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Helix Bundle Loops Determine Whether Histidine Kinases Autophosphorylate in *cis* or in *trans*

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Abstract

Bacteria frequently use two-component signal transduction pathways to sense and respond to environmental and intracellular stimuli. Upon receipt of a stimulus, a homodimeric sensor histidine kinase autophosphorylates and then transfers its phosphoryl group to a cognate response regulator. The autophosphorylation of histidine kinases has been reported to occur both in *cis* and in *trans*, but the molecular determinants dictating which mechanism is employed are unknown. Based on structural considerations, one model posits that the handedness of a loop at the base of the helical dimerization domain plays a critical role. Here, we tested this model by replacing the loop from *Escherichia coli* EnvZ, which autophosphorylates in *trans*, with the loop from three PhoR orthologs that autophosphorylate in *cis*. These chimeric kinases autophosphorylated in *cis*, indicating that this small loop is sufficient to determine autophosphorylation mechanism. Further, we report that the mechanism of autophosphorylation is conserved in orthologous sets of histidine kinases despite highly dissimilar loop sequences. These findings suggest that histidine kinases are under selective pressure to maintain their mode of autophosphorylation, but they can do so with a wide range of sequences.

Introduction

Organisms must sense and respond to their environments to survive. In many cases, organisms use membrane-bound protein kinases to directly sense environmental stimuli and, in response, phosphorylate substrates that can effect cellular changes. These phosphorylation events are typically reversible, allowing for temporal control of protein function. In bacteria, the predominant phosphorylation-based systems are two-component signal transduction pathways, which involve a sensor histidine kinase and its cognate substrate, a response regulator.[1] Following activation by an input signal, a kinase homodimer autophosphorylates on a conserved histidine. The phosphoryl group is then transferred to a cognate response regulator, which can often trigger a transcriptional response inside cells. Many histidine kinases are bifunctional and, in addition to their kinase activity, exhibit phosphatase activity toward their cognate regulators, allowing a system to reset once the input signal is removed.[2] Using these systems, prokaryotes can respond to a large and diverse array of stimuli including light, carbon sources, quorum signals, antibiotics, and others.[3] Most bacteria encode dozens of paralogous histidine kinases and response regulators, with some encoding hundreds.[4]

The typical histidine kinase is an integral membrane homodimer with an extracytoplasmic sensory domain linked to a cytoplasmic transmitter domain through a transmembrane helix. Sensory domains are often members of the PAS or GAF family, but they show low sequence similarity owing to the diversity of inputs they recognize.[5] In contrast, the transmitter region of the kinase is highly conserved and always consists of a DHp (*d*imerization and *h*istidine *p*hosphotransfer) domain linked to a CA

(*c*atalytic and *A*TP binding) domain (Fig. 1a). The DHp domain includes two α-helices that mediate homodimerization through the formation of a four-helix bundle.[6] The CA domain forms an α/β-sandwich that binds ATP and catalyzes autophosphorylation of an exposed histidine residue in the DHp domain.[7]

Early studies of the model histidine kinase EnvZ from *Escherichia coli* demonstrated that it autophosphorylates in *trans*, as the CA domain from one subunit of a homodimer phosphorylates the DHp domain of the other subunit (Fig. 1b).[8] *E. coli* kinases NtrB, AtoS, and CheA; *Staphylococcus aureus* AgrC; and *Agrobacterium tumefaciens* VirA also autophosphorylate in *trans*,[9–13] and all histidine kinases were initially assumed to function similarly. However, the kinases *Thermotoga maritima* HK853, *S. aureus* PhoR, and *E. coli* ArcB were recently shown to autophosphorylate exclusively in *cis* (Fig. 1b), that is, the CA domain from one subunit autophosphorylates the DHp domain of the same subunit (although these kinases still homodimerize).[14,15] Whether these two mechanisms of autophosphorylation have functional consequences for signaling and what determines whether a kinase autophosphorylates in *cis* or in *trans* are currently unknown.

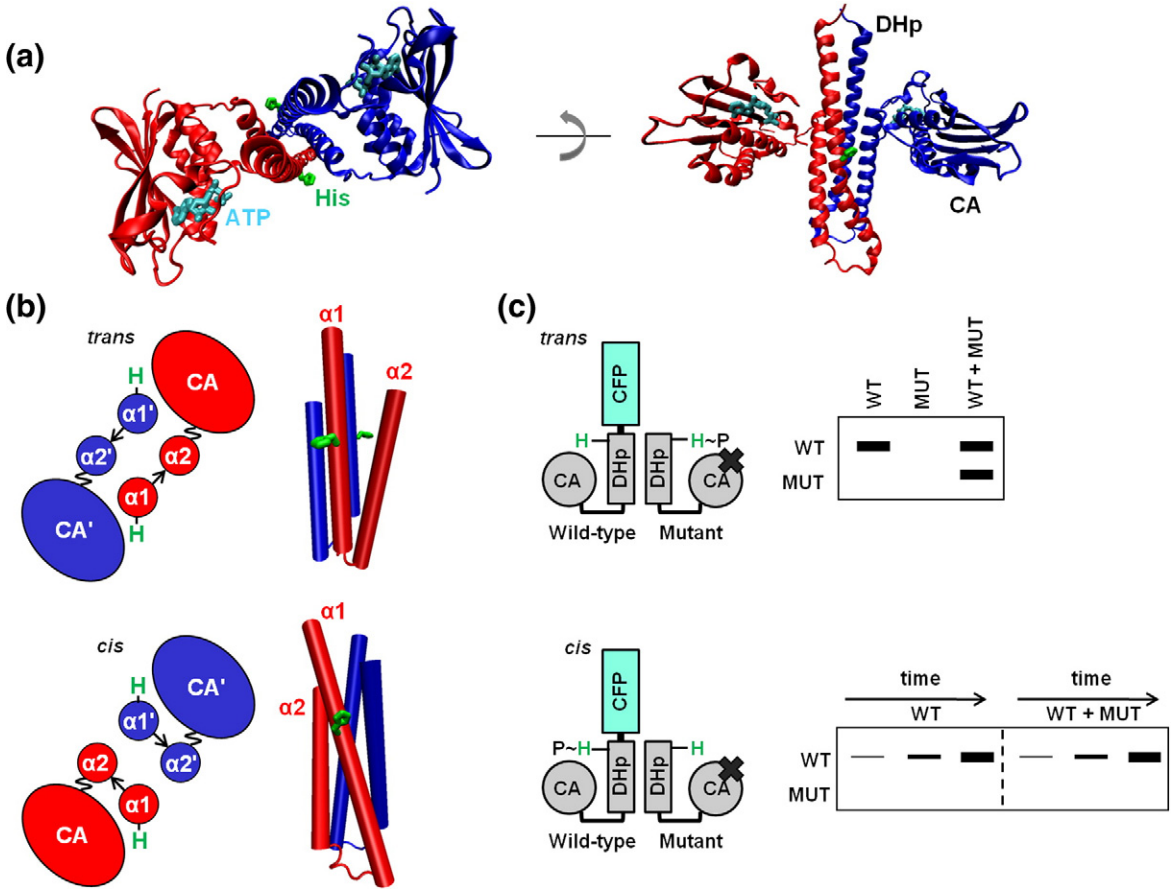

Fig. 1. Histidine kinases autophosphorylate in *cis* or in *trans*. (a) The cytoplasmic region of a histidine kinase [Protein Data Bank (PDB) ID 2C2A] consists of conserved DHp and CA domains. The histidine site of phosphorylation on the DHp domain and the ATP analog bound by the CA domain are shown in stick form in green and cyan, respectively. The kinase shown autophosphorylates in *cis*. (b) Cartoon (left) looking down the four-helix bundle (right) of the DHp domain dimer. The α1 and α2 helices in the DHp domain are labeled, with the prime symbol (′) symbol denoting the opposite chain (top, PDB ID 3ZRX; bottom, PDB ID 2C2A). The loop at the base of the DHp domain is depicted by an arrow, and the linker between the DHp and CA domains is depicted as a wavy line to reflect the mobility of the CA domain. Depending on loop handedness in the DHp domain, the CA domain is closer to either the histidine on the same chain (*cis*) or the histidine on the opposite chain (*trans*). (c) Schematic of the assay to test in *cis versus* in *trans* autophosphorylation. A wild-type (WT) histidine kinase homodimer is mixed with excess mutant (MUT) histidine kinase homodimer unable to bind ATP. Autophosphorylation within the heterodimer is initiated by the addition of radiolabeled ATP, and the chains in the dimer are then separated by SDS-PAGE. In the heterodimer, either the WT or the MUT chain is labeled, depending on whether the kinase autophosphorylates in *cis* or in *trans*, respectively. In addition, WT homodimer, also present in the WT + MUT mixture, undergoes autophosphorylation. To confirm autophosphorylation in *cis*, we compared the kinetics of the WT and WT plus excess MUT reactions.