk | FusR | P _{z0461} | Fucose represses transcriptional output | [107] |
| Fumarate | <i>E. coli</i> | DcuSZ | OmpR | P _{ompC} | Chimeric DcuS-EnvZ SHK. < 5-fold activation | [66] |
| Glucose-6-phosphate | <i>E. coli</i> | UhpB | UhpA | P _{uhp799} | Requires UhpC inner-membrane accessory protein | [61,108] |
| L-Glutamate | <i>P. aeruginosa PAO1</i> | AauS | AauR | P _{aauJ} | Very weakly activated by aspartate, glutamine, and asparagine | [109] |
| Heme (extracellular) | <i>S. aureus</i> | HssS | HssR | P _{hrtA} | >100-fold activation | [110] |
| Indole | <i>E. coli</i> | BacS | BacR | P _{acdD} | Presence of CpxAR TCS amplified the indole response | [111] |
| Malate | <i>B. subtilis</i> | YufL | YufM | P _{maeN381} | 100-fold activation | [112] |
| Methanol | <i>P. denitrificans/E. coli</i> | FliS-EnvZ | OmpR | P _{ompC} | 2-fold activation | [65] |

| Input | Native organism(s) | SHK | RR | Output promoter | Performance notes | Reference |
|---------------------------------------------------------------------------------------|----------------------------------|----------|--------|-----------------|-----------------------------------------------------------------------------------------------------|-----------|
| Pyruvate (extracellular) | <i>E. coli</i> | BtsS | BtsR | P_{xyjY} | Active in uropathogenic <i>E. coli</i> during urinary-tract infections | [113] |
| Ribose | <i>E. coli</i> | Trg-EnvZ | OmpR | P_{ompC} | Chimeric SHK. 20-fold activation | [71] |
| D-xylose (extracellular) | <i>C. beijerinckii</i> | LytS | YesN | P_{xyf} | D-xylose recognized by outer membrane transporter-like protein XyIFII | [114] |
| Styrene | <i>Pseudomonas</i> sp. strain Y2 | StyS | StyR | P_{styA} | StyS has non-canonical HK-REC-HK structure | [115] |
| Inter-bacterial communication signals | | | | | | |
| AIP-I (autoinducer peptide) | <i>S. aureus</i> | AgrC | AgrA | P_{agrB} | AIP-II inhibits AgrC kinase activity | [116] |
| CAl-1 ((S)-3-hydroxytridecan-4-one) | <i>V. cholerae</i> | CqsS | LuxO | P_{qpp4} | Requires intermediate phosphotransfer protein LuxU | [117] |
| CSP (Competence Stimulating Peptide) | <i>S. gordonii</i> | ComD | ComE | P_{comC} | | [118] |
| ComX (extracellular pheromone) | <i>B. subtilis</i> | ComP | ComA | P_{srfA} | | [119] |
| Antibiotics | | | | | | |
| β -lactams | <i>V. cholerae</i> | VxrA | VxrB | P_{nuu} | Generally activated by cell envelope damage | [120] |
| Linearmycins (intra-membrane) | <i>B. subtilis</i> | LnrJ | LnrK | P_{lnrL} | Can also detect the antifungal polyene amphoterin B. | [121,122] |
| Vancomycin (extracellular) | <i>S. coelicolor</i> | VanS | VanR | P_{vanJ} | Phosphorylated VanR additionally activates the VanSR operon | [123] |
| Antimicrobial peptides | | | | | | |
| Antimicrobial peptides produced by the oral pathogen <i>S. mutans</i> (extracellular) | <i>Streptococcus</i> sp. A12 | PefK | PefR | P_{pef} | Nearly 100-fold activation | [124] |
| Bacitracin (extracellular) | <i>B. subtilis</i> | LiaS | LiaR | $P_{lia(opt)}$ | 1000-fold activation. Activation occurs in a 1-2 h pulse. Requires accessory membrane protein LiaF. | [125] |
| Nisin (extracellular) | <i>L. lactis</i> | NishK | NisR | P_{nisA} | 1000-fold activation. | [126] |
| Subtilin (extracellular) | <i>B. subtilis</i> | SpaK | SpaR | P_{spas} | 110-fold induction | [126,127] |
| Oligosaccharides | | | | | | |
| Arabinogalactan | <i>B. thetaiotaomicron</i> | BT0267 | BT0267 | P_{BT0268} | BT0267 is a hybrid TCS wherein the SHK and RR are fused | [128] |
| Chondroitin sulfate | <i>B. thetaiotaomicron</i> | BT3334 | BT3334 | P_{BT3334} | BT3334 is a hybrid TCS | [128] |
| Mucin glycans | <i>P. aeruginosa</i> | GacS | GacA | P_{gacY} | Mucin glycans are sensed via the accessory histidine kinase RetS | [129] |
| Oligo-Arabinose | <i>B. thetaiotaomicron</i> | BT0366 | BT0366 | P_{BT0365} | BT0366 is a hybrid TCS | [130] |
| Proteins | | | | | | |

| Input | Native organism(s) | SHK | RR | Output promoter | Performance notes | Reference |
|----------------------------------------------------------------------------------------------------------------|-----------------------------|-------------------|---------------------|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| PilA | <i>P. aeruginosa</i> | PilS | PilR | P_{pilA} | PilA is the major Type IV pilin protein | [131] |
| Host signals | | | | | | |
| Antimicrobial peptides, divalent cation limitation, and acidic pH produced by mammalian hosts during infection | <i>S. Typhimurium</i> | PhoQ | PhoP | P_{virK} | 70-fold activation in <i>E. coli</i> | [132] |
| Epinephrine, norepinephrine | <i>E. coli O157:H7</i> | QseC | QseB | P_{fhdD} | 2-fold activation by epinephrine. 1.5-fold repression by norepinephrine. Also activated by bacterial autoinducer 3. | [133] |
| Indole-3-acetic acid (auxin) | <i>P. phytofirmans PsJN</i> | IacS | IacR1 | P_{iacA} | Dioxindole-3-acetic acid amplifies the signal | [134] |
| 2-isopentenyladenine (cytokinin) | <i>X. campestris</i> | PcrK | PcrR | P_{ctrA} | 3-fold induction | [135] |
| Phenolics, monosaccharides, and acidic pH present at plant wounds | <i>A. tumefaciens</i> | VirA | VirG | P_{vir} | Monosaccharide sensing requires the periplasmic accessory protein ChvE. | [136] |
| Trans-zeatin (cytokinin) | <i>A. thaliana, E. coli</i> | AQ ₁ * | PhoP ₁ * | P_{ngxB} | AQ ₁ * is a fusion of the sensing domain of <i>A. thaliana</i> AHK4 and <i>E. coli</i> PhoQ. This TCS is insulated against phosphorylation cross-talk with all <i>E. coli</i> TCSs | [70] |