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Unequal twins: Unraveling the reaction mechanism of dimeric histidine kinases

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Histidine kinases (HKs), together with their partner proteins, the response regulators (RRs), form the ubiquitous two-component systems that are global players in control and adjustment of microbial lifestyle. Although their basic function (*i.e.* the transfer of a phosphate group from the HK to its RR partner) is simple to articulate, deciphering the molecular details of this process has proven anything but simple, especially when quantitative aspects come into play. Bouillet *et al.* report a series of elegant and sophisticated experiments to quantitatively understand HK functions, clearing up several open questions and providing a new strategy for future work in the field.

All organisms must be able to sense and to respond to the living conditions in their habitats and changes therein, and so they need ways to collect and "compute" these external stimuli, whether they be threats such as drought or variations in pH, temperature, or salinity (causing osmotic stress) or to resources, such as the migration in the direction of an energy (sugar) source. Many of these inputs feed into two-component systems (TCSs) to transmit information to the cell metabolism. Although the inputs can vary widely, the outputs of the TCSs are less variable; most common is the induction of tactic or phobic response or the regulation of gene expression. Studies on TCSs have been instrumental for our understanding of signal transduction, as is documented by the large number of literature reports, *e.g.* (1–4), yet questions regarding their function remain.

Despite the capability to respond individually to many different stimuli, the mode of activation of HKs and the interaction between an HK and its cognate RR is outstandingly robust and highly specific, minimizing or even preventing cross-talk between components from other TCSs (5). Most HKs form homodimers through interactions in between the amphipathic, antiparallel double helical motifs, where the eponymous histidine residue is located, resulting in a four-helix bundle. The common understanding of HKs is that they function in this dimeric formation, although reports suggest that some HKs may act as monomers (6). The four-helix bundle interacts with a catalytic ATP-binding domain (Fig. 1), responsible for the initial phosphorylation event. Stimulation of the sensory domain, fused N-terminally to the beginning of the antiparallel helix motif, is generally thought to trigger a *trans*-phosphorylation event in which the ATP-binding domain of protomer 1 approaches the histidine of protomer 2, and this residue is phosphorylated by transfer of the ATP γ-phosphate. It is, however, worth noting that *cis*-phosphorylation is difficult to rule out because the complexes of the homodimers are dynamic in separation and dissociation. Signal transmission is then accomplished through complex formation between the activated HK and its cognate RR, allowing the phosphate group to be transferred to an aspartate group in the RR. After phosphate transfer, the complex falls apart, and the now activated RR takes over by, for example, forming dimers or binding promoter-regulating elements. TCSs may also act in chains with HKs and RRs in repetitive function (phospho-relay systems) such that an RR activates an HK that in turn activates another RR.

Despite this seemingly straightforward mechanism, many questions remain. For example, are one or both histidines in the homodimer phosphoraylated? Previous measurements have generally observed 50–70% modification, but whether that means the phosphate transfer occurs at only one of the two protomers or at both protomers of some homodimers and not in others is not known. Crystal structures of HKs have revealed a structural asymmetry, suggesting that the protein is probably not able to form productive complexes to phosphorylate both histidines simultaneously and implying the existence of negative cooperativity, in which an initial phosphorylation hinders phosphorylation of the second protomer. It is also known that ADP promotes the reverse reaction, suggesting that any diphosphorylated species, if formed, would be quickly lost. However, these assumptions have not been tested in a quantitative manner.

The study by Bouillet *et al.* (7) approaches these unknowns about single-site, double-site, or (negatively) regulated cooperative phosphorylation, starting with the role of ADP molecules present that could hamper the histidine phosphorylation. A scrutinizing search revealed a quite significant amount of ADP prowling around, being formed as a by-product upon γ-phosphate transfer, but also, fairly high levels of ADP were found in commercial ATP batches, whether from impurities or being formed from ATP hydrolysis. Initial kinetic assays using the HK CpxA from *Escherichia coli* with varying ADP concentrations showed that these minor amounts of ADP diminish the phosphorylation by ~50%, a result that might be mistakenly interpreted as negative cooperativity.

The methods, rightly selected by the authors, were "Golden Gate cloning" (8) and the "SpyTag/SpyCatcher" method (9) to selectively link modified HK monomers covalently into dimers of choice (Fig. 1). In this way, Bouillet *et al.* generated various combinations of HK containing mutations in which the instrumental histidine is removed (H248A) or ATP binding is

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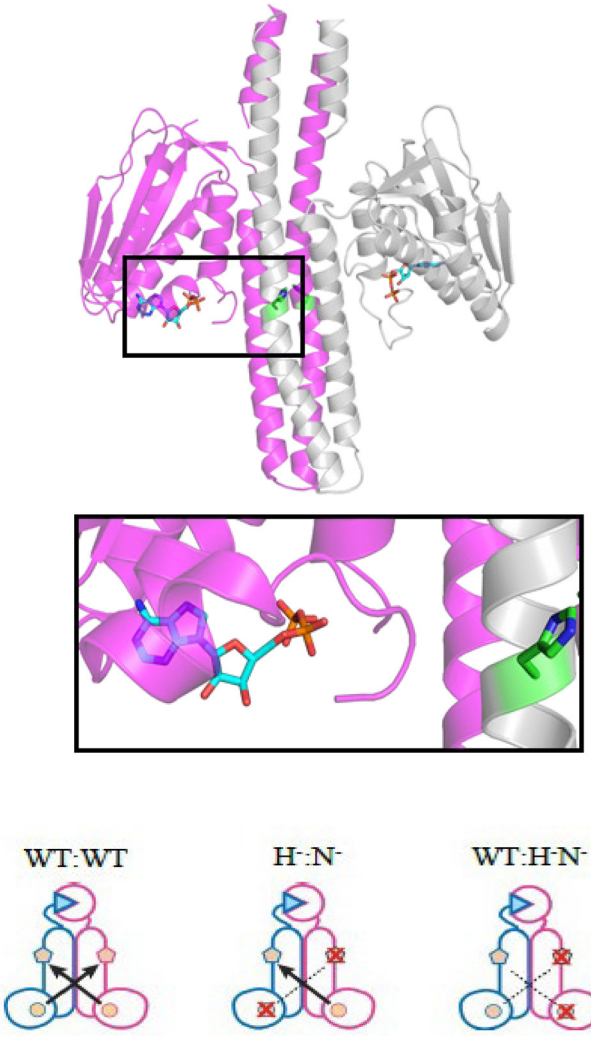

Figure 1. A model-testing selectivity in fused homodimeric HK CpxA. *Top*, three-dimensional structure of CpxA from *E. coli* (Protein Data Bank code 4BIU) with (*below*) an *enlarged view* (*black square*) onto the helical structure, highlighting the functional histidine of one protomer (*green*) and the ATP molecule (*cyan*) of the other protomer; the sensory domains (not shown) would be fused to the N-terminal ends of the helices. The picture shows the homodimeric arrangement of antiparallel helices of each monomer in *pink* and *gray*; the lateral α-helical/β-sheet domains include the CA (ATP-binding domain). *Bottom*, schematic diagram for testing autophosphorylation in CpxA mutant proteins (adapted from Bouillet *et al.* (7)). Phosphorylation reactions in *trans* are depicted as *solid arrows* pointing from the CA domain of one protomer to the DHp domain of a second protomer. Phosphorylation reactions disrupted by an N356K mutation in the CA domain (N^-) or an H248A mutation in the DHp domain (H^-) are indicated by *dotted lines*, with mutated residues indicated by *red crosses*. The *cartoon* depicts the fused protomers, covalently linked through the SpyCatcher (*circle*) and SpyTag (*blue triangle*) sequences.

abolished (N356K). Their fused dimer constructs included (i) two unmodified WT protomers, (ii) an H248A protomer connected to an N356K protomer, and (iii) a WT protein linked to a doubly modified protomer.

The obtained results from these constructs obey the predictions: in construct i, phosphorylation is found on both protomers, with 50% of histidines modified overall; construct ii yields a clear *trans*-phosphorylation complex, and construct iii shows no phosphorylation at all (thereby negating a *cis*-phosphorylation for this HK). Furthermore, with an ATP-recycling system in place, the authors observed 100% labeling. With these data and the previous ADP results in hand, the authors were finally able to develop a full kinetic model of the process, including initial ADP concentrations as well as the relative binding affinities of ATP and ADP for the unphosphorylated and phosphorylated sites. The results clearly demonstrate that structural asymmetry does not necessarily cause negative cooperativity.

The positive results from Bouillet *et al.* help to clarify several outstanding questions in the TCS field and further prove that their approach is a versatile method for studying HKs that are distinguished for their *trans*-phosphorylation. But it is much further-reaching, as nature shows us that protein dimerization is a widely distributed blueprint for many receptor types, and especially the SpyTag/SpyCatcher protocol now allows combining at will modified monomers of the same protein to elucidate molecular details of their collective activities.

Conflict of interest—The author declares that he has no conflict of interest with the contents of this article.

Abbreviations—The abbreviations used are: TCS, two-component system; HK, histidine kinase; RR, response regulator.

References

1. Buschiazzo, A., and Trajtenberg, F. (2019) Two-component sensing and regulation: how do histidine kinases talk with response regulators at the molecular level? *Annu. Rev. Microbiol.* **73,** 507–528 CrossRef Medline
2. Simon, M. I., Crane, B. R., and Crane, A. (eds) (2007) *Methods in Enzymology: Two-component Signaling Systems, Part A*, Vol. 422, Elsevier, Amsterdam
3. Simon, M. I., Crane, B. R., and Crane, A. (eds) (2007) *Methods in Enzymology: Two-component Signaling Systems, Part B*, Vol. 423, Elsevier, Amsterdam
4. Simon, M. I., Crane, B. R., and Crane, A. (eds) (2010) *Methods in Enzymology: Two-component Signaling Systems, Part C*, Vol. 471, Elsevier, Amsterdam
5. Hübschmann, T., Jorissen, H. J. M. M., Börner, T., Gärtner, W., and Tandeau de Marsac, N. (2001) Phosphorylation of proteins in the light-dependent signalling pathway of a filamentous cyanobacterium. *Eur. J. Biochem.* **268,** 3383–3389 CrossRef
6. Rivera-Cancel, G., Ko, W., Tomchick, D. R., Correa, F., and Gardner, K. H. (2014) Full-length structure of a monomeric histidine kinase reveals basis for sensory regulation. *Proc. Natl. Acad. Sci. USA* **111,** 17839–17844 CrossRef Medline
7. Bouillet, S., Wu, T., Chen, S., Stock, A. M., and Gao, R. (2020) Structural asymmetry does not indicate hemi-phosphorylation in the bacterial histidine kinase CpxA. *J. Biol. Chem.* **295,** 8106–8117 CrossRef Medline
8. Engler, C., Kandzia, R., and Marillonnet, S. (2008) A one pot, one step, precision cloning method with high throughput capability. *PLoS ONE* **3,** e3647 CrossRef Medline
9. Keeble, A. H., Banerjee, A., Ferla, M. P., Reddington, S. C., Anuar, I. N. A. K., and Howarth, M. (2017) Evolving accelerated amidation by SpyTag/SpyCatcher to analyze membrane dynamics. *Angew. Chem. Int. Ed. Engl.* **56,** 16521–16525 CrossRef Medline