Full-Length Structure of a Sensor Histidine Kinase Pinpoints Coaxial Coiled Coils as Signal Transducers and Modulators

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SUMMARY

Two-component systems (TCSs), which comprise sensor histidine kinases (SHK) and response regulator proteins, represent the predominant strategy by which prokaryotes sense and respond to a changing environment. Despite paramount biological importance, a dearth exists of intact SHK structures containing both sensor and effector modules. Here, we report the full-length crystal structure of the engineered, dimeric, blue-light-regulated SHK YF1 at 2.3 Å resolution, in which two N-terminal light-oxygen-voltage (LOV) photosensors are connected by a coiled coil to the C-terminal effector modules. A second coaxial coiled coil derived from the N-termini of the LOV photosensors and inserted between them crucially modulates light regulation: single mutations within this coiled coil attenuate or even invert the signal response of the TCS. Structural motifs identified in YF1 recur in signal receptors, and the underlying signaling principles and mechanisms may be widely shared between soluble and transmembrane, prokaryotic, and eukaryotic signal receptors of diverse biological activity.

INTRODUCTION

Excitability, i.e., the ability to perceive and respond to signals, is a basic hallmark of life. Two-component systems, which comprise sensor histidine kinase (SHK) and cognate response regulator (RR), are the most widespread and important signal transduction systems in prokaryotes but also occur in certain eukaryotes, notably in Saccharomyces cerevisiae and Arabidopsis thaliana (Gao and Stock, 2009; Capra and Laub, 2012). Sensor histidine kinases commonly consist of an N-terminal stimulus-specific sensor module and a C-terminal effector module, which comprises the dimerization/histidine phosphotransfer (DHp) and catalytic/ATP-binding (CA) domains. Most sensor histidine kinases catalyze three distinct phosphotransfer reactions: auto-phosphorylation at the eponymous histidine residue within the DHp domain and both phosphorylation and dephosphorylation of the cognate RR; certain SHKs apparently lack the phosphatase activity (Gao and Stock, 2009). While the sensor modulates all three elementary reactions, the biological response is determined by net kinase activity, i.e., the balance between antagonistic phosphorylation and dephosphorylation of the RR (Russo and Silhavy, 1993).

SHKs exhibit remarkably diverse combinations of sensor and effector modules (Finn et al., 2006; Aravind et al., 2010), which is particularly evident for the two most frequently occurring two-component system (TCS) signaling modules, HAMP (Hulko et al., 2006) and Per-ARNT-Sim (PAS) domains (Szurmant et al., 2007; Möglich et al., 2009b). The plethora of combinatorial arrangements affords immense functional versatility and arguably accounts for the wide recurrence of TCS. Although it is unclear whether SHKs employ universal signal transduction mechanisms, to some extent, these mechanisms are evidently shared, as certain sensor and effector modules are functionally exchangeable. In particular, we previously replaced the oxygen-sensitive PAS-B domain of the Bradyrhizobium japonicum FixL SHK with the flavin-mononucleotide (FMN)-binding light-oxygen-voltage (LOV) photosensor domain from Bacillus subtilis YtvA (Figure 1A; Möglich et al., 2009a). The resultant, engineered SHK YF1 phosphorylates its cognate RR FixJ with near-FixL activity in the dark; however, upon blue-light absorption, a covalent bond forms between the FMN chromophore and cysteine 62 within the LOV sensor (Christie et al., 1998; Herrou and Crosson, 2011), and YF1 phosphatase activity is enhanced, which results in a more than 1,000-fold decrease of net kinase activity.

High-resolution structures of isolated sensor (Gao and Stock, 2009), DHp (Tomomori et al., 1999), and CA (Bilwes et al., 1999) domains have greatly contributed to elucidating the molecular architecture of SHKs. Two landmark crystal structures of the entire effector module from the Thermotoga maritima SHK HK853 (Marina et al., 2005; Casino et al., 2009) revealed that the DHp and CA domains are connected by flexible hinges and thus can adopt different spatial orientations (Albanesi et al., 2009), depending on functional state. Arguably due to this flexibility, full-length SHKs containing both sensor and effector modules have so far largely eluded structural characterization. The mechanism by which the signal is transmitted from the sensor
to the effector modules, in particular, the precise molecular nature of the connection between sensor and effector, has thus remained unclear. Only during the review of this manuscript, the structure of the VicK SHK, comprising both sensor and effector moieties, has been reported (Wang et al., 2013).

We now report the crystal structure of the sensor histidine kinase YF1 at full length. Via site-directed mutagenesis, we identify residues that govern signal response and net kinase activity and that can be modified to reprogram the properties of the TCS (e.g., to invert its response to signal).

RESULTS

Structure of the Blue Light-Regulated Histidine Kinase YF1

Crystals of native and selenomethionine-substituted YF1 were obtained by sitting-drop vapor diffusion in the dark in space group P6_522 and diffracted X-rays to 3.1 and 2.3 Å resolution, respectively. The structure of YF1 was solved by molecular replacement, confirmed by selenium single-wavelength anomalous dispersion, and refined at 2.3 Å resolution to R_work = 17.87% and R_free = 21.70% (Table 1). Within each asymmetric unit, two YF1 molecules assemble into an elongated, parallel dimer, which comprises 4,420 Å² of solvent-accessible surface area (Figure 1B). The dimer interface is formed by an intricate α-helical spine, which traverses the entire molecule and consists of three segments. An N-terminal parallel coiled coil (denoted A₀α; amino acids [aa] 9–22) is inserted into the interface between the two LOV domain cores (aa 23–127). Coaxial with the A₀α coiled coil, the C-terminal Jₓ helices of the LOV domain (aa 128–147) form a second coiled coil that is contiguous with N-terminal helices of the DHp domains (aa 148–217), which dimerize as an antiparallel four-helix bundle (Tomomori et al., 1999). A pronounced kink of ~35° within the N-terminal region of the DHp domain probably arises from intermolecular packing within the YF1 crystal lattice (Figure S1A available online). Notably, the globular LOV photosensor and CA domains (aa 218–375) are situated laterally to the helical spine and make no direct contact with each other.

The two LOV domains adopt the characteristic PAS fold (Crosson and Moffat, 2001; Möglich et al., 2009b), which comprises a five-stranded antiparallel β sheet (strands Aβ, Bβ, Gβ, Hβ, and Iβ) and four α helices (Ca, Da, Eα, and Fα). The absence of a covalent bond between the FMN and C62 confirms that YF1 assumes its fully dark-adapted state (Figure S1B). When crystalized as a truncated construct (aa 20–147) lacking the additional, N-terminal A₀α helix (Möglich and Moffat, 2007), the same LOV domain showed closely similar tertiary structure but differences in the dimer interface. In the YF1 structure, the A₀α helices assemble into a coiled coil at the LOV-domain interface and are stabilized by interactions among the hydrophobic residues I9, L13, I16, and L20 and by mostly intermolecular contacts with the β sheets of the adjacent LOV domains (Figures 2A and S2). Prominently, the aliphatic side chains of V15 and A19 protrude into a hydrophobic cavity lined by V27 and I29 in strand Aβ, M111 and I113 in strand Hβ, and by Y118 and V120 in strand Iβ. In contrast to these hydrophobic, nondirectional interactions, the C terminus of A₀α is precisely oriented by a pair of intra- and intermolecular hydrogen bonds to the adjacent LOV domains. Residue D21 forms an intramolecular hydrogen bond to the backbone of residue Q44 within helix Ca, and H22 forms an intermolecular hydrogen bond with residue D109 within strand Hβ of the opposite LOV domain. The junction between the LOV domain and the C-terminal Jₓ helix is provided by the conserved
residues D125, I126, and T127 (DIT motif; Möglich et al., 2009a), which are engaged in five hydrogen bonds with each other and the backbone of W103 in strand H161 (Figures 2B and S2). Thus precisely coordinated, the Jx helices form a coiled coil via hydrophobic interactions of their C-termini (L136, L139, L143, and V146), whereas their N-termini are splayed apart. As the Jx helices are conjoined with helices of the DHp domain, a continuous α-helical linker is established between the LOV photosensor and effector modules of the YF1 SHK.

The DHp domain comprises two long helices (aa 148–181) and α2 (aa 189–214) that are connected by a short hairpin (aa 182–188) in clockwise direction, also found in EnvZ (Tomo­mori et al., 1999), but different from the counterclockwise direction in HK853 (Marina et al., 2005; Casino et al., 2009; Figures S3A and S3B). In both YF1 monomers, the phosphoaccepting histidine 161 complexes a sulfate ion via its N6 atom, which is thought to mimic phosphorylation (Marina et al., 2005); the YF1 structure may thus represent a pseudophosphorylated state of the SHK. The C-terminal CA domains adopt the canonical mixed αβ sandwich fold (Bilwes et al., 1999) and are attached to the DHp domains by unstructured loops (Figure 3). Due to the clockwise orientation of the DHp helices, the CA domains are in closer proximity to the phosphoaccepting residues H161 within the opposite monomer than to those within the same monomer, which argues for autophosphorylation in trans (Casino et al., 2009). Although the YF1 crystals were grown in the presence of excess ATP, only the CA domain of monomer A binds ADP via residues within the conserved N, F, G1, and G2 sequence regions (Parkinson and Kofoid, 1992; Figure S3C). In monomer B, the nucleotide-binding loop (aa 310–337) adopts a divergent conformation, in which residues D318 and M335 obstruct the nucleotide-binding pocket and residues T329, T330, and K331 point outward (Figure S3D). The difference in nucleotide binding between monomers A and B is accompanied by partial unwinding of the α2 helix within the DHp domain of monomer B and by an altered orientation of their CA domains relative to the DHp domains (Figure 3). While the CA domains largely occupy equivalent regions in space, they are rotated by ~58.7° and translated by 3.5 Å relative to another. The distance between the ADP cofactor (atom Pβ) in monomer A to H161 (atom Ni) in monomer B amounts to 23.0 Å; a comparable distance of 21.8 Å between the ADP nucleotide and the phosphoaccepting histidine is found in the structure of HK853, which undergoes autophosphorylation in cis (Marina et al., 2005; Casino et al., 2009). When modeling an ADP nucleotide into the binding pocket of the CA domain in monomer B of YF1, a distance of 12.8 Å is obtained to the phosphoaccepting H161 of monomer A (Figure 3B). A similar CA orientation and ADP-histidine distance (12.9 Å) are observed in the complex structure of HK853 and its response regulator (Protein Data Bank [PDB] 3DGE; Casino et al., 2009). The orientation of the CA domain in monomer B of YF1 may thus be predisposed to binding the response regulator and subsequent steps. While it is difficult to confidently ascribe functional states to the two CA conformations and orientations presently observed in the YF1 structure, they at least demonstrate the inherent flexibility of SHKs.

**Table 1. Data Collection and Refinement Statistics**

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*Highest resolution shell is shown in parentheses.

Coiled Coils as Key Modulators of Signal Transduction

A survey of diverse PAS domains suggested that signal transduction generally originates in structural changes within the central β sheet and concomitant modulation of the affinity between the outer face of the sheet and its interacting partner(s), which frequently is (are) an α helix (Möglich et al., 2009b). In particular, in the monomeric LOV2 domain from Avena sativa phototropin 1 (ASLOV2), the C-terminal Jx helix packs on the outer face of the β sheet (Halavaty and Moffat, 2007); blue-light absorption leads to a weakening of this interaction and to dissociation and unfolding of Jx (Harper et al., 2003). Intriguingly, A’α of YF1 is in a location equivalent to that of Jx in ASLOV2, although A’α derives from the juxtaposed LOV domain and runs in the opposite direction (Figure S5). To test the notion that A’α is involved in signal transduction, we generated mutants of YF1 and measured their activity and dependence on light using the pDusk-DsRed plasmid (Ohlendorf et al., 2012). Briefly, this plasmid encodes YF1 and its cognate RR FixJ, which drive expression of the fluorescent reporter DsRed in Escherichia coli, thus allowing facile measurements of net kinase activity (Figure 4A). In the reference construct YF1, blue-light absorption results in 10.3-fold decreased fluorescence compared to dark conditions. To ascertain that the in vivo fluorescence observed in the pDusk background indeed reflects the activity of YF1 variants, we compared the levels of intracellular, soluble protein of these variants to...
wild-type YF1 by western blotting (Figures 4B and S4). All YF1 variants shown in Figure 4A were expressed to similar or at most slightly lower extent as the wild-type.

Mutations within Aα of hydrophobic residues that mediate the coiled coil interaction either attenuated light responsiveness (L13A) or completely abolished activity, as in the case of I16A,

Figure 2. The Aα and Jα Coiled Coils
(A) The N-terminal Aα coiled coil. The Aα helices are engaged in hydrophobic interactions among I9, L13, I16, and L20 and numerous, mostly intermolecular contacts to the adjacent LOV domains. Residues V15 and A19 within Aα protrude into a hydrophobic cavity lined by V27, I29, M111, I113, Y118, and V120 within the juxtaposed LOV β sheet. At the C terminus of Aα, residue D21 forms an intramolecular hydrogen bond to the backbone of Q44 and residue H22 forms an intermolecular hydrogen bond to D109.

(B) The C-terminal Jα coiled coil. The Jα helices display hydrophobic interactions among L136, L139, L143, and V146. Residues D125, I126, and T127 within the DIT motif at the base of Jα form five hydrogen bonds with each other and with the backbone of W103 in strand Hβ of the LOV domain.

Figure S2 presents an extended view of the LOV β sheet and helices Aα and Jα.

Figure 3. Orientation of the DHp and CA Domains
(A) The distance from the ADP cofactor (atom Pβ) within the CA domain of monomer A to the phosphoaccepting histidine 161 (atom Nδ) of monomer B is 23.0 Å (dashed line). The CA domain of monomer B occupies a similar spatial position relative to the DHp domain, but its angular orientation differs. The positions and orientations of the CA domains A and B relative to the DHp domains are related by a 58.7° rotation around and 3.5 Å translation along the screw-rotation axis indicated (solid line). The dashed circle highlights partial unwinding of the α2 helix of the DHp domain in monomer B.

(B) Nucleotide binding in the CA domain of monomer B was modeled by superposing a copy of the ADP-binding CA domain of monomer A (light orange, denoted CA A). In this model, the distance between ADP and H161 is only 12.8 Å. The views of YF1 in (A) and (B) are aligned with respect to the α1 and α2 DHp helices of monomers A and B, respectively.

(A) and (B) of Figure S3 compare the architecture of the DHp domains in YF1 to that in HK853 (Marina et al., 2005); (C) and (D) show close-up views of the YF1 CA domains of monomer A and B.
L20A, and L20I. Replacement of residues V15 and A19 by other aliphatic residues (V15A and A19V) largely removed light responsiveness and resulted in constitutive activity. Whereas the mutations D21A, H22L, D109L (Avila-Pérez et al., 2009), and D109E resulted in constitutive activity, the variants D21G and H22P displayed inverted signal responses with moderate impairment of light responsiveness (I126A and I126D). The mutagenesis data corroborate the pivotal role of the Jz helices in YF1 provide a rigid conduit, along which signals are propagated from sensor to effector over extended molecular distances, presumably as quaternary structural changes (Matthews et al., 2006; Möglich et al., 2009b).

DISCUSSION

Structure-Informed Model for Signal Transduction

We previously proposed the rotary-switch mechanism, which posits that signals are propagated from sensor to effector as torque movements within a coiled coil linker (Möglich et al., 2009a). In support of this model, the YF1 structure now reveals a continuous helical spine, which provides an axle around which rotary motions may occur. Crucially, the axes of the A’z and Jx coiled coils are precisely aligned, which is conducive to transmitting torque motions between them and to the C-terminal effector module. Additional coiled coils could serially concatenate and coaxially align additional N-terminal sensor domains, thereby providing the structural rationale for signal integration in multi-input signal receptors (Möglich et al., 2010). A molecular model for signal transduction in YF1 is now afforded by comparison to the structure of the Pseudomonas putida LOV protein SB1 (Circolone et al., 2012). While SB1 lacks a C-terminal effector module, it shares with YF1 high sequence similarity (41.7% identity within aa 25–127 of YF1) and a similar overall fold; crucially, SB1 was crystallized in its fully light-adapted state. Thus, we generated a homology model for the light-adapted state of the YF1 LOV photosensor on the basis of the SB1 structure and compared it to the experimentally determined dark-adapted structure (Figure 5; Movies S1 and S2). In the framework of this model, the LOV domains would rotate upon light absorption by about 15° around the axes indicated in Figure 5A (solid lines); remarkably, the swivel point for this rotation is at the interface between the LOV β sheet and the A’z coiled coil, which our functional data identify as a hub for signal modulation. The A’z helices would tilt and thereby increase their crossing angle from 29° in the dark-adapted state to almost 70° in the light-adapted state.
The quaternary structural transition of the LOV domains would further entail a rotation of the C-terminal $J_a$ helices around the dimer axis and a concomitant increase of their crossing angle from $33^\circ/C14$ in the dark-adapted state to $49^\circ/C14$ in the light-adapted state. The increase of the helix-crossing angle would in turn induce left-handed torque and lead to a supertwisting of the left-handed $J_a$ coiled coil. Notably, the rotary-switch mechanism we propose is compatible with two recent models for signal transduction in SHKs. In the model advanced by Coles, Lupas, and colleagues (Ferris et al., 2012), signals would induce rotary movements within the antiparallel coiled coil of the DHp domain, akin to the torque motions we implicate for YF1; resultant subtle structural rearrangements would modulate the interaction between DHp and CA domains and thus regulate net kinase activity. The helix-cracking model (Dago et al., 2012) envisions signal-induced structural perturbations, which lead to partial unfolding of helix $a_2$ of the DHp domain, as we indeed observe in monomer B of our structure (Figure 3); as a consequence, the mobility of the CA domain would be enhanced, enabling it to associate with and phosphorylate the active-site histidine. Our structure, our functional data, and the rotary-switch mechanism are consistent with both models, and we thus cannot discriminate between them. In fact, the two models are not in contradiction, and signal transduction may well rely on aspects of both. Whereas our data implicate torque movements in signal transduction from sensor to effector, in other SHKs, evidence for piston-type movements has been obtained (Cheung and Hendrickson, 2009; Falke and Erbse, 2009; Moore and Hendrickson, 2009). Apparently, multiple and ingenious mechanisms are at play in the regulation of SHKs and other signal receptors.

Recurring Motifs in Signal Receptors

YF1, although an engineered SHK, is representative of diverse naturally occurring signal receptors (Finn et al., 2006), including numerous PAS-linked (cf. Figure 5 of Möglich et al., 2009a) and LOV-linked (Purcell et al., 2007; Swartz et al., 2007) species. Notably, the structural principles evidenced in the photoreceptor YF1 also apply to the prevalent transmembrane chemoreceptors: helices and coiled coils corresponding to $J_a$ traverse the plasma membrane and thereby connect the physically separated extracellular (or periplasmic) sensor and intracellular effector modules. Indeed, helix rotation has been implicated in the signal transduction of certain transmembrane SHKs (Hulko et al., 2006) and signal receptors (Moukhametzianov et al., 2006). The architecture of YF1 recurs in diverse signal receptors, including the transmembrane SHK CitA (Sevvana et al., 2008) and soluble bacteriophytochrome red-light sensors (Yang et al., 2008; Figures 6 and S6). Although the molecular details somewhat differ, in all cases, sensor domains interact through their $\beta$ sheets with a central $\alpha$-helical spine that connects to a C-terminal effector module. Intriguingly, these similarities are limited neither to SHKs nor to prokaryotes, as evidenced by the recent structure of the heterodimeric complex of the transcription factors CLOCK and BMAL1 (Huang et al., 2012) from mouse (Figures 6 and S6). Despite their eukaryotic provenance and a different effector module, consisting of an N-terminal helix-loop-helix domain, the PAS-A domains of CLOCK and BMAL1 embrace a coiled coil highly reminiscent of $A_0a$ in YF1. In line with the structural resemblance, certain mutations of residues in the interface between the PAS-A domains of BMAL1 and CLOCK lead to functional impairment (Huang et al., 2012). Thus, structural principles identified in YF1 are widely shared across

Figure 5. Structure-Based Model for Signal Transduction

(A) The YF1 LOV photosensor domains in their dark-adapted state as determined by x-ray crystallography (left) are compared to a homology model of their light-adapted state (right), based on the structure of the Pseudomonas putida SB1 LOV protein (Circolone et al., 2012). Light absorption could induce quaternary structural rearrangements that culminate in a supertwist of the C-terminal coiled coil (arrows). Structures were aligned with respect to the LOV core domains (residues 29–126). The dimer axis is shown as a dashed line; screw-rotation axes describing the motions of the LOV core domains are shown as solid lines. (B) Light absorption could increase the crossing angle of the $J_a$ helices from $33^\circ$ to $49^\circ$, thus increasing the left-handed supertwist of the coiled coil. Figure S5 shows conserved modes of interaction between the $\beta$ sheet of LOV domains and flanking helices. Movies S1 and S2 show an animation of the signal-transduction model depicted in (A).
Structure
Full-Length Structure of a Sensor Histidine Kinase

Figure 6. Recurring Structural Motifs in Signal Receptors
(A) A schematic of the YF1 LOV photosensor domains (yellow shapes) and the Aα and Jα coiled coils (blue and green cylinders). Signals may be propagated as rotary movements within the Jα helices to the C-terminal effector modules (red arrows).
(B–D) Closely related structural configurations with sensor domains embracing coiled coils corresponding to Aα are widely observed in diverse signal receptors (e.g., in bacteriophytochrome red-light sensors [B], Pseudomonas aeruginosa BpHP (Yang et al., 2008), in bacterial chemoreceptors [C], Klebsiella pneumoniae CtxA (Sevvana et al., 2008), and in the mouse CLOCK:BMAL1 complex [D] (Huang et al., 2012)). Notably, in contrast to the other receptors, CLOCK and BMAL1 form a heterodimer are of eukaryotic origin and have N-terminal helix-loop-helix effector modules.

Figure S6 shows the four structures of signal receptors in cartoon representation.

EXPERIMENTAL PROCEDURES

Molecular Biology and Protein Expression
The gene encoding YF1 was amplified by PCR from an earlier expression construct in the pET-28c vector (Möglich et al., 2009a). Ligation into the pET-41a expression vector (Novagen, Merck) via restriction sites derived from the plasmid pDusk-DsRed (Ohlendorf et al., 2012) via PCR by appending a myc epitope (EQKLISEEDL) to the C terminus of YF1. Site-directed mutants of YF1 were generated in the background of the reporter plasmid pDusk-myc-DsRed according to the QuickChange protocol (Invitrogen, Life Technologies). The identity of all constructs was confirmed by DNA sequencing (GATC Biotech).

For protein expression, pET-41a-YF1 was transformed into E. coli BL21 CmpX13 cells (Mathes et al., 2009). A 5 ml overnight culture was used to inoculate 500 ml Luria broth (LB) media containing 50 μg ml⁻¹ kanamycin and 50 μM riboflavin. Cells were grown at 37°C and 225 rpm to an optical density at 600 nm (OD₆₀₀) of 0.6, at which point expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). After incubation for 4 hr at 37°C and 225 rpm, cells were harvested by centrifugation and resuspended in 20 ml buffer A (50 mM Tris-HCl pH 8.0, 20 mM NaCl, 20 mM imidazole, protease inhibitor cocktail Complete Ultra [Roche Diagnostics]). Cells were lysed by sonication, and the suspension was cleared by centrifugation. The supernatant was loaded on a 5 ml Ni²⁺-chelate affinity column (GE Healthcare) using an AKTAprime plus chromatography system (GE Healthcare) and washed with ten column volumes (CV) buffer A followed by ten CV buffer B (50 mM Tris-HCl pH 8.0, 1 M NaCl), His2−tagged YF1 protein was eluted from the resin with an imidazole gradient from 20 mM to 1 M imidazole over 12 CV. Fractions containing pure YF1 were identified on the basis of analysis by polyacrylamide gel electrophoresis and pooled. After dialysis against twice 2 l buffer C (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 10% [v/v] glycerol), the protein solution was concentrated in a 10,000-molecular weight cutoff spin concentrator (Pall Corporation) to ~40 mg ml⁻¹ and stored at −80°C. Protein concentration was determined with an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies) using an extinction coefficient of 12,500 M⁻¹ cm⁻¹ at 450 nm (Möglich et al., 2009a).

Selenomethionine-substituted (SeMet) YF1 protein was produced as described (Doublié, 1997). Briefly, CmpX13 cells containing pET-41a-YF1

homodimeric and heterodimeric, soluble and transmembrane, single-input and multi-input, and prokaryotic and eukaryotic signal receptors that combine disparate sensor and effector modules in different topologies (N→C versus C→N). We suggest that the structural correspondence entails similar signaling mechanisms.

Helical connectors (Anantharaman et al., 2006), coiled coils, and signal-induced quaternary structural changes (e.g., rotary switching) represent versatile concepts (Möglich et al., 2009b) that enable the combination of various sensor and effector modules and could at least partially account for the enormous diversity of such combinations found in nature (Finn et al., 2006). The YF1 structure confirms that coiled coils obviate the need for direct contact or shape complementarity between sensors and effectors. The design of novel sensor-effector combinations—be it by evolution (Capra and Laub, 2012), be it by rational engineering—thus greatly simplifies to correctly fusing α helices and coiled coils, which link sensor and effector modules. Moreover, since even single mutations within the sensor (e.g., H22P in YF1) suffice to completely alter the signal response of receptors at the physiological level, environmental stimuli can be rapidly rewired to achieve a novel cellular adaptation.
were grown in 500 ml M9 minimal media supplemented with 50 μg ml⁻¹ kanamycin, 50 μM riboflavin, 0.4% (w/v) glucose, 0.1 mM CaCl₂, 2 mM MgSO₄, and 1 mg ml⁻¹ thiamine. Protein expression was induced at OD₅₆₀ of 0.6 by adding 1 mM IPTG, 100 mg ml⁻¹ each of the L-amino acids isoleucine, leucine, lysine, phenylalanine, threonine, and valine, and 60 mg ml⁻¹ selenomethionine. Protein purification was performed as described above.

Crystallographic and Data Collection

Crystals of native YF1 were grown by sitting-drop vapor diffusion in the dark. One microliter of a solution containing 40 mg ml⁻¹ YF1, and 1 mM ATP/MgCl₂ in buffer C was mixed with 1 μl reservoir solution (0.1 M potassium fluoride, 2 M (NH₄)₂SO₄, 2 M (NH₄)₂SO₄, 25% [v/v] glycerol). Crystallization of SeMet YF1 was conducted under the same conditions but yielded thick, coin-shaped single crystals of up to 300 μm diameter.

Monochromatic oscillation X-ray diffraction data were collected at 100 K and a wavelength of 0.9816 Å for both native and SeMet samples on beamline 14.1 at the BESSY II electron storage ring (Berlin-Adlershof) (Mueller et al., 2012), which is operated by the Helmholtz-Zentrum Berlin. Native crystals of YF1 diffracted X-rays to 3.07 Å resolution and were indexed in space group P6₂₂₂ (179) with unit cell dimensions of a = b = 105.14 Å and c = 443.57 Å. SeMet YF1 crystals diffracted X-rays to 2.30 Å resolution and were also indexed in space group P6₂₂₂ (179) with slightly different unit cell dimensions of a = b = 105.16 Å and c = 441.80 Å. Indexing, integration, and scaling were performed with the XDS program (Kabsch, 2010) through the XDSAPP interface (Krug et al., 2012).

Structure Determination and Analysis

We first solved the structure of YF1 on the basis of the native diffraction data by molecular replacement (MR). Search models for the DHp and CA domains were derived from structures of T. maritima HK853 (Marina et al., 2005; Casino et al., 2009; PDB 3DGE and 2C2A, respectively) with the CHAINSAW program (Stein, 2008). Using the PHASER software (McCoy et al., 2005), two monomers of the isolated B. subtilis YtvA LOV domain (PDB 2PR5, aa 26–127) (Möglich and Moffat, 2007), one copy of the DHp search model, and two copies of the CA search model were placed within the unit cell. Initial refinement of the MR solution yielded R-factors of Rwork = 44.32% and Rfree = 48.83%. For calculation of Rwork, 50% of reflections across all resolution shells were randomly assigned and used only for validation. Based on the resulting electron density, the structure was automatically rebuilt with the BUCCANEER (Cowtan, 2012) program from the CCP4 suite (Winn et al., 2011). When diffraction data from SeMet YF1 crystals became available, the MR solution was confirmed by selenium single-wavelength anomalous dispersion (Sheldrick, 2008) and can be found with this article online at http://dx.doi.org/10.1016/j.str.2013.04.024.

Model building was done with COOT (Emsley and Cowtan, 2004); structure refinement was initially done with Refmac5 (Murshudov et al., 1997) and at later stages with PHENIX (Adams et al., 2002). The final model was obtained with the XDSAPP interface and can be found with this article online at http://dx.doi.org/10.1016/j.str.2013.04.024.

Activity Assays

Site-directed mutants of YF1 in the pDusk-myc-DsRed background were transformed into E. coli BL21 CmpX13. Three 5 ml LB cultures each were incubated overnight at 37°C and 225 rpm, either in the dark or under constant blue light (100 μW cm⁻²). DsRed fluorescence and OD₆₀₀ were measured with a Tecan M200 plate reader (Tecan Group) in 96 well µClear plates (Greiner BioOne), as described (Ohlendorf et al., 2012). Fluorescence excitation and emission wavelengths were set at 554 ± 9 nm and 591 ± 20 nm, respectively. Data were normalized to the fluorescence observed for YF1 under dark conditions and represent the averages of three biological replicates ± SD.

Expression of YF1 and its mutants within the pDusk-myc-DsRed background was confirmed by western blotting. The cell pellet from 1 ml of above bacterial culture was lysed by addition of the nonionic detergent B-PER according to the manufacturer’s protocol (Thermo Fisher Scientific). The amount of B-PER was normalized to OD₆₀₀ of the cell cultures to achieve equal concentrations of solubilized protein. After removal of insoluble debris by centrifugation, the supernatant was separated by SDS-PAGE and analyzed by western blotting using anti-c-myc primary and alkaline phosphatase-conjugated anti-rabbit immunoglobulin G secondary antibodies (Sigma-Aldrich).

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, two movies, and one PDB session file and can be found with this article online at http://dx.doi.org/10.1016/j.str.2013.04.024.

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R.P.D. and A.M. conceived the project and designed experiments. R.P.D. carried out molecular biology, protein purification, crystallization, collection of diffraction data, structure determination, and activity assays. M.B. participated in crystallization, collection of diffraction data, and structure determination. T.G. discovered and analyzed several mutant variants of YF1. A.M. supervised the study. R.P.D. and A.M. interpreted data and wrote the manuscript.

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