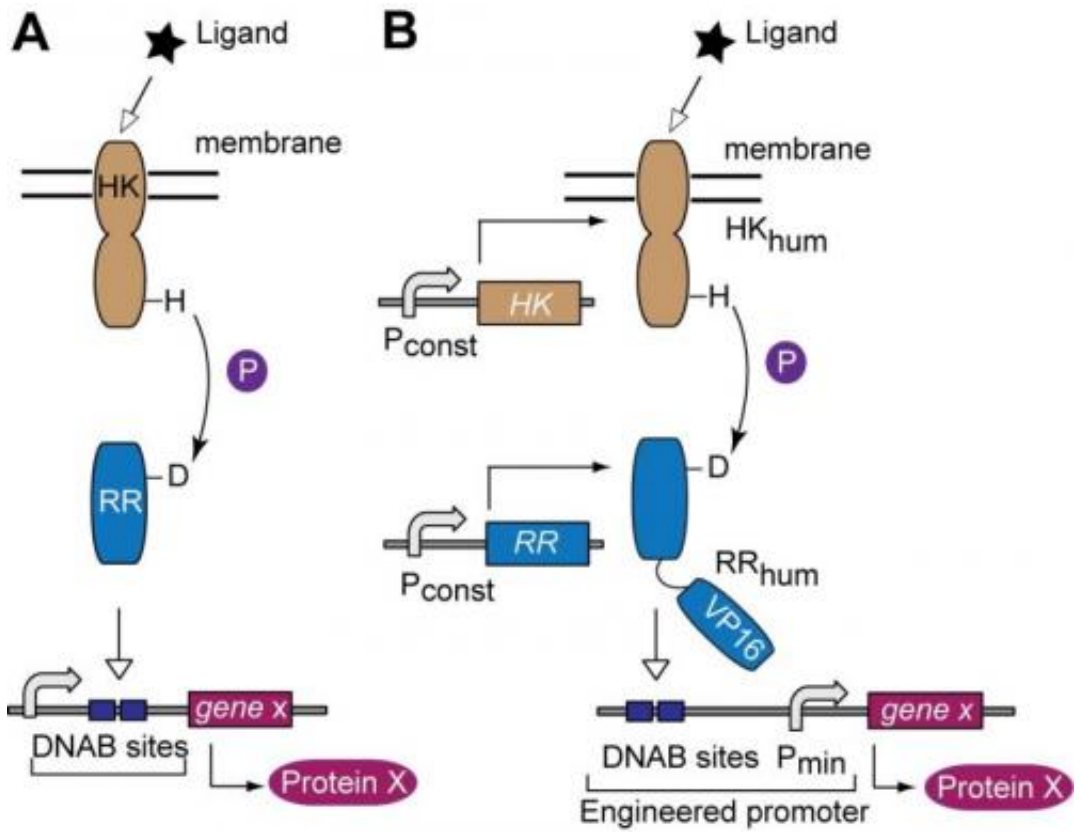
Synthetic biology, genetic engineering and you: Two-component signaling pathways as elements in synthetic circuit design

November 25 2014, by Stuart Mason Dambrot



Schematics of native and transplanted two-component signaling pathways. (A) The native pathway consists of a receptor histidine kinase protein, which senses and propagates the signal to a cognate response regulator that regulates gene expression. (B) The envisioned adaptation to the mammalian host. Subscript "hum" indicates human-optimized codon sequence. DNAB, DNA binding. Pconst, constitutive mammalian promoter. P, phosphate; Pmin, minimal mammalian promoter; VP16, VP16 transactivator domain. Credit: Hansen J et al.

(2014) Transplantation of prokaryotic two-component signaling pathways into mammalian cells. *Proc Natl Acad Sci* USA 111 (44):15705-15710.

(Phys.org) —Two of the most exciting areas of science and technology, synthetic biology and genetic engineering, have just taken a step towards a brave new future in which large-scale synthetic biological circuits composed of bioengineered logic gates, orthogonal to (that is, independent of) the host in which they operate, will enable a range of applications that include biosensors, gene expression control, cell motility, programmable gene circuits for cell physiology control, and other sophisticated gene circuits. This capability is based on the use of *two-component regulatory system* – basic stimulus-response coupling mechanisms that allow organisms to sense and respond to changes in many different environmental conditions. These systems consist of a membrane-bound *histidine kinase* that senses a specific environmental stimulus and a corresponding *response regulator* that mediates the cellular response, primarily through differential expression of target genes. ((A *histidine kinase*, or HK, is a multifunctional, typically transmembrane, protein involved in signal transduction across the cellular membrane; a *response regulator*, or RR, protein is the second component in two-component signal transduction systems.)

A particularly promising type of two-component regulatory system – *two-component signaling pathways* – are the prevalent signal processing modality in prokaryotes and are also found in low eukaryotes and plants, but absent from vertebrate cells. Recently, scientists in the Department of Biosystems Science and Engineering at Eidgenössische Technische Hochschule Zürich (ETH Zurich, or Swiss Federal Institute of Technology Zurich), Basel, Switzerland transplanted two-component pathways into a mammalian host, demonstrating that these pathways could be partially reconstituted in mammalian cell culture and used for

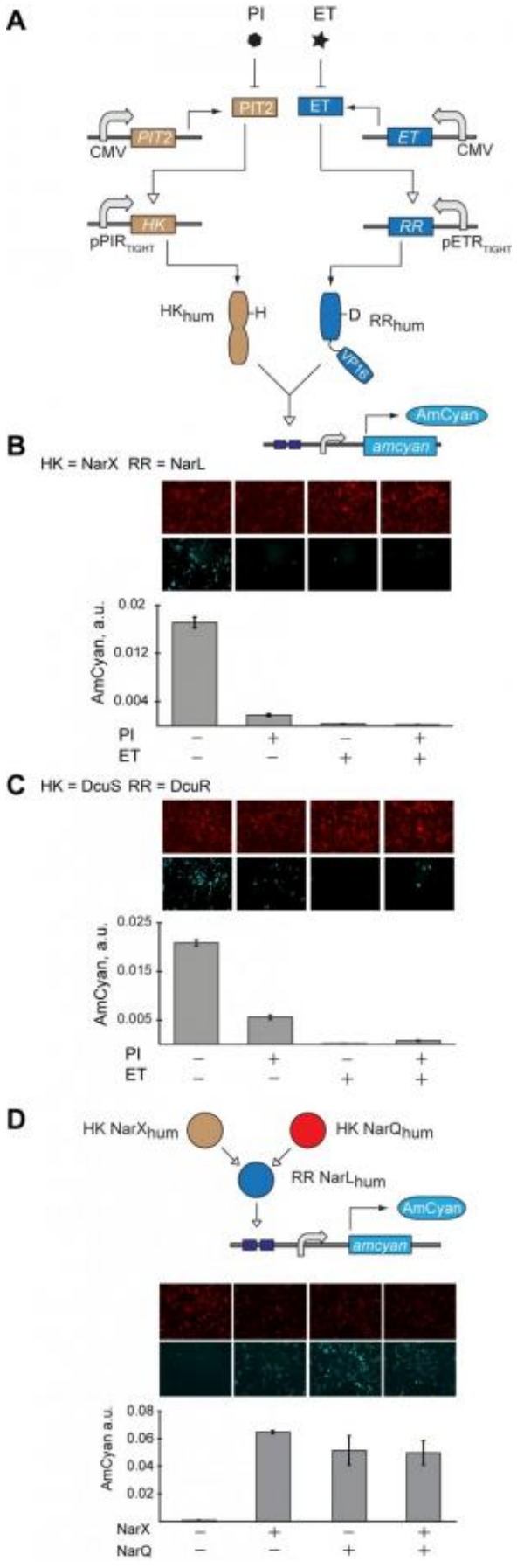
programmable control of gene expression. They found that the core biochemical processes are maintained, and while the capacity to sense chemical ligands is diminished or obscured and the preservation of basic biochemical processes during mammalian pathway transplantation is not guaranteed, they were able to use the pathways for multi-input gene regulation and show that they can be used as building blocks for gene expression control in mammalian cells, thereby creating new investigative possibilities in synthetic circuit design.

Prof. Dr. Yaakov (Kobi) Benenson discussed the paper that he, graduate student Jonathan Hansen and their co-authors published in *Proceedings of the National Academy of Sciences*. "The key step was to think about this possibility," Benenson tells *Phys.org*. "The idea occurred to me in the year 2007, when I was a Bauer Fellow at the Harvard FAS Center for Systems Biology. Michael Laub[1,2], at the time also a Bauer fellow, worked on two-component signaling pathways in prokaryotes. I was exposed to this area through his research, and discussed with him the possibility of implementing these pathways in human cells." It was several years before Benenson and his group members started the project in their new lab at ETH Zurich. "The practical challenge to investigating whether the elements of prokaryotic two-component pathways are operational in mammalian cells was to first read a large volume of papers to identify the candidate pathways for trans-kingdom transplantation, and then to redesign them such that operation in mammalian cells was possible." The scientists built on their experience with mammalian gene circuit design to make a few informed decisions that turned out to be correct.

Benenson lists several other challenges the researchers faced, the first being using the pathways for multi-input gene regulation and showing that they can serve as a rich source of orthogonal building blocks for gene expression control in mammalian cells. The two challenges here, he notes, was to ensure that there was no crosstalk between the transplanted

components, and that the observed prokaryote behavior is recapitulated in mammalian cells. Secondly, he continues, was implementing two-input logical AND-like gene regulation in mammalian cells. "Here in particular," Benenson says, "the challenge was the pathway's high sensitivity to low expression levels of the histidine kinase receptor. This made it difficult to achieve a low *off* state."

The third issue Benenson describes was based on implementing the three conditions – preserving pathway internal operations, pathway components being orthogonal to the host, and host to pathway components – needed to preserve basic biochemical processes during mammalian transplantation. "Given the vast differences in just about any aspect of biological processes between the two hosts, there was no guarantee that the processes occurring in bacteria would take place in mammalian cells," Benenson explains.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Logic with TCS. (A) NOR-gate circuit schematics comprising antibiotic-regulated HK and RR genes. (B) Quantitative data for antibiotic-regulated NarXL pathway. (C) Quantitative data for antibiotic-regulated DcuSR pathway. PI and ET are at 10 μg/mL and 4 μg/mL, respectively. Plasmid composition and output values are in SI Appendix, Tables S13 and S14. (D) (Top) Schematic representation of an OR gate between NarX and NarQ. (Bottom) Quantitative data and representative images. Plasmid composition and output values are in SI Appendix, Tables S15 and S16. In all panels the images are shown with red pseudocolor indicating DsRed Transfection marker, and cyan pseudocolor indicating AmCyan output. The resultant data are presented as mean ± SD of biological triplicates. Credit: Hansen J et al. (2014) Transplantation of prokaryotic two-component signaling pathways into mammalian cells. *Proc Natl Acad Sci* USA 111 (44):15705-15710.

The scientists addressed these challenges though a number of insights and innovations. "One approach was to perform accurate trans-kingdom transplantation by codon optimization and placing the expression of the pathway genes under mammalian promoters," Benenson recounts. (A *codon* is a sequence of three nucleotides that together form a unit of genetic code in a DNA or RNA molecule.) "Another key innovation was the development of the regulated promoter controlled by the phosphorylated response regulators, where we used a novel minimal core promoter sequence developed in our lab as well as experimenting with the number of binding site repeats upstream of this core promoter – and it turned out that having two or more binding sites is essential for strong induction." Benenson adds that research[3,4,5] about synthetic two-component signaling in plants published by the June Medford Lab was valuable resource – especially in clarifying the role of nuclear localization signals. (A nuclear localization signal, or NLS, is an amino acid sequence that tags a protein for import into the cell nucleus by

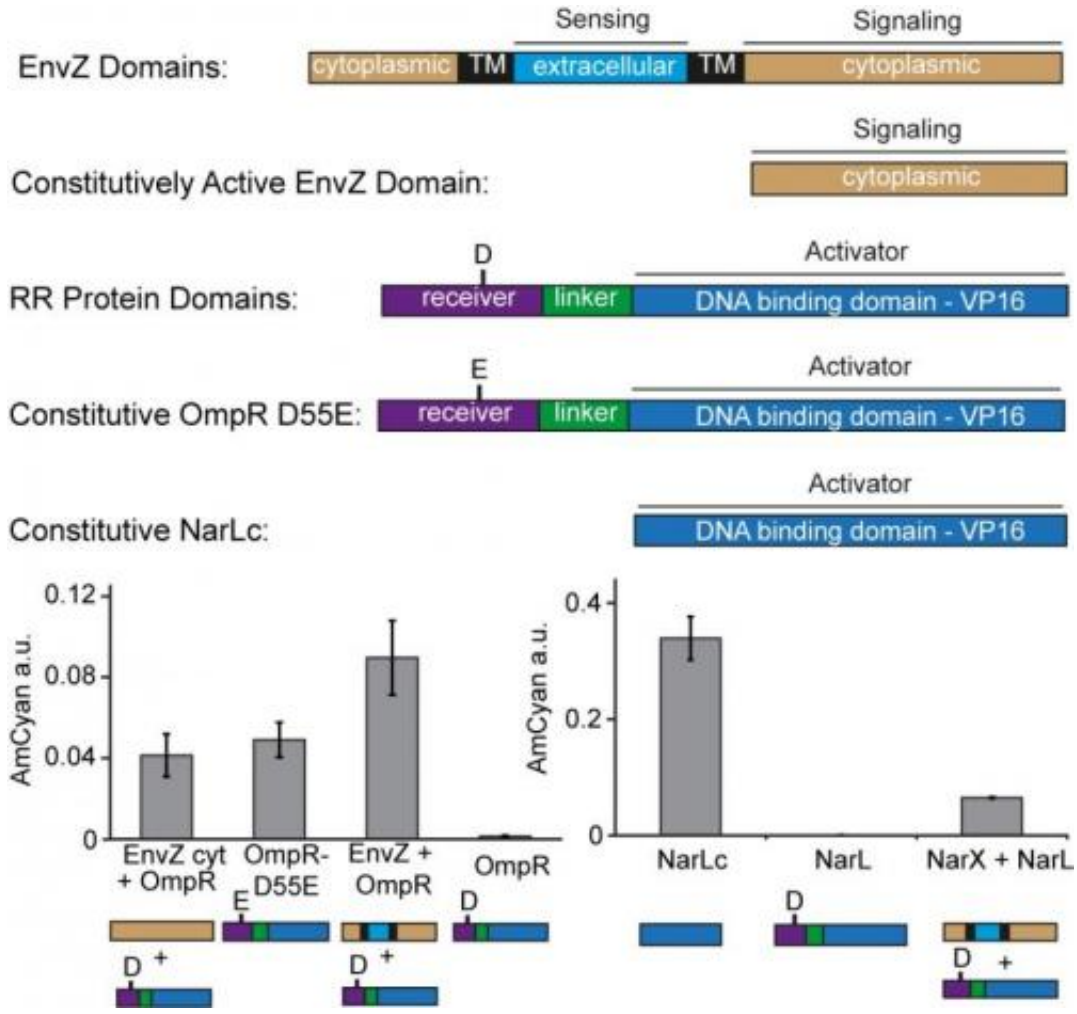
nuclear transport.)

Benenson discussed several key aspects of the study detailed in their paper, the first being the finding that in mammalian cells, core prokaryotic two-component biochemical processes are maintained but the capacity to sense chemical ligands is diminished or obscured. "We observed constitutive strong signaling via the pathway once both the histidine kinase and the response regulator components were constitutively expressed," he explains. "In prokaryotes, signaling is typically induced upon external stimulation." This led the scientists to speculate that there are multiple cytoplasmic and environmental components in mammalian cells and their growth medium that might stimulate the HK receptors. "More research is needed to understand this phenomenon in full," Benenson adds.

A fascinating aspect of two-component signaling is the support for complex logic signal integration in mammalian cells. "The two-component system naturally lends itself to performing two-input logic, because the expression of both histidine kinase and response regulator genes is required for downstream gene activation. While the natural way to control the pathway is via appropriate ligand or stimulus, another way to utilize this feature is by controlling the expression of these genes via gene regulatory tools, for example transcriptional regulation. In addition, having multiple pathways operating in parallel allows scaling of this approach to larger logic cascades.

In their paper, the scientists tie the two preceding points together by discussing the ability of two-component signaling to implement AND, NOR, and OR gates using constitutive and inducible histidine kinases and response regulators. "The core requirement of two-components naturally lends itself to an AND-like logic behavior," Benenson notes. "By appropriate wiring of upstream gene regulation, the gate can be converted into the NOR gate; OR gates are possible with pathways of

known cross-reactivity – for example, when two different histidine kinase receptors activate the same response regulator."



Activity of mutant TCS components. Full-length, truncated, and mutant TCS genes are shown with each protein domain color-coded and labeled. Experimental data are given below. Each bar represents mean ± SD of a biological triplicate. The output values for NarX + NarL and NarL are from Fig. 3B; they are displayed again for side-by-side comparison. Plasmid composition and output values are in SI Appendix, Tables S17 and S18. TM, transmembrane domain. Credit: Hansen J et al. (2014) Transplantation of prokaryotic two-component signaling pathways into mammalian cells. *Proc Natl Acad Sci* USA 111 (44):15705-15710.

The researchers also state in their paper that their findings open new avenues in synthetic circuit design, citing the example of using histidine kinases as biosensors for cytoplasmic metabolites *if* cytoplasmic metabolites or media components can be responsible for histidine kinase activation. Benenson expands on this and gives additional examples of novel synthetic biology and genetic engineering technologies and applications that may be possible due to their results. "Apart from potential use as biosensors, new avenues arise due to the fact that these pathways enable AND, OR and NOR logic," he tells *Phys.org*. "Due to the multiple existing pathways that exhibit minimal cross-talk, large logic circuits can be envisaged via cascading of multiple pathways." Specifically, he points out that while building AND gates in mammalian cells is difficult, they are very useful because they produce an output when multiple conditions hold simultaneously – so having the building blocks of two-component pathways will facilitate the construction of such gates for precise actuation in mammalian cells. "In addition, we showed that certain response regulator mutants can act as efficient constitutive activators, meaning that the plethora of response regulators can therefore generate large sets of orthogonal transcriptional activators for gene circuits of increasing sophistication."

When asked if a longer-term implication of their study might be that an ability to implement robust logic circuits in mammalian cells could lead to a novel translational approach to medical protocols, Benenson replied, "I think that this novel methodology will mesh with existing synthetic biology tools to enable better, more robust, and more programmable gene circuits for rational control of cell physiology, including the control of cell fate or the detection of pathological cell states. Being a signaling cascade, transplanted two-component pathways might improve the speed of information processing in these circuits and enable new ways to sense metabolites." That said, he points that metabolite sensing will have to be experimentally demonstrated.

As to the planned next steps in their research, the scientists plan to investigate the sensory function of HKs, which Benenson says was obscured in their experiments to date. "We also plan to test how building blocks derived from the two-component pathways can lead to the construction of large synthetic circuits," he adds. "In the long run, it would be intriguing to see if more complex pathways based on two-component systems, such as chemotaxis, can be similarly transplanted and perhaps coupled with cell motility machinery." (*Chemotaxis* is movement of somatic cells, bacteria, and other single-cell or multicellular organisms in response to a chemical stimulus.)

Regarding other areas of research that might benefit from their study, Benenson tells *Phys.org* that their discovery that key steps of two-component signaling are functional in human cells "can enable a clean cell-based model system for researchers who investigate these pathways. Usually," he adds, "such studies involved labor-intensive purification of protein components, because it is difficult to study these pathways in isolation in bacteria where tens of pathways coexist. We showed that the components can be expressed in human cells, and cross-talk as well as mutant behavior mirrors those found in prokaryotes. We also found one instance of previously-unreported crosstalk, and we suspect that it might also manifest itself in prokaryotes. This," he concludes, "would be an interesting area to pursue."

More information: Transplantation of prokaryotic two-component signaling pathways into mammalian cells, *Proceedings of the National Academy of Sciences*, (2014) vol. 111 no. 44 15705-15710, 2014, doi:10.1073/pnas.1406482111

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[2]Specificity in Two-Component Signal Transduction Pathways, *Annual Review of Genetics* (2007) Vol. 41, pp 121–145, doi:10.1146/annurev.genet.41.042007.170548

[3]Developing a Synthetic Signal Transduction System in Plants, *Methods in Enzymology*, Academic Press, 2011, Volume 497, Synthetic Biology, Part A, Chapter 25, Pages 581-602

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[5]Engineering Key Components in a Synthetic Eukaryotic Signal Transduction Pathway, *Molecular Systems Biology* (2009) 5:270, doi:10.1038/msb.2009.28