Published in final edited form as:
Curr Opin Microbiol. 2010 April ; 13(2): 113–115. doi:10.1016/j.mib.2010.02.003.

Two-component signal transduction

Robert B Bourret and
Department of Microbiology & Immunology, University of North Carolina, Chapel Hill, NC 27599-7290, USA bourret@med.unc.edu

Ruth E Silversmith
Department of Microbiology & Immunology, University of North Carolina, Chapel Hill, NC 27599-7290, USA silversr@med.unc.edu

1986 was a very good year for research on signal transduction in bacteria, with the publication of two landmark papers that reflected progress in biological research at the time. DNA sequencing was a relatively new technology (subject of the 1980 Nobel Prize), but manual sequencing techniques had become widespread enough to be routinely performed in individual laboratories. Geneticists who had studied various regulatory systems for years could determine the sequences of their favorite genes and deposit that information in publicly accessible databases. As a result, it suddenly became apparent that many diverse and seemingly unrelated processes were in fact controlled by closely related pairs of regulatory proteins with similar amino acid sequences. This connection was independently recognized and published by multiple research groups beginning in 1985, and in 1986 Nixon, Ronson, and Ausubel coined the phrase "two-component regulatory systems" to describe the discovery [1]. At the time, protein phosphorylation (to be the topic of the 1992 Nobel Prize) was the subject of vigorous investigation in eukaryotes, but had only been confirmed in bacteria less than a decade before and was not yet known to be widespread in prokaryotes [2]. Thus the demonstration by Ninfa and Magasanik, also in 1986, that the two-component regulatory system controlling nitrogen assimilation utilizes protein phosphorylation was a major step forward [3]. The dual 1986 discoveries of amino acid sequence similarity and protein phosphorylation in two-component systems sparked an expanding field of investigation that continues vigorously to this day. This entire issue of *Current Opinion in Microbiology* is devoted to reviewing the current state of knowledge concerning two-component signal transduction.

The prototypical two-component regulatory system is composed of a sensor kinase and a response regulator, assembled according to a modular design. A typical sensor kinase consists of a variable N-terminal input domain connected to conserved C-terminal domains that catalyze phosphorylation. A typical response regulator consists of a conserved N-terminal receiver domain and a variable C-terminal output domain. Thus information flow through the system begins with detection of an environmental stimulus by an input domain, which in turn causes the conserved portion of the sensor kinase to encrypt the data as a covalently attached phosphoryl group. The phosphoryl group is then transferred to the receiver domain of the response regulator, which acts as a switch to control the function of the output domain, thus generating an appropriate adaptive response to the stimulus. Hydrolysis of the phosphoryl group resets the system to respond to additional stimuli. Through this process, phosphoryl

groups flow from ATP to a histidine of a sensor kinase, then to an aspartate of a response regulator, and finally are released as inorganic phosphate.

After more than two decades of research, a great deal is known about the conserved features that define membership in the two-component family of proteins and endow two-component systems with their characteristic properties. Hundreds of specific two-component systems are under active investigation in various laboratories. In contrast to the humble beginnings of DNA sequencing, tens of thousands of two-component system proteins have been identified through genome sequences. However, in spite of extensive knowledge concerning shared properties and the ease with which two-component systems can be identified, it remains difficult to predict much about two-component systems that have not been studied experimentally. In particular, many factors that define differences between two-component systems (e.g. stimulus detected, response controlled, specificity between sensor kinase/response regulator pairs, interactions between domains within sensor kinases or response regulators, interactions with auxiliary proteins, circuit architecture, reaction kinetics, subcellular localization, etc.) are relatively poorly understood.

In previous collections of reviews on two-component regulatory systems [4–6], it was appropriate to focus on specific systems, particularly the paradigms that served as the foundation of the field. However, the field now has achieved a scale such that description of individual systems is neither practical nor provides an informative perspective. Instead, this collection is organized by general features of two-component systems and works through the signaling pathway from beginning to end. A noteworthy aspect is that each review includes a box listing key unanswered questions or directions for future research.

The first section consists of three reviews on sensor kinases. Wayne Hendrickson and Jonah Cheung describe input domains of sensor kinases. The recent determination of of numerous structures, obtained both by targeted efforts and through structural genomics projects, has dramatically increased understanding of stimulus detection. Jerry Hazelbauer and Wing-Cheung Lai review what can be learned from the receptors involved in bacterial chemotaxis. Chemoreceptors are an enlightening special case in which the input proteins are not covalently connected to the sensor kinase. Finally, Rick Stewart discusses the conserved "transmitter module" portion of sensor kinases, consisting of the domains responsible for binding ATP, catalyzing phosphorylation of the histidine, and dimerization.

The second section includes three reviews on response regulators. Bob Bourret describes the structure and function of receiver domains. Michael Galperin's review provides an overview of the diverse collection of output domains to which receiver domains can be connected. Michael Galperin's lab maintains an online database with information on tens of thousands of response regulators (http://www.ncbi.nlm.nih.gov/Complete_Genomes/RRcensus.html). Finally, Ann Stock and Rong Gao discuss the mechanisms by which receiver domains control output function.

The third section has two reviews on phosphatases. Many sensor kinases exhibit phosphatase activity toward response regulators, but remarkably little is known about this phenomenon. In particular, the reaction mechanism has not been characterized and which or how many sensor kinases have phosphatase activity cannot currently be predicted. Linda Kenney debates whether or not the phosphatase activity of sensor kinases observed *in vitro* is important *in vivo*. Some two-component systems rely on other proteins to stimulate response regulator dephosphorylation. Ruth Silversmith summarizes auxiliary phosphatases of the CheC/CheX/FliY, CheZ, Rap, and Spo0E families.

The fourth section contains five reviews about networks. Few actual two-component systems are as simple as the prototypical scheme described at the beginning of this overview. A

prominent example is multistep phosphorelays that include histidine phosphotransferase proteins in addition to sensor kinases and response regulators. Mark Goulian describes the diversity of circuit achitectures adopted by two-component systems, and also summarizes computer simulation efforts to infer the information-processing properties that result from different designs. Jim Hoch and Hendrik Szurmant explain how sensor kinases and response regulators can identify their partners with high fidelity in a cell that may have tens or hundreds of other two-component systems operating in parallel. Barry Wanner and Yi-Ju Hsieh describe the *Escherichia coli* Pho regulon, which is cross-regulated by at least two other two-component systems. Alan Wolfe explores the physiological roles of small molecule phosphodonors (primarily acetyl phosphate) that can bypass sensor kinases and contribute phosphoryl groups directly to response regulators. Finally, Eleonora García Véscovi, Mariela Sciara, and María Castelli discuss the nascent field of spatial localization of sensor kinases and response regulators within bacterial cells.

The fifth section consists of four reviews highlighting the biological significance of two-component systems, which are found in all three domains of life (Bacteria, Archaea, Eukarya). Igor Zhulin, Kristin Wuichet, and Brian Cantwell review the phyletic distribution and evolutionary origins of two-component systems. Igor Zhulin's lab maintains an online database with information on tens of thousands of two-component system proteins (http://mistdb.com/). Many two-component systems control functions important for microbial pathogenesis. Eduardo Groisman and Varsha Raghavan discuss recent discoveries in a subset of two-component systems important for symbiotic or pathogenic relationships with mammalian hosts. Two-component systems have attacted interest as potential targets for new classes of drugs. Ryutaro Utsumi, Yasuhiro Gotoh, Yoko Eguchi, Takafumi Watanabe, Sho Okamoto, and Akihiro Doi summarize efforts to target bacterial two-component systems. Finally, Alex Ninfa outlines the potential for modules of two-component systems to be used in synthetic biology applications.

The sixth and final section consists of one review on methods and in a sense, ends the story where it began. Technological developments such as DNA sequencing revealed the existence of two-component systems 25 years ago. Birgit Scharf briefly summarizes current experimental methods specifically adapted to the particular features of two-component systems. Her overview should be an invaluable overview to newcomers entering the field. In the future, technologies such as structural genomics, rapidly improving imaging methods, and techniques to characterize single cells may drive new discoveries about two-component systems.

A few words about nomenclature are appropriate before turning you, the reader, loose. We have not imposed a uniform set of terms on the reviews in this collection, but rather have let the authors' words reflect the diversity of terminology in current use. Thus, "two-component regulatory systems" are often called "two-component systems" (TCS) or "two-component signal transduction" (TCST or 2CST). "Sensor kinases" (SK) are also variously referred to as "histidine kinases" (HK), "sensor histidine kinases", "histidine protein kinases" (HPK) or "protein histidine kinases" (PHK). "Response regulators" (RR) has achieved widespread agreement, but "receiver domains" are sometimes termed "phosphoacceptor domains". "Autophosphorylation" can refer to either the reaction of sensor kinases with ATP or the reaction of response regulators with small molecule phosphodonors, depending on context. "Phosphotransfer" refers to the movement of phosphoryl groups between histidine and aspartate residues on sensor kinases, receiver domains, and histidine phosphotransferases, and is intentionally avoided when describing the transfer of phosphoryl groups from ATP or other phosphodonors to protein, or from protein to water.

Finally, we would like to thank the expert authors of this collection, who enthusiastically agreed to participate in this project, contributed high quality reviews in a timely manner, and cheerfully

revised their manuscripts in response to editorial suggestions. It is a compelling measure of the vitality and rapid progress of current research on two-component systems that we learned a great deal ourselves by reading these reviews. We hope you will as well. Enjoy!

Biographies

Bob Bourret is Professor and Director of Graduate Studies in the Department of Microbiology & Immunology at the University of North Carolina, Chapel Hill. He brings a microbiology and genetics perspective to research on the molecular mechanisms of signal transduction in two-component regulatory systems and jointly operates a lab with Ruth Silversmith.

Ruth Silversmith is Research Associate Professor in the Department of Microbiology & Immunology at the University of North Carolina, Chapel Hill. She brings a biochemistry and biophysics perspective to research on the molecular mechanisms of signal transduction in two-component regulatory systems and jointly operates a lab with Bob Bourret.

References

1. Nixon BT, Ronson CW, Ausube FM. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. Proc Natl Acad Sci U S A 1986;83:7850–7854. [PubMed: 3020561]
2. Cozzone AJ. Protein phosphorylation in prokaryotes. Annu Rev Microbiol 1988;42:97–125. [PubMed: 2849375]
3. Ninfa AJ, Magasanik B. Covalent modification of the *glnG* product, NRI, by the *glnL* product, NRII, regulates the transcription of the *glnALG* operon in *Escherichia coli*. Proc Natl Acad Sci U S A 1986;83:5909–5913. [PubMed: 2874557]
4. Hoch, JA.; Silhavy, TJ., editors. Two-component signal transduction. Washington, D.C.: American Society for Microbiology; 1995.
5. Inouye, M.; Dutta, R., editors. Histidine kinases in signal transduction. San Diego, CA: Academic Press; 2002.
6. Utsumi, R., editor. Bacterial signal transduction: Networks and drug targets. Austin, TX: Landes Bioscience; 2008.