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Structure
Review

Structure and Signaling Mechanism of Per-ARNT-Sim Domains

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Per-ARNT-Sim (PAS) domains serve as versatile sensor and interaction modules in signal transduction proteins. PAS sensors detect chemical and physical stimuli and regulate the activity of functionally diverse effector domains. In contrast to this chemical, physical, and functional diversity, the structure of the core of PAS domains is broadly conserved and comprises a five-stranded antiparallel β sheet and several α helices. Signals originate within the conserved core and generate structural and dynamic changes predominantly within the β sheet, from which they propagate via amphipathic α-helical and coiled-coil linkers at the N or C termini of the core to the covalently attached effector domain. Effector domains are typically dimeric; their activity appears to be largely regulated by signal-dependent changes in quaternary structure and dynamics. The signaling mechanisms of PAS and other signaling domains share common features, and these commonalities can be exploited to enable structure-based design of artificial photosensors and chemosensors.

Introduction

Per-ARNT-Sim (PAS) domains, first identified by sequence homology in the *Drosophila* proteins *per*iod and *sin*gle-*m*inded, and the vertebrate *a*ryl hydrocarbon *r*eceptor *n*uclear *t*ransporter (ARNT) (Hoffman et al., 1991; Nambu et al., 1991), are widespread components of signal transduction proteins where they serve as universal signal sensors and interaction hubs. PAS domains occur in all kingdoms of life (Finn et al., 2006) and regulate processes as diverse as nitrogen fixation in rhizobia (David et al., 1988), phototropism in plants (Christie et al., 1998), circadian behavior in insects (Nambu et al., 1991), and gating of ion channels in vertebrates (Morais Cabral et al., 1998). In common with other signal transduction systems (Pawson and Nash, 2003), proteins containing PAS domains are modular: PAS sensor (input) domains detect a wide variety of physical and chemical stimuli and regulate, in response, the activity of effector (output) domains such as catalysis or DNA binding.

The Pfam database (version 23.0, July 2008) includes more than 21,000 entries annotated as PAS domains (Finn et al., 2006). Of these, 81%, 13%, and 6% derive from bacterial, eukaryotic, and archaeal proteins, respectively. PAS domains comprise 100–120 amino acids and exhibit low pairwise sequence identity (Finn et al., 2006). Some PAS domains bind cofactors such as metabolites, ions, heme and flavin nucleotides, but for most no cofactor has been identified. It is likely that many PAS domains exert their physiological role in the absence of any cofactor. Frequently, PAS domains mediate interactions between proteins (Huang et al., 1993). PAS domains are covalently linked to and regulate the activities of a wide range of different effector domains (Figure 1). The most frequent class is formed by sensor histidine kinases of prokaryotic two-component signaling systems. Other widely represented effector domains include serine/threonine kinases, guanylate cyclases, phosphodiesterases, transcription factors, ion channels, and chemotaxis proteins. In almost all cases, PAS domains are covalently linked to the N termini of their effector domains, but in a few examples they are linked to the C termini of their effector domains, for example in the Sim protein (Nambu et al., 1991). There is no example in which a PAS domain is inserted into an effector domain. Often, a protein contains several PAS domains or combines PAS domains with other domains commonly involved in signal transduction such as GAF domains (Aravind and Ponting, 1997). Thus, interactions between PAS and other sensor and effector domains are critical to signal transduction.

A detailed treatment of the physiology and of specific classes of PAS proteins is provided in a number of review articles (Crosson et al., 2003; Mascher et al., 2006; Szurmant et al., 2007; Taylor and Zhulin, 1999). Here, we compare the three-dimensional structures of 47 PAS domains, and discuss models for signal transduction applicable to both natural and designed PAS proteins from a structural perspective. How are signals detected by PAS domains and propagated to effector domains? How can a single class of PAS domains regulate the activity of many and structurally diverse effector domains? Are there common, recurring principles that give rise to a general signaling mechanism?

Structure Of PAS Domains

PAS Domains and the PAS Fold

PAS motifs were originally identified as homologous regions of ~50 amino acids in the proteins Per, ARNT, and Sim (Nambu et al., 1991). Additional conserved residues immediately C terminal to that region were identified subsequently as PAC motifs (Ponting and Aravind, 1997) or S_2 boxes (Zhulin et al., 1997). The first three-dimensional structure of a PAS domain, that of photoactive yellow protein (PYP) from *Halorhodospira halophila* (Borgstahl et al., 1995), showed that the PAS and

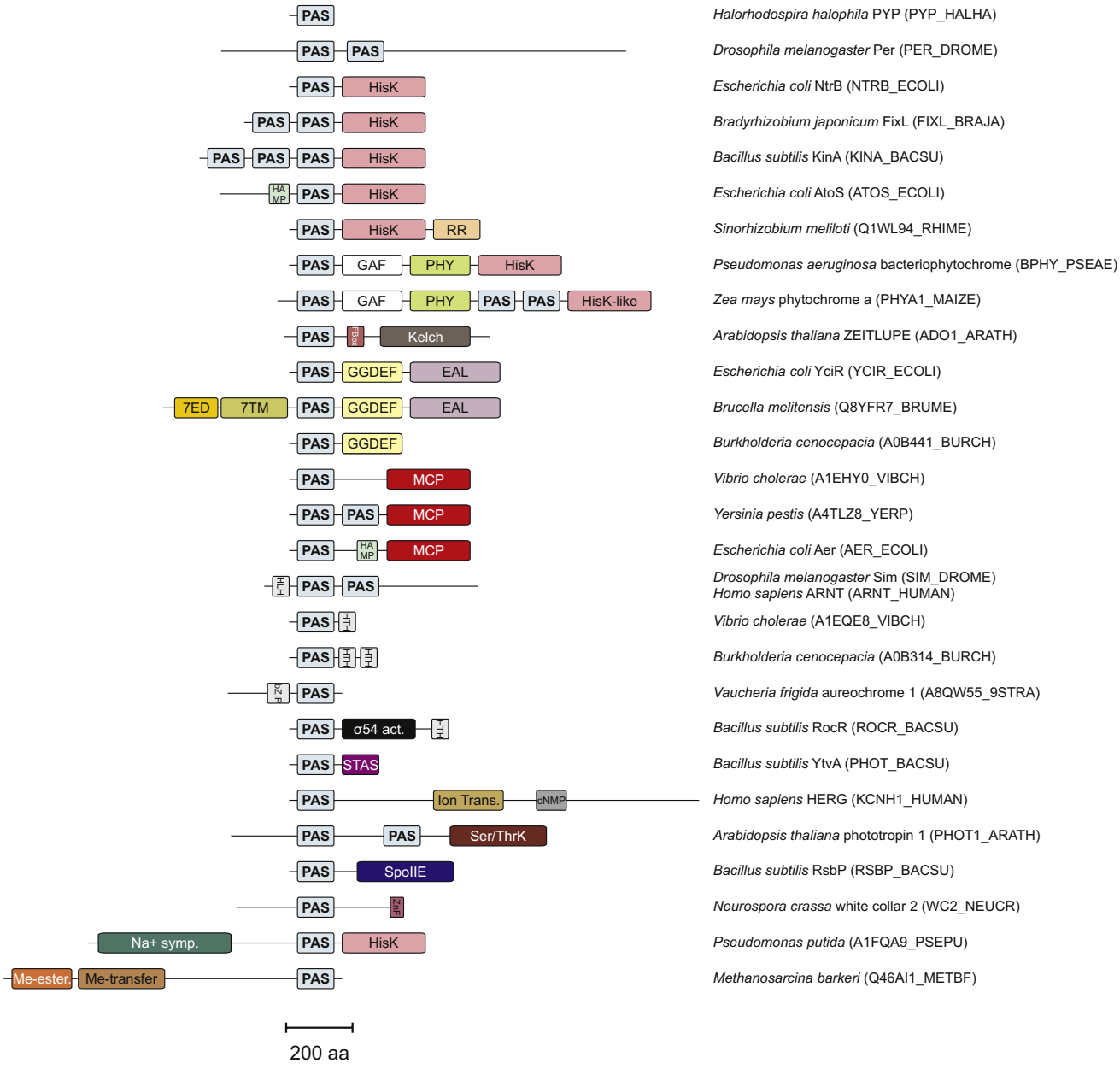


Figure 1. Diversity of PAS Proteins

Architectures of typical proteins containing PAS domains according to Pfam (Finn et al., 2006). Proteins are drawn approximately to scale; the scale bar indicates 200 amino acids. Characteristic representatives are listed with their UniProt identifiers (UniProt Consortium, 2008). Domain abbreviations are supplied in Table S2.

PAC motifs adopt a single globular fold of ~100 residues, now known as the PAS domain (Hefti et al., 2004).

Novel PAS domains are routinely identified and annotated by sequence homology to a seed of known PAS domains (Finn et al., 2006). Distant relatives can be detected using sensitive profile-method searches (Taylor and Zhulin, 1999) as implemented in the popular PSI-BLAST (Altschul et al., 1997) or HMMER (Eddy, 1998) programs. Identification is complicated by the relatively low level of sequence homology among PAS domains; the pairwise sequence identity is below 20% on average (Finn et al., 2006). Consequently, some PAS domains will not be recognized as such (false negatives), and other domains will be misannotated as PAS domains (false positives).

The 47 PAS domains whose structures have been deposited in the Protein Data Bank (PDB) through April 2009 show essentially the same overall fold as PYP, the PAS fold (Table 1). As illustrated in Figure 2A for the PAS A domain of *Azotobacter vinelandii* NifL (Key et al., 2007), the canonical PAS fold comprises a central antiparallel β sheet with five strands Aβ, Bβ, Gβ, Hβ, and Iβ, and several α helices, denoted Cα, Dα, Eα, and Fα, flanking the sheet. The strands of the β sheet are in the topological order B-A-I-H-G, that is, 2-1-5-4-3 (Figure 2B). We refer to the region

Table 1. PAS Domain Structures

Protein	Organism	PDB[a]	Cofactor[b]	Reference[c,d]
PYP	*Rhodospirillum centenum*	1MZU	*p*-Coumaric acid	(Rajagopal and Moffat, 2003)
PYP	*Halorhodospira halophila*	1NWZ	*p*-Coumaric acid	(Borgstahl et al., 1995; Getzoff et al., 2003)
Neochrome PAS B	*Adiantum capillus-veneris*	1G28	FMN	(Crosson and Moffat, 2001; Crosson and Moffat, 2002)
Phot1 PAS A	*Chlamydomonas reinhardtii*	1N9L	FMN	(Fedorov et al., 2003)
Phot1 PAS B	*Avena sativa*	2V0U	FMN	(Halavaty and Moffat, 2007; Harper et al., 2003)
Phot1 PAS A	*Arabidopsis thaliana*	2Z6C	FMN	(Nakasako et al., 2008)
Phot2 PAS A	*A. thaliana*	2Z6D	FMN	(Nakasako et al., 2008)
YtvA	*Bacillus subtilis*	2PR5	FMN	(Möglich and Moffat, 2007)
Vivid	*Neurospora crassa*	2PD7	FAD	(Zoltowski et al., 2007)
NifL PAS A	*Azotobacter vinelandii*	2GJ3	FAD	(Key et al., 2007)
MmoS PAS A, B	*Methylococcus capsulatus*	3EWK	FAD	(Ukaegbu and Rosenzweig, 2009)
FixL PAS B	*Bradyrhizobium japonicum*	1XJ3	Heme	(Gong et al., 1998; Key and Moffat, 2005)
FixL	*Sinorhizobium meliloti*	1D06	Heme	(Miyatake et al., 2000)
DOS PAS A	*Escherichia coli*	1V9Z	Heme	(Kurokawa et al., 2004; Park et al., 2004)
GSU0935	*Geobacter sulfurreducens*	3B42	Heme	(Pokkuluri et al., 2008)
GSU0582	*G. sulfurreducens*	3B47	Heme	(Pokkuluri et al., 2008)
DctB PAS A, B	*S. meliloti*	3E4O	C_3, C_4 sugars	(Zhou et al., 2008)
DcuS PAS A	*E. coli*	3BY8	C_4 sugars	(Cheung and Hendrickson, 2008; Pappalardo et al., 2003)
CitA PAS A	*Klebsiella pneumoniae*	2J80	Citrate	(Reinelt et al., 2003; Sevvana et al., 2008)
Q87T87	*Vibrio parahaemolyticus*	2QHK	Glycerol[e]	—
Q5V5P7 PAS C	*Haloarcula marismortui*	3BWL	1H-indole-3-carbaldehyde[e]	—
RHA05790 PAS B	*Rhodococcus jostii*	3FG8	3-Phosphonooxy-butanoic acid[e]	—
PhoQ	*E. coli*	3BQ8	$Metal^{2+}$	(Cheung et al., 2008)
PhoQ	*Salmonella typhimurium*	1YAX	$Metal^{2+}$	(Cho et al., 2006)
HIF2α PAS B	*Homo sapiens*	1P97, 3F1O	N-[2-nitro-4-(trifluoromethyl) phenyl]morpholin-4-amine[e]	(Erbel et al., 2003; Scheuermann et al., 2009)
ARNT PAS B	*H. sapiens*	1X0O	—	(Card et al., 2005; Scheuermann et al., 2009)
PAS kinase PAS A	*H. sapiens*	1LL8	—	(Amezcua et al., 2002)
NCoA-1/SRC-1 PAS B	*H. sapiens*	1OJ5	—	(Razeto et al., 2004)
Per PAS A, B	*Drosophila melanogaster*	1WA9	—	(Yildiz et al., 2005)
HERG	*H. sapiens*	1BYW	—	(Morais Cabral et al., 1998)
LuxQ PAS A, B	*Vibrio harveyi*	2HJE	—	(Neiditch et al., 2006)
BphP	*Deinococcus radiodurans*	2O9C	—	(Wagner et al., 2005; Wagner et al., 2007)
BphP3	*Rhodopseudomonas palustris*	2OOL	—	(Yang et al., 2007)
BphP	*Pseudomonas aeruginosa*	3C2W	—	(Yang et al., 2008)
CphI	*Synechocystis sp.*	2VEA	—	(Essen et al., 2008)
H-NOXA	*Nostoc punctiforme*	2P04	—	(Ma et al., 2008)
KinA PAS A	*B. subtilis*	2VLG	—	(Lee et al., 2008)
TyrR	*E. coli*	2JHE	—	(Verger et al., 2007)
NR(II)	*V. parahaemolyticus*	3B33	—	—
Q87SR8	*V. parahaemolyticus*	2P7J	—	—
PhoR PAS A	*B. subtilis*	3CWF	—	—
Q5V4P0	*H. marismortui*	3FC7	—	—
Q74DN1 PAS A	*G. sulfurreducens*	2R78	—	—

[a] Where several PDB coordinate files are available for a given protein, the structure with the highest resolution is analyzed.
[b] Only cofactors bound directly by the PAS domain are listed. A dash indicates that no cofactor has been identified.
[c] Explicit citations are given in Supplemental Data.
[d] A dash indicates that coordinates have been deposited in the PDB but no publication is available.
[e] As observed in the crystal structure; uncertain whether physiologically relevant ligand.

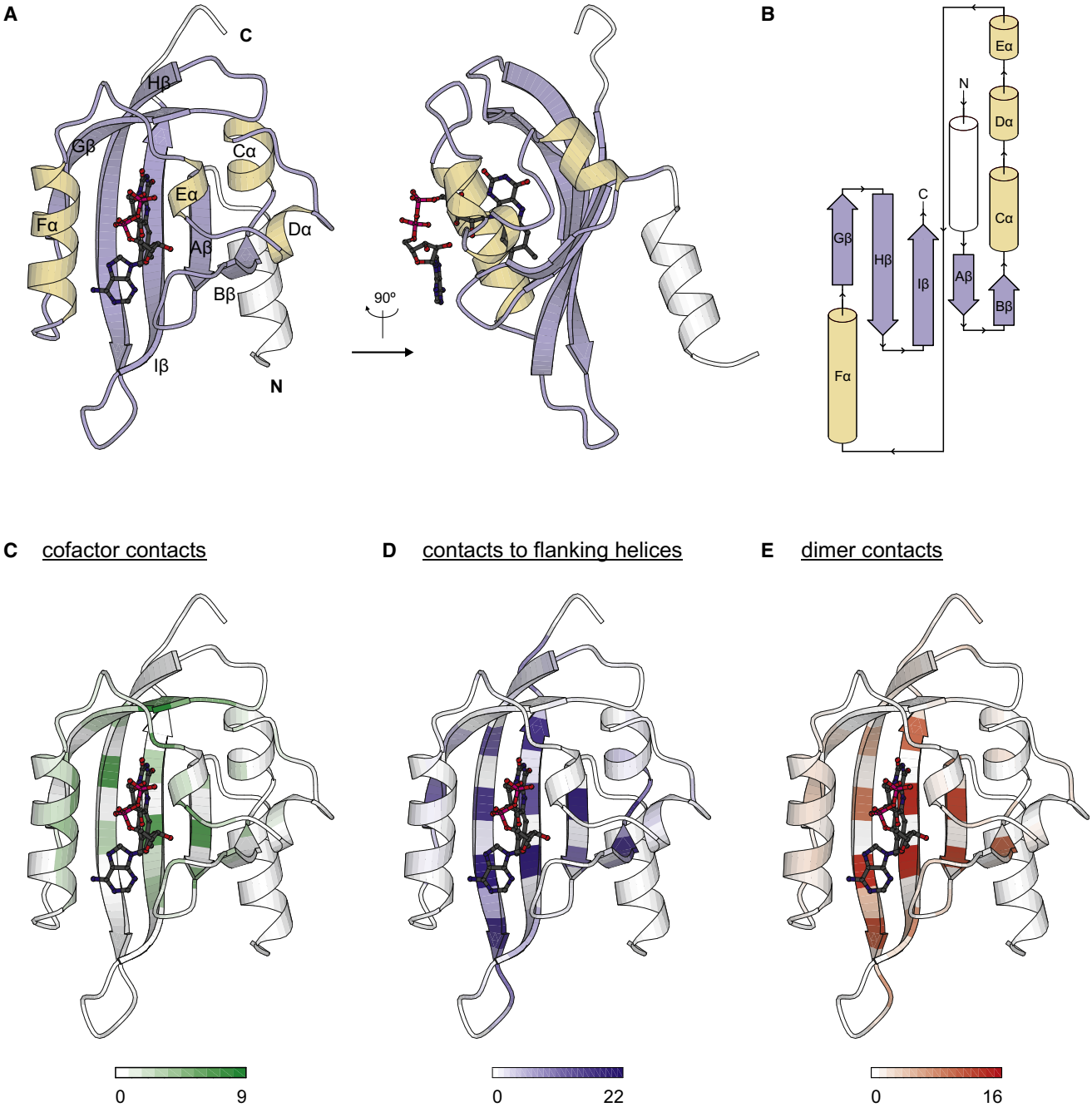


Figure 2. The PAS Domain Fold

(A) The three-dimensional structure of the PAS A domain of *Azotobacter vinelandii* NifL (2GJ3) shows the canonical PAS fold with secondary structure elements Aβ to Iβ. A flavin adenine dinucleotide cofactor is bound in a cleft formed by the β sheet and helices Eα and Fα. An N-terminal flanking α helix is shown in white.

(B) Topology diagram of 2GJ3. β strands are arranged in the order 2-1-5-4-3.

(C) Residues involved in cofactor binding in 11 different PAS domains mapped onto the structure from (A). Color indicates number of structures in which a given residue forms a ligand contact.

(D) Residues forming intramolecular or intermolecular contacts to N- or C-terminal flanking α helices. A total of 34 PAS structures were analyzed, and the color code indicates the number of times a certain residue makes a contact. Closely similar results are obtained when only intramolecular contacts to flanking helices are considered.

(E) Residues involved in dimerization of 26 different PAS domains mapped onto the structure from (A). Color code indicates the number of structures in which a given residue contributes to forming the dimer interface.

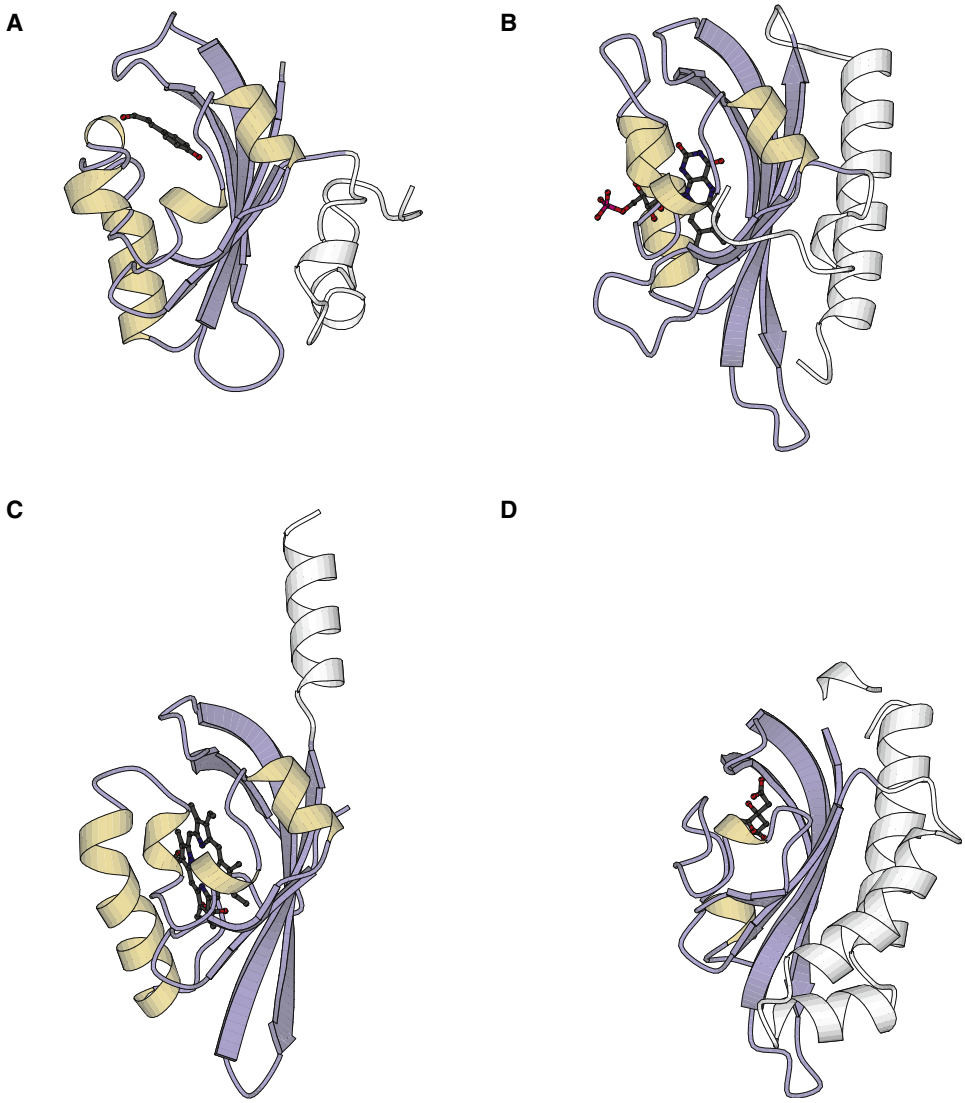


Figure 3. Diversity of PAS Domain Structures
Three-dimensional structures of the PAS domains of (A) *H. halophila* PYP (1MWZ), (B) *A. sativa* phototropin 1 (2V0U), (C) *B. japonicum* FixL (1XJ3), and (D) *K. pneumoniae* CitA (2J80). Secondary structure elements are colored as in Figure 2A. Other PAS domain structures are shown in Figure S3.

comprising α-helical and β strand secondary structure elements from Aβ through Iβ as the PAS core, and to N- or C-terminal extensions to the core as flanking regions. Multiple PAS domains within one protein are labeled alphabetically from the N to the C terminus, for example PAS A and PAS B. Individual PAS domain structures are referred to by their PDB identifier (Table 1).

Diversity of PAS Domains

These 47 PAS domain structures derive from proteins with quite different effector domains, and respond to diverse chemical signals such as the concentration of metabolites or physical stimuli such as light. Structural superposition reveals that the central β sheet is the most conserved region (Figure 3). A dendrogram based on structural relatedness is given in Figure S1 (available online). On average, the root-mean-square deviation (rmsd) for the β sheet backbone atoms between two PAS domain structures is (1.9 ± 0.6) Å (Figure S2). In contrast to the β sheet, the orientation, length, and number of intervening α helices vary considerably, as for example in the PAS A domain of *Vibrio harveyi* LuxQ (2HJE), which lacks helices Dα and Eα (Neiditch et al., 2006). The defining structural feature of the PAS core is therefore the five-stranded antiparallel β sheet in the topological order 2-1-5-4-3.

From the pairwise structure superpositions, we generated a multiple sequence alignment of the PAS domains (Figure 4). Although the length of the β strands is well-conserved among PAS domains, loops and the region between strands Bβ and Gβ, comprising helices Cα, Dα, Eα, and Fα of the core, vary markedly in length and structure.

Relationship to Other Signaling Domains

The term *l*ight-*o*xygen-*v*oltage (LOV) domain was introduced to refer to two tandem PAS-like domains in plant phototropins (Crosson et al., 2003; Huala et al., 1997). Because LOV domains

are clearly classed as PAS domains by sequence and structure, the term LOV domain is currently restricted to a particular subset of PAS photosensors that bind flavin nucleotides and display phototropin-like photochemistry.

Recently, certain PAS-like domains were said to adopt a distinct PDC fold (*P*hoQ-*D*cuS-*C*itA) (Cheung et al., 2008). However, the corresponding structures superpose well with authentic PAS structures over the defining feature of the PAS fold, the central β sheet (Figures 3 and S1). The rmsd values for the β sheet atoms between structures from the PDC subset and other PAS structures are (2.1 ± 0.5) Å, and not significantly higher than the values obtained for all PAS domains (Figure S2). Structural differences are largely confined to α-helical elements, and such differences are found between other apparently authentic members of the PAS family. We conclude that to the extent there is a distinct PDC fold, it constitutes a subset of the PAS fold.

Cache domains were first identified as mostly extracellular domains of diverse prokaryotic and animal signaling proteins. Based on sequence similarity, it was suggested that Cache domains might assume a fold similar to PAS domains (Anantharaman and Aravind, 2000). This suggestion was recently confirmed by the structure of a Cache domain from a bacterial chemotaxis protein (2QHK). Sequence analysis reveals additional conserved regions C terminal to the original Cache motif (Anantharaman and Aravind, 2000), which are also closely similar to those in PAS domains, specifically at the end of strand Iβ (Möglich et al., 2009). Based on structure and sequence, it appears that Cache domains also constitute a subset of the PAS fold.

Despite limited sequence homology (Finn et al., 2006), PAS domains share remarkably similar three-dimensional folds with GAF domains (Ho et al., 2000). The core of GAF domains usually comprises a six-stranded antiparallel β sheet with strand topology 3-2-1-6-5-4, corresponding to that of the PAS β sheet with an additional strand inserted between strands 2 and 3. Notably, several GAF core domains have five-stranded antiparallel β sheets, for example structures 3CIT and 2VZW (Podust et al., 2008). An α helix lies between strands 3 and 4, and additional α helices often lie between strands 4 and 5 (Ho et al., 2000). The GAF domain annotation also encompasses α-helical segments N- and C-terminal flanking the core, yet the GAF core itself is of a size and fold closely similar to the PAS core. PAS and GAF domains are linked to similar classes of effector domains (Galperin, 2004), further underlining their relatedness and implying that they share a common evolutionary origin (Anantharaman et al., 2001; Ho et al., 2000). We distinguish here between PAS and GAF domains following the sequence-based domain annotations in the Pfam database (Finn et al., 2006), but caution that in some cases these might be in error. It is not yet established whether PAS and GAF domains employ the same signaling mechanisms, or merit the retention of separate domain classifications.

Cofactor Binding

Several PAS domains bind cofactors either covalently or noncovalently (Table 1). In some PAS sensors these constitute the signal to which the protein responds, for example the citrate sensor CitA (Sevvana et al., 2008). For other PAS domains the cofactor directly mediates signal detection, for example where flavin cofactors absorb blue light (Crosson et al., 2003) or a heme cofactor binds oxygen (Key and Moffat, 2005). Some PAS domains also bind a range of chemically distinct and nonnatural ligands with high affinity (Scheuermann et al., 2009). Promiscuous binding of distinct ligands might be integral to the physiological function of certain PAS domain, such as ARNT (Hoffman et al., 1991).

We analyzed a representative subset of PAS domains that bind flavin nucleotide, *p*-coumaric acid, heme, and different carbon metabolites, respectively (Table S1). Despite the wide chemical diversity of these ligands, most are bound in a spatially conserved cleft formed by the inner surface of the β sheet and helices Eα and Fα (Figures 2C and 3). Interestingly, the protein region around helices Eα and Fα is also among the structurally least conserved parts of the entire PAS core. Part of the structural diversity among PAS domains must thus arise from accommodation of diverse cofactors in different proteins. A similar cofactor binding site is found in GAF domains, which further substantiates the close relatedness of PAS and GAF domains (Ho et al., 2000; Wagner et al., 2005).

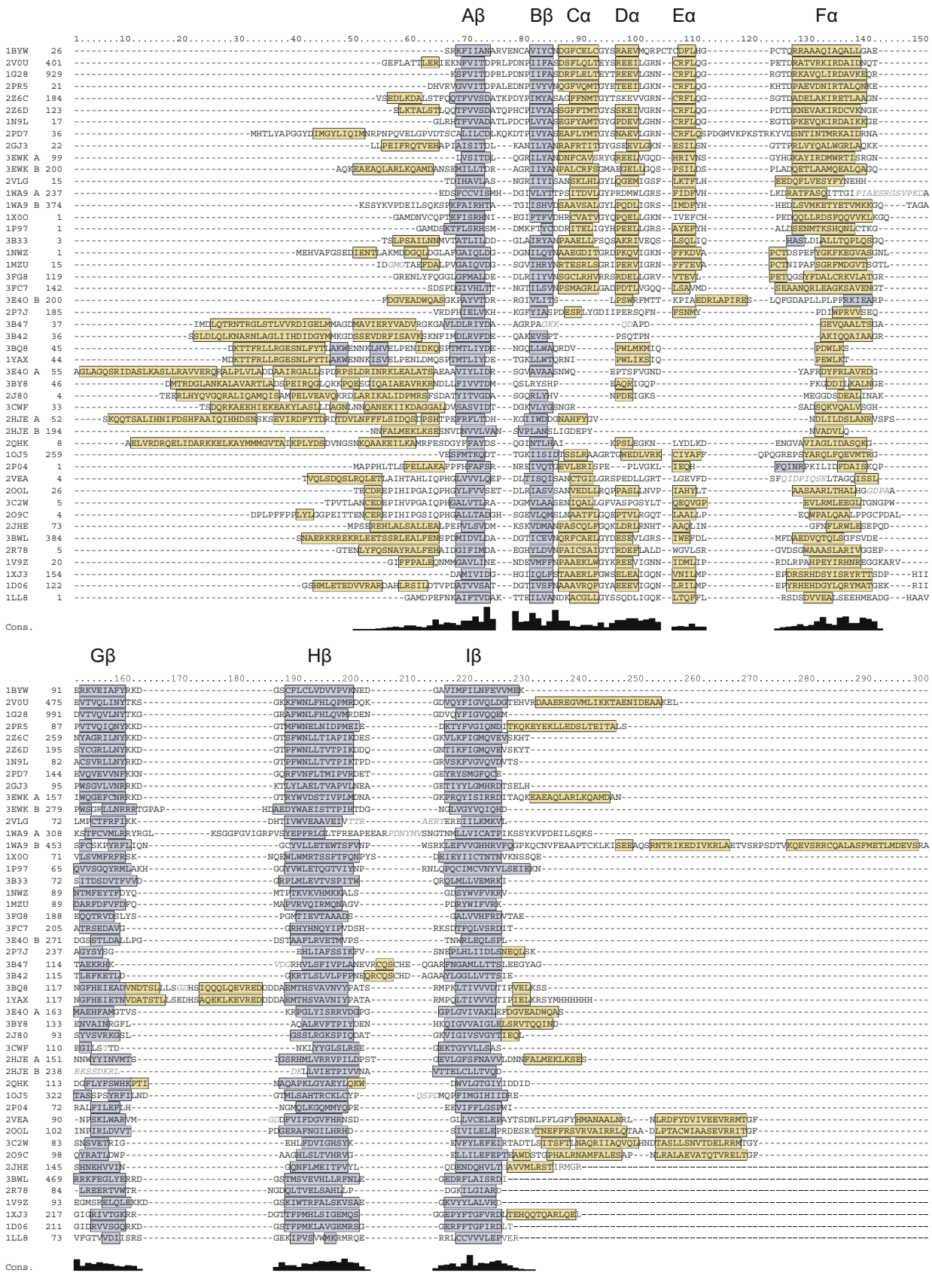
In the PAS B domain of NCoA-1/SRC-1, a peptide ligand is bound on the surface of the core domain between strand Bβ and helices Cα and Dα (Razeto et al., 2004). Interestingly, in the crystal structures of *Bacillus subtilis* KinA (Lee et al., 2008b) and *Drosophila melanogaster* Per (Yildiz et al., 2005) protein loops of one PAS molecule are inserted between helices Eα and Fα of another PAS molecule. Several PAS domains, such as those of *V. harveyi* LuxQ (Neiditch et al., 2006) and bacteriophytochromes (Wagner et al., 2005), do not directly bind cofactors but associate with other sensor domains that do so.

Flanking Regions

Most PAS domains form part of larger proteins and are covalently linked to effector and other domains (Figure 1). In most such proteins, especially those of prokaryotic origin, the linkers between the PAS core and other domains are short, usually 20–40 amino acids (Finn et al., 2006). When we analyzed such linkers within a large group of PAS-histidine kinases, we observed only low levels of sequence homology (Möglich et al., 2009). However, linker lengths fell into distinct classes differing by multiples of seven residues (i. e., 7, 14, or 21). Further, hydrophobicity showed a remarkable heptad residue periodicity, indicating that these linkers form amphipathic α helices and coiled coils (see Figure 5 of Möglich et al., 2009).

We extended this analysis to PAS domains that are linked N terminally to guanylate cyclase (GGDEF) domains (Pei and Grishin, 2001) (Figure 1). The linkers between PAS and GGDEF domains also display the characteristic heptad pattern of hydrophobicity characteristic of α-helical coiled coils (McLachlan and Stewart, 1975) (Figure 5). Strikingly, in about 85% of the 2074 proteins analyzed the linkers between the PAS and GGDEF domains have the identical length, suggesting that structural requirements for the linker are more stringent than those for PAS-histidine kinases. The remaining 15% of PAS-GGDEF proteins mostly have linker sequences that are extended by multiples of 7 residues.

A heptad pattern of hydrophobic residues is also observed for the linkers between tandem PAS domains (R.A.A., A.M., and K.M., manuscript in preparation). In contrast to the PAS-histidine kinase and PAS-GGDEF linkers, these linkers are shorter or


Aβ
Bβ
Cα
Dα
Eα
Fα
Gβ
Hβ
Iβ
Cons.

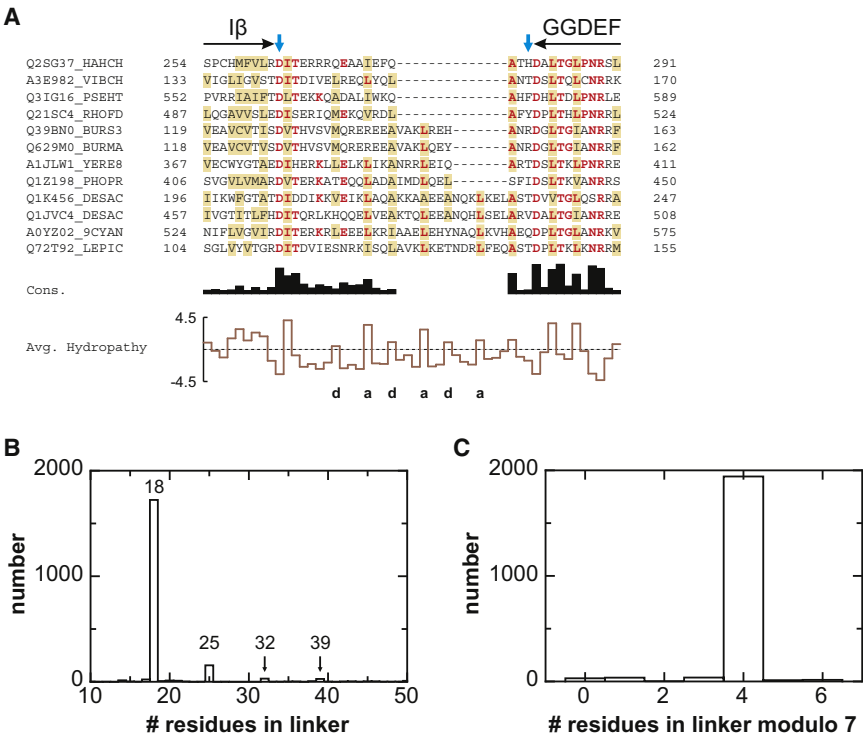


Figure 5. Helical Domain Linkers in PAS-GGDEF Proteins

(A) Multiple sequence alignment of the linker region between PAS and GGDEF domains. A total of 12 out of 2074 sequences are shown and labeled with their UniProt identifiers. Residues conserved in more than 50% of all 2074 sequences are highlighted in bold red, positions with more than 50% hydrophobic residues by brown shading. Plots below the alignment indicate average sequence conservation and hydropathy. Hydrophobic positions are labeled a and d according to coiled-coil nomenclature (McLachlan and Stewart, 1975).

(B) Length distribution of linkers between PAS and GGDEF domains. Lengths were determined according to the alignment as the number of residues between the indicated positions (blue arrows).

(C) Modulo 7 of the distribution shown in (B). Ninety-four percent of all sequences fall into the length class $7n + 4$.

longer by multiples of three or four amino acids, which is consistent with an α-helical linker but not necessarily with a coiled coil.

Taken together, these data imply that short linkers between PAS sensor and effector domains are structured and adopt an α-helical structure; many form coiled coils.

Do PAS domain structures provide direct evidence for helical or coiled-coil linkers? Initially, structural studies largely focused on PAS core domains and employed short protein constructs lacking any flanking regions. Several more recent structures of longer constructs show well-defined extensions to their cores (Figure 3). In contrast to the common PAS fold shared by their core, individual PAS domains differ in the structure of those flanking regions. Strikingly, the vast majority of flanking regions adopt an α-helical conformation (Figure 4). Such flanking helices occur both at the N terminus of the PAS core, such as in the *A. vinelandii* NifL PAS A domain (2GJ3) (Key et al., 2007), and at the C terminus, such as the prominent Jα helix in the *Avena sativa* phototropin 1 PAS B (LOV 2) domain (2V0U) (Halavaty and Moffat, 2007). The sequences of these flanking helices frequently are amphipathic, in agreement with the above analysis. Flanking helices either extend from the PAS core or pack on the outer surface of the β sheet, where they are stabilized mainly through hydrophobic interactions (Figure 2D). Thus, residues in the β sheet alternate between those that make cofactor contacts via its inner surface (Figure 2C) and those that make contacts with flanking α helices via its outer surface (Figure 2D). Residues located in extended or helical regions of the PAS core usually do not contribute to contact formation with flanking helices.

α Helices are also present at the N termini of many effector domains regulated by PAS domains, such as in GGDEF domains (Chan et al., 2004), the helical DHp subdomain of histidine kinases (Marina et al., 2005), and methyl-accepting chemotaxis proteins (Alexander and Zhulin, 2007). Direct fusion of these helices to the C-terminal linker helix could result in a single, long signaling helix (Anantharaman et al., 2006), a coiled coil, or a helical bundle (Möglich et al., 2009).

PAS Domain Oligomers

PAS domains promote formation of dimers and higher-order oligomers of many proteins (Huang et al., 1993; Pongratz et al., 1998; Taylor and Zhulin, 1999). Although prokaryotic PAS proteins and domains form homo-oligomers, eukaryotic PAS domains also form hetero-oligomers, such as the *Neurospora crassa* white-collar proteins (Froehlich et al., 2002). The presence of PAS domains can dictate the association specificity of their effector domains. For example, the basic helix-loop-helix domain of ARNT homo-dimerizes as an isolated domain but forms a heterodimer with the aryl hydrocarbon (dioxin) receptor when covalently linked to its PAS domain (Pongratz et al., 1998).

PAS monomers can pack together in quite different ways to form dimers (Ayers and Moffat, 2008). Several PAS domains form parallel dimers, so that the N termini of each monomer are proximal (Key et al., 2007; Kurokawa et al., 2004; Ma et al., 2008). Others form antiparallel dimers (Fedorov et al., 2003; Nakasako et al., 2008), yet others adopt intermediate orientations (Ayers and Moffat, 2008). Some PAS domains, such as PAS B from *Bradyrhizobium japonicum* FixL (Ayers and Moffat, 2008) and PAS A from *B. subtilis* KinA (Lee et al., 2008b), adopt several different quaternary structures under the same solution conditions. This suggests that the interface between PAS monomers is plastic. Several relative monomer orientations differ only

Figure 4. Structure-Based Multiple Sequence Alignment of PAS Domains

Sequences of PAS domains were aligned with respect to their three-dimensional structures and are indicated by their PDB identifiers (Table 1). α Helices and β sheets are marked by brown and blue shading. Secondary structure elements within the PAS core are labeled. Residues shown in gray italic were not resolved in the structures.

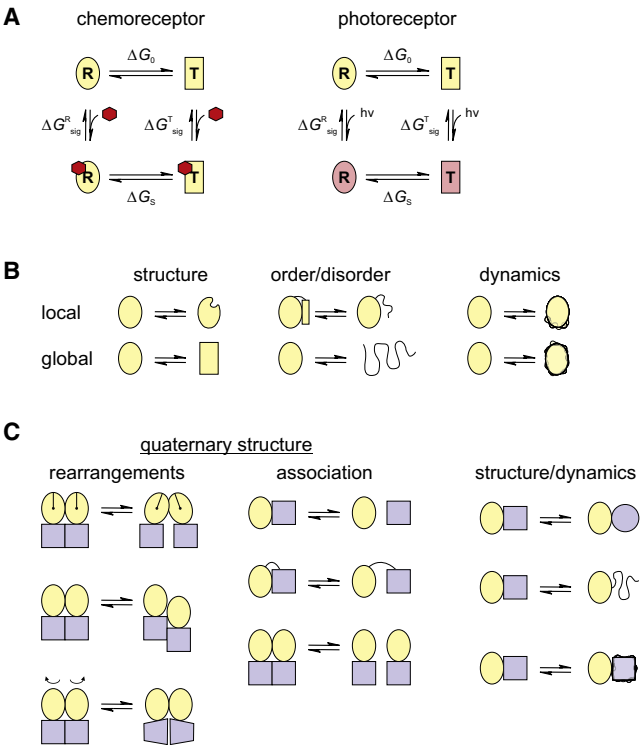


Figure 6. Signaling by PAS Domains

(A) Thermodynamic cycle for signal transduction by PAS domains. A protein is in equilibrium between states *T* and *R*, which differ in biological activity. Presence of a signal alters the free energies of states *T* and *R*, and thus shifts the equilibrium between them. Depending on the sensor domain, signal can correspond to binding of a ligand, absorption of a photon, or changes in redox potential or electrical field.

(B) Models for signal transduction within PAS domains. Signal might induce local or global changes in structure and dynamics in the PAS domain.

(C) Models for signal propagation to effector domains (blue squares). The activity of oligomeric effector proteins is frequently regulated by quaternary structural changes. In addition, regulation might depend on signal-induced structural and dynamic changes within the effector domain.

slightly in free energy or, to put it another way, a small change in free energy of stabilization of the dimer interface could produce a large quaternary structural change.

Despite displaying a wide range of possible monomer orientations, residues comprising the dimer interface are largely conserved in structural location and overlap with those forming contacts to flanking helices (compare Figures 2D and 2E). Most PAS domains form homodimers and heterodimers through a patch of hydrophobic residues on the outer surface of their β sheet. Electrostatic interactions between charged residues in opposing β sheets can influence the quaternary structure (Card et al., 2005). In many prokaryotic PAS dimer structures, flanking helices N terminal and C terminal to the PAS core also contribute to the interface (e.g., structures 1D06, 1V9Z, 2GJ3, 2J80, 2P04, 3B42, 3B47, 3BQ8, 3BY8, 3E4O). Flanking helices frequently associate with each other into α-helical bundles and pack on the β sheets to form intramolecular and intermolecular contacts, as in *Sinorhizobium meliloti* DctB (3E4O) (Zhou et al., 2008).

Oligomerization is a necessary component of function for many PAS sensor proteins, as in histidine kinases that dimerize to achieve phosphorylation in *trans* (Yang and Inouye, 1991). The proper orientation of monomers might also be necessary for function and regulation. Mutation of residues either within the PAS β sheet or in the N- or C-terminal flanking regions can modulate function while maintaining the oligomeric state (Miyatake et al., 2000).

We propose that a general role for PAS domains is to modulate the affinity of proteins for an identical protein (homo-oligomerization) or another protein (hetero-oligomerization). For the subset of PAS domains that serve as sensors, modulation of affinity becomes signal dependent. Oligomerization, structural promiscuity, and the ability of PAS domains to homodimerize and heterodimerize thus provide specificity and accommodate complex spatial and temporal regulation in cellular signaling networks. Signal-induced changes in quaternary structure might play a key role in signal transduction.

Signaling Mechanism Of PAS Domains

Thermodynamics of Signaling

Signaling is inherently thermodynamic in nature. The presence of a signal alters either the intramolecular affinity of one part of a protein or domain for another through a change in tertiary structure and dynamics; or the intermolecular affinity of one protein or domain for another through a change in quaternary structure and dynamics; or through both, of course.

As a model, consider the first case for a simple allosteric protein. In the absence of signal, a single protein be in equilibrium between two pre-existing states, say $[T]_0$ and $[R]_0$, with equilibrium constant L_0 (Figure 6A) (Monod et al., 1965). For example, structural heterogeneity in the ground state exists for the active-site cysteine of photosensory PAS domains (Fedorov et al., 2003), for the Jα helix of *A. sativa* phototropin 1 PAS B (Yao et al., 2008), and in α helices at the dimer interface of a bacteriophytochrome (Yang et al., 2008). The states *T* and *R* are assumed to differ in their biological activity and the free energy difference between them is

$$\Delta G_0 = -RT \ln L_0,$$

where $L_0 = [T]_0/[R]_0$. The presence of signal S shifts the equilibrium constant between the states from L_0 to L_S. Then,

$$\Delta G_S = -RT \ln L_S,$$

where $L_S = [T]_S/[R]_S$. The free energy derived from the signal, $\Delta\Delta G_{sig}$, is therefore

$$\Delta\Delta G_{sig} = \Delta G_S - \Delta G_0 = \Delta G^T_{sig} - \Delta G^R_{sig},$$

where ΔG^T_{sig} and ΔG^R_{sig} are the signal-induced free energy changes within the *T* and *R* states, respectively (Figure 6A). $\Delta\Delta G_{sig}$ is available to modulate the structure, dynamics, and activity of the protein. If for example the *T* state is less active than the *R* state and $L_S < L_0$, then the signal produces an increase in biological activity; if *T* is less active than *R* and $L_S > L_0$, then signal decreases biological activity.

In a chemoreceptor, the *T* and *R* states differ in their affinity for a chemical signal such as a small molecule; in a redox sensor, in

their affinity for an electron; in a photoreceptor, in their response to absorption of a photon; and in a voltage sensor, in their response to an electric field. A characteristic of photoreceptors is that $\Delta\Delta G_{sig}$ can in principle be large, up to the energy of the photon itself (e. g. ~64 kcal mol^{-1} for a photon of 450 nm wavelength). In practice, most of the energy of the photon is dissipated as vibrational energy or heat, and hence only a fraction of the photon energy is available for signaling. For example, when the PAS B (LOV 2) domain of *A. sativa* phototropin 1 absorbs a photon in the blue, $\Delta\Delta G_{sig}$ is only ~4 kcal mol^{-1} (Yao et al., 2008). For chemoreceptors $\Delta\Delta G_{sig}$ is determined by the difference in ligand affinity between the *T* and *R* states (e.g., 4.1 kcal mol^{-1} for an affinity difference of 10^3, say between 1 nM and 1 μM). An upper limit to $\Delta\Delta G_{sig}$ for chemoreceptors is given by the free energy for binding of small molecule ligands to proteins, which usually ranges from 3 to 15 kcal mol^{-1} (Liu et al., 2007).

This thermodynamic view also emphasizes that the magnitude of a signal is most usefully measured in kcal mol^{-1}, not in Å. Indeed, structural changes that are both localized in spatial extent and small in magnitude accompany photon absorption by several photosensory PAS domains (Crosson and Moffat, 2002; Fedorov et al., 2003; Halavaty and Moffat, 2007; Möglich and Moffat, 2007; Zoltowski et al., 2007). Conversely, large structural changes in which groups of atoms move by many Å are not necessarily accompanied by large changes in free energy, as we have just seen in the case of certain quaternary structural changes. In an extreme case the effect of a signal might be purely entropic in nature and produce an alteration in dynamics with no alteration in the mean atomic positions. However, we caution that within a crystal lattice, packing forces might prevent signal-induced conformational changes from manifesting their full extent (Fedorov et al., 2003; Halavaty and Moffat, 2007; Möglich and Moffat, 2007; Zoltowski et al., 2007).

This simple model can be extended to the induced-fit case (Koshland et al., 1966), or to oligomeric proteins in which quaternary structural changes, such as domain rearrangements or association reactions, accompany the presence of a signal.

Signal Detection by PAS Domains

Almost all PAS domains bind their cofactors within their core (Figure 2), thereby ensuring precise coordination, and specificity and longevity of the signaling complex. As illustrated schematically in Figure 6B, signals can be propagated within PAS domains as a combination of conformational and dynamic changes. Such changes can be either local or global.

Structures of several PAS domains in the presence and absence of signal reveal that signal-induced conformational changes are small and concentrated in the cofactor binding site and its vicinity. For example, light absorption by certain photosensory PAS domains results in chromophore isomerization, as in PYP (Genick et al., 1997), or formation of a covalent adduct between the chromophore and the protein, as in phototropin PAS (LOV) domains (Crosson and Moffat, 2002; Fedorov et al., 2003; Salomon et al., 2001). Binding of diatomic ligands to the heme cofactor of certain PAS domains induces conformational changes in residues forming the cofactor binding pocket (Key and Moffat, 2005). The PAS domain of *N. crassa* Vivid was reported to respond to light and redox potential, thus integrating two stimuli (Zoltowski et al., 2007). Interestingly, in most PAS domains that have been studied in detail, signals apparently propagate to and through the central β sheet and ultimately to spatially remote effector domains, where they modulate biological activity. Time-resolved crystallography revealed structural changes in the β sheet of PYP following blue light absorption (Rajagopal et al., 2005). On the timescale of nanoseconds to seconds, conformational changes propagate to a conserved cap on the Cα helix, and from the cap to an N-terminal pair of short helices. All are packed on the outer surface of the β sheet, which also undergoes smaller conformational changes. Signal-induced structural and dynamic changes in the region of the β sheet have also been reported for the PAS domains of *N. crassa* Vivid (Zoltowski et al., 2007), plant phototropins (Halavaty and Moffat, 2007; Harper et al., 2003; Yao et al., 2008), *B. japonicum* FixL (Key and Moffat, 2005), *B. subtilis* YtvA (Möglich and Moffat, 2007), and *Klebsiella pneumoniae* CitA (Sevvana et al., 2008). The β sheet of PAS domains might be malleable, as seen for the ARNT PAS B domain where a single mutation populates an alternative conformation with a 3-residue slip in strand Iβ (Evans et al., 2009). The central role of the β sheet in signal propagation concurs with its pronounced importance in cofactor binding and dimerization of PAS domains (Figure 2).

The initial signal upon photon absorption or ligand binding might have a variety of effects, any one of which constitutes a signal. The affinity of the outer surface of the β sheet for whatever it interacts with might be lowered. If, as is commonly the case, the outer surface interacts with N- or C-terminal helical flanking regions (Figure 2D), these might dissociate from that surface, unfold, and then bind to other locations on the PAS domain or elsewhere. Examples include the N-terminal helical caps of PYP (Rajagopal et al., 2005) and Vivid (Zoltowski et al., 2007), and the Jα helix of phototropin-like PAS domains (Harper et al., 2003), which are packed on the outer surface of the β sheet in the dark but become disordered in the light. Likewise, citrate binding to CitA from *K. pneumoniae* induces partial unfolding of an N-terminal helix (Sevvana et al., 2008). The newly exposed outer surface of the β sheet might have a higher affinity for another peptide. If the β sheet forms part of a dimer interface (Figure 2E), the stability of that interface is altered, potentially leading to changes in quaternary structure and dynamics.

At the C terminus of strand Iβ, many PAS domains possess a highly conserved DIT sequence motif (Möglich et al., 2009). The aspartate (or in some examples glutamate) residue usually forms a salt bridge to a lysine or arginine residue in the GH loop, and the threonine residue forms a hydrogen bond to a backbone amide in strand Hβ (e.g., structures 1BYW, 1D06, 1G28, 1N9L, 1XJ3, 2GJ3, 2PD7, 2PR5, 2QHK, 2V0U, 2Z6C, 2Z6D, 3BWL, 3BY8, 3FG8). We propose that this provides a structural basis for coupling the PAS core to its C-terminal flanking region (and to the N-terminal flanking region, in close proximity). Effector domains are usually covalently connected to the C terminus of PAS domains (Figure 1).

Signal Transduction to Effector Domains

How are signals further propagated to effector domains, and how do they modulate biological activity? Confident answers await high-resolution structures of full-length proteins comprising both PAS sensor and effector domains, in the presence

and absence of signal. Presumably due to their inherent flexibility, full-length PAS signaling proteins have largely eluded efforts to determine their structure at atomic resolution.

The tertiary structural uniformity of PAS core domains is in stark contrast to the wide sequence diversity of effector domains, which in turn adopt very different tertiary structures (Figure 1). This immediately argues against signaling mechanisms that rely on specific tertiary structural recognition between PAS core and effector domains. An important clue to how protein activity is regulated is provided by the observation that known effector domains act as homo- or hetero-oligomers, mainly dimers. Prominent examples include histidine kinases (Szurmant et al., 2007), serine/threonine kinases such as plant phototropins (Christie et al., 1998), phosphodiesterases such as *E. coli* DOS (Kurokawa et al., 2004), transcription factors such as the *N. crassa* white-collar proteins (Froehlich et al., 2002), and chemotaxis receptors such as *E. coli* Aer (Taylor and Zhulin, 1999). In oligomeric proteins, signal modulates the association equilibrium between monomers or individual domains, and hence their quaternary structure (Figure 6C). Signal-induced quaternary structural changes have been identified in numerous PAS proteins (Ayers and Moffat, 2008; Kurokawa et al., 2004; Möglich and Moffat, 2007; Nakasako et al., 2008; Neiditch et al., 2006; Scheuermann et al., 2009; Sevvana et al., 2008; Zhou et al., 2008). Quaternary structure rearrangements, for example association, piston, pivot, and rotation movements (Matthews et al., 2006), are also compatible with signaling across membranes in transmembrane proteins.

As discussed above, structural and sequence analysis indicates that the linkers between many PAS domains and their effector domains adopt an α-helical (and often coiled-coil) conformation. Due to their large persistence lengths ($\sim$100 and 150 nm, respectively) (Wolgemuth and Sun, 2006), such α helices and coiled coils behave as rigid rods at the molecular level (Anantharaman et al., 2006). Signals originating within PAS domains at one end of a helix or coiled coil could thus be propagated over long distances to remote effector domains at the other end. Crystal structures of *S. meliloti* DctB (3E4O) (Zhou et al., 2008), *V. harveyi* LuxQ (2HJE) (Neiditch et al., 2006), and bacteriophytochromes (2VEA, 3C2W) (Essen et al., 2008; Yang et al., 2008) provide examples of how α helices connect several sensor domains. Individual domains are arranged along a continuous α-helical spine. Structures of full-length PAS signaling proteins might reveal that effector domains are coupled to PAS sensors in similar ways.

In addition to quaternary structure changes, signal is likely to lead to changes in tertiary structure and dynamics of the sensor and effector domains. Such a mechanism also applies to monomeric proteins and could be mediated by different linkers between PAS sensor and effector domains. It is not clear to which extent these mechanisms are realized in natural proteins. However, such mechanisms are certainly relevant for recently designed, synthetic PAS proteins (see below).

Common Themes in Signal Transduction

Do all PAS domains employ essentially the same signaling mechanism, or does each PAS domain behave differently? It is challenging to reconcile the wide range of currently available data on PAS domain structure and signaling with a single canonical signaling mechanism. However, many aspects recur in different PAS domains. All PAS domains share a similar three-dimensional core structure, and in many the β sheet and flanking helices N and C terminal to the core play central roles in signal transduction. Effector domains are usually connected to the C terminus via short α-helical and coiled-coil linkers and function as oligomers. These commonalities argue for a common predecessor, an ancestral PAS domain. However, presence of a signal might induce multiple dynamic and structural changes in PAS domains that can be harnessed in different ways to regulate effector domain activity (Figures 6B and 6C). Consequently, divergent signaling mechanisms might have evolved. Still, at least some PAS domains must share key features of their signaling mechanisms, as demonstrated by our recent work where we replaced the oxygen-sensing PAS domain of the *B. japonicum* FixL kinase with a photosensory PAS domain (Möglich et al., 2009). The resultant chimeric protein retained the catalytic efficiency of FixL but responded to blue light instead of oxygen. This success implies a high degree of similarity in the mechanisms of the parent chemosensor and the chimeric photosensor.

Recently, PAS blue-light sensors were also used to control the activity of proteins that are normally not coupled to PAS domains (Lee et al., 2008a; Strickland et al., 2008). By deliberately mimicking the modular composition and domain structure of natural PAS proteins (Figure 1), target proteins were put under the control of blue light by covalently linking them to the C termini of flavin-based PAS photosensor domains via a helical linker. Functional light-regulated proteins were obtained with surprising ease; it was not necessary to synthesize and screen large numbers of variants. However, the effect of light on protein activity was modest, on the order of 2- to 3-fold (Lee et al., 2008a; Strickland et al., 2008).

Similar design approaches also apply to other PAS sensors that detect changes in the concentration of small molecules, electrical field, or redox potential. Fusion to suitable PAS domains thus facilitates the rational design of synthetic chemosensors and photosensors.

The signal transduction mechanisms and strategies realized in PAS sensors could also apply to a larger group of modular signaling proteins. One type of sensor domain, such as PAS, can regulate the activity of very different effector domains (Figure 1). Conversely, in certain natural signaling proteins, different sensor domains, such as PAS, GAF (Aravind and Ponting, 1997), or BLUF (Gomelsky and Klug, 2002) domains, are found to regulate the activity of the same class of effector domain, such as histidine kinases or guanylate cyclases (Finn et al., 2006). These observations imply that these classes of sensor domains are at least in part interchangeable and follow similar signaling mechanisms. This might reflect a common evolutionary origin (Anantharaman et al., 2001). We have argued that the structural diversity among sensor and effector domains makes mechanisms involving tertiary-structure-specific contacts unlikely. In an extension of our findings for PAS proteins (Figure 5), sensor and effector domains are linked in many classes of signaling proteins by short amphipathic α helices and possibly coiled coils (Anantharaman et al., 2006). Signals could be transmitted along such linkers as a combination of changes in dynamics and tertiary and quaternary structure. These broader findings suggest that signaling principles are similar over diverse classes of signaling proteins. As a corollary,

strategies that recently led to the successful design of artificial PAS photoreceptors (Lee et al., 2008a; Möglich et al., 2009; Strickland et al., 2008) could also apply to many other protein families.

SUPPLEMENTAL DATA

Supplemental Data include three figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://www.cell.com/structure/supplemental/S0969-2126(09)00334-7.

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Note Added in Proof

Recently, Wu et al. (Nature (2009) *461*, 104–108) fused the GTPase Rac to the C terminus of the *A. sativa* phototropin 1 LOV2 domain and thus put Rac activity under the control of light. The synthetic photosensor was expressed in fibroblasts and could control cell motility by blue light.