Allostery and protein plasticity: the keystones for bacterial signaling and regulation

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Abstract

Bacteria sense intracellular and environmental signals using an array of proteins as antennas. The information is transmit- ted from such sensory modules to other protein domains that act as output effectors. Sensor and effector can be part of the same polypeptide or instead be separate diffusible proteins that interact specifically. The output effector modules regulate physiologic responses, allowing the cells to adapt to the varying conditions. These biological machineries are known as signal transduction systems (STSs). Despite the captivating architectural diversity exhibited by STS proteins, a universal feature is their allosteric regulation: signal binding at one site modifies the activity at a physically distant site. Allostery requires protein plasticity, precisely encoded within their 3D structures, and implicating programmed molecular motions. This review summarizes how STS proteins connect stimuli to specific responses by exploiting allostery and protein plasticity. Illustrative examples spanning a wide variety of protein folds will focus on one- and two-component systems (TCSs). The former encompass the entire transmission route within a single polypeptide, whereas TCSs have evolved as separate dif- fusible proteins that interact specifically, sometimes including additional intermediary proteins in the pathway. Irrespective of their structural diversity, STS proteins are able to modulate their own molecular motions, which can be relatively slow, rigid-body movements, all the way to fast fluctuations in the form of macromolecular flexibility, thus spanning a continuous protein dynamics spectrum. In sum, STSs rely on allostery to steer information transmission, going from simple two-state switching to rich multi-state conformational order/disorder transitions.

Keywords Allostery · Protein dynamics · Bacterial signaling · Regulation · Phosphorylation · Two-component systems

Introduction

Bacteria are single or multi-cellular organisms (Lyons and Kolter 2015) that succeeded in colonizing almost every niche on Earth. As all living entities, bacteria have the capacity to sense inner and environmental cues triggering specific physiologic responses, which tend to homeosta- sis and adaptation. These sensory/regulatory processes, also known as signaling, signal transduction, or informa- tion metabolism, are ultimately based upon defined protein structural rearrangements that occur at the molecular scale. Each bacterial cell harbors an array of different proteins that act as antennas, able to detect a broad spectrum of signals: temperature, pH, peptides, lipids, sugars, gases, redox-active species, light, etc. (Stock et al. 1990; Galperin 2005; Ulrich et al. 2005; Watts et al. 2019). The information is most often communicated to other biomolecules (such as other proteins or protein domains, or yet nucleic acids), resulting in the regulation of gene expression or enzyme activity, ultimate agents of the adaptive response (Harshey et al. 2003). Such signal transduction systems (STSs) can be simple, engaging few components, or rich inter-communicating multi-protein networks (Francis and Porter 2019).

That bacteria detect such a wide spectrum of stimuli is consistent with the equally wide diversity of STS sensor domains that evolved in these microorganisms (Cheung and Hendrickson 2010; Ortega et al. 2017). However, a universal feature shared by all STSs, irrespective of their sequence variability, is their *allosteric regulation*, a capac- ity encoded within their 3D structures. Allostery makes signal transduction possible, transforming STS proteins into information-processing devices: signal detection is intimately linked to allosteric rearrangements of the sensory proteins that, according to the presence/absence of the signal, populate alternate ground states (such as in discrete alternative conformations) and/or states with strongly mod- ulated dynamic features (such as in order/disorder transitions). The on/off regulation of STS proteins is allosteric, meaning that the stimuli bind to an *allosteric* site that controls the output activity of a spatially distant *orthosteric* site, which carries out functional output activities (Fenton 2008; Motlagh et al. 2014). The physical or chemical signals that bind to the allosteric site are often called *allosteric effectors*, a term not to be confused with the output effector activities of the whole STS pathway. The orthosteric site is often a catalytic center, such as in histidine kinases, or in response regulators containing enzymatic output effector domains. Orthosteric sites can also present themselves as specific binding surfaces, engaged in protein:protein or protein:DNA associations, such as in methyl-accepting chemotaxis protein receptors, one-component sensory systems, or yet in most monodomain response regulators (CheY-like) and in those harboring DNA-binding output effector domains.

Two mechanisms have been put forward over the years to describe the transition pathways connecting the end-states of allosteric proteins (Changeux 2012), mechanisms that conform well to the workings of signaling proteins: either the sensed stimuli induce conformational changes on a previously "inactive" sensory protein ("induced-fit") hence modifying the active site or the stimuli stabilize a particular conformational state of the protein's active site that precedes the signal, a state that is sampled by the apo protein with a certain rate and that becomes stabilized once the signal is present ("conformational selection"). The flow of information in STS pathways may happen within a single polypeptide, when the sensor and effector domains are covalently fused, or with intermediary diffusible protein modules taking part of the information relay. Some of these intermediary players are rigid "transferring" units, while others comprise allosteric regulation circuits of their own. Small molecules might also act as diffusible connectors: noncovalent binding of second messengers is an important means of bridging sensory proteins to output effectors (Jenal et al. 2017; Yoon and Waters 2021). However, the most paradigmatic mode that bacteria evolved in order to relay information among diffusible components is the covalent modification of STS proteins by phosphoryl groups, which are transferred in tan- dem: the same phosphoryl group is subsequently transferred from ATP to the first STS component and then to the other downstream components in the pathway (Buschiazzo and Trajtenberg 2019).

This review attempts to summarize some of the recent advances about bacterial signal transduction systems focusing on the molecular details of STS proteins as allosteric machines. Bacterial STSs can be broadly classified in four groups: one-component systems, two-component systems (and related phosphorelay and chemotactic systems), phosphoenolpyruvate:carbohydrate phosphotransferase systems, and extra-cytoplasmic function sigma factors (Deutscher et al. 2014; Jung et al. 2018). Bacteria also possess addi- tional signaling elements such as

eukaryotic-like protein kinases and membrane-anchored enzymes bearing extracel- lular sensory domains (Galperin 2004). In each and every one of these STSs, allosteric regulation underlies their capacity to transmit information. In the context of this fascinating diversity of STSs, and for the sake of conciseness, we shall limit our analyses to one- and two-component systems as illustrative examples.

Signal transmission within single sensory proteins: plasticity in one-component systems.

One-component systems (OCSs) showcase the simplest and more expanded protein architecture used by bacteria to sense signals and orchestrate regulated responses. OCSs are cytoplasmic proteins that harbor a DNA-binding domain (DBD) including a helix-turn-helix motif—or variations of it—and a ligand-binding domain (LBD) within the same polypeptide. The LBD in OCSs—also referred to as effector-binding domain, we shall keep the LBD denomination to avoid con- fusion with the *output effector domain* of response regulators, vide infra—mediates homo-dimerization, irrespective of the presence of the signal. The LBD is key to the signaling function of OCSs, acting as the sensory domain with the ligand being the input signal. Ligand binding to its specific site at the LBD allosterically modulates the DNA-binding properties of the DBD in a signal-dependent way, ultimately effecting gene expression (Ulrich et al. 2005). OCSs ensure swift responses to oscillations of a wide variety of intra- cellular metabolites, which can also reflect environmental variations through the action of channels and transporters (Fig. 1a).

Information transmission by repositioning the structure of whole domains

The most classical and easy-to-picture paradigm of allostery involves atomic coordinates shifts of the structure physically connecting the allosteric and orthosteric sites. In other words, according to the occupation of the allosteric site by the signal, structurally distinct ground states of the active functional site result. The entire protein or domains shift via rigid-body motions (Daily and Gray 2009) in the micro- to milliseconds range. Members of the Fur (ferric uptake regulator) superfamily of OCSs are widespread homodimeric

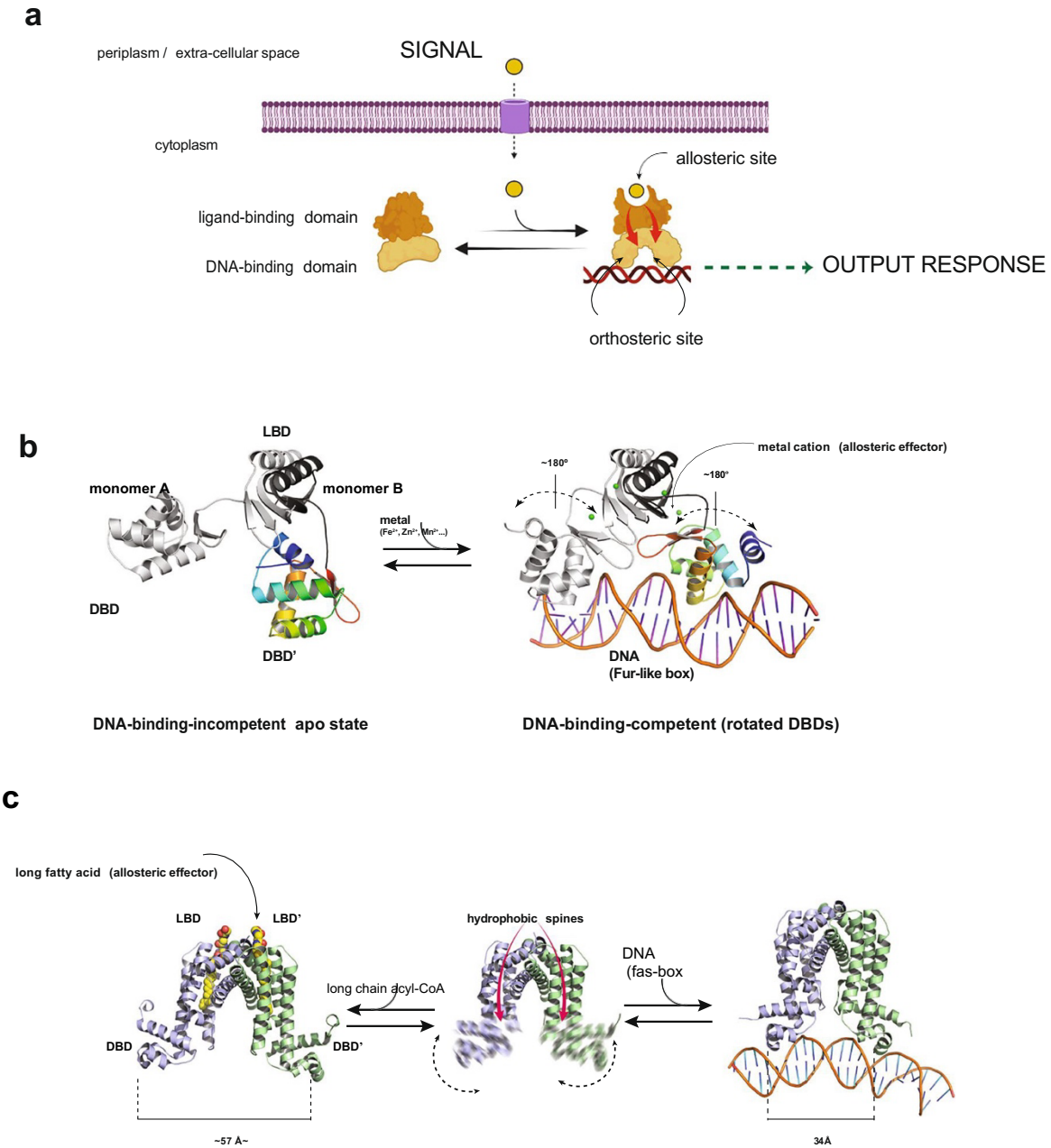

Fig. 1 Allostery and protein plasticity in one-component systems. **a** Schematic drawing of one-component signal transduction systems in bacterial cells. The signal (yellow sphere) is sensed by the ligand- binding domain (LBD) via association into the allosteric site. This results in conformational rearrangements at the distant orthosteric site, in this case located at an output DNA-binding domain (DBD). Allosteric coupling pathways are depicted as red arrows, effecting the transition from an "inactive" apo protein (left) towards an "activated" holo-protein (right). Created with BioRender.com **b** illustration of a two-state switching allosteric mechanism, engaging rigid- body motions. Cartoon representations of the crystal structures of apo (PDB ID 4RAY) and metal-bound holo (PDB 4RB1) Fur MSR-1 from *Magnetospirillum gryphiswaldense*. The holo form exhibits in this case bound Mn^{2+} cations and was solved in complex with its cognate DNA Fur-box. Each monomer in the dimer is distinguished with tones of gray and domain labels with a prime symbol. Only one DNA-binding domain (DBD) is colored with a blue-to-red ramp from N-to-C-terminus, so that the rigid-body rotation is clearer comparing the apo to the holo form. Allosterically induced rotations of *ca.* 180° are observed on both DBDs as indicated, bringing their conformation into DNA-binding-competent geometry (Deng et al. 2015). **c** Allosteric transitions can implicate high protein dynamics with side/ main chain flexibility in one-component systems. Cartoon representations of the crystal structures of FasR, a TetR-like transcription factor from *M. tuberculosis*. The allosteric effect of long-chain fatty acids that bind within the LBD (leftmost panel, PDB 6O6N) rigidifies an otherwise flexible apo form (mid panel, adapted from PDB 6O6O) by completing a hydrophobic spine and rendering a DNA-binding- incompetent geometry. 6O6O is here schematically blurred to high- light regions of higher flexibility in TFRs, constituting a multi-state conformational ensemble. DNA selects the proper conformation and stabilizes the DNA-bound form (rightmost panel, PDB 6O6N), achieving the proper distance between DBD helices that insert into the major groove (Lara et al. 2020)

regulators that control iron homeostasis in bacteria, as well as other metals (zinc, manganese, nickel, etc.). Fur, among several other OCSs, is an excellent illustration of this allosteric coupling mechanism. Fe^{2+} is bound by the dimerization LBD into a pocket that directly bridges this domain to the output response DBD, allosterically inducing a pronounced rotation. This rigid-body motion aligns the DNA-recognition helices of the DBD helix-turn-helix motif into a competent geometry to bind DNA through its major groove (Fig. 1b). It is in its Fe^{2+}-bound, holo form, that Fur is enabled to bind to specific Fur-boxes in the promoters of target genes (Bradley et al. 2020), repressing the transcription of genes coding for proteins involved in iron uptake and assimilation—more infrequently, activatory roles have also been reported (Delany et al. 2004). Apo Fur may be more flexible than initially thought, explaining why relatively few apo structures are available, among which variable inter-domain geometries are observed (Sarvan et al. 2018). Thus, the dis- tinction with dynamic allosteric mechanisms—described next—appears now to be less clear-cut, and might all be specific cases of a more general, common mechanistic scenario.

Information transmission by modulating protein dynamics

The allosteric mechanism might also involve changes of the proteins' conformational flexibility. Such dynamic features refer to pico- to nanosecond motions of amino acid side chains and/or main chain, around a defined average structure. These motions are not directly observable by ground-state microscopic approaches such as X-ray crystallography or cryo-electron microscopy, although they can be deduced from parameters that are refined with those techniques, e.g., atomic displacement factors (temperature or B factors) or local resolution figures. NMR spectroscopy has become a powerful method to characterize dynamic allosteric transmission with great detail, providing both atomic-level information on chemical environments and quantitative conformational dynamics data. A whole range of illustrative examples comes from another group of OCSs, the TetR family of regulators (TFRs) (Yu et al. 2010). TetR was the first factor describing an OCS architecture that has since been recognized as one of the most numerous transcription factor families in prokaryotes. TetR mediates resistance to tetracycline, by sensing the antibiotic and thereafter inducing the expression of a gene encoding a tetracycline efflux pump in Gram-negative bacteria (Orth et al. 2000). TFRs are homodimeric OCSs, most of which act as repressors, inducing the expression of target genes via ligand-binding- dependent DNA dissociation. Recent work with FasR, a TFR from *Mycobacterium tuberculosis*, further confirms how these OCSs' allostery relies on strong dynamic modulation, in the form of order/disorder transitions that link the signal sensing to the output DNA-binding sites (Lara et al. 2020). *M. tuberculosis* FasR is unusual in that it acts as a transcriptional activator of *fas I* and *acpS* genes encoding fatty acid biosynthesis enzymes, key actors in the lipid metabolism and cell wall homeostasis of this pathogen. FasR binds long- chain acyl-CoAs through a deep hydrophobic tunnel open on both ends within the sensor LBD. A physically continuous spine of hydrophobic residues that connects the protein- folding cores of both domains of FasR was identified and seen to be conserved in all TFRs. The hydrophobic spine is interrupted precisely by the ligand-binding cavity, such that only in the ligand-bound condition is the spine completed by the ligand itself (Fig. 1c). The allosteric mechanism at play in TFRs appears to be based on a disordered (flexible, no ligand bound) to ordered transition, with the latter stabilized in a DNA-incompetent conformation, as observed in *M. tuberculosis* FasR (Lara et al. 2020) and TetR (Kamionka et al. 2004; Reichheld et al. 2009). Another TFR, QacR from *Staphylococcus aureus*, binds a range of different drugs inducing drug resistance. The integration of crystallographic and NMR data (Takeuchi et al. 2019) in principle upholds a two-state model, which would thus be more similar to Fur (vide supra). However, differently sized drugs trigger a linear range of inducive effects, and NMR relaxation data indicate that apo QacR is in equilibrium between repressive and inducive conformations constituting a conformational selection scenario (Takeuchi et al. 2019) thus ruling out a simple two-state model. Furthermore, revisiting available structures of wild-type QacR and engineered mutants, the hydrophobic spine can be readily identified, with different ligands completing it to different extents. A continuum of dynamic modulation for information transmission, implying different levels of disorder/flexibility, is thus present among the several thousand TFR systems that have evolved in bacteria. Considering OCSs and other signaling systems alike, this idea of a continuum in the way that proteins achieve allosteric transition is a bridging concept between induced fit and conformational selection, mechanisms that are not essentially dissimilar at the molecular level.

Signal transmission through diffusible modules: two-component systems and derived phosphorelay networks

Two-component systems (TCSs) process signal information by using at least two different proteins that act as diffusible modules. Now the "wire" is not physically connected; instead one component talks to the other via specific non- covalent interactions, ultimately warranting the signal-to- output linkage (Jacob-Dubuisson et al. 2018; Buschiazzo and Trajtenberg 2019). However discontinuous, this scheme has been extremely successful, with the evolutionary selection of thousands of TCSs in prokaryotes, fungi, and plants, enabling cells to sense an extremely diverse range of signals (Fig. 2a). TCSs trigger signal-dependent responses that include powerful transcriptional regulation, but that also go beyond, activating/inhibiting the catalytic activity of enzymes as diverse as (di)nucleotide cyclases,

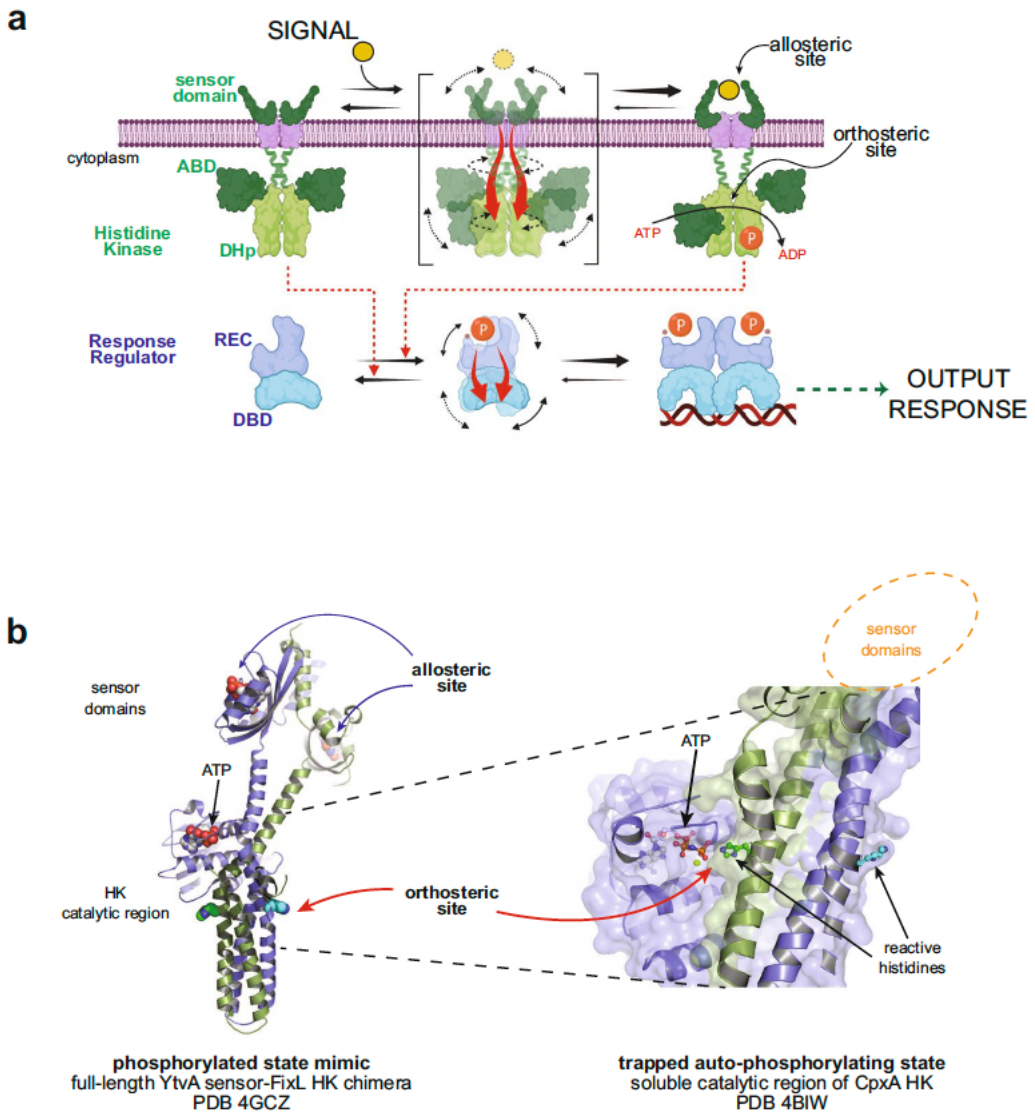

Fig. 2 Allostery and protein plasticity in two-component systems. **a** Schematic drawing of two-component signal transduction systems in bacterial cells. The triggering signal is detected by the first protein component, a histidine kinase (HK). Stimuli are usually environmental (yellow sphere), yet intracellular cues can also be perceived by intracytoplasmic sensor domains, where the allosteric site is located. Conformational rearrangements are coupled to the orthosteric site, in this case located at the HK catalytic reaction center. Allosteric coupling pathways are depicted as red arrows (also for panels (a) and (b)). The conformational transition goes from a kinase-off/ phosphatase-on state (upper, left) to kinase-on/phosphatase-off state (upper, right) that can autophosphorylate using ATP. A coiled-coil structure or S-helix serves as a transmission gear, highlighted in the transition state of the HK (upper, mid panel), affecting the catalytic center on the central "dimerization and histidine phosphotransfer" domain (DHp) and the mobility of the ATP-binding domains (ABDs). The downstream output response is executed by a distinct second protein component, the response regulator (RR). The non-covalent link is guaranteed by phosphoryl-transfer from the P~His on the HK, to the reactive Asp on the RR's receiver (REC) domain. This phosphorylation allosterically shifts the equilibrium from the RR's "inactive" state (lower, left) to a phosphorylated "activated" state (lower, right). The allosteric coupling to favor homo-dimerization and out- put response domain (in this case DNA-binding DBD) activation is highlighted in the lower, central panel. HK activities on the RR are depicted as red dotted arrows. Created with BioRender.com, **b** cartoon representations of histidine-kinases highlighting the spatial separation between the sensory allosteric sites and the orthosteric site engaged in catalysis. To the left, a functional full-length HK shows the overall location of key sites, with the right panel closing up on the orthosteric site trapped while performing auto-phosphorylation catalysis. Both panels correspond to crystal structures of different HKs as indicated, both belonging to the same family (HisKA). Signal-dependent changes at the allosteric site provoke reorganizations of the orthosteric site, ultimately regulating HK-catalyzed auto-phosphorylation, RR phosphoryl transfer, and P~RR dephosphorylation

phosphodiesterases, or methylesterases, among many others (Gao et al. 2019). Even other sorts of responses are also controlled by TCSs via the regulation of protein:protein associations. Chemotactic motility is an amazing example of the latter, wherein phosphorylated CheY binds to the flagellar motor, promoting a switch of its rotation orientation (Bi and Sourjik 2018; Muok et al. 2020). The constellation of TCSs spans from the simplest cases comprising just one histidine kinase (HK) and its cognate response regulator (RR) to much more complex phosphorelay combinations that include one or more intermediary components (Stephenson and Hoch 2002; Wall et al. 2018) and/or lateral networking of multiple phosphorelay pathways that cross-talk in defined ways (Francis and Porter 2019; Jung et al. 2019).

So, how is information transmission ensured between two or more separate protein components, and how is protein plasticity once again pivotal in such paths? It is the HK that acts as the signal-sensory component of TCSs, to then interact with the partner that executes the output response of the system, namely a specific RR. HKs bear one or more sensor domains, extremely variable among different HKs. Two additional domains are always present and show detect- able homology among all HKs: a dimerization and histidine phosphotransfer domain (DHp) and an ATP-binding domain (ABD). The latter is mostly known as catalytic and ATP-binding domain (CA), even though catalysis depends on its concerted action with the DHp. The orthosteric site of HKs is delimited to precise regions of the DHp and ABD (Fig. 2b) involved in catalyzing three different reactions: (i) autophosphorylation of a conserved histidine (His) on the DHp, by transferring the γ-phosphate of ATP, which is delivered to the reaction center bound to the ATP-binding pocket within the swinging ABD; (ii) phosphoryl-transfer from the P~His to a conserved aspartate (Asp) on the RR partner; and, (iii) phosphate hydrolysis, actively dephosphorylating the P~Asp of the P~RR. These two reactions, RR phosphorylation and P~RR dephosphorylation, depend on the association of the HK's DHp with the reactive Asp-containing domain of the RR known as the receiver domain (REC), thus generating a two-protein reaction center. The ABD makes additional contacts with the RR in some cases but not within the active site (Casino et al. 2009; Xie et al. 2020). Phospho- transferase and phosphatase activities catalyzed by the same enzyme, make of HKs an example of paradoxical enzymes (Hart and Alon 2013). This counterintuitive mechanism ensures robustness, guaranteeing the same level of response (i.e., concentration of P~RR) irrespective of the variable concentrations of HK and RR in the cell (Shinar et al. 2007). Paradoxical enzymes require exquisite regulation to avoid futile cycles, a regulation in HKs that is controlled by signal- effected allostery.

Allostery in TCSs can be best understood by considering it to be a two-tiered regulatory phenomenon. On a first level, the HK and the RR each encode allosteric workings that regulate their own orthosteric sites. A second level of regulation takes place with the interaction between HK and RR, each partner playing the role of an allosteric effector on the other. We shall emphasize how phosphorylation of both HKs and RRs stabilize specific structural configurations, ultimately ensuring the efficient transfer of information among diffusible modules.

Coiled-coils can be long-range carriers of information…if they are not too stable

The active site in HKs is under allosteric regulation from the distant sensor domains. Input signals in the form of chemical ligands or physical modifications perceived by the HKs' sensor domains act as the allosteric effectors. Connecting the sensor domains with the HK's catalytically active region, a coiled-coil works as an effective allosteric route, exploit- ing its structural features. The central domain, including the DHp in all HKs, is α-helical, and α-helices are known to span approximately seven residues (positions a through g) every two turns. When positions a and d are both occupied by hydrophobic residues, their side chains stick out from the same side of the helix and tend to interact with neighbor helices comprising similar heptad-repeating hydrophobic patterns. This side-by-side packing results in a left-handed superhelix or coiled-coil. Shifts at one end of a coiled-coil can be mechanically transmitted into substantial positional shifts at distantly located residues, exploiting the stiffness of α-helices and sequence-encoded heptad-repeat deviations. Non-ideal heptad repeats, bearing polar residues at a/d positions and/or insertions, reduce the coiled-coil's energetic stability and enable conformational switching for allosteric regulation. HK sequences comprise a coiled-coil structure along the DHp four-helix bundle where the reactive histidine is located and through an N-terminal two-helix extension that connects to the sensor domain(s). Such coiled-coil region was presciently identified as a key functional element on the basis of HKs sequence

analyses (Singh et al. 1998). This two-helix coiled-coil segment has also been named helix Jα (Möglich et al. 2009) or S-helix (Anantharaman et al. 2006), indeed exhibiting non-ideal heptad-repeat motifs. Additional S-helices, when more than one sensory or signal-transmission domains are present, connect the intervening domains (Lesne et al. 2018).

The S-helix is the means to transmit positional information downstream, by helical rotation and helix axis translation following signal-effected coiled-coil rearrangements. Rotational rearrangements of S-helices had been proposed to underlie HK activation in quorum sensing (Neiditch et al. 2006) and light perception (Möglich et al. 2009) pathways, based on functional experiments. A first direct observation of this allosteric route involving S-helix coiled-coil rearrangements was obtained with X-ray structures of *Bacillus subtilis* DesK, a thermosensor, in different conformational/signaling states (Albanesi et al. 2009; Saita et al. 2015; Trajtenberg et al. 2016; Imelio et al. 2017) and then confirmed in several other cases including NarX (Huynh et al. 2013), NarQ (Gushchin et al. 2017), and NsaS (Bhate et al. 2018). Further, supporting evidence for a universal coiled-coil-borne allosteric mechanism has been provided with elegant experiments on light-sensitive (Berntsson et al. 2017b, 2017a; Engelhard et al. 2017) and virulence-regulating (Wang et al. 2014; Lesne et al. 2016, 2017, 2018) HKs. Coiled-coil formation/stabilization typically drives HKs to kinase-off/phosphatase-on conformations (Fig. 3a), whereas coiled-coil disruption/destabilization leads to kinase-on/ phosphatase-off states (Trajtenberg et al. 2016; Gushchin et al. 2017). Transmission can also travel through such a coiled-coil in the opposite direction. The phosphorylated RR P~DivK—member of the *Caulobacter crescentus* DivJ- DivK-PleC phosphorelay that controls cell identity during asymmetric cell division—binds to the DHp domain of the DivL HK and, via DivL's S-helix, triggers the allosteric upstream reorganization of its own sensory PAS domains (Childers and Shapiro 2015), a key event for subsequent DivL-dependent inhibition of a second phosphorelay— CckA-ChpT-CtrA—involved in differentiation and cell-cycle progression.

Information transmission via coiled-coil segments in signaling proteins goes beyond HKs. In the TCS-related realm of chemotaxis, analogous coiled-coil destabilization/stabilization switching has been put forward as the molecular basis of allostery connecting MCP perception of attractant/repellent molecules and downstream control of the CheA HK activity (Bartelli and Hazelbauer 2016). RRs provide with additional examples, like in DgcR from Leptospira biflexa, which possesses a coiled-coil segment connecting the phosphorylatable REC domain to the output diguanylate cyclase (DGC) domain. REC phosphorylation modulates allosterically the DGC domains in the dimer via a register shift and extension of the coiled-coil (Fig. 3b), which repositions the inter-domain hinge, eventually trig- gering c-di-GMP production (Teixeira et al. 2021).

In sum, marginally stable coiled-coils act as information carriers, and their shifts provide mechanical energy to switch among conformational/functional states.

Covalent stabilization of protein conformations by phosphorylation: a particular case of allostery?

The classic definition of allosteric regulation excludes covalent modification of proteins as allosteric effectors (Fenton 2008), considering that such effectors must bind reversibly onto their regulatory site. However, obvious parallels between covalent and non-covalent modifications can be drawn. Protein phosphorylation, even though covalent, is eventually reversible and can thus be considered a special case of binding with very slow dissociation rates (enhanced by dedicated phosphatases in some scenarios). Indeed, the phosphorylation of the reactive aspartate on the REC domain of RRs allosterically regulates the conformation of remote functional sites within the same domain and eventually also the configuration of additional output effector domains when present (Bourret 2010; Gao et al. 2019).

Analogously to the picture described earlier for OCSs, according to which a continuum of protein dynamics under- lines allosteric regulation mechanisms, the REC domain in TCS RRs is again a fascinating example of encoded allostery. REC allosteric modulations range from discrete structural switching of key residues' positions all the way to essentially dynamic order/disorder transitions. Both case scenarios are controlled by the phosphorylatable aspartate reaction center that acts as the allosteric site. This site is phosphorylated by the cognate HK upstream, hence connecting the HK-sensed input signal to the RR-mediated response. Examples of positional shifts in RECs triggered by Asp phosphorylation were observed early on (Zhu et al. 1997; Hastings et al. 2003)

and designated as the Y–T coupling mechanism. A conserved threonine that is part of the P~Asp environment propagates a phosphorylation-triggered conformational rearrangement to a nearby tyrosine, ultimately channeling an allosteric modulation to distant, solvent-exposed areas. The latter are typically defined by the β4-α4 and β5-α5 loops and part of the α4-β5-α5 surface (for the standard nomenclature of RR secondary structure elements see Gao et al. (2019)), which act as the orthosteric functional sites of RECs: their allosteric alteration typically drives the domain to interact with other proteins exerting response functions, or with itself, forming homo-oligomeric species (dimers or tetramers most often) critical to gain out- put function attributes. Correlated to REC-mediated dimer- ization, phosphorylation-dependent allostery also triggers the reconfiguration of output effector domains. For exam- ple, RRs that comprise DBDs often exhibit these attached to the REC domain in the inactive state and then unleashed ready to bind DNA upon REC phosphorylation. The antibiotic resistance regulator VraR from *S. aureus* is an example of this (Leonard et al. 2013). VraR in its inactive form is monomeric and exhibits a "closed" configuration, with the output response DNA-binding domains tightly associated to the N-terminal REC and thus impeded to bind DNA. Upon phosphorylation by VraS, coupling is observed between the P~Asp acting as allosteric site and two different areas of the REC domain: while one promotes homo-dimerization, the other one liberates the DBD to trigger the response (Fig. 3c). Further confirming this, engineered perturbations of the DBD:REC interface result in enhanced RR phosphorylation

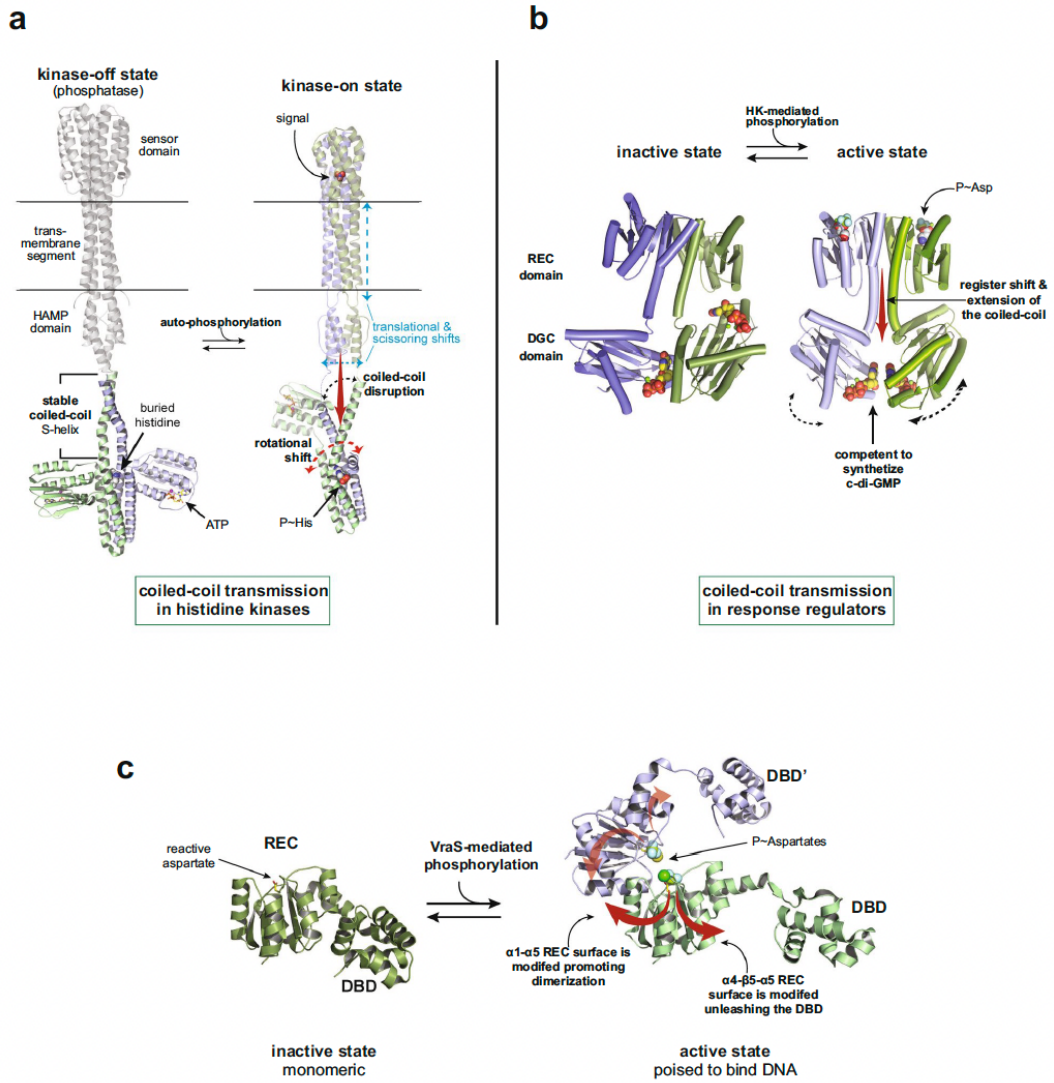

Fig. 3 Allostery in TCSs: transmitter coiled-coils and phosphorylation as allosteric effector. **a** Coiled-coils serve as transmission gears in two-component systems (see also panel (**b**)). A full-length HK was built with two systems from the same HK family, merging them in a plausible configuration for illustrative purposes: *E. coli* NarQ for the extracellular and trans-/juxta-membrane region [PDBs 5JEQ for the inactive state, 5IJI for the active state (Gushchin et al. 2017)]; and *B. subtilis* DesK for the intracytoplasmic catalytic region [PDBs 5IUN for the inactive state, 5IUM for the active state (Trajtenberg et al. 2016)]. In the kinase-off/phosphatase-on state (top panel), a stabilized coiled-coil maintains the HK rigid and unable to auto-phosphoryl- ate. Upon signal perception, the coiled-coil is disrupted provoking a rotational shift that liberates the ABDs and exposes the reactive His (bottom panel). **b** Cartoon representations of the RR DgcR from *L. interrogans*. Upon phosphorylation of the REC domain in the inactive state (left, PDB 6ZXB), a coiled-coil connecting to the diguanylate cyclase (DGC) output response domain undergoes a register shift and extension, activating the RR (right, PDB 6ZXC) (Teixeira et al. 2021). **c** Phosphorylation as an allosteric effector in two-component systems, an example of response regulator plasticity. Unphosphorylated VraR (left, PDB 4GVP) exhibits a "closed" configuration, with the output response domain associated to the REC. Upon phosphorylation, allosteric coupling affects two areas within the REC, stabilizing a dimeric form with free DNA-binding domains to exert transcriptional regulation (right, PDB 4IF4) (Leonard et al. 2013)

(Barbieri et al. 2010), consistent with the fact that allosteric coupling drives reciprocal effects between allosteric and orthosteric sites.

NMR relaxation analyses of the REC showcase other examples where phosphorylation-triggered allostery in RRs occurs through more dynamic pathways as compared to the paradigmatic Y–T coupling mechanism. The *Escherichia coli* NtrC is a TCS RR that plays a key role in nitrogen metabolism regulation. The non-phosphorylated inactive state of NtrC REC comprises not one but a heterogeneous collection of conformers that sample the active conformation as well, even though barely populated (Volkman et al. 2001). Phosphorylation stabilizes this single, well-defined active conformation from among the inactive mixture, without the requirement of positional shifting of connected residues, and in this way reinterpreting the Y–T coupling paradigm (Villali et al. 2014). This more general perspective, of a flexible non-phosphorylated REC, inactive for downstream signaling functions, allows for a better understanding of alternative activatory allosteric effectors beyond Asp phosphorylation. In the hypoxia sensor NtrYX TCS from *Brucella abortus*, the RR NtrX exhibits meta-active conformations independent of phosphorylation, including a homodimeric species with a similar-to-active configuration of the β4-α4 loop (Fernandez et al. 2015). The active form of the HK NtrY selects the meta-active conformation of NtrX, phosphorylating it and stabilizing its active conformation. A similar picture had been reported in *B. subtilis* DesKR, where the HK-RR interaction stabilizes a pre-existent active state of DesR promoting its dimerization and transcriptional activity (Trajtenberg et al. 2014).

Concluding remarks

Allostery has been referred to as the "second secret of life," the first one being the genetic code (Monod 1970). Allosteric regulation is only feasible because of protein plasticity, which must be distinguished from disorder in that it is a form of defined structural flexibility encoded within the protein's sequence, selected during evolution. Signal transduction systems rely on such protein plasticity to produce controlled physiologic responses upon receiving information of varying environmental and intracellular conditions. The presence/ absence of a signal induces motion in sensor modules or yet selects certain configurations of them that are being sampled over time. The conceptual framework is the same for these two protein dynamics situations, i.e., signal-triggered induced fit or conformational selection. The only difference between two-state switching and multi-state sampling is the actual energy landscape selected for each signaling protein through evolution. In the shallow multi-minima scenario, allostery may regulate protein flexibility rather than the

fixing of specific alternate atomic coordinates. The combination of these two extremes into a continuum of protein plasticity might probably be the actual case in most systems (Motlagh et al. 2014), with relevant protein motions happening in the pico/nanosecond scales of vibrations all the way to micro/millisecond of entire domain shifts. Upstream protein configuration rearrangements are transmitted down- stream to effector modules, which in turn also suffer structural rearrangements that are conducive to the execution of a physiologic response. This coupling is the very definition of allostery. Although the spectrum of existing sensory and output effector domains is tantalizing, the mechanisms by which signal transduction occurs show common regulatory features including protein plasticity, allostery, intermolecular interactions, and tandem phosphoryl transfers. Understanding these mechanisms at the molecular level is not only instrumental to make new breakthroughs in biotechnological and biomedical fields such as in drug design and synthetic biology, but also to shed light on how life has managed to thrive for billions of years.

Funding This study received funding from ANII (grant FCE_1_2017_1_136291) and from the Unit of Integrative Microbiology of Zoonotic Agents IMiZA, Joint International Units program, Institut Pasteur/Institut Pasteur de Montevideo.

Conflict of interest The authors declare no competing interests.

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