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Review Article

Gas and light: triggers of c-di-GMP-mediated regulation

Zhaoqing Yu[1,2], Wei Zhang[1], He Yang[1], Shan-Ho Chou[1], Michael Y. Galperin[3], Jin He[1,*]

[1]National Key Laboratory of Agricultural Microbiology and Hubei Hongshan Laboratory, College of Life Science and Technology, Huazhong Agricultural University, 1 Shizishan Street, Wuhan, Hubei 430070, PR China
[2]Institute of Agro-Product Processing, Jiangsu Academy of Agricultural Sciences, 50 Zhongling Street, Nanjing, Jiangsu 210014, PR China
[3]National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 8600 Rockville Pike, Bethesda, MD 20894, USA
*Corresponding author. National Key Laboratory of Agricultural Microbiology & Hubei Hongshan Laboratory, College of Life Science and Technology, Huazhong Agricultural University, 1 Shizishan Street, Hongshan District, Wuhan, Hubei 430070, PR China. Tel: +86-27-87280670; Fax: +86-27-87280670;
E-mail: hejin@mail.hzau.edu.cn
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Abstract

The widespread bacterial second messenger c-di-GMP is responsible for regulating many important physiological functions such as biofilm formation, motility, cell differentiation, and virulence. The synthesis and degradation of c-di-GMP in bacterial cells depend, respectively, on diguanylate cyclases and c-di-GMP-specific phosphodiesterases. Since c-di-GMP metabolic enzymes (CMEs) are often fused to sensory domains, their activities are likely controlled by environmental signals, thereby altering cellular c-di-GMP levels and regulating bacterial adaptive behaviors. Previous studies on c-di-GMP-mediated regulation mainly focused on downstream signaling pathways, including the identification of CMEs, cellular c-di-GMP receptors, and c-di-GMP-regulated processes. The mechanisms of CME regulation by upstream signaling modules received less attention, resulting in a limited understanding of the c-di-GMP regulatory networks. We review here the diversity of sensory domains related to bacterial CME regulation. We specifically discuss those domains that are capable of sensing gaseous or light signals and the mechanisms they use for regulating cellular c-di-GMP levels. It is hoped that this review would help refine the complete c-di-GMP regulatory networks and improve our understanding of bacterial behaviors in changing environments. In practical terms, this may eventually provide a way to control c-di-GMP-mediated bacterial biofilm formation and pathogenesis in general.

Keywords: c-di-GMP, diguanylate cyclase, c-di-GMP-specific phosphodiesterase, sensory domains, NO sensors, photoreceptors

Introduction

Unlike intracellular bacteria that inhabit stable ecological niches, most free-living bacteria face complex and rapidly changing ecological environments. To survive, these bacteria need to be able to monitor changes in various physical or chemical parameters around them and respond quickly to adapt to various environments (Mascher et al. 2006). As a result, they have evolved complex signal transduction systems. Research in this field allowed discovering several different bacterial signal transduction systems, including, among others, the two-component signal transduction systems (Stock et al. 2000), one-component transcriptional regulators (Ulrich et al. 2005), the alternative sigma factor regulatory systems (Mascher 2013), methyl-accepting chemotaxis receptor protein (MCP)-based chemosensory systems (Miller et al. 2009, Ortega et al. 2017), and protein kinase cascades (Shi et al. 1998, Pereira et al. 2011). However, most of these signal transduction pathways act in a relatively straightforward way and typically regulate only certain classes of downstream targets. In contrast to these systems, second messenger signaling pathways are characterized by a multitude of potential receptors, comprising a new and constantly expanding field of bacterial signaling pathways (Römling et al. 2013, He et al. 2020, Lowey et al. 2020, Stülke and Krüger 2020).

The second messenger systems are important components of the signal transduction networks. In eukaryotes, they can be roughly divided into four categories: nucleotides, lipids, gases, and free radicals or ions (Newton et al. 2016). In bacteria, there are mostly nucleotide second messengers, specific nucleotide derivatives that are not used in cellular nucleic acid synthesis (Stülke and Krüger 2020). Nucleotide second messengers found in bacteria include guanosine-(penta- or tetra-)phosphate ((p)ppGpp), cyclic adenosine monophosphate (cAMP), cyclic di-adenosine monophosphate (c-di-AMP), cyclic di-guanosine monophosphate (c-di-GMP), cyclic GMP–AMP (cGAMP), and some others (Lau et al. 2020, Lowey et al. 2020). They act to bridge bacterial signal perception with cellular response(s). When the cell surface receptors (or receptor domains) receive extracellular signals (first messengers), they can affect the catalytic activities of various intracellular enzymes, including cyclic nucleotide synthases and hydrolases, resulting in changes in the concentrations of certain nucleotide molecules, which serve as the "second messengers." Alterations of the second messenger levels affect their binding to downstream receptors, which regulate specific physiological functions of bacteria.

Signal transduction systems that rely on nucleotide second messengers have distinct advantages. First, the concentrations of nucleotide second messengers are directly controlled by their metabolic enzymes, which, upon receiving a specific signal, can rapidly change the cellular concentrations of the respective second messenger, which, in turn, would alter the properties (activity, conformation, and/or oligomeric state) of their receptor(s). Second, cellular receptors of nucleotide second messengers in bacteria are typically abundant and diverse, enabling them to regulate downstream pathways in multiple ways (expression,

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enzymatic activity, and binding properties). Such a signal transduction system allows the bacteria to participate in continuous sensing and/or coordinated regulation of a single biological process (Orr et al. 2016, Stülke and Krüger 2020). Therefore, bacteria can rely on the nucleotide second messenger-mediated signal transduction systems to enhance their environmental adaptability.

c-di-GMP is a cyclic dinucleotide present in a wide variety of bacteria (Römling et al. 2013). Its synthesis and degradation are regulated by diguanylate cyclases (DGCs) containing the GGDEF domain (which typically contains the Gly-Gly-Asp/Glu-Glu-Phe sequence motif) and c-di-GMP specific phosphodiesterases (PDEs) containing either EAL or HD-GYP domains, so named after their conserved sequence motifs, Glu-Ala-Leu in case of the EAL domain and His-Asp and Gly-Tyr-Pro motifs in case of the HD-GYP domain. DGCs and PDEs are collectively referred to as c-di-GMP metabolic enzymes (CMEs) (Dahlstrom and O'Toole 2017, Jenal et al. 2017).

CMEs typically act as mediators that transform environmental signals into c-di-GMP concentration changes, thereby participating in signal transduction and regulating bacterial behaviors. Signaling CMEs can be divided into two categories based on the way they sense signals.

(1) Many signaling CMEs combine sensory domains and an enzymatic domain in a single protein, and can directly respond to various signal stimuli including gases, light, redox state, temperature, and chemical compounds; these CMEs can be referred to as one-component systems (Ulrich et al. 2005). They are thought to be the predominant mode of sensing in bacteria, and this type of CMEs is also the most easily recognized by bioinformatics.
(2) However, some CMEs can still respond to stimuli despite having no sensory domains themselves. This usually happens in one of two ways. First, part of the CMEs receive signals through protein–protein interactions with proteins containing the sensory domains. Second, other CMEs act as downstream response regulators in two-component systems or more complex multicomponent phosphorelay systems (e.g. chemosensory cascades) and alter their enzymatic activities in response to the transfer of the phosphoryl group to upstream phosphoacceptor domains.

Although the specific mechanisms of signal sensing in signaling CMEs are different, the common feature is that after receiving signals, these CMEs adjust their catalytic activities through conformational changes, thereby altering the concentrations of c-di-GMP in bacteria. Concentration fluctuations are then sensed by specific downstream receptors, which in turn regulate multiple bacterial physiological functions (Wang et al. 2016, Valentini and Filloux 2019), including biofilm formation (Ha and O'Toole 2015, Teschler et al. 2022), motility (Sun et al. 2020), cell differentiation (Lori et al. 2015, Kaczmarczyk et al. 2020), phage resistance (Junkermeier and Hengge 2021, Sellner et al. 2021), and virulence factors expression (Fu et al. 2018, Hall and Lee 2018).

Because of the rich regulatory functions of c-di-GMP, the downstream components of c-di-GMP-dependent signaling pathways, such as the nature of c-di-GMP receptors and the organization of c-di-GMP-regulated networks, have been studied in detail for more than 35 years. However, studies of the upstream signals that the CMEs respond to are relatively limited, and relevant reviews are even rarer. The lack of comprehensive information on the kinds of signals modulating the c-di-GMP levels affects our understanding of the c-di-GMP regulatory networks and hinders the studies of the environmental adaptability of bacteria.

Considering the variety of the sensing capabilities of signaling CMEs and the paucity of comprehensive reviews of this subject, we believe that there is a need for a focused and in-depth analysis of the upstream signaling pathways that control bacterial c-di-GMP levels. Here, we have focused on the sensory domains capable of sensing gas and light to modulate the activity of CMEs. We discuss their various types, structures, and regulatory mechanisms, hoping to promote further research in this important area of signal transduction.

Diversity of sensory domains in bacterial CMEs

CMEs, which catalyze synthesis and hydrolysis of c-di-GMP, are widespread in the microbial world. While the presence of such enzymes in archaea and eukaryotes is limited to just a few cases, such as *Methanocella arvoryzae* MRE50 and *Dictyostelium discoideum*, respectively (Chen and Schaap 2012), CME genes are found in the genomes of all bacterial phyla sampled so far (Galperin 2005, Römling et al. 2013). These enzymes are encoded by most free-living bacteria and even by obligately intracellular vector-borne pathogens of the order *Rickettsiales* that have genome sizes under 900 kb and cause such diseases as human ehrlichiosis and Potomac horse fever. Remarkably, most of these enzymes combine the enzymatic (DGC or PDE) domains with N-terminal regions that in many cases have been recognized as ligand-binding sensory domains.

In fact, the early discovery of c-di-GMP as a component of the signal transduction machinery was partly due to the presence of the PAS domain in the *Komagataeibacter xylinus* CMEs (Tal et al. 1998, Chang et al. 2001). Indeed, a great majority of CMEs are coupled to such signaling domains as PAS (named after Per, ARNT, and Sim proteins) (Huang et al. 1993), GAF (the common domain in cGMP-specific and cGMP-stimulated PDEs, adenylate cyclases, and *Escherichia coli* FhlA) (Hurley 2003), and REC (the receiver domain of two-component response regulators). Interestingly, the PAS and GAF domains adopt similar topologies (Ho et al. 2000), and ligand-binding pockets of both can accommodate cofactors such as heme and flavin (Gilles-Gonzalez and Gonzalez 2004). These cofactors endow sensing specificity to the sensory domains, allowing proteins containing these domains to sense diatomic gases, light, redox state, and other signals (Taylor and Zhulin 1999).

In the past several years, a wide variety of other sensory domains have been identified in CMEs. These include, among others, the globin-coupled sensor (GCS) domains (Wan et al. 2009, Patterson et al. 2021), periplasmic calcium channel and chemotaxis (CACHE) domains (Upadhyay et al. 2016, Giacalone et al. 2018), periplasmic (extracytoplasmic) cyclases/histidine kinases-associated sensory extracellular (CHASE) domain and gamma-proteobacterial periplasmic sensor (GAPES) domain series, CHASE1 through CHASE5 and GAPES1 through GAPES4 (Hengge et al. 2015), as well as a dozen of membrane-associated sensor (MASE) integral membrane domains, from MASE1 (Nikolskaya et al. 2003, Pfiffer et al. 2019) to MASE12 (Galperin and Chou 2022, Martín-Rodríguez et al. 2022). A given CME may contain a single sensory domain that would sense a specific signal (or a group of related ligands), or multiple sensory domains for signal amplification, attenuation, or integration of multiple environmental signals. The diverse combinations of various sensory domains in CMEs can be expected to help the cells to specifically and precisely regulate the c-di-GMP concentrations, thus conferring upon the bacteria high adaptability to environmental changes.

Table 1 shows the distribution of 112 sensory domains, which include 96 distinct domains and several sequence models for

Table 1. Diversity of sensory domains found in association with GGDEF, EAL, and/or HD-GYP domains.[a]

Domain name[b]	Pfam ID	COG[c]	PDB ID	PSSM, aa[d]	Domain name origin	GGDEF only	EAL only	GGDEF + EAL	HD-GYP	GGDEF + HD-GYP	Ligands	Reference
Extracellular (periplasmic) domains												
4HB_MCP_1	PF12729	–	5XUA	181	Four-helix bundle of methyl-carrier proteins	+	+	+	+	+	Citrate, fumarate, succinate, pyrene	Hong et al. (2019)
7TMR-DISMED2	PF07696	–	3JYB	127	7TM Receptors with diverse intracellular signaling modules, extracellular domain 2	+	+	+	–	–	Ca^{2+}, carbohydrates	Jing et al. (2010)
7TMR-HDED	PF07697	COG1480	–	219	7TM-HD extracellular domain	–	–	–	+	–	–	
ABC_sub_bind	PF04392	COG2984	6HNI	293	ABC transporter substrate binding protein	+	–	+	+	–	Tyr?	Bradshaw et al. (2019)
Cache3/Cache2	PF17201	–	–	298	Calcium channels and chemotaxis receptors fused domains 3 and 2	+	–	+	–	–	–	
CBM_2	PF00553	–	2CWR	101	Carbohydrate binding module	–	–	–	+	–	Chitin	Nakamura et al. (2008)
CBM_4_9	PF02018	–	1GUI	134	– same –	+	–	–	–	–	Cellulose, xylan	Boraston et al. (2002)
CHASE	PF03924	COG3614	3T4J	184	Cyclases/histidine kinases associated sensory extracellular domain	+	+	+	+	–	Cytokinin	Hothorn et al. (2011)
CHASE2	PF05226	COG4252	–	264	– same –	+	+	+	+	–	–	
CHASE3	PF05227	COG5278	3VA9	138	– same –	+	–	+	–	–	Pyrene	Zhang et al. (unpublished data)
CHASE4	PF05228	COG3322	–	139	– same –	+	–	+	+	+	–	
CHASE5	PF17149	–	–	108	– same –	+	–	+	–	–	Arg?	
CHASE7	PF17151	–	–	187	– same –	+	–	–	–	–	Taurocholate	
CHASE8	PF17152	–	–	102	– same –	+	+	+	–	–	–	
CHASE9	PF17153	–	–	116	– same –	–	+	–	–	–	–	
CSS-motif	PF12792	COG4943	–	209	Conserved Cys-Ser-Ser motif	–	+	–	–	–	DsbA, DsbB	
DAHL	PF19443	–	–	221	Double all-helical ligand-binding	+	+	+	–	–	Asp, Arg, Ile, fucose, galactose, mannose	
dCache					Double CACHE (Calcium channel and chemotaxis receptor) domain						Dicarboxylates	
dCache_1	PF02743	–	3LID	238	– same –	+	+	+	+	+	Amino acids, pH, diamines, purines, betaine, succinate	Zhang and Hendrickson (2010)
dCache_2	PF08269	–	5G4Z	297	– same –	+	–	+	–	+	C2 and C3 carboxylates	Brewster et al. (2016)
dCache_3	PF14827	–	5IS1	235	– same –	+	+	+	+	–	–	Kim et al. (2016)
DICT[e]	PF10069	COG4250	–	126	Diguanylate cyclases and two-component systems	+	+	+	+	–	Light?	
DUF3365	PF11845	–	5B82	167	Domain of unknown function	+	–	+	–	+	c-type heme, redox	Motomura et al. (2017)

Table 1. Continued

Domain name[b]	Pfam ID	COG[c]	PDB ID	PSSM, aa[d]	Domain name origin	GGDEF only	EAL only	GGDEF + EAL	HD-GYP	GGDEF + HD-GYP	Ligands	Reference
GAPES1	PF17155	–	–	274	Gammaproteobacterial periplasmic sensor domain	+	–	–	–	–	Autoinducer-2 (AI-2)	
GAPES2	PF17156	–	–	204	– same –	+	–	–	–	–	–	
GAPES3	PF17154	–	–	121	– same –	+	+	+	–	–	–	
GAPES4	PF17157	–	–	98	– same –	+	–	+	–	–	–	
LapD_MoxY_N	PF16448	–	3PJV_D	124	N-terminal periplasmic domain of LapD and MoxY	+	+	+	–	–	LapG protein, methanol	Navarro et al. (2011)
LuxQ-periplasm	PF09308	COG1879	3C30	239	Periplasmic domain of LuxQ	–	–	+	–	–	LuxP protein	Slama and Hendrickson (unpublished data)
PBP1_AmiC (Peripla_BP_5)	PF13433	COG0683	1PEA	363	Periplasmic binding protein AmiC-type	+	–	+	–	–	Acetamide, other amides	Pearl et al. (1994)
PBP1_ABC_LivBP (Peripla_BP_6)	PF13458	COG0683	1Z15	342	Periplasmic binding protein LivBP-type	+	–	+	–	–	Leu, Ile, Val	Trakhanov et al. (2005)
Peripla_BP_3	PF13377	COG1609	1JYE	160	Periplasmic binding protein-like domain	+	+	+	+	–	Ribose, galactose, glucose 6-phosphate	Bell et al. (2001)
Peripla_BP_4	PF13407	COG4203	3UUG	259	– same –	+	–	–	–	–	Fructose, galactose	Hu et al. (2013)
Phosphonate-bd	PF12974	COG3221	5LQ5	243	Phosphonate-binding	+	–	+	+	+	Phosphates, phosphonates	Bisson et al. (2017)
PilJ/NarX	PF13675	COG3850	6GCV	112	Nitrate-binding domain of McpN	+	–	+	–	–	Nitrate, nitrite	Martín-Mora et al. (2019)
PBPb (SBP_bac_3)	PF00497	COG0834	2LAO	221	Bacterial extracellular solute-binding proteins, family 3	+	+	+	+	+	Amino acids	Oh et al. (1993)
sCache					Single CACHE (Calcium channel and chemotaxis receptor) domain							
sCache_2	PF17200	COG4564	3UB6	153	– same –	+	–	+	+	+	Urea, propionate, malate, pyruvate	Goers et al. (2012)
sCache_3_2	PF17203	–	–	140	– same –	–	–	+	–	–	Citrate, malate	
sCache_3_3	PF17202	–	–	107	– same –	+	–	+	+	–	–	
sCache_4	PF09984	–	5O7J	146	– same –	+	–	+	–	–	–	Ali-Ahmad et al. (2017)
TarH	PF02203	–	2LIG	152	Ligand-binding domain of the bacterial aspartate receptor	+	–	+	–	–	Asp, Glu, Ser, citrate, 4-hydroxybenzoate	Milburn et al. (1991)
Integral membrane domains												
5TM-5TMR_LYT	PF07694	COG3275	–	170	5TM Receptors of the LytS-YhcK type, 5 TM	+	–	+	+	+	Pyruvate	
7TM-7TMR_HD	PF07698	–	–	190	7TM Receptor with intracellular HD hydrolase	–	–	–	+	–	–	
7TMR-DISM_7TM	PF07695	–	–	207	7TM Receptors with diverse intracellular signaling modules, 7 TM domain	+	+	+	+	–	–	

Table 1. Continued

Domain name[b]	Pfam ID	COG[c]	PDB ID	PSSM, aa[d]	Domain name origin	GGDEF only	EAL only	GGDEF + EAL	HD-GYP	GGDEF + HD-GYP	Ligands	Reference
AA_permease (SLC12)	PF00324	COG0531	–	415	Amino acid permease, 9TM	–	–	+	–	–	Amino acids	
AA_permease_2	PF13520	COG0531	5J4I	427	Amino acid permease, 12 TM	+	–	+	+	+	Amino acids	Ilgü et al. (2016)
Ammonium_transp	PF00909	COG0004	6EU6	399	Ammonium channel transporter Amt, 9–11 TM	+	–	+	–	–	NH_4^+	Pflüger et al. (2018)
DUF4084	PF13321	–	–	304	Domain of unknown function, 9–10 TM	+	–	+	–	–	–	
DUF4118	PF13493	COG2205	2KSF	107	Transmembrane domain of KdpD, 4 TM	+	+	+	–	–	–	Maslennikov et al. (2010)
HisKA_7TM	PF16927	–	–	221	N-terminal 7TM region of histidine kinase, 7TM	+	+	+	+	+	Autoinducer-1 (AI-1)	
MASE1	PF05231	–	–	299	Membrane-associated sensor domain, 5 TM	+	+	+	–	–	–	
MASE2	PF05230	–	–	89	– same –, 6 TM	+	–	+	–	–	–	
MASE3	PF17159	–	–	226	– same –, 5 TM	+	+	+	+	–	–	
MASE4	PF17158	–	–	239	– same –, 8 TM	+	–	+	–	–	–	
MASE5	PF17178	–	–	192	– same –, 6 TM	+	–	–	–	–	–	
MHYT	PF03707	COG3300	–	54 (x3)	Met-His-Tyr-Thr motif, 6 TM	+	+	+	–	–	NO, nitrate	
PTS_EIIC	PF02378	COG1455	–	315	Phosphotransferase system, EIIC domain, 9–10 TM	–	+	–	–	–	–	
Intracellular (cytoplasmic) domains												
BLUF[e]	PF04940	–	2BYC	89	Blue light using FAD	–	+	+	–	–	FAD	Jung et al. (2005)
CBS[e]	PF00571	COG0517	2RC3	57 (x2)	Regulatory domain in cystathionine-beta synthase	+	+	+	+	+	Adenine derivatives	Dong et al. (unpublished data)
C_GCAxxG_C_C	PF09719	–	1H21	115	Putative redox-active protein with a CGAxxG motif	–	–	+	–	–	c-type heme	Abreu et al. (2003)
cNMP_binding	PF00027	COG0664	2ZCW	89	Cyclic nucleotide-binding domain	+	+	+	+	–	Cyclic NMPs	Agari et al. (2008)
CZB[e]	PF13682	–	–	64	Chemoreceptor zinc-binding domain	+	+	+	–	–	Zinc	
Diacid_rec	PF05651	COG3835	–	131	Sugar diacid recognition domain	+	–	+	–	–	Sugar acids	
DUF484	PF04340	–	3E98	219	Domain of unknown function	+	–	–	–	–	–	JCSG (unpublished data)
DUF1631	PF07793	–	–	742	– same –	+	+	+	–	–	–	
DUF1883	PF08980	–	2B1Y	86	– same –	+	–	–	–	–	–	Nocek et al. (unpublished data)
DUF2892	PF11127	–	–	66	– same –	+	–	+	+	–	–	
DUF3330[e]	PF11809	–	–	69	– same –	–	+	–	–	–	–	
DUF3369	PF11849	COG3437	–	168	– same –	+	+	+	+	–	–	
DUF3391	PF11871	COG2206	–	136	– same –	–	–	–	+	–	–	
FHA	PF00498	COG1716	1G6G	66	Forkhead-associated domain	+	+	+	+	–	pThr, pTyr	Durocher et al. (2000)
FIST/NosP	PF08495	COG3287	–	129	F-box and intracellular signal transduction	+	+	+	+	+	NO	
FIST_C/NosP	PF10442	COG3287	–	135	– same –	+	+	+	+	+	NO	

Table 1. Continued

Domain name[b]	Pfam ID	COG[c]	PDB ID	PSSM, aa[d]	Domain name origin	GGDEF only	EAL only	GGDEF + EAL	HD-GYP	GGDEF + HD-GYP	Ligands	Reference
GAF	PF01590	COG2203	5VIV	133	Common domain in cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA	+	+	+	+	+	Biliverdin, cGMP, phycocyanobilin (+O_2, CO, NO)	Baloban et al. (2017)
GAF_2	PF13185	COG1956	4MN7	137	– same –	+	+	+	+	+	O_2, CO, NO	Kim et al. (2014)
GAF_3	PF13492	COG2203	3EEA	129	– same –	+	+	+	+	+	–	Zhang et al. (unpublished data)
HDOD[e]	PF08668	COG1639	3M1T	196	HD-related output domain	+	+	+	+	–	–	JCSG (unpublished data)
Hemerythrin	PF01814	COG2703	4XPX	128	Hemerythrin HHE cation binding domain	+	+	+	+	–	O_2	Chen et al. (2015)
HNOBA	PF07701	–	–	215	Heme NO binding associated domain	+	–	+	–	–	Oxygen, NO	
Laminin_G_3[e]	PF13385	–	4DQA	151	Laminin globular domain	+	+	+	–	–	Arabinan, O-glycans	JCSG (unpublished data)
MEDS	PF14417	–	–	160	Methanogen/methylotroph, DcmR sensory domain	+	+	+	+	–	Dichloromethane	
NIT	PF08376	–	4AKK	228	Nitrate- and nitrite sensing domain	+	–	+	–	–	Nitrate, nitrite	Boudes et al. (2012)
NMT1	PF09084	COG0715	2X26	216	NMT1/THI5 protein domain	+	–	+	–	–	Alkanesulfonate	Beale et al. (2010)
PAS	PF00989	COG2202	1KOU	113	Common domain in Period circadian protein (Per), Ah receptor nuclear translocator protein (ARNT), and Single-minded protein (Sim).	+	+	+	+	+	FAD, FMN, heme, 4-hydroxycinnamic acid (+O_2, CO, NO)	van Aalten et al. (2002)
PAS_2	PF08446	COG4251	6G20	107	– same –	+	–	+	–	–	–	Schmidt et al. (2018)
PAS_3	PF08447	–	5SY7	89	– same –	+	+	+	+	+	–	Wu et al. (2016)
PAS_4	PF08448	–	–	110	– same –	+	+	+	+	+	Aromatic compounds	
PAS_7	PF12860	–	–	115	– same –	+	+	+	–	–	–	
PAS_8	PF13188	–	–	65	– same –	+	+	+	+	+	O_2, CO, NO	
PAS_9/LOV	PF13426	–	–	102	– same –	+	+	+	+	+	Light, O_2, voltage	
PHY[e]	PF00360	COG4251	2VEA	178	Phytochrome region	+	–	+	–	–	Red light	Essen et al. (2008)
PilZ	PF07238	–	2L74	102	Type IV pili biosynthesis protein	+	+	+	+	–	c-di-GMP	Habazettl et al. (2011)
PocR	PF10114	–	–	155	Ligand binding domain of transcriptional regulator PocR	+	+	+	+	+	1,2-propanediol	
Protoglobin	PF11563	–	4ZVA	149	Globin sensor domain	+	+	+	+	–	O_2, CO, NO	Tarnawski et al. (2015)
RsbRD_N	PF14361	–	–	104	N-terminal domain of the stressosome component RsbRD	+	–	+	–	–	–	

Table 1. Continued

Domain name[b]	Pfam ID	COG[c]	PDB ID	PSSM, aa[d]	Domain name origin	GGDEF only	EAL only	GGDEF + EAL	HD-GYP	GGDEF + HD-GYP	Ligands	Reference
SnoaL_3	PF13474	COG4319	3CNX	121	SnoaL-fold domain 3	+	–	+	+	–	–	JCSG (unpublished data)
T2SSE_N/MshEN	PF05157	–	5HTL	108	Type II secretion system protein E, N-terminal domain	+	–	–	+	–	c-di-GMP	Wang et al. (2016)
TackOD1	PF18551	–	–	188	Thaumarchaeal output domain 1	+	–	–	–	–	–	
TPR_1	PF00515	COG0457	2KC7	34	Tetratricopeptide repeat						–	Eletsky et al. (unpublished data)
TPR_2	PF07719	COG0457	4XI0		– same –	+	–	–	–	–	–	Zeytuni et al. (2015)
TPR_4	PF07721	–	–		– same –	+	–	–	–	–	–	
TPR_7	PF13176	–	–		– same –	+	+	+	–	–	–	
TPR_8	PF13181	–	–	33	– same –	+	+	+	+	–	–	
TPR_10	PF13374	–	–		– same –	+	–	+	–	–	–	
TPR_12	PF13424	–	3ESK	77	– same –	+	+	+	+	+	–	Kajander et al. (2009)
TPR_16	PF13432	–	–	68	– same –	+	–	+	–	–	–	
TPR_MalT	PF17874	–	–		MalT-like TPR region	+	–	+	–	+	–	
V4R	PF02830	COG1719	2OSD	62	Vinyl 4 reductase	+	–	+	–	–	Hydrocarbons	JCSG (unpublished data)
YceI	PF04264	COG2353	3HPE	118	YceI-like domain	–	–	+	–	–	Isoprenoids, fatty acids	Sisinni et al. (2010)
YkuI_C[e]	PF10388	–	2BAS	166	C-terminal domain of YkuI	–	+	–	–	–	–	Minasov et al. (2009)
Y_Y_Y	PF07495	–	4A2M	65	Tyr-x-Tyr-x-Tyr sequence motif	+	–	+	+	–	Heparin?	Lowe et al. (2012)
Total number						**94**	**52**	**88**	**48**	**25**		

[a]The table indicates presence (+) or absence (–) of the respective domain combination in the Pfam database as of 12-12-2022. Domain combinations found in a single protein in UniProt were ignored. For domain counts, additional ligands, and references, see Table S1 (Supporting Information). Additional references for some of these domains can be found in the recent review by Matilla et al. (2022). Some domain combinations in the EAL-only column are listed as absent despite being annotated as such in UniProt because all the respective entries contain a diverged GGDEF domains.
[b]Domain names in Pfam (alternative names are in parentheses). The respective entries can be retrieved from InterPro using the https://www.ebi.ac.uk/interpro/entry/pfam/PFxxxxx/format, e.g. https://www.ebi.ac.uk/interpro/entry/pfam/PF12729/for 4HB_MCP_1
[c]COG database (https://www.ncbi.nlm.nih.gov/research/cog/) entries that include this domain. A dash in this and other columns indicates the absence of data.
[d]Length (in amino acid residues) of the domain sequence model in the CDD database (https://www.ncbi.nlm.nih.gov/cdd).
[e]This domain is usually found at the C-termini of the respective CMEs.

PAS, GAF, and tetratricopeptide repeat (TPR) domains [Table S1 (Supporting Information) contains actual domain counts but they are variable]. As sensor domains are often promiscuous, being shared by CMEs, MCPs, sensor histidine kinases, and other bacterial receptors such as adenylate cyclases and serine/threonine protein kinases (Zhulin et al. 2003), this list shows a significant overlap with the one that was compiled recently for sensor kinases, chemoreceptors and transcriptional regulators (Matilla et al. 2022). Analysis of the sensory domain composition of the signaling CMEs reveals that their distribution is quite uneven.

First, the number of signal sensory domains coupled to c-di-GMP metabolic domains is found to vary widely in different bacteria. CMEs are widely distributed in *Proteobacteria*, especially in *γ-Proteobacteria*. The number of CMEs encoded by each genome of these bacteria often reaches dozens, and most of them contain CMEs with signal sensory domains. As an example, the genome of *Pseudomonas aeruginosa* PAO1 encodes 43 CMEs, of which 16 CMEs contain PAS or GAF domains (Valentini and Filloux 2016). In contrast, bacteria from the phyla Firmicutes and Actinobacteria generally encode only a few CMEs. The number and type of signaling proteins contained in bacteria usually depend on the phylogenetic position, lifestyle, and environment of the bacterium (Galperin 2005). Thus, a wide diversity of signaling CMEs has been frequently seen in some opportunistic pathogens that face complex environments (Randall et al. 2022).

In addition to the differences in the number of CMEs encoded in different bacteria, many CMEs differ in the number of sensory domains per molecule. The composition of the sensory domains in a given CME appears to determine their specific role(s) in the bacterium. Table 1 clearly shows that the EAL- and HD-GYP-containing PDEs usually contain fewer sensory domains compared to their numbers in the GGDEF domain-containing DGCs and the GGDEF–EAL hybrid proteins. The relative paucity of sensory domains in EAL-only PDEs, as well as the typically lesser number of EAL-only protein-encoding genes in most genomes compared to the number of the GGDEF domain proteins (Galperin 2005), may indicate that EAL-containing PDEs serve primarily as a sink for the c-di-GMP molecules, non-specifically lowering its cellular levels. Likewise, HD-GYP-containing PDEs rarely contain multiple sensory domains (Galperin and Chou 2022). In contrast, the complex sensory domain network of GGDEF–EAL hybrid proteins may allow them to quickly respond to extracellular or intracellular signals by switching their enzymatic activities (DGC to PDE and back) and thereby control the c-di-GMP levels and c-di-GMP-mediated responses. The fountain model proposed by Sarenko et al. (2017) can well explain these results, in which some of these CMEs form cellular c-di-GMP pools while others perform specific local functions.

In conclusion, the distribution of sensory domains in CMEs is related to the complexity of the environment and specific functional characteristics of related proteins, which fully reflects the important role of signaling CMEs in bacterial signal transduction and environmental adaptation.

Regulation of c-di-GMP levels via heme-based gas-sensing domains

Gaseous molecules are ubiquitous in the environment and have high cell membrane permeability. They can serve as nutrients [e.g. carbon dioxide (CO_2)], terminal electron acceptors [e.g. oxygen (O_2)], or just act as signal molecules [e.g. nitric oxide (NO)] to regulate physiological processes in bacteria. Gas-sensing proteins in bacteria usually rely on cofactors such as heme, iron–sulfur cluster, or nonheme iron to capture gaseous molecules; heme-based regulators appear to predominate (Aono 2008).

Heme-based gas-sensing proteins typically use the iron-bound form of heme *b* (protoporphyrin IX) as a cofactor in their active site to exploit the redox-switching properties of iron for signal transduction. The iron atom present at the center of the heme porphyrin is able to coordinate six ligands: four nitrogen atoms at the center of the heme porphyrin ring, side chain of a His or Cys residue of the protein, and, finally, an exogenous ligand or another amino acid side chain (Farhana et al. 2012). When the gaseous molecule acting as an exogenous ligand associates with (or dissociates from) the sixth binding site of the heme–Fe complex, the coordination structure of the heme iron changes, causing accompanying changes in the surrounding protein. These structural changes generate a signal that can be transduced to the functional domain, ultimately enabling various crucial physiological functions to be switched on or off (Shimizu et al. 2015). The ability to switch between the coordination states of heme iron is essentially the basis for the signal transduction of heme-based gas-sensing proteins. Meanwhile, the gaseous molecules recognition specificity of heme-based gas-sensing proteins depends on the interaction of the amino acid residues around the heme group with ligands (Jain and Chan 2003).

A variety of CMEs with heme-based gas-sensing domains have been identified in bacteria that regulate cellular c-di-GMP concentrations in response to the presence of certain gases. Here, we mainly discuss the gas-sensing domains in CMEs that are sensitive to O_2 and NO, both of which are of physiological significance in bacteria. We have chosen not to discuss the effects of carbon monoxide (CO) binding since (i) CO is not a physiological axial ligand in bacteria, and (ii) CO-specific sensory domains are typically found in transcription regulators, such as CooA from *Rhodospirillum rubrum* or RcoM from *Burkholderia xenonorans*, and do not include any known CMEs (Shimizu et al. 2015).

O_2-sensing domains

O_2 is one of the most abundant gases in the environment and has important effects on many physiological processes of bacteria, including biofilm formation (Mashruwala et al. 2017), motility (Taylor et al. 1999), respiration, chemotaxis (Muok et al. 2019), and virulence. The ability to sense changes in O_2 availability is essential for many bacteria to carry out physiological switching and to counteract oxidative stress induced by reactive oxygen species (ROS). This is especially true for pathogens because many niches inside the host are hypoxic compared to the natural environment. Monitoring O_2 concentrations enables these bacteria to respond quickly by readjusting gene expression programs in the face of environmental changes, thereby facilitating the switch between aerobic and anaerobic metabolism. Some CMEs containing O_2-sensing domains have been identified in bacteria, and these enzymes help bacteria sense the O_2 concentrations in the environment and convert it into a c-di-GMP concentration signal, thereby inducing bacterial behavioral responses and enhancing their environmental adaptability (Wan et al. 2009, Burns et al. 2017).

Currently, the reported O_2-sensing domains in bacterial CMEs can be divided into two categories—one is the heme-containing PAS domain (heme-PAS domain), and the other is the heme-containing GCS domain (heme-GCS domain). There are some differences between the two O_2-sensing mechanisms in bacteria as described below.

Heme-PAS domains

PAS domains are important signaling modules that are widely distributed in prokaryotes and eukaryotes (Taylor and Zhulin 1999). The core structure of the PAS domain is well-conserved across species and consists of five antiparallel β-strands (A_β, B_β, G_β, H_β, and I_β) and four α-helices (C_α, D_α, E_α, and F_α) (Fig. 1A) (Möglich et al. 2009). Some PAS domains rely on cofactors to directly sense environmental signals such as light, gases, and redox state and subsequently transmit the signal to the functional domain; while the other PAS domains do not bind any ligands, but indirectly respond to signals through the mediation of protein–protein interaction (Huang et al. 1993).

The heme-PAS domain belongs to the former group and can accomplish ligand-dependent switching of a variety of functional domains, including histidine kinases (HKs), MCPs, CMEs, and basic helix-loop-helix DNA-binding modules (Dioum et al. 2002). Among identified signaling CMEs, the heme-PAS domains were mostly reported in bacterial PDEs.

As early as 2000, the PDE *Ec*DosP from *E. coli* was found to function as an O_2 sensor (Delgado-Nixon et al. 2000). It contains two N-terminal PAS domains, but only the first PAS domain can bind heme. The unliganded heme Fe complex in *Ec*DosP inhibits catalytic activity, while ligand binding to the heme–Fe(II) complex can alleviate this inhibition (Tanaka and Shimizu 2008). In the absence of any external ligands, both the heme–Fe(III) and Fe(II) complexes of *Ec*DosP are in a six-coordinated low-spin state (Tomita et al. 2002). In the ferric form, heme iron is attached to the side chain His77 (proximal ligand) and a water molecule (distal ligand); when reduced to the ferrous form, the distal axial ligand is changed from a water molecule to Met95 on the FG-loop (encompassing residues 86–97 between the F_α-helix and G_β-strand) (Fig. 2). Binding of exogenous axial ligand O_2 molecules to the heme Fe(II) complex is dependent on the dissociation of Met95 from the heme plane (Kurokawa et al. 2004, Park et al. 2004). Thus, the ligand binding process is accompanied by a change in protein conformation, which relieves inhibition through intramolecular signal transduction to enhance the PDE activity (Shimizu 2013).

*Kx*PDEA1 from *K. xylinus* is also a PDE containing a heme-PAS domain, although quite different from *Ec*DosP in terms of structure and enzymatic properties. For example, the heme-free form of apo-*Kx*PDEA1 does not retain high catalytic activity like apo-*Ec*DosP but completely loses its activity. This suggests that the presence of the heme–Fe complex in this protein is critical for maintaining an active site structure suitable for optimal catalysis and that the PDE activity of *Kx*PDEA1can be activated only when the ligand dissociates from the heme–Fe(II) complex, rather than binds it. In addition, the heme–Fe(II) complex of *Kx*PDEA1 is not in the six-coordinated state; rather, it is in the five-coordinated state, i.e. more common in heme-PAS proteins. This allows O_2 molecules to bind to the vacant distal site of the complex without any ligand exchange (Tomita et al. 2002). The above differences illustrate the diversity of the signal transduction mechanisms that rely on binding O_2 molecules.

Heme-GCS domain

Heme-GCS domain was first discovered in *Bacillus subtilis* and *Halobacterium salinarium* as a heme sensor that controls aerotaxis and is widely distributed in bacteria, archaea, fungi, and even some protozoa (Hou et al. 2000, Vinogradov et al. 2005). The crystal structures of GCS proteins show that the GCS domain is usually in a dimer form with a canonical α-helical rich globin fold and a heme cofactor in a hydrophobic cavity formed by the fold (Fig. 1B) (Keppner et al. 2020). Compared to myoglobin and hemoglobin, GCS has a shortened globin fold that lacks a complete D-helix and partial E-helix, these changes appear to favor O_2 sensing, as opposed to O_2 transport (Martínková et al. 2013, Walker et al. 2017).

The heme-GCS domain is usually located at the N-terminus of the proteins and is fused to the C-terminal domains with activities such as MCPs, HKs, or CMEs. However, although the proximal histidine linked to the heme is absolutely conserved, the sequence similarity of proteins containing the heme-GCS domain is generally not very high. The currently characterized signaling CMEs with a heme-GCS domain are mainly DGCs, including *Ec*DosC from *E. coli* (Fig. 2) (Tuckerman et al. 2009), *Dp*DGC from *Desulfotalea psychrophila* (Sawai et al. 2010), *Bp*GReg from *Bordetella pertussis*, and *Pc*GCS from *Pectobacterium carotovorum* (Burns et al. 2017). These enzymes need to be in the form of multimers to exhibit catalytic activities. Some GCSs possess a middle domain that was demonstrated to adopt a four-helix bundle structure containing a short π-helix and form a dimer in the crystal structure. These GCSs transmit the ligand-binding signals sensed by the N-terminal GCS domain to the C-terminal DGC domain through this unique middle domain and orientate the three domains through the π-helix of the middle domain, resulting in a compact structure with the DGC activity (Walker et al. 2020). Like in the heme-PAS domain, the redox state and linkage of heme in the heme-GCS domain can also modulate the catalytic activities of the downstream domains. GCS proteins with a heme–Fe(II) complex (Fig. 1B) typically have some basal DGC activity, which gets enhanced by the binding of the O_2 ligand. Binding of O_2 to the heme causes subtle rearrangements of the heme pockets, changes in the helix flexibility, and rotation around the globin–dimer interface, and even changes in the oligomerization state of the protein, thereby regulating the DGC activity (Burns et al. 2016). Furthermore, some distal globin residues, like residues Phe42, Tyr43, Ala68 (*Ec*GReg)/Ser68 (*Bp*GReg), and Met69 in the distal heme pockets of *Ec*GReg and *Bp*GReg, exhibit certain effects on the DGC activities of GCS proteins (Wan et al. 2017).

Bacteria exploit differences in O_2-sensing domains

Except for the differences in the coupling of functional domains, there are also some differences in the O_2 binding properties of the heme-PAS domain and the heme-GCS domain. These are mainly manifested in (1) different binding sites. Most PAS sensors use Arg residues on the FG-loop to bind O_2, while GCS sensor binds O_2 mainly through Tyr residues in helix B (Martínková et al. 2013); (2) different binding affinities. The PAS sensor has a weaker binding affinity for O_2 than the GCS sensor. The dissociation constants of the heme–Fe(II) complexes in the PAS sensors and GCS sensors are 12–340 μM and 0.077–14 μM, respectively (Kitanishi et al. 2010, Nakajima et al. 2012); and (3) different catalytic effects on the functional domains. Binding of O_2 to the heme of the PAS domain usually reduces the PDE activity of the CME (except for *Ec*DosP), while binding of O_2 to the heme of the GCS domain can greatly enhance the DGC activity of the respective enzyme.

Based on the above characteristics, these heme-based sensors are able to carry out corresponding functions in different environments. PAS sensors can discern the presence of O_2 and activate genes related to aerobic metabolism; while the GCS sensors can respond to hypoxia and activate associated genes (Martínková et al. 2013). The *Ec*DosCP regulatory system serves as a very interesting model to explain how bacteria use these differences in O_2 sensors to adapt to local O_2 concentrations and maintain c-di-GMP homeostasis (Fig. 3). The genes encoding *Ec*DosC and *Ec*DosP are normally coexpressed during stationary phase by the *dosCP*

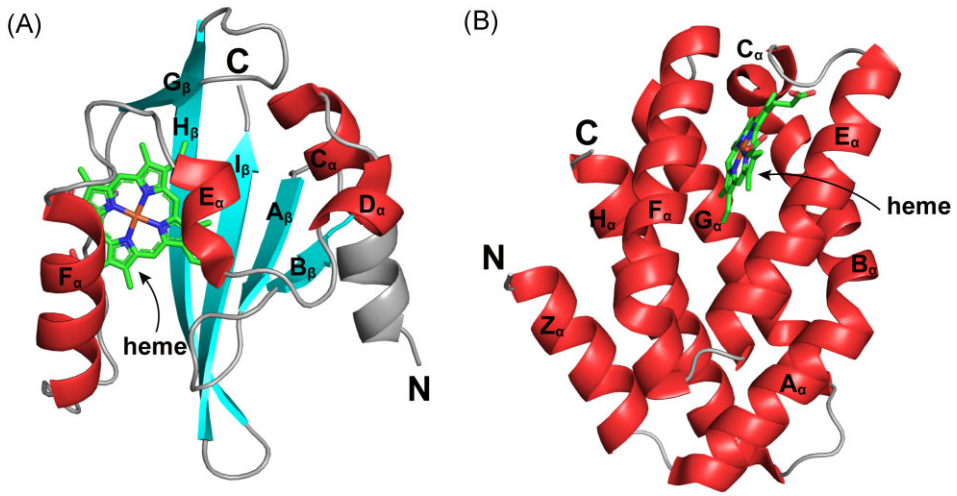

Figure 1. Crystal structures of two types of heme-based O_2 sensors. (A) Crystal structure of the heme-containing PAS domain from *Escherichia coli* *Ec*DosP (PDB entry: 1V9Z) (Kurokawa et al. 2004). Its five β-strands (A_β, B_β, G_β, H_β, and I_β) and four flanking α-helices (C_α, D_α, E_α, and F_α) are labeled as indicated. (B) Crystal structure of the heme-containing GCS domain from *Escherichia coli* *Ec*DosC (PDB entry: 4ZVB) (Tarnawski et al. 2015). Each monomer contains eight α-helices, which are named Z_α, A_α, B_α, C_α, E_α, F_α, G_α, and H_α according to the classical globin nomenclature. The heme ligand in each domain is indicated by arrows.

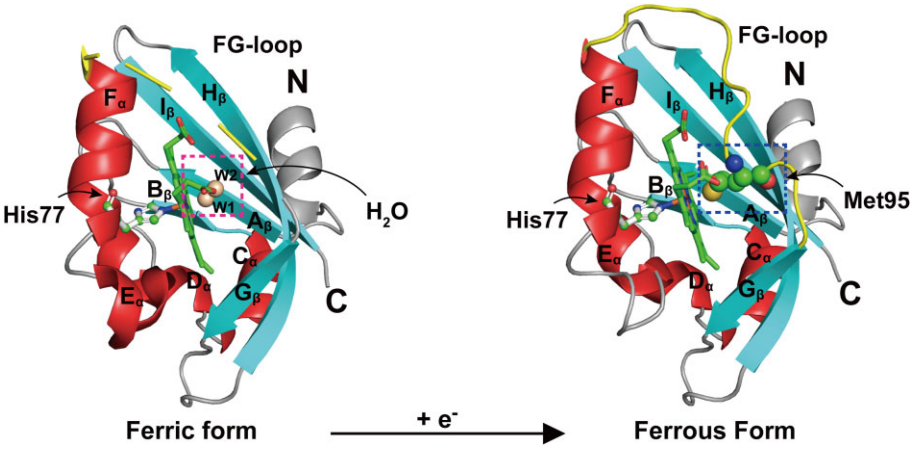

Figure 2. Redox-induced changes at the distal heme site of *Ec*DosP. *Ec*DosP is O_2-dependent and its activity is regulated by the transition between the ferric form (PDB entry:1V9Y) (Kurokawa et al. 2004) and ferrous form (PDB entry:1V9Z) (Kurokawa et al. 2004) of the heme-PAS domain, a process accompanied by the change of distal axial ligands. When the heme-PAS domain is in the ferric form, the distal axial ligand of its complex is a water molecule W1, stabilized by another water molecule W2 (marked by the magenta dashed box); when it is reduced to the ferrous form, the distal axial ligand of its complex is changed to Met95 (marked by the blue dashed box) of the FG-loop (shown in yellow). Also indicated is the iron-binding His77.

operon (Tuckerman et al. 2009). Both *Ec*DosC and *Ec*DosP are heme proteins with O_2-sensing domains and c-di-GMP metabolic domains. *Ec*DosC is a DGC that fuses an N-terminal GCS domain to a C-terminal GGDEF domain, whereas *Ec*DosP is a PDE with two PAS domains, a degenerated GGDEF domain with an EGTQF active site motif, and an EAL domain (Fig. 3A). *Ec*DosC, *Ec*DosP, ribonuclease E (RNase E), polynucleotide phosphorylase (PNPase), and some other components, including several degradosome-associated proteins and RNAs, were reported to form an oxy-degradosome (Gilles-Gonzalez and Sousa 2019). Bacteria selectively activate *Ec*DosC or *Ec*DosP according to the O_2 concentrations to regulate cellular c-di-GMP levels. Changes in c-di-GMP levels affect the activity of PNPase, the receptor for c-di-GMP in this large enzyme complex, which in turn affects the processing or degradation of associated RNAs to control the effects at the post-transcriptional level (Fig.3B) (Tuckerman et al. 2011).

A similar regulatory mechanism may take place in bifunctional CMEs. The enzymatic activities of these proteins are determined by the ligands binding to their sensory domains. For example, *Pd*DcpG from *Paenibacillus dendritiformis* possesses GCS, GGDEF, and EAL domains and exhibits dual functions of DGC and PDE (Patterson et al. 2021). *Pd*DcpG relies on the GCS domain to bind different gaseous molecules to achieve differential regulation of downstream PDE/DGC activity: when the GCS domain is in the Fe(II)–NO state, its DGC activity is activated; whereas when the GCS domain is in the Fe(II)–O_2 state, the PDE activity is activated, allowing bacteria to control biofilm formation in response to different gaseous environments (Patterson et al. 2021). Bifunctional CMEs like *Pd*DcpG containing multiple sensory domains are abundant in bacteria, but the signal transduction mechanisms behind them remain obscure. It is tempting to speculate that similar regulatory mechanisms might function in other bifunctional CMEs.

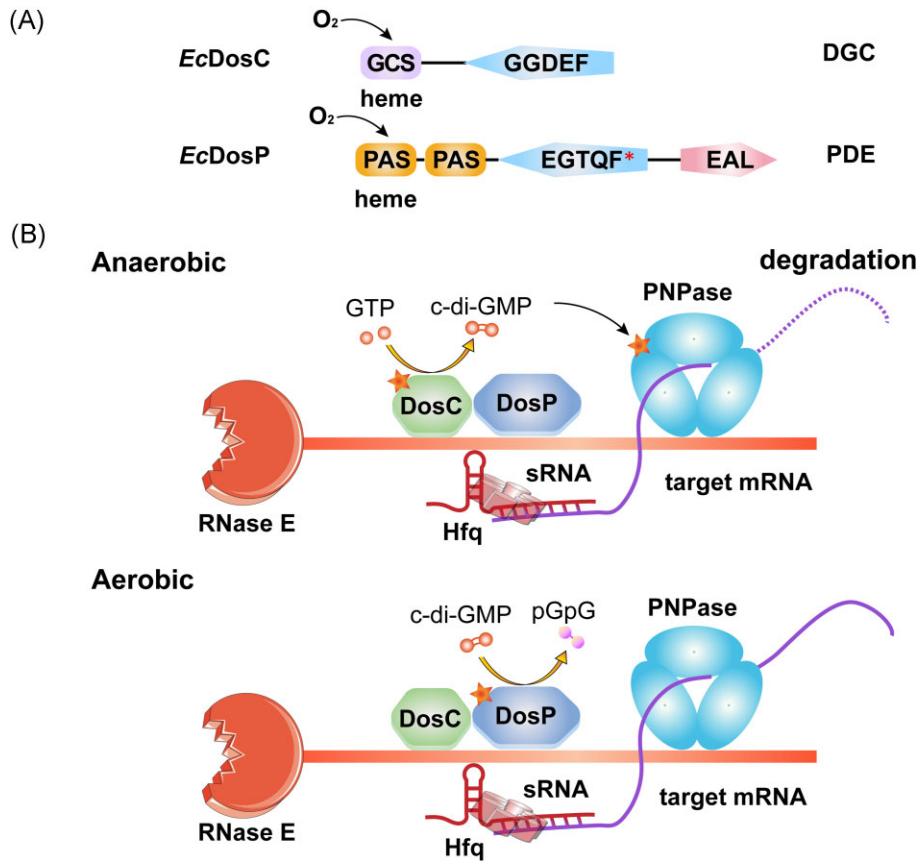

Figure 3. O_2 sensors *Ec*DosC and *Ec*DosP mediate c-di-GMP-dependent RNA processing in *Escherichia coli*. (A) Domain organization of *Ec*DosC and *Ec*DosP. *Ec*DosP contains a degenerate GGDEF domain with the EGTQF motif at its active site. (B) Possible scheme for O_2-dependent RNA degradation in the *Ec*DosCP complex (based on Tuckerman et al. 2011). Under anaerobic conditions (upper panel), *Ec*DosP and *Ec*DosC are unliganded (deoxygenated), and the DGC activity of *Ec*DosC is activated (marked with an asterisk), producing c-di-GMP to activate the receptor PNPase in the RNA degradation complex. Under aerobic conditions (lower panel), *Ec*DosP and *Ec*DosC are liganded (oxygenated) and the PDE activity of *Ec*DosP is induced (marked with an asterisk). *Ec*DosP hydrolyzes c-di-GMP to pGpG, which drastically decreases PNPase activity. mRNAs that depend on O_2 for preservation and degradation may be selected by a mechanism involving sRNAs and Hfq, where sRNAs serve as mediators to recognize target mRNAs, and the RNA chaperone Hfq catalyzes this hybridization.

Sensing O_2 through changes in the redox state

While heme-containing proteins discussed above sense O_2 through direct binding of O_2 molecules, some proteins can monitor the change in the environmental O_2 concentrations indirectly, by sensing the change of the redox state of the electron transport chains.

*Kx*DGC2 from *K. xylinus* (Qi et al. 2009), *Av*NifL from *Azotobacter vinelandii* (Hill et al. 1996), and *Ec*Aer from *E. coli* (Taylor 2007) are all capable of sensing O_2 concentrations indirectly by utilizing a PAS domain that binds a redox-sensitive flavin adenine dinucleotide (FAD) cofactor. The redox state of FAD determines the signaling output of these PAS sensors. In addition to the FAD-binding PAS domains, there are other domains that can help bacteria sense changes in O_2 concentrations, such as the bacterial hemerythrin domain that may be present in either DGCs (Schaller et al. 2012) or PDEs (Kitanishi et al. 2020).

Hemerythrin domains typically have characteristic sequence motifs that provide ligand residues for the nonheme diiron site that binds O_2 molecules and undergoes autoxidation. The diiron site is capable of cycling between diferric and diferrous forms depending on the O_2 concentrations, thereby affecting the catalytic activities of the downstream domains. Existing research data show that such CMEs generally have higher catalytic activities in the reduced ferrous form compared to the oxidized ferric form (Kitanishi 2022).

Several years ago, a new class of PDEs that can respond to redox state has been described. These proteins combine the EAL domain with the periplasmic Cys-Ser-Ser (CSS)-motif domain that contains two highly conserved Cys residues flanked by two transmembrane segments. Such PDEs appear to use the disulfide–dithiol transition in the CSS domain as a redox switch that regulates the PDE activity of the EAL domain (Herbst et al. 2018). The CSS–EAL domain combination is encoded in five copies in both *E. coli* and *Salmonella enterica* and in three copies in *P. aeruginosa*. A recently described variant of the CSS-motif domain, referred to as the CSS_CxxC domain, contains two extra Cys residues. PDEs combining this domain with the EAL domain are encoded in a single copy in *Shewanella*, *Vibrio*, and some other species (Martín-Rodríguez et al. 2022). The discovery of such signaling CMEs illustrates the complexity of bacterial signal transduction pathways and suggests that there might be additional sensors of O_2 and/or redox state.

NO-sensing domains

NO has been previously referred to as a double-edged sword in many physiological and pathological processes in a variety of organisms (Mocellin et al. 2007). That characterization is even more true for bacteria, so their ability to sense NO has a clear physiological significance. On the one hand, as a toxic gas, NO can diffuse

freely and has a wide range of sources. It may come from bacteria, e.g. as an intermediate in the denitrification process of bacterial reduction of nitrate and nitrite, or from the oxidation of L-arginine by bacterial NO synthases; at the same time, NO may be produced by host macrophages as a line of defense against bacterial infection (Spiro 2007, Crane et al. 2010). This requires some bacteria, especially pathogens facing chronic exposure to high concentrations of NO, to have a mechanism to monitor and eliminate NO (Williams et al. 2018). On the other hand, low concentrations of NO have been demonstrated to act as a signaling molecule to regulate bacterial community behaviors, such as biofilm formation (Hossain et al. 2017), quorum sensing (Urbano et al. 2018), and symbiotic relationships (Wang et al. 2010).

C-di-GMP has been shown to be involved in regulating NO-responsive bacterial behaviors through some heme-based NO sensors (Rinaldo et al. 2018). These NO sensors can sense NO concentrations to regulate the catalytic activity of signaling CMEs. They are mainly divided into two categories: the heme-nitric oxide/oxygen (H-NOX) protein family, which has been intensively studied, and the NO-sensing protein (NosP) family that has been discovered in recent years (Bacon et al. 2017, Williams and Boon 2019). They are described in detail below.

H-NOX domain

The H-NOX protein, a heme-protein identified in bacteria, is homologous to the eukaryotic NO sensor soluble guanylyl cyclase and can bind diatomic gaseous molecules (Cary et al. 2006). Its crystal structure was first solved in *Caldanaerobacter subterraneus*, which showed that the H-NOX family possesses a novel fold comprising an N-terminal helical subdomain and a C-terminal subdomain. Among them, the N-terminal subdomain is composed of five helices (A_α–D_α and K_α), the C-terminal subdomain is composed of a four-stranded antiparallel β-sheet and two helices (E_α and F_α) (Fig. 4), with the heme cofactor buried deep between the two subdomains and stabilized by a conserved His residue (His102) and three highly conserved residues (Tyr131, Ser133, and Arg135) in the YxSxR motif of F_α (Nioche et al. 2004, Pellicena et al. 2004).

H-NOX can appear as a domain in signaling proteins or as a free protein adjacent to other signaling partners. In the obligate anaerobic bacteria, H-NOX with a distal pocket hydrogen-bonding network often appears as a domain at the C-terminus of MCPs, which can not only bind to NO and CO molecules but also has the ability to bind to O_2 tightly. In aerobic or facultative anaerobic bacteria, H-NOX is often used as an independent protein associated with HKs or CMEs, which can only stably bind NO and CO, but not O_2 (Bacon et al. 2017, Guo and Marletta 2019). This strict discrimination against O_2 ligands is necessary for a selective NO sensor because under aerobic conditions, O_2 concentrations in cells typically far exceed the NO concentrations (Boon and Marletta 2005, Plate and Marletta 2013a). Differences in ligand-binding capacity also provide the basis for bacteria to selectively transmit signals in cells. It is worth mentioning that all currently identified bacterial H-NOXs have high affinity for NO at nanomolar to femtomolar levels, and most of the known physiological functions are related to NO sensing, highlighting their important role as NO sensors in signal transduction (Table 2).

A possible mechanism by which NO activates H-NOX proteins can be described as follows: due to the special interaction of some amino acid residues of H-NOX with heme, the heme cofactor of H-NOX is in a severely distorted conformation before binding the NO molecules. Upon binding NO, the Fe–His bond between the heme–Fe(II) complex and the histidine ligand is broken, followed by relaxation of the heme cofactor and conformational rearrangement, ultimately causing activity changes of the functional domain or a partner protein (Olea et al. 2010, Herzik et al. 2014, Hespen et al. 2016). As common signaling partners of H-NOX, some CMEs are, therefore, also regulated by NO, and we refer to these CMEs as H-NOX-associated c-di-GMP metabolic enzymes (HaCMEs). The genes encoding HaCMEs are mostly found in the genomes of *γ-Proteobacteria*. Some HaCME-encoding genes are adjacent to *hnoX*, and the two genes are cotranscribed in the same operon; some HaCME-encoding genes are not in the *hnoX* operons, but their encoded products serve as response regulators that form two-component systems with H-NOX-associated histidine kinase (HaHK), whose gene is adjacent to *hnoX* (Plate and Marletta 2013a). Due to the different composition of the *hnoX* operons, there are certain differences in the specific mechanisms of how NO/H-NOX regulates c-di-GMP, so they can be divided into the following two categories (Fig. 5):

1) When the *hnoX* gene is adjacent to the gene encoding HaCME, binding of NO to the H-NOX protein can directly affect the protein–protein interaction between H-NOX and HaCME to either inhibit the DGC activity of HaCME or stimulate its PDE activity, causing the down-regulation of the cellular c-di-GMP concentrations, eventually leading to biofilm dispersal (Fig. 5A).
2) When the *hnoX* gene is adjacent to the gene encoding HahK, binding of NO to the H-NOX protein first inhibits the autophosphorylation activity of HaHK, and then blocks the downstream transfer of the phosphoryl group (the process in a hybrid two-component system is also dependent on histidine phosphotransfer protein (Hpt) as a mediator), changing the phosphorylation state of the downstream HaCME, thereby inhibiting the PDE activity of HaCME or activating its DGC activity, which results in increased cellular c-di-GMP concentrations in bacteria and promotes biofilm formation (Fig. 5B). Since many *hnoX* operons contain a *hahK* gene, this multicomponent signaling system has been reported in a variety of bacteria (Table 3). In addition, HaHK may also have other phosphate transfer acceptors than HaCME in some bacteria. Taking *Shewanella oneidensis* as an example, its H-NOX/HaHK system has two other phosphotransfer acceptors besides SoHnoB (HaCME), namely SoHnoD and SoHnoC. SoHnoD contains a degenerated HD-GYP domain and does not itself have the ability to hydrolyze c-di-GMP. However, it can fine-tune the catalytic activity of SoHnoB through allosteric effects in different phosphorylation states to control the cellular concentrations of c-di-GMP; SoHnoC is a transcriptional regulator that controls the expression of some genes in the NO-signaling network, thus creating a transcriptional feedback loop, which could further modulate NO-response dynamics (some bacteria, such as *Vibrio cholerae*, have this multicomponent signaling network, except for the HnoC homolog) (Plate and Marletta 2013b). Compared with the classical H-NOX/HaHK system containing a unique response regulator HaCME, the proteins mentioned above form a more complex multicomponent phosphotransfer regulatory network for NO signaling (Fig. 5B) (Plate and Marletta 2012).

From the available data, NO seems to mediate the dispersion of bacterial biofilm through the simpler H-NOX system, while the multilevel regulation of the more complicated H-NOX system helps bacteria increase adhesion and biofilm formation (Table 3). However, considering that the H-NOX system model is still in its

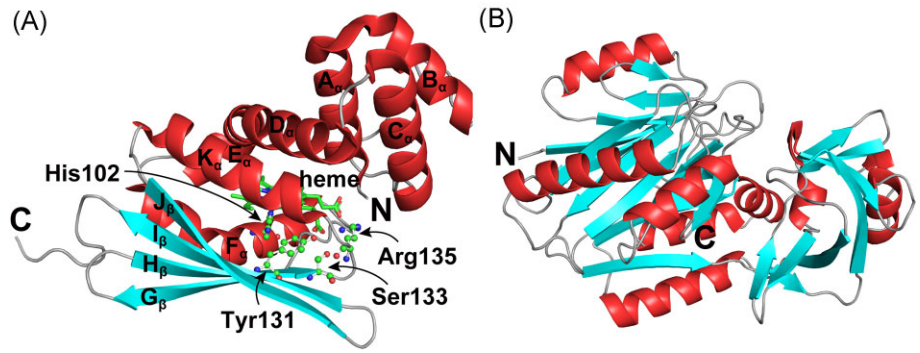

Figure 4. Crystal structures of two classes of heme-based NO sensors. (A) Crystal structure of the H-NOX domain from *Caldanaerobacter subterraneus* (PDB entry: 5JRU) (Hespen et al. 2016). The H-NOX fold consists of seven α-helices (A_α–F_α, and K_α) and a four-stranded antiparallel β-sheet (G_β, H_β, I_β, and J_β). Located on the α-helix F_α, His102 is the proximal axial ligand for heme iron and is highly conserved across all H-NOX domains. Tyr131, Ser133, and Arg135 are strictly conserved residues in the YXSXR motif. The heme ligand is also indicated by arrows. (B) Predicted structure of *Pseudomonas aeruginosa* NosP (PA1975), obtained from the AlphaFold website (https://alphafold.ebi.ac.uk/entry/Q9I2D0). This model contains 10 α-helices and 21 β-sheets.

Table 2. NO dissociation rate constants for H-NOX and NosP proteins

Protein	Species	k_{off} (NO) (× 10^{-4} s^{-1})	Reference
*Tt*H-NOX	*Thermoanaerobacter tengcongensis*	5.6 ± 0.5	Boon et al. (2005)
*Lp*H-NOX1	*Legionella pneumophila*	10.3 ± 1.4	Boon et al. (2006)
*Lp*H-NOX2	*Legionella pneumophila*	21.8 ± 0.5	Boon et al. (2006)
*Vf*H-NOX	*Vibrio fischeri*	21 ± 0.6	Wang et al. (2010)
*Sw*H-NOX	*Shewanella woodyi*	15.2 ± 3.5	Liu et al. (2012)
*Pa*H-NOX	*Pseudoalteromonas atlantica*	8.9 ± 3.6	Arora and Boon (2012)
*Vh*H-NOX	*Vibrio harveyi*	4.6 ± 0.9	Henares et al. (2012)
*Vp*H-NOX	*Vibrio parahaemolyticus*	4.3 ± 0.5	Ueno et al. (2019)
*Sd*H-NOX1 (Sde_3804)	*Saccharophagus degradans*	97.0 ± 1.8	Guo et al. (2018)
*Sd*H-NOX2 (Sde_3557)	*Saccharophagus degradans*	3.3 ± 0.6	Guo et al. (2018)
*Pa*NosP	*Pseudomonas aeruginosa*	1.8 ± 0.5	Hossain and Boon (2017)
*Lp*NosP	*Legionella pneumophila*	< 2	Bacon et al. (2018)
*Vc*NosP	*Vibrio cholerae*	4.6 ± 0.1	Hossain et al. (2018)
Soluble guanylyl cyclase	Bovine lung	3.6 ± 0.8	Stone and Marletta (1994)

early stage, and some experiments were also performed extracellularly or under conditions of excessive NO, whether this is true in bacterial physiological settings remains to be further explored. Furthermore, with the discovery of orphan H-NOX, bifunctional H-NOX, and other NO sensors, there is a growing realization that the NO regulatory networks in bacteria might be quite complicated (Mukhopadyay et al. 2016, Guo et al. 2018).

Orphan H-NOXs whose genes are not adjacent to any partner genes, found in a few bacteria, also have the potential to regulate the activities of the components in another typical H-NOX system in the same bacteria. For example, in addition to the typical H-NOX/HaHK pair, the genome of the marine bacterium *Saccharophagus degradans* encodes an orphan H-NOX protein *Sd*H-NOX2, which also has the function of binding gaseous molecules and inhibiting HaHK activities, but compared with the conventional H-NOX (*Sd*H-NOX1), this protein has a smaller NO dissociation rate and a weaker binding to the kinase (Guo et al. 2018). This property may help increase the duration of intracellular NO-induced signaling and prolong kinase inhibition. Therefore, *S. degradans* may use the dual H-NOX system to help bacteria more flexibly regulate downstream responses in the face of complex environments. Since this type of orphan H-NOX can affect the autophosphorylation activity of HaHK in the H-NOX/HaHK pair, it may also affect the activities of the downstream response regulators (presumably HaCMEs) in this signaling system. In conclusion, some bacteria encoding both an orphan H-NOX and an H-NOX/HaHK pair may have more complex mechanisms of NO-responsive regulation of the c-di-GMP concentrations.

In recent years, some H-NOX proteins have also been found to act as both heme-dependent NO sensors and heme-independent redox sensors, realizing the regulation of downstream signaling protein activity under the dual conditions of NO binding and cysteine oxidation (Mukhopadyay et al. 2016, Mukhopadhyay et al. 2020). For example, in the genomes of γ-*Proteobacteria*, which include many well-known pathogens, some conserved Cys residues are present in about half of the H-NOX domains, although such H-NOXs still remain to be experimentally characterized (Bacon et al. 2017). Considering that the partners of these bifunctional H-NOXs may be HaCMEs, further research would be required to figure out whether their bifunctionality affects the concentrations of bacterial c-di-GMP and exhibits physiological significance in regulating bacterial biofilm formation.

NosP (FIST) domain

Although H-NOX is the primary NO sensor for many bacteria, there are still many bacteria that lack the H-NOX domain but are still able to respond to low concentrations of NO. Therefore, there appear to be other, additional NO sensors in these

Table 3. H-NOX- and NosP-mediated regulation of c-di-GMP signaling.

Species	Pathway	Domains composition of CME	NO effect				Reference
			HK activity	CME activity	c-di-GMP level	Biofilm	
Legionella pneumophila	H-NOX/HaCME	GGDEF and an inactive EAL	None	DGC↓	↓	↓	Carlson et al. (2010)
Dinoroseobacter shibae	H-NOX/HaCME	GGDEF	None	DGC↓	↓	ND	Bedrunka et al. (2018)
Shewanella woodyi	H-NOX/HaCME	GGDEF and EAL	None	DGC↓ PDE↑	↓	↓	Liu et al. (2012)
Agrobacterium vitis	H-NOX/HaCME	GGDEF and EAL	None	DGC– PDE↑	↓	ND	Williams et al. (2018)
Shewanella oneidensis	H-NOX/HaHK/HaCME	REC, PAS and EAL	↓	PDE↓	↑	↑	Plate and Marletta (2012)
Vibrio cholerae	H-NOX/HaHK/HaCME	REC and EAL	↓	ND	ND	ND	Mukhopadyay et al. (2016)
Pseudoalteromonas atlantica	H-NOX/HaHK/HaCME	REC and an inactive HD-GYP	↓	May alter its interaction with other CMEs	ND	ND	Arora and Boon (2012)
Silicibacter sp. TrichCH4B	H-NOX/HaHK/Hpt/HaCME	REC and GGEDF	↓	DGC↑	↑	↑	Rao et al. (2015)
Legionella pneumophila	NosP/NaHK/NaCME	REC, PAS, GGDEF and EAL	↑	DGC↓ PDE↑	↓	↓	Fischer et al. (2019)
Shewanella oneidensis	NosP/NaHK/H-NOX/HaHK/HaCME	REC, PAS and EAL	*So*NaHK↓ *So*HaHK↑	PDE↑	↓	↓	Nisbett et al. (2019)

↑ indicates an increase in enzyme activity, c-di-GMP concentration, or biofilm formation, while ↓ indicates the opposite and - indicates no change.
ND, not determined.

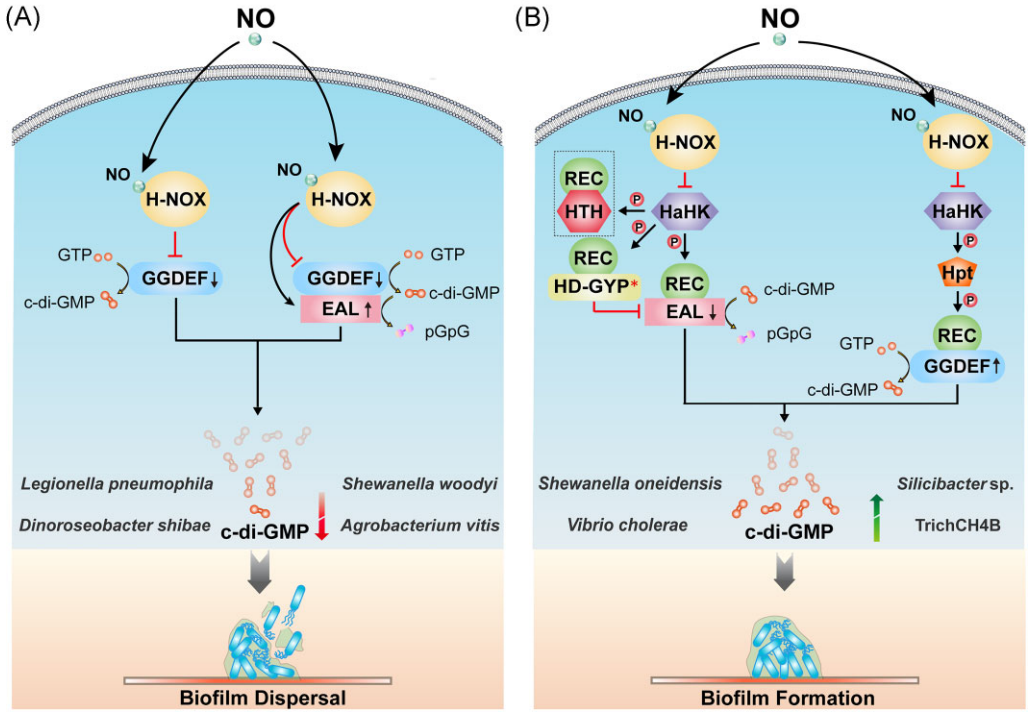

Figure 5. Mechanisms of NO-induced biofilm dispersal or formation via H-NOX domain. CMEs in bacteria function normally in the absence of NO. However, when bacteria are exposed to a certain concentration of NO, it would affect the activities of some CMEs, changing the cellular c-di-GMP concentrations and affecting the formation of bacterial biofilm. (A) NO may directly affect the protein–protein interaction between H-NOX proteins and HaCMEs, thereby altering their catalytic activities. When such HaCME has only a separate GGDEF domain (in some bacteria, it may have an additional degenerated EAL domain), the binding of NO to H-NOX inhibits the DGC activity of such HaCME. When the HaCME contains both GGDEF and EAL domains, binding of NO to H-NOX will maintain or even down-regulate the DGC activity of this HaCME, or activate its PDE activity. These signal events would reduce the cellular c-di-GMP concentrations in bacteria, ultimately leading to biofilm dispersal. (B) NO may also affect the interaction of H-NOX protein with HaHK, thereby affecting the transfer of the phosphoryl group to indirectly regulate the activity of response regulator HaCME. Such HaCME proteins usually fuse the phospho-signaling receptor REC domain and the GGDEF/EAL domain. Binding of NO to H-NOX protein inhibits the autophosphorylation of HaHK, hindering the downstream transmission of the phosphoryl group, and changes the phosphorylation state of HaCME, thereby inhibiting the PDE activity or activating the DGC, resulting in an elevated c-di-GMP level, which ultimately promotes the formation of bacterial biofilms. ∗ indicates domain degeneration and a lack of catalytic activity. The arrows on the c-di-GMP metabolic domains represent an increase or decrease in activities of the corresponding enzymes. The protein shown in the dashed box in Fig. 5(B) is HnoC, which may not be present in the signaling networks of some bacteria.

bacteria. *P. aeruginosa* belongs to this group of bacteria, which does not encode any H-NOX protein, but still responds to NO for regulating biofilm formation (Barraud et al. 2006, Cutruzzolà and Frankenberg-Dinkel 2016).

In the past, a variety of proteins, including MCPs such as *Pa*BdlA (Morgan et al. 2006, Barraud et al. 2009) and some CMEs such as *Pa*DipA (Roy et al. 2012), *Pa*NbdA (Li et al. 2013), and *Pa*GcbA (Petrova et al. 2015) among others, have been identified in *P. aeruginosa* that were implicated in NO-mediated biofilm dispersal; however, none of them was confirmed as the primary NO sensor. Later, a new NO-sensing heme protein, named NosP, was identified. Upon mutating the relevant components of the NosP signaling pathway in *P. aeruginosa*, the biofilms formed by the mutant were found to no longer disperse in response to NO, confirming that NosP is a bacterial NO sensor (Hossain and Boon 2017).

The NosP protein is currently annotated as the F-box intracellular signal transduction (FIST) protein (Borziak and Zhulin 2007), and its predicted structure consists of more than 20 β-strands and several α-helices) (Fig. 4B). Compared to H-NOX, which often occurs in eukaryotes, NosP has been seen almost exclusively in bacteria (Williams and Boon 2019). Most NosPs are encoded as standalone proteins, although some NosPs appear as domains that are coupled to well-known MCP, HK, and CME signaling domains (Bacon et al. 2017, Hughes et al. 2022). The binding characteristics of NosP domains are currently not well-known, although previous studies have shown that the ligand-binding properties of NosP are consistent with it being a dedicated NO sensor, which can bind NO and CO molecules but cannot form stable ferrous–oxy complexes (Hossain and Boon 2017, Hossain et al. 2018).

The NO-sensing mechanism of NosP is similar to that of H-NOX to a certain extent: when bound to NO, it replaces the original histidine ligand with a NO molecule as an axial ligand to form a five-coordinated heme complex (Olea et al. 2010, Bacon et al. 2018). The dissociation rate of NO from NosP is usually much slower than that from H-NOX (Table 2). This difference indicates that H-NOX and NosP may be sensitive to different concentrations of NO or may play different roles in bacterial physiology.

The NO/NosP system can also regulate cellular c-di-GMP concentrations in a manner similar to the second mode of regulation of NO/H-NOX/c-di-GMP: as a component of a multicomponent signaling system, NosP binding of NO molecules affects the kinase activity of NosP-associated histidine kinase (NaHK), thereby controlling the phosphate flux of related signaling pathways, ultimately regulating the activities of NaCMEs (NosP-associated c-di-GMP metabolic enzymes) and affect cellular c-di-GMP concentrations (Williams et al. 2018, Fischer et al. 2019). But unlike

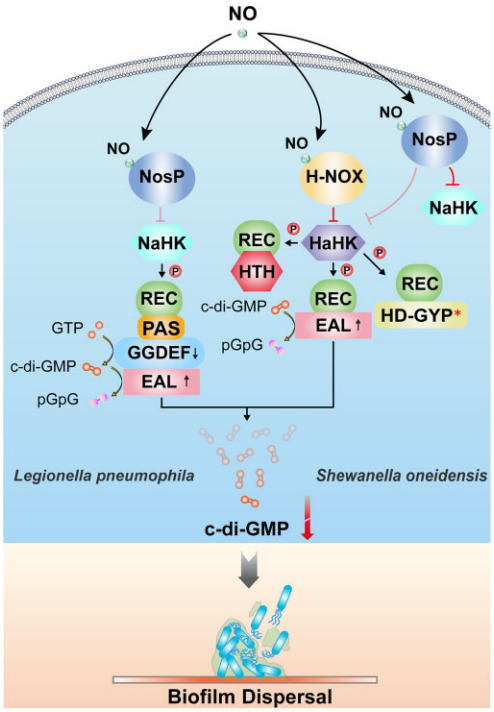

Figure 6. Mechanisms of NO-induced biofilm dispersal via the NosP domain. Possible NosP signaling pathway in *Legionella pneumophila* (left, based on Fischer et al. 2019). Binding of NO to *Lp*NosP weakens the interaction between *Lp*NosP and *Lp*NahK and diminishes the inhibitory effect of *Lp*NosP on the autophosphorylation of *Lp*NaHK. *Lp*NaHK can thus transfer the phosphoryl group to the downstream bifunctional *Lp*NaCME, which exhibits reduced DGC activity and increased PDE activity, causing a decrease in the cellular c-di-GMP concentrations, and ultimately leading to biofilm dispersal. *So*NosP is a master regulator of the multicomponent No/c-di-GMP signaling network in *Shewanella oneidensis* (right, based on Nisbett et al. 2019). When the bacteria are not exposed to NO, iron-free *So*NosP strongly inhibits the autophosphorylation activity of *So*NaHK and *So*HaHK, thereby preventing downstream components of the phosphate transport chain from being phosphorylated. However, when NO is present, *So*NosP attenuates the inhibitory effect on *So*HaHK, enabling the transfer of the phosphoryl group to *So*HaCME and enhancing the PDE activity of *So*HaCME to induce biofilm dispersion. ∗ indicates that the domain is degraded and lacks catalytic activity. The arrows on the c-di-GMP metabolic domains represent an increase or decrease in activities of the corresponding enzymes.

H-NOX, iron-free NosP has a strong inhibitory effect on NaHK (Rao et al. 2017, Fischer et al. 2019). When NO binds to NosP, it weakens the original inhibition of NaHK's autophosphorylation activity by NosP, allowing the phosphoryl group to be delivered to the downstream components (Fig. 6) (Price et al. 2007, Nisbett et al. 2019). It is worth noting that the current research on the NO/NosP system is limited, so this conclusion may be revised or expanded when more cases appear in the future.

Cross-talk between H-NOX and NosP systems

Although studies of H-NOX and NosP systems are still limited to only a few bacteria, they already detected a cross-talk between the H-NOX and NosP systems. For example, the NosP system was found to add regulation upstream of the H-NOX system in *S. oneidensis* (Nisbett et al. 2019). When bacteria were not exposed to NO, *So*NosP without the linking ferrous ion was able to bind to *So*NaHK or even *So*HaHK, thereby strongly inhibiting their autophosphorylation activities and resulting in the inability of downstream components of the phosphate transport system to be phosphorylated. When *So*HnoB cannot be phosphorylated, its PDE activity cannot be activated, and unphosphorylated *So*HnoD will simultaneously inhibit the activity of *So*HnoB, resulting in an increase in the cellular concentrations of c-di-GMP. In contrast, when bacteria are exposed to NO, although *So*NosP still maintains its inhibitory effect on *So*NaHK and *So*HaHK after binding NO, the addition of NO would weaken the control effect of *So*NosP on *So*HaHK. Moreover, *So*H-NOX does not significantly inhibit the autophosphorylation activity of *So*HaHK in the absence of a significant stoichiometric excess of NO-bound *So*H-NOX (Price et al. 2007). Therefore, this achieves a certain degree of relief of *So*HaHK inhibition compared to the absence of NO, resulting in increased phosphate flux to downstream targets of the H-NOX signaling pathway, such as *So*HnoD and *So*HnoB, and promoting the *So*HnoB PDE activity to reduce the cellular c-di-GMP levels (Fig. 6) (Plate and Marletta 2012). As a master regulator in the multicomponent signaling system, *So*NosP can not only regulate the NosP/NaHK signaling pathway but also exert a regulatory effect upstream of the H-NOX/HaHK signaling pathway, enabling the two systems to establish an antagonistic relationship in a push–pull mechanism (Nisbett et al. 2019). Based on these results, the existing NO/H-NOX model of *S. oneidensis* was revised and a new NO/NosP/H-NOX pathway was established (Plate and Marletta 2012, Nisbett et al. 2019). However, considering that the reports of NosP regulating the H-NOX pathway are still limited, it could be only a single case. Besides *S. oneidensis*, other bacteria also have H-NOX, HnoB, HnoD, and HnoC homologs, but a complete NO signaling network has not been demonstrated in these bacteria, so we still retain the original regulatory model in Table 3.

Bioinformatic analysis of the distribution of H-NOX and NosP revealed that, in addition to *S. oneidensis*, many bacteria, especially Gram-positive ones, possess both H-NOX and NosP systems. Whether there is a regulatory relationship between the H-NOX and NosP systems in these bacteria as well, deserves further exploration. Beyond that, there are many questions to be answered. For example, the H-NOX of some bacteria can simultaneously act as a NO sensor and redox sensor. Does NosP affect the NO signaling pathway mediated by this type of H-NOX? Also, a subset of bacteria encodes neither H-NOX nor NosP. Do these bacteria sense NO, and, if so, how? In conclusion, the puzzle of the bacterial NO-sensing signaling pathways has not been fully resolved, and more research is needed in this field.

Regulation of c-di-GMP levels via light-sensing domains

Response to light was previously thought to be exclusive to photosynthetic bacteria, but recent studies have found that genes encoding photoreceptor proteins are also common in the genomes of nonphotosynthetic bacteria (van der Horst et al. 2007, Elías-Arnanz et al. 2011). Light is essential for photosynthetic bacteria to conserve energy but for other bacteria, light can also serve as a cue for optimal orientation and direction. In addition, light sensing is especially important for some pathogens. Because light can affect host immune responses and susceptibility by regulating circadian rhythms, pathogenic bacteria might benefit from the ability to adjust their behaviors in response to light signals in order to better infect the host (Verma et al. 2020).

A total of seven photoreceptor families have been found in bacteria (Table 4), namely phytochromes (Phys), light oxygen voltage

Table 4. Well-characterized bacterial photoreceptor families.

Type	Chromophore	Spectral sensitivity	Reference
Phy	Linear tetrapyrrole bilin	Mainly red/far-red light photoreceptors (Cyanobacteriochromes have a broader spectrum)	Kraiselburd et al. (2017)
LOV	FMN/FAD/riboflavin	Blue light	Herrou and Crosson (2011)
BLUF	FAD	Blue light	Kraiselburd et al. (2017)
PYP	*p*-coumaric acid	Blue/UV-A light	Haker et al. (2003), Purcell and Crosson (2008)
Rhodopsin	Retinal	Visible region (400–700 nm)	Ernst et al. (2014)
Cryptochrome	FAD/pterin/flavin antenna	Blue/UV-A light	Geisselbrecht et al. (2012)
OCP	Carotenoid	Blue–green light	Muzzopappa and Kirilovsky (2020)

Table 5. Characteristics and distribution of selected photosensory DGC/PDEs from bacteria.

Sensor type	Protein name	Species	Domains	Light dependence (*in vitro*)	Reference
Phy	*Rs*BphG1 (Bph)	*Rhodobacter sphaeroides*	PAS-GAF-PHY-GGDEF-EAL	DGC activity is red light-dependent	Tarutina et al. (2006)
	*Id*PadC (Bph)	*Idiomarina* sp. A28L	PAS-GAF-PHY-GGDEF	DGC activity is red light-dependent	Gourinchas et al. (2017)
	*Xo*BphP (Bph)	*Xanthomonas oryzae* pv. *oryzae*	PAS-EAL-GAF-PHY-PAS	PDE activity is red light-dependent	Verma et al. (2020)
	SyCph2 (Cph)	*Synechocystis* sp. PCC 6803	GAF-GAF-GGDEF-EAL-GAF-GGDEF	PDE activity is red light-dependent; DGC activity is blue light-dependent	Savakis et al. (2012)
	*Te*SesA/*Te*SesB/*Te*SesC (Cphs)	*Thermosynechococcus elongatus*	SesA: PAS-GAF-GGDEF SesB: GAF-GGDEF-EAL SesC:PAS-PAS-PAS-PAS-GAF-PAS-GGDEF-EAL	DGC activity of SesA/SesC is blue light-dependent; PDE activity of SesB is teal light-dependent; PDE activity of SesC is green light-dependent	Enomoto et al. (2015)
LOV	*Se*SL2	*Synechococcus elongatus*	REC-PAS-PAC-LOV-GGDEF-EAL	PDE activity is blue light-dependent	Cao et al. (2010)
BLUF	*Kp*BlrP1	*Klebsiella pneumoniae*	BLUF-EAL	PDE activity is blue light-dependent	Barends et al. (2009)
	*Mm*BldP	*Magnetococcus marinus*	BLUF-EAL	PDE activity is blue light-dependent	Ryu et al. (2017a)
	*Rp*PapA–*Rp*PapB complex	*Rhodopseudomonas palustris*	PapA: EAL PapB: BLUF	PapA interacts with PapB; PDE activity of PapA is blue light-dependent via PapB BLUF domain	Kanazawa et al. (2010)

(LOV) proteins, blue light sensing using flavin (BLUF) proteins, photoactive yellow proteins (PYPs), rhodopsins, cryptochromes, and orange carotenoid proteins (OCPs) (van der Horst and Hellingwerf 2004). Among them, the most studied families are PYPs, Phys, LOV, and BLUF proteins, which all participate in the signal transduction from light signals to c-di-GMP concentration change signals (Table 5).

Phys—more than simple red photoreceptors

Phys are photoreceptors that utilize a linear bilin tetrapyrrole as a chromophore and are mainly present in plants, algae, fungi, and bacteria (Davis et al. 1999, Yu and Fischer 2019). The classic Phy photosensory core usually contains a conservative PAS-GAF-PHY light sensor module (the PHY domain is a phytochrome-specific domain likely belonging to the GAF family). Among them, GAF domain is the core, that binds chromophores to form biliary adducts (Aravind and Ponting 1997). The PAS and PHY domains are involved in biliary lyase activity and reversible photoconversion, respectively (Nagano 2016). In addition, some special structures are formed between the three domains, such as a figure-eight knot, a tongue-like structure, and a long centrally located α-helix, which further helps the transduction of light signals (Auldridge and Forest 2011, Fischer et al. 2020).

Considering the special taxonomic status of cyanobacteria, we further divided the bacterial Phys into bacteriophytochromes (Bphs) and cyanobacterial Phys (Cphs). The former are usually linked to biliverdin IXα as a chromophore through a conserved Cys residue upstream of the PAS domain, while the latter are mainly linked to phycocyanobilin (PCB) through a Cys residue in the GAF domain (Rockwell and Lagarias 2010).

Bphs use the PAS-GAF-PHY core structure to sense red light

Bphs typically act as photoswitches through reversible light conversion between the red-absorbing state (P_r) and far-red-

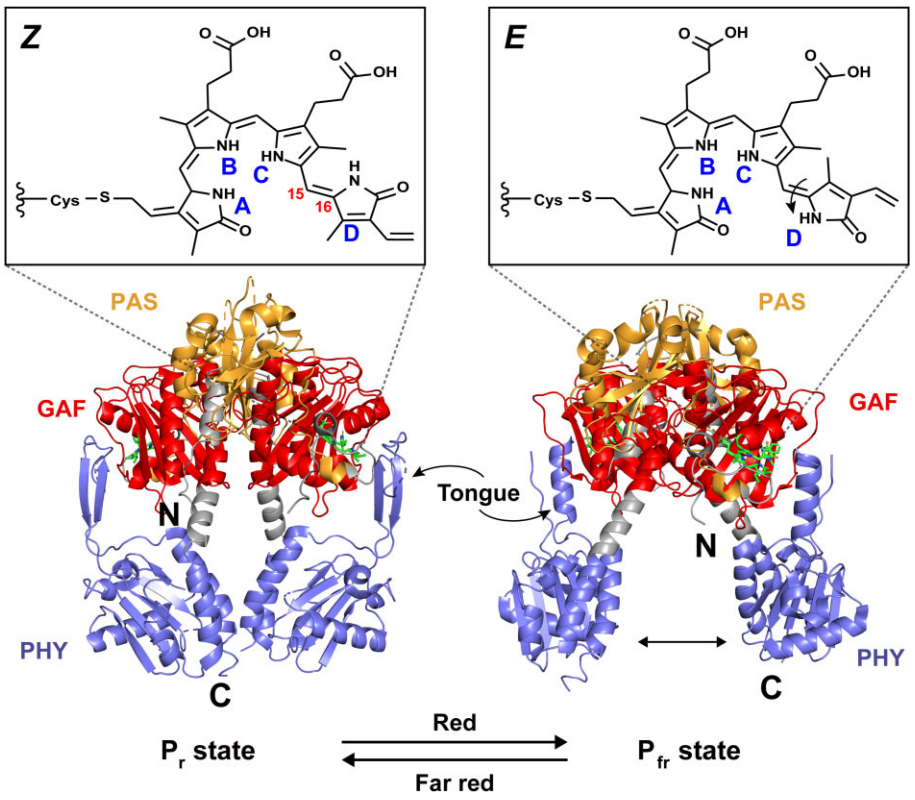

Figure 7. Light-driven changes in the structure and spectral properties of *Deinococcus radiodurans* Bph. The biliverdin chromophore contains four pyrrole rings, named A, B, C, and D rings. Under the irradiation by red and far-red light, biliverdin undergoes reversible Z/E isomerization around the C15/C16 double bond in the methine bridge between the C ring and the D ring, resulting in the rotation of the D ring and causing atomic rearrangements in the chromophore-binding pocket, which in turn leads to repositioning of the PHY domain and refolding of the tongue-like structure therein. In the P_r state, the tongue-like region appears as a β-sheet (PDB entry:4O0P) (Takala et al. 2014), while in the P_{fr} state, it transforms into an α-helix (PDB entry:4O01) (Kurokawa et al. 2004).

absorbing state (P_{fr}) in cells (Fig. 7) (Takala et al. 2014). Their N-terminus conform to the classical Phy model, with complete PAS, GAF, and PHY domains. The C-termini of some Bphs also contain a domain related to c-di-GMP metabolism that helps bacteria regulate c-di-GMP levels in response to light signals. Such Bphs are present in both photosynthetic and nonphotosynthetic bacteria (Table 5).

*Rs*BphG1 was the first Bph with a nonkinase photoactivated enzymatic activity and also the first potentially bifunctional enzyme involved in c-di-GMP synthesis and hydrolysis. It is a photoreceptor protein from the photosynthetic bacterium *Rhodobacter sphaeroides* (recently renamed *Cereibacter sphaeroides*) that contains both GGDEF and EAL domains (Fig. 8A). When expressed at full length, the protein exhibited only light-independent PDE activity (Tarutina et al. 2006). In addition, *Rs*BphG1 expressed in *E. coli* was found to be partially cleaved into two species; the smaller species was identified as an EAL domain with PDE activity, while the larger species lacking an EAL domain exhibited biliverdin-dependent and light-activated DGC activity. The authors speculated that the bifunctional CME *Rs*BphG1 utilizes an "EAL lock" and a corresponding special unlocking mechanism to help bacteria decide whether DGC activity is required to be activated under specific conditions (Fig. 8B) (Tarutina et al. 2006). As previously mentioned, sensory domains are more widely distributed in GGDEF–EAL hybrid proteins, and this model can help us better understand the role of bifunctional GGDEF–EAL proteins in the dynamic regulation of the c-di-GMP levels.

Cphs use simpler photoreceptor domains

Cyanobacteria, as photosynthetic prokaryotes, regulate their motility behaviors in response to light signals in order to grow under optimal conditions (Yang et al. 2018). This process may also involve the participation of c-di-GMP whose levels are regulated by cyanobacterial photoreceptor CMEs (Fig. 9A) (Savakis et al. 2012).

The photoreceptor domain composition of Cphs appears to be more diverse than that of Bphs. Canonical Cphs (e.g. Cph1), like Bphs, have a complete PAS-GAF-PHY architecture at the N-terminus, and in some cases, Cphs are knotless Phys that differ from the classical model. Knotless Phys do not have a typical figure-eight knot structure due to the lack of a PAS domain, but they retain the ability to sense light, such as Cph2-like Phys and cyanobacteriochromes (CBCRs) (Fushimi and Narikawa 2019).

Cph2-like Phys do not have a PAS domain but usually have multiple GAF domains. Some of these GAF domains contain conserved Cys residues that can covalently bind the chromophore, while others contain no such Cys residues and are homologous to the PHY domain. They utilize these domains to form a conserved GAF-PHY structure for P_r/P_{fr} photoconversion (Montgomery and Lagarias 2002). Compared to Cph2-like Phys, CBCRs, the main cyanobacterial photoreceptors, have simpler domain components for light sensing. They lack the PAS and PHY domains and only require the GAF domains to sense various light qualities covering the entire ultraviolet (UV)-to-visible spectrum (Fushimi and Narikawa 2019, Villafani et al. 2020).

Both classes of knotless Phys have been shown to convert light signals into c-di-GMP concentration change signals, which are

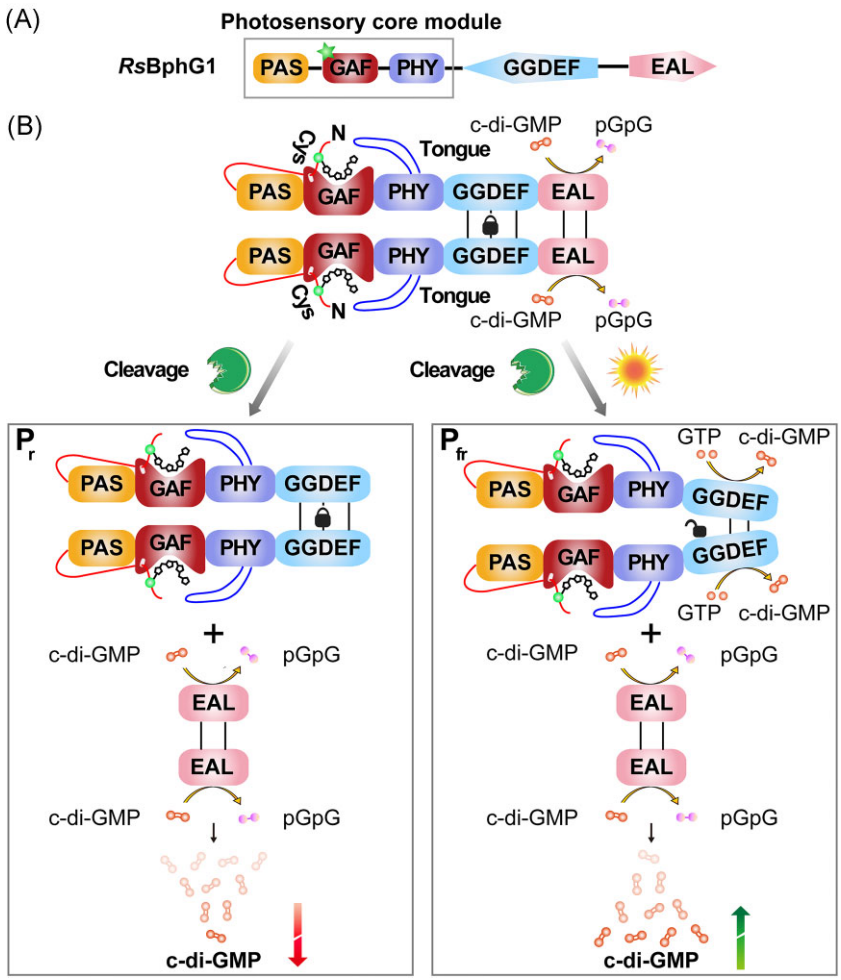

Figure 8. The photoreceptor protein *Rs*BphG1 from *Rhodobacter sphaeroides* is involved in regulating c-di-GMP levels in bacteria. (A) Schematic representation of the domain composition of *Rs*BphG1 that includes the classic PAS-GAF-PHY photosensory module and the GGDEF–EAL functional modules. Green stars represent the biliverdin chromophore. (B) An "EAL lock" model can be applied to explain how *Rs*BphG1 modulates its catalytic activity in response to light signals (based on Tarutina et al. 2006). The EAL domain and the linker between GGDEF and EAL of *Rs*BphG1 tend to homodimerize, although dimerization of the EAL domains is not required for PDE activity. Protein–protein interaction between the EAL domains can form an "EAL lock" to restrict the mobility of the upstream GGDEF domains. In this process, homodimer formation of GGDEF domains is necessary to receive signals from the sensory domain to undergo a conformational change and complete the transition from a nonproductive state with low or no enzymatic activity to a productive state with high activity. When "EAL lock" is present, the GGDEF domain cannot obtain enough mobility to change conformation, so DGC activity is inhibited; in other words, *Rs*BphG1 gets locked in PDE mode. However, bacteria may use a cleavage mechanism to unlock this "EAL lock," eventually splitting full-length *Rs*BphG1 into two proteins. The smaller species was identified as the EAL domain and the linker with PDE activity, while the larger species was the rest of *Rs*BphG1. When the unlocked *Rs*BphG1 is not activated by light, its GGDEF domain is in a nonproductive state; when activated by light, the conformation of its GGDEF domain will shift to a productive mode, increasing the cellular c-di-GMP concentrations. *Rs*BphG1 usually exists as a tetramer or a higher-order oligomer, but was drawn as a dimer in this figure for ease of presentation.

involved in regulating cyanobacterial motility behaviors (Blain-Hartung et al. 2021, Nakane et al. 2022). In *Thermosynechococcus elongatus*, multiple CBCRs form a color-sensitive and highly specific c-di-GMP signaling network to synergistically induce cyanobacterial aggregation or dispersal (Enomoto et al. 2015). They can even appear as two modules in a single protein for different light signals to help cyanobacteria achieve flexible regulation of cellular c-di-GMP concentrations, such as SyCph2 from *Synechocystis*, which contains six domains in the order GAF-GAF-GGDEF-EAL-GAF-GGDEF. The first GGDEF domain is degenerated and loses its ability to synthesize c-di-GMP, but the remaining c-di-GMP metabolic domains can function accordingly (Fig. 9B). SyCph2 is a hybrid photoreceptor with an N-terminal module for receiving red/far-red light and a C-terminal module with CBCR functionality sufficient for photoconversion between green and blue absorbing states. When irradiated with blue light, the downstream GGDEF domain can be activated to promote c-di-GMP synthesis, resulting in hindered cyanobacterial motility (Fig. 9C) (Wallner et al. 2020); but when irradiated with white light, the long wavelength contribution of white light absorbed by the N-terminal GAF module abolishes blue light-induced motility inhibition, resulting in induced cyanobacterial motility (Savakis et al. 2012). Moreover, SySlr1143, an interaction partner of SyCph2, was also shown to regulate cyanobacterial motility. It is a highly active DGC containing a nonphotoactive GAF domain and a GGDEF domain, where the GGDEF domain can interact with the EAL domain of SyCph2 independently of light, and mutation of *slr1143* affects the motility behavior of *Synechocystis* under red light. Since SySlr1143 itself is not photosensitive, it likely forms a photochemically active protein complex with SyCph2 and

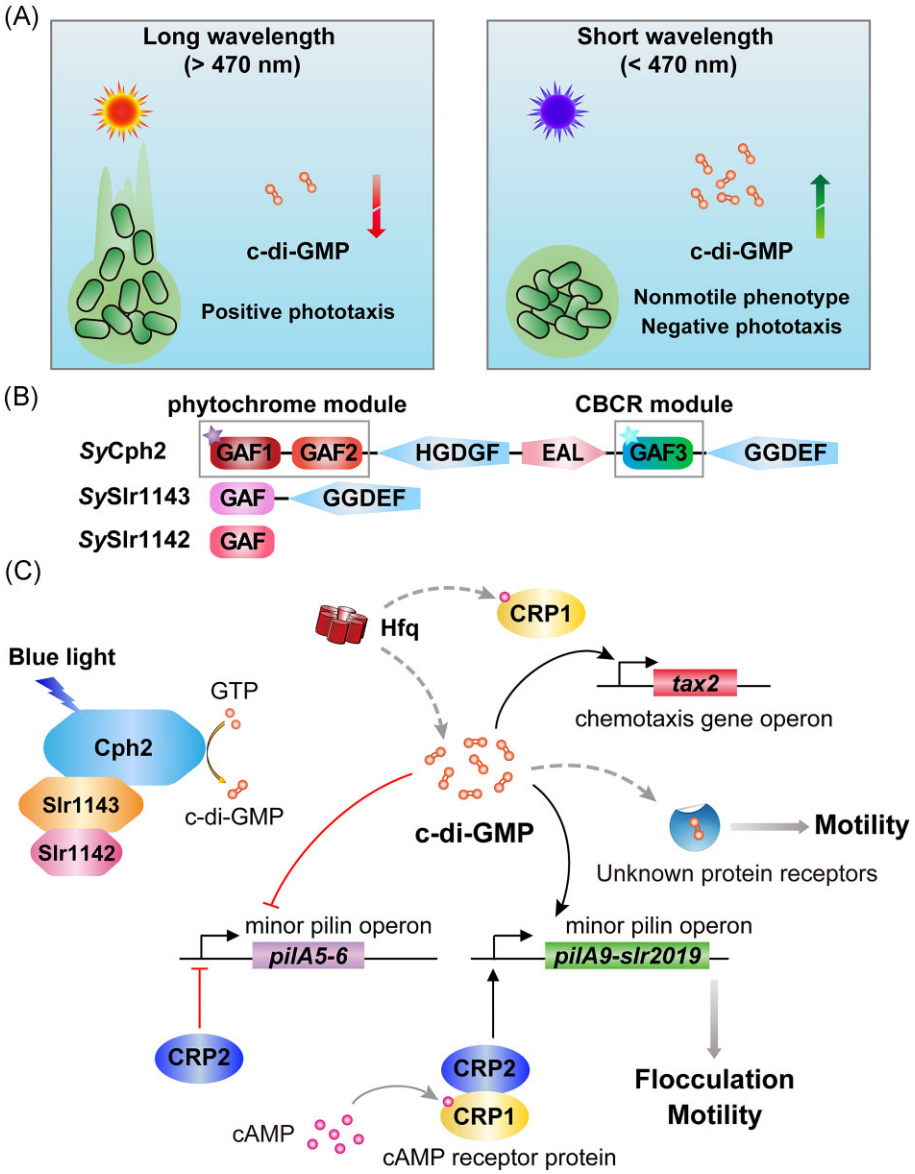

Figure 9. c-di-GMP is involved in regulating *Synechocystis* motility. (A) Motility behaviors of *Synechocystis* sp. Its phototaxis is spectrally dependent, and the phototaxis behaviors have a transition point at a wavelength of about 470 nm. When the wavelength is higher than 470 nm, the cells show positive phototaxis, while when the wavelength is lower than 470 nm, the cells either do not move (e.g. under blue light) or exhibit negative phototaxis (e.g. from ultraviolet light) (Fiedler et al. 2005, Chau et al. 2017). This process may involve c-di-GMP, since artificial degradation of c-di-GMP in *Synechocystis* activates bacterial motility, while synthetic c-di-GMP inhibits phototaxis (Savakis et al. 2012, Wallner et al. 2020). (B) Schematic illustration of the domain composition of SyCph2 and its interaction partners that regulate phototaxis in *Synechocystis*. The PCB chromophore (purple star) and tetrapyrrole chromophore (cyan star) are covalently bound to the GAF1 and GAF3 domains of SyCph2, respectively, conferring SyCph2 light-sensing capabilities. (C) SyCph2-dependent model of *Synechocystis* blue light avoidance behaviors (based on Wallner et al. 2020). SyCph2, SySlr1143, and SySlr1142 may be able to form a protein complex that can flexibly adjust cellular c-di-GMP concentrations in response to different light signals. Under blue light irradiation, the C-terminal GGEDF domain of SyCph2 is activated to synthesize c-di-GMP. Elevated levels of c-di-GMP affect the expression of minor pilin operon (*pilA5-pilA6* and *pilA9-slr2019*) and chemotaxis gene operon (*tax2*), in particular, up-regulated expression of *pilA9-slr2019* significantly induces flocculation of *Synechocystis*, and this operon has also been shown to play an important role in the phototaxis motility of *Synechocystis*. Besides, the c-di-GMP receptor CdgR has been recently identified in cyanobacteria and demonstrated to control the cell size (Zeng et al. 2023). The possible existence of yet unknown c-di-GMP receptors that could regulate cyanobacterial motility remains to be investigated. Furthermore, this c-di-GMP-dependent signaling network also appears to crosstalk with the cAMP signal transduction system and the Hfq regulatory system due to the involvement of two CRP-like transcription factors, SyCRP1 (a cAMP receptor protein) and SyCRP2 (a potential c-di-GMP-dependent transcription factor lacking the key amino acids for binding cAMP) (Fu et al. 2021). The dotted lines represent mechanisms that have yet to be studied.

SySlr1142 (consisting of a single GAF domain encoded by a gene in the same operon as *slr1143*), to help cyanobacteria regulate c-di-GMP concentrations in response to different light signals. However, the exact mechanism behind it remains unknown and more research is required (Angerer et al. 2017a). These cases reflect the complexity of regulatory networks of cyanobacterial photoreceptor proteins, and it is because of them that cyanobacteria can improve their ability to adapt to different depths in the water.

Bacteria have more blue light than red light photoreceptors

In addition to red light-responsive systems, there are four major classes of blue-light photoreceptors that rely on flavins as chromophores in bacteria: LOV proteins, BLUF proteins, PYPs, and cryptochromes (Fig. 10). They can mediate bacterial responses to blue light, including photosynthesis (Metz et al. 2012), motility (Yang et al. 1995), DNA repair, stress response (Avila-Pérez et al. 2006), and regulation of development. Blue light usually inhibits the growth of bacteria. Therefore, exposure to blue light can trigger the dispersion of bacterial biofilms, helping bacteria migrate to a more favorable environment to survive (Halstead et al. 2016, Kahl et al. 2020). c-di-GMP has also been found to play an important role in the regulation of blue light signals in the first three types of photoreceptors.

LOV domains

Proteins containing LOV domains are the most widely distributed blue light-sensitive proteins in bacteria, fungi, and plants (Losi et al. 2002). The LOV domain belongs to the PAS domain superfamily, and its core domain consists of a five-stranded antiparallel β-sheet (A_β, B_β, G_β, H_β, and I_β) with helical linker elements (C_α, D_α, E_α, and F_α), which are flanked by variable and often helical N- and/or C-terminal extensions (Ncap or Ccap) (Fig. 10A) (Henry and Crosson 2011, Hart and Gardner 2021). The core domain of the LOV is capable of forming an internal pocket for noncovalent binding of flavin [most commonly flavin mononucleotide (FMN), occasionally FAD (He et al. 2002) or riboflavin (Rivera-Cancel et al. 2014)] as a chromophore. The form of flavin chromophore appears to depend on the expression system, expression conditions, and the characteristics of the domain itself (Dorn et al. 2013)). There is a conserved GXN**C**RFLQ motif that binds flavin on the E_α of LOV, and the Cys residue in this motif can form a covalent flavin-Cys thiol adduct with the flavin C_{4a} atom when irradiated by blue light. This is the only criterion for the LOV domain to be photosensitive, and it is also the basis for participating in photocycling and signal transduction. At this point, the protein is in a "light state" that differs from the "dark state" in terms of spatial and electronic properties (strain, degrees of freedom, and intramolecular interactions), resulting in a rearrangement of the protein structure, accompanied by changes in protein dynamics, ultimately enabling regulation of downstream functional domain activity (Seifert and Brakmann 2018). Among them, antiparallel β-sheets and helical ends flanking the core domain play a major role in this process (Möglich and Moffat 2007).

Similar to the domain organization described in other bacterial signaling proteins, LOV proteins typically have a sensory domain at the N-terminus and a functional domain at the C-terminus. About half of the functional domains of LOV proteins are HK domains. In addition, the c-di-GMP metabolic domains occupy the second place, accounting for about 20%, which also means that some LOV proteins can sense blue light signals to regulate cellular c-di-GMP levels (Crosson et al. 2003, Herrou and Crosson 2011). One such LOV protein, *Se*SL2, was identified in *Synechococcus elongatus*. In addition to the LOV domain, *Se*SL2 contains a GGDEF and an EAL domain and exhibits blue light-induced PDE activity *in vitro* (Cao et al. 2010). It is worth emphasizing that the LOV–GGDEF–EAL construct is one of the most common domain organizations in bacterial LOV proteins, suggesting that regulation of cellular c-di-GMP concentrations by light may be a general regulatory mechanism in bacteria.

BLUF domain

Another class of blue-light sensing domains is the BLUF domain which utilizes FAD as a chromophore and is present in unicellular eukaryotes and about 10% of prokaryotes (Losi and Gärtner 2008). BLUF domains are small, compact photosensitive modules that form a ferredoxin-like protein fold with two α-helices aligned parallel to a five-stranded mixed β-sheet (Fig. 10B) (Anderson et al. 2005, Jung et al. 2006). Furthermore, a more variable helical C-cap is often stacked against the β-sheet on the side opposite the N-terminal helices (Conrad et al. 2014). In addition to the preferential noncovalent binding of the FAD chromophore, the BLUF domain, like LOV, can also bind other types of natural flavins under certain conditions (Laan et al. 2004). The uniqueness of the photochemical reaction of the BLUF domain is that its photoactivation process is not accompanied by a major structural change of the chromophore but merely rearranges the hydrogen bonds around the chromophore, resulting in a reversible red shift of the absorption spectrum of the BLUF domain (Kennis and Mathes 2013). In addition, among all blue light photoreceptors utilizing the flavin chromophore, BLUF proteins are also the only photoreceptor family showing photo-induced proton-coupled electron transfer (Park and Tame 2017).

BLUF domains can occur in single- or multidomain proteins, with 70% of BLUF proteins being represented by individual BLUF domains. BLUF–EAL fusions are the second most common construct (14%), such as *Kp*BlrP1 from *Klebsiella pneumoniae* (Gomelsky and Hoff 2011). In solution, the isolated BLUF domain of *Kp*BlrP1 is in stable monomeric form, while full-length *Kp*BlrP1 is in dimeric form. Under blue light irradiation, the BLUF domain of one subunit of the antiparallel *Kp*BlrP1 dimer absorbs photons, activating the EAL domain of the second subunit through allosteric communication transmitted through the conserved domain–domain interfaces, and hydrolyzes c-di-GMP (Barends et al. 2009).

However, most YcgFs in enteric bacteria, although containing a similar domain composition to *Kp*BlrP1, have no enzymatic activity in the EAL domain (Gomelsky and Hoff 2011). *Ec*YcgF is a direct antagonist of the MerR-like regulator *Ec*YcgE, and the two encoding genes are located adjacent to each other. *Ec*YcgF changes its conformation after blue light irradiation and then releases *Ec*YcgE from bound DNA by interacting with *Ec*YcgE, which ultimately affects the expression of bacterial biofilm formation-related proteins. Interestingly, not only the expression of *ycgF* and *ycgE* is strongly induced by low-temperature conditions, but the light-induced dimerization of *Ec*YcgF also exhibits a significant temperature dependence (Tschowri et al. 2009, Nakasone et al. 2010). This indicates that the *Ec*YcgE/*Ec*YcgF signaling pathway involves not only the response to light but also the perception of temperature, which can help *E. coli* to survive better in aquatic environments other than the original host. In fungi, some Phys use the property that temperature can alter the rate of dark reversal to rely on light to sense temperature, acting as "temperature sensors" (Yu et al. 2019). It has been found that the optimal growth temperature of some bacteria varies under different light conditions (Mesquita et al. 2019), and the photosensitivity of some bacteria is temperature-dependent (Gomelsky and Hoff 2011, Dorey et al. 2019). These results imply that there is a cross-talk between the bacterial light signaling and the temperature signaling pathway.

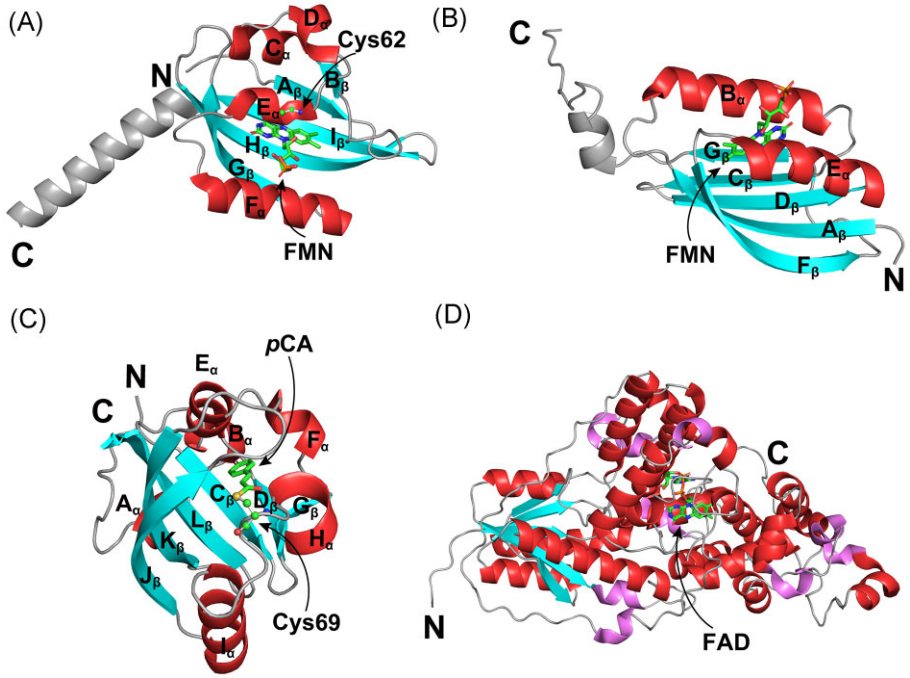

Figure 10. Structures of blue light photoreceptors in bacteria. (A) Crystal structure of the LOV domain of *Bacillus subtilis* YvtA (PDB entry: 2PR5) (Möglich and Moffat 2007). The YvtA core domain consists of a five-stranded antiparallel β-sheet (A_β, B_β, G_β, H_β, and I_β) and four α-helices (C_α, D_α, E_α, and F_α), with a short linker helix at the C-terminus of the core domain. Critical Cys62 are highlighted and drawn in ball-and-stick. (B) Crystal structure of the BLUF domain of *Rhodobacter sphaeroides* AppA with FMN replacing the FAD cofactor of the native AppA (PDB entry: 1YRX) (Anderson et al. 2005). It contains two α-helices (B_α and E_α) and a five-stranded mixed β-sheet (A_β, C_β, D_β, G_β, and F_β). (C) Crystal structure of *Ectothiorhodospira halophila* PYP (PDB entry: 2PHY) (Borgstahl et al. 1995). PYP consists of a central six-stranded β-sheet (C_β, D_β, G_β, J_β, K_β, and L_β) and six α-helices (A_α, B_α, E_α, F_α, H_α, and I_α). Key Cys69 covalently binding the chromophore are highlighted and drawn in ball-and-stick. (D) Protein fold of the *Synechocystis* sp. PCC6803 cryptochrome DASH (PDB entry: 1NP7) (Brudler et al. 2003). It contains several 3_{10} helices (magenta) in addition to α-helices (red) and β-sheets (cyan). The chromophores in each domain are also indicated by arrows.

Pyp

PYP is a water-soluble protein and a class of small cytoplasmic blue light receptors. It was originally discovered in *Halorhodospira halophila*, and more than 100 bacteria are now known to encode PYPs (Schmidt 2017, Kim et al. 2020). PYP is the structural archetype of the PAS domain superfamily with an α/β folded structure consisting of a central six-stranded β-sheet packed by six α-helices (Borgstahl et al. 1995, Pellequer et al. 1998). PYP covalently binds the *p*-coumaric acid (*p*CA) chromophore to a conserved Cys residue, thereby conferring blue/UV light sensitivity to PYP. This chromophore is in a deprotonated *trans* form in the dark. After photoexcitation, the chromophore undergoes photoisomerization from *trans* to *cis*, followed by protonation to achieve electrostatic neutrality. This results in a rearrangement of the hydrogen-bonding network, ultimately leading to a change in the overall conformation of the protein, which alters protein function (Imamoto and Kataoka 2007).

Due to the limited distribution of PYP, there is currently limited evidence for its association with c-di-GMP. In the genome of the gammaproteobacterium *Idiomarina loihiensis*, isolated from deep-sea samples and not expected to use photoreceptors, a gene encoding a PYP homolog is located next to a gene encoding the GGDEF domain. This protein has photochemical properties of the PYP family, and inhibition of bacterial biofilm formation was observed when bacteria were exposed to light (van der Horst et al. 2009). In addition, a protein combining PYP and GGDEF domains has been found in the thermophilic photosynthetic purple sulfur bacterium *Thermochromatium tepidum*, suggesting a possible functional link between PYPs and c-di-GMP (Kyndt et al. 2005).

Blue light photoreceptors may play different roles

Bacteria use abundant blue light photoreceptors to detect the blue light signals in the environment, regulating a wide range of biological responses. From the distribution of these blue photoreceptors, it is likely that their functions can be replaced by each other. For example, LOV homologs are generally absent in obligate anaerobes, obligate intracellular parasites, and extremophilic microbes that are infrequently stimulated by light. But there are also no LOV homologs in *Enterobacterales* and *Vibrionales*, which may have been exposed to light multiple times in their respective living environments. Instead, they encode BLUF proteins to take over blue light-sensing functions. At the same time, the results of bioinformatics analysis showed that the functional domains of BLUF proteins in bacteria were highly similar to those of LOV proteins, further confirming the previously mentioned opinion that the two blue photoreceptors may have overlapping functions (Krauss et al. 2009).

This conclusion seems to apply to other blue-light photoreceptor families as well. Compared with LOV- and BLUF-based blue photoreceptors, PYPs and cryptochromes have a very limited distribution. However, some halophilic bacteria that do not encode either LOV or BLUF, such as *H. halophila* and *Halochromatium salexigens*, have been found to encode PYP-containing proteins instead (Kumauchi et al. 2008). Therefore, the examples of c-di-GMP metabolic domains as the functional domains of blue-light photoreceptors may not be limited to LOV- and BLUF-containing proteins, and are likely to be experimentally confirmed in the other two classes of blue-light photoreceptors in the future. This also suggests that some bacteria with different blue light photoreceptors may share a common blue light avoidance mechanism.

At the same time, some bacteria encode more than one class of blue-light photoreceptors. For example, *R. sphaeroides* and *Burkholderia phytofirmans* possess both LOV-, BLUF-, and PYP-based photoreceptors (Kumauchi et al. 2008). From the available clues, although both LOV and BLUF domains are capable of coupling to c-di-GMP metabolic domains, LOV tends to be coupled to GGDEF–EAL (this coupling accounts for 20% of bacterial LOV proteins) (Herrou and Crosson 2011), while BLUF tends to couple to the EAL domain (this coupling constitutes 14% of bacterial BLUF proteins) (Gomelsky and Hoff 2011). Therefore, in these bacteria, although the blue light photoreceptors can all sense blue light to control c-di-GMP concentrations, they may play different roles in regulating the specific processes of the c-di-GMP signaling pathway.

Photosensitive CMEs have broad application prospects in synthetic biology

The light signals appear to be more controllable, safer and gentler than other inducers of bacterial behaviors and metabolism. Therefore, some researchers began to use photosensitive CMEs to explore the feasibility of regulating cellular c-di-GMP levels in bacteria and to develop tools for optogenetic applications (Ryu et al. 2017a,b, Angerer et al. 2017b). To a certain extent, light can help bacteria make important lifestyle-changing decisions. For example, it can help bacteria switch between the single-cell planktonic state and the attached biofilm state; in some pathogens, light can also affect bacterial virulence. However, a deeper understanding of the physiological significance of bacterial phototactic behaviors and the molecular mechanisms of light-dependent signal transduction is still required if we want to interfere with the lifestyle of bacteria to better control bacterial virulence and biofilms.

Summary and outlook

As the external environment changes, bacteria are challenged with fluctuations in various chemical and physical parameters, which pose a serious threat to the integrity and metabolic state of cells (Klinkert and Narberhaus 2009, Cornforth and Foster 2013). Therefore, the ability to sense and respond to environmental signals is a key factor for bacteria to adapt to an ever-changing environment to survive and reproduce. As an important nucleotide second messenger, c-di-GMP is a mediator that bridges signal perception with the cellular response and regulates the adaptive behaviors of bacteria. This process has been shown to benefit bacteria to improve their ability to infect hosts (Fu et al. 2018), fight environmental stress (Li et al. 2018), and establish symbiotic relationships (Rao et al. 2015), among others.

This review focused on those sensory domains that can sense gas and light to modulate CME activities, whether they achieve this function through direct sensing or indirect regulation. We summarized their signal transduction mechanisms and the corresponding phenotypic outputs and compared the differences between sensory domains responding to the same signals, which improved our understanding of bacterial adaptation behaviors. The existence of some unique domains (e.g. orphan H-NOX) and some bifunctional CMEs mentioned above illustrates the complexity of the bacterial signal transduction networks. Their physiological significance and related regulatory mechanisms are going to remain a key research direction in bacteriology for the foreseeable future.

In addition to the stimuli mentioned in our article, bacterial CMEs can sense and respond to other signals, such as nutrients, chemicals (Bernier et al. 2011, Heo et al. 2019), temperature (Almblad et al. 2021), and so on. In addition, the enzymatic properties of CMEs themselves can also determine whether they are affected by factors such as pH (Koestler and Waters 2014), ionic strength (Tamayo et al. 2005), and other signals. Due to the limitation of article length, they have not been discussed here.

In order to reduce the virulence of pathogens and solve the problems of antibiotic-resistant bacteria caused by biofilm formation in the medical field, researchers are trying to develop small molecule inhibitors as new antibacterial drugs targeting the c-di-GMP signaling pathway. These inhibitors mostly work by acting on the potential targets, like CMEs and c-di-GMP receptors, to block c-di-GMP signaling, but they often lack good selectivity in terms of biological activities and there is still a huge space for exploration in terms of chemical structure diversity (Xuan et al. 2021). We, therefore, advocate a new strategy, i.e. "understand what bacteria want and then use environmental signals to modulate c-di-GMP-mediated bacterial behaviors" (Galperin 2018). This strategy not only lifts the aforementioned limitations, but also helps prevent direct confrontation with bacteria during the use of traditional methods such as antibiotics, and avoiding damage to the human (animal) host (Galperin 2018). Thus, further understanding of the complete c-di-GMP-mediated signal transduction pathway and its roles in bacterial physiology and host–pathogen interactions may also provide good new targets for rational drug design.

More importantly, mastering the specific mechanisms by which bacteria sense and respond to signals, as well as the corresponding phenotypic outputs, is also beneficial to the development of new biosensors suitable for synthetic biology to a certain extent. These signaling CME-derived biosensors, especially related optogenetic tools, will enable rapid and reversible modulation of bacterial cellular c-di-GMP concentrations, thereby regulating bacterial physiological activities with a higher spatiotemporal resolution. Researchers can use these biosensors to regulate bacterial adaptation behaviors by exerting specific signals, so as to induce the formation of biofilms that can be used as living catalysts (Halan et al. 2012) and improve the colonization efficiency of intestinal probiotics (He et al. 2018).

However, there are still many problems in the practical application of the above strategy. First, different bacteria may respond differently to the same environmental signals, making it difficult to model the link between the initial signal and the final phenotypic output. Second, bacteria face a rapidly changing environment in the process of infecting the host, and not all the signals are controllable. Finally, and most importantly, the regulatory behavior of bacteria is inherently very complex. Notably, there are other enzymes (such as oligoribonucleases, which serve as degrading enzymes for the linear intermediate pGpG in c-di-GMP signaling) that can affect c-di-GMP concentrations, and their activities are also affected by environmental signals (Orr et al. 2018). In addition to regulating c-di-GMP levels, the same environmental signals can also regulate other cyclic dinucleotide messengers (e.g. c-di-AMP) and even other signal transduction pathways (Zhulin et al. 2003, Rao et al. 2010). Moreover, there is also a cross-talk between these second messenger networks and other signal transduction systems. For example, the DGC activity of Lcd1 from *Leptospira interrogans* and the PDE activity of Bd1971 from *Bdellovibrio bacteriovorus* can both be activated upon binding of cAMP to their sensory domains (Cadby et al. 2019, da Costa Vasconcelos et al. 2017); c-di-GMP and (p)ppGpp, the messenger molecule in the stringent response, appear to be antagonistic (Boehm et al. 2009). These problems all reduce the feasibility of the practical application of this strategy to control bacterial

behavior. Therefore, there is still a long way to go before we can truly domesticate bacteria, and more research is needed in this field.

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Supplementary data

Supplementary data are available at FEMSRE online.

Conflicts of interest statement. None declared.

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