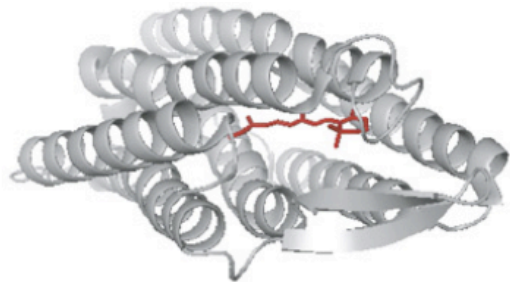


Light-sensing mechanisms in prokaryotes

An overview and possible
applications

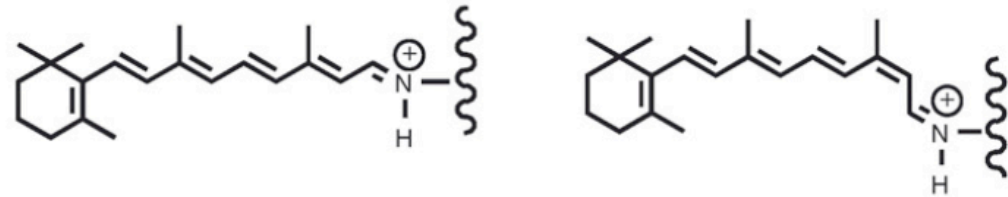
Major classes of protein domains

Protein domain



Chromophore

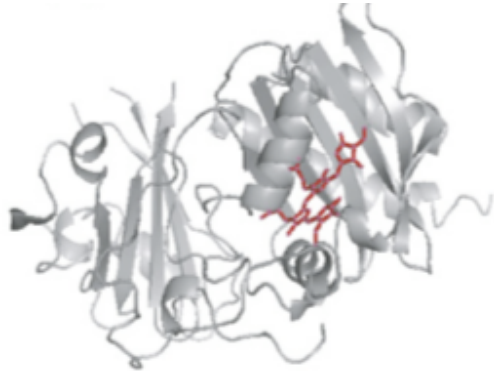
retinal



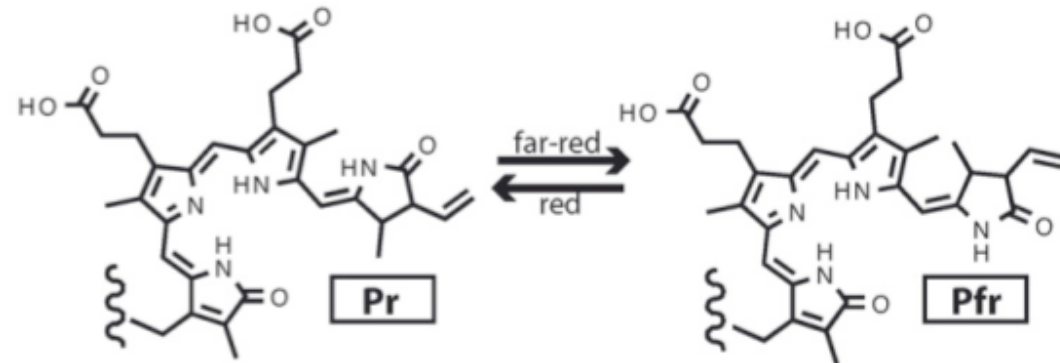
Bacteriorhodopsin :

- First found in haloarchaea
 - Quite an easy mechanism to create a proton gradient through the use of light (through the isomerisation of retinal)
 - Absorbing mostly green light
- Some Bacteriorhodopsin-like genes have been found in other organisms. There are named **proteorhodopsins**. They have been expressed in *E.Coli*

Bacteriophytochrome



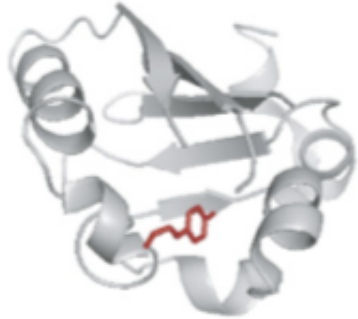
Bilin derivative



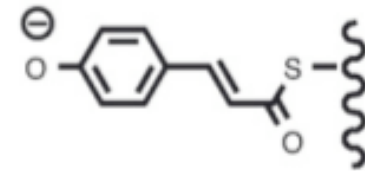
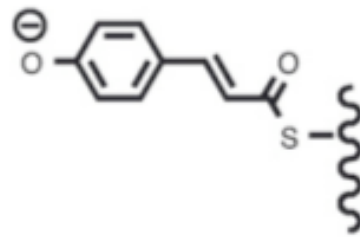
Bacteriophytochrome:

- Found in many different types of bacteria
- Based on the isomerisation of the chromophore
- Most output domains are HK and EAL-GGDEF (involved in cyclic di-GMP hydrolysis)
- Absorbing red and far-red light

PYP



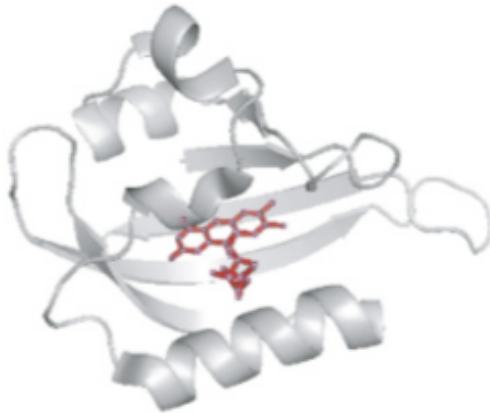
p-coumaric acid



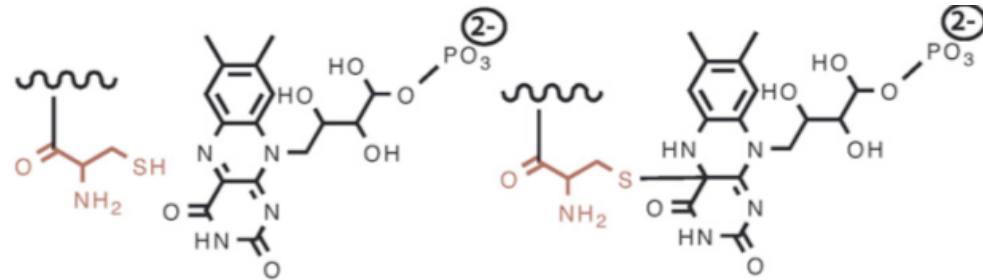
PYP domain:

- Based on the isomerisation of coumaric acid
- Mostly absorb blue light (maximum at 446 nm)
- Usually single-domained but can also form hybrids with HisKA, GGDEF or EAL output domains

LOV



Flavin



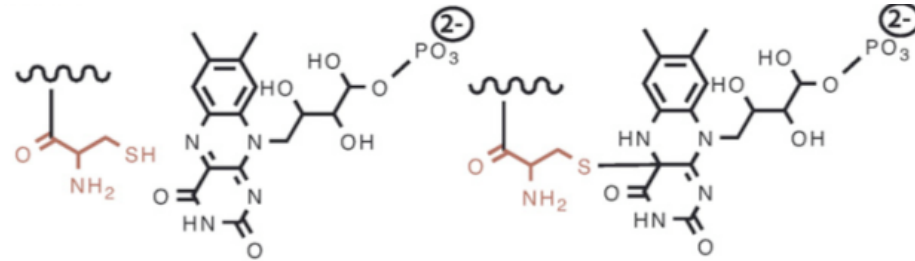
LOV-family:

- First discovered in plant phototropin and more recently in bacteria
- Based on the transitional formation of a covalent bond between the cysteine and the chromophore (flavin)
- It is a subclass of the PAS domain (large class of sensing and protein interaction based on the use of a co-factor)
- Named after the input signal of this domain (Light, Oxygen and Voltage)
- *B. Subtilis* has a LOV domain (in photoreceptor YtvA)

BLUF



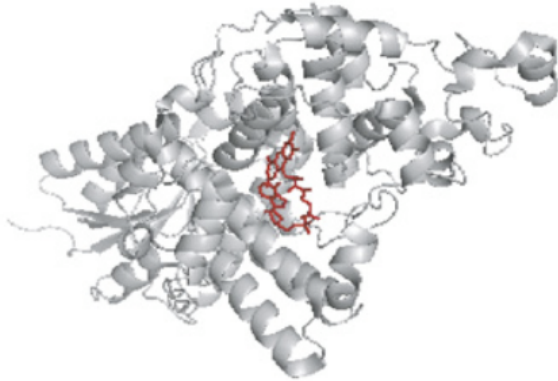
Flavin



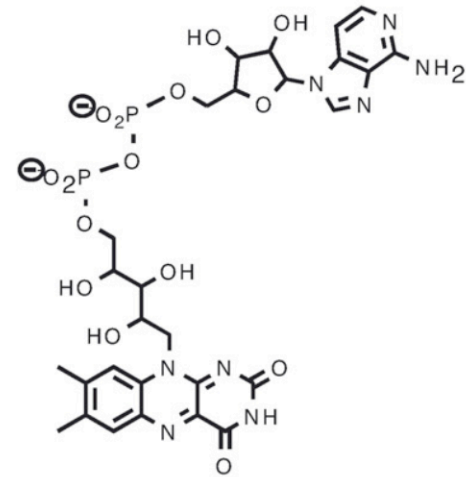
BLUF-domain:

- Similar structure with the PAS domain
- Found in *Rhodobacter sphaeroides*
- The name comes from Blue Light sensing Using Flavin
- Logically, this domain senses blue light
- *E. Coli* has a BLUF domain (more precisely the YgcF photoreceptor protein)

Cryptochrome



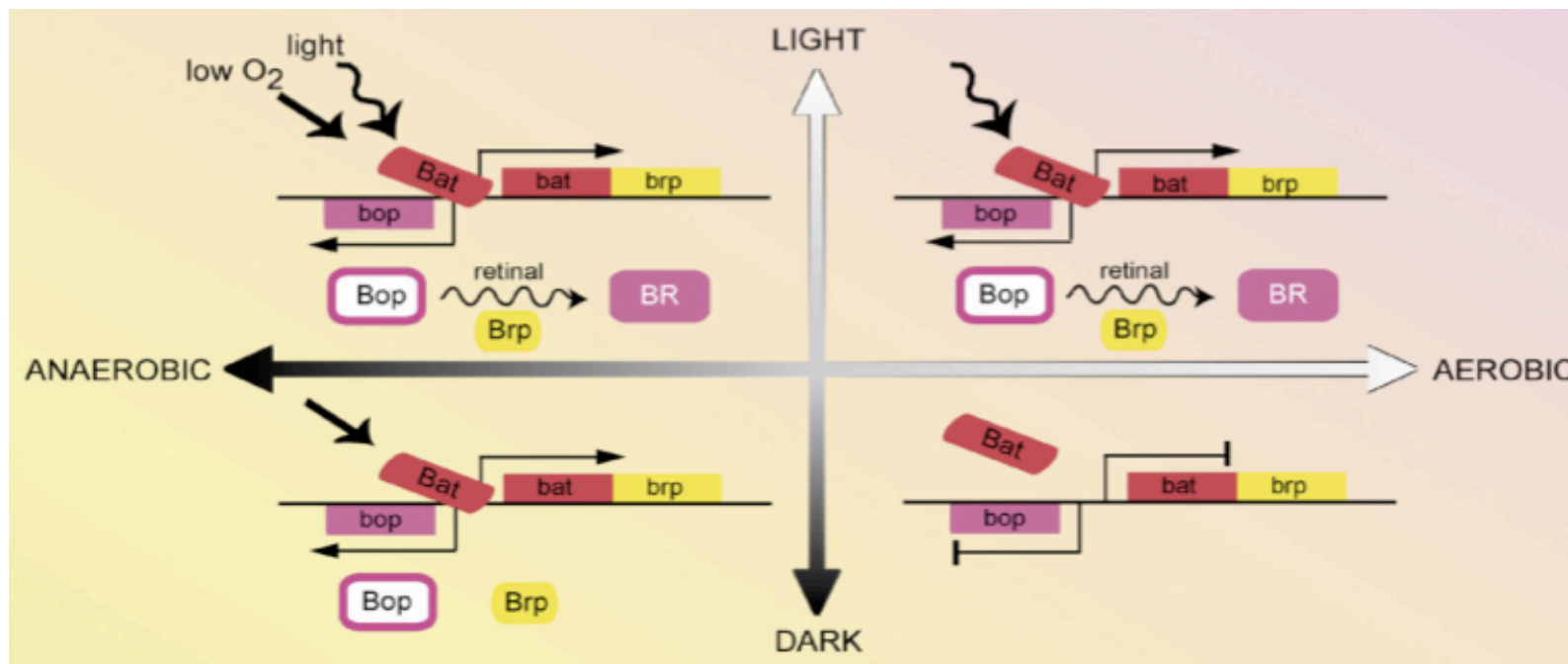
FAD



Cryptochrome:

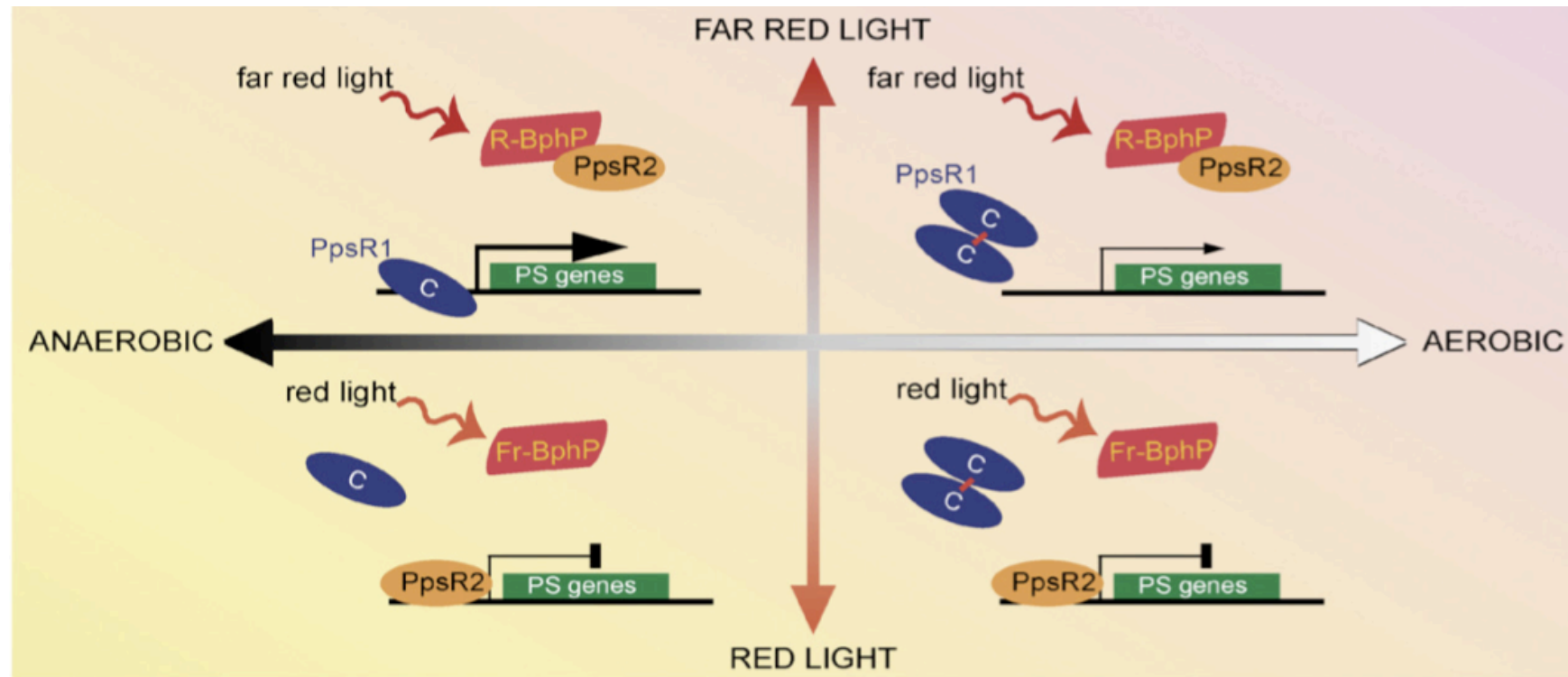
- Found in many different organisms, like plants and animals as well as in bacteria
- The chromophore is a flavin combined with pterin
- Absorbs in the violet-blue domain of the spectrum
- Light activates a phosphorylation which then might have gene regulation output

BR regulation in *H. Salinarum*



We notice that in fully aerobic and/or dark conditions, the bacteria doesn't synthesise the protein

Bacteriophytochrome and PS regulation in *C. Bradyrhizobium*



Once again we see that the expression of the photosystems genes are regulated by light and oxygen conditions

Examples of bacteria with domains

	BLUF	LOV	PYP	Phytochrome	Sensory rhodopsin		BLUF	LOV	PYP	Phytochrome	Sensory rhodopsin
<i>Acidovorax avenae</i>	2	1		1		<i>Nitrosococcus oceani</i>		1			
<i>Acidovorax</i> sp. JS42	1					<i>Nitrospira multiformis</i>		1			
<i>Acinetobacter baumannii</i>	1					<i>Novosphingobium aromaticivorans</i>		2			
<i>Acinetobacter</i> sp. ADP1	4					<i>Oceanicola batsensis</i>				1	
<i>Agrobacterium tumefaciens</i>				3		<i>Oceanicola granulosus</i>	1			1	
<i>Alkalilimnicola erlichii</i>		1		1		<i>Oceanobacillus iheyensis</i>		1			
<i>Alpha proteobacterium</i> HTCC2255	1					<i>Oceanobacter</i> sp. RED65	1	1			
<i>Azoarcus</i> sp. EbN1						<i>Polaromonas</i> sp. JS666	2				
<i>Bacillus subtilis</i> subsp. <i>Subtilis</i>		1				<i>Pseudoalteromonas atlantica</i>	2			1	
<i>Bdellovibrio bacteriovorus</i>	1			1		<i>Pseudoalteromonas tunicata</i>	2				
<i>Blastopirellula marina</i> DSM 3645				1		<i>Pseudomonas aeruginosa</i>				1	
<i>Brucella melitensis</i>		1				<i>Pseudomonas entomophila</i>				1	
<i>Erythrobacter</i> sp. NAP1		1		1		<i>Rhodoferax ferrireducens</i>	1				
<i>Escherichia coli</i>	1					<i>Rhodopirellula baltica</i>					
<i>Exiguobacterium sibiricum</i>					1	<i>Roseovarius</i> sp. HTCC2601				1	
<i>Flavobacterium johnsoniae</i>				2		<i>Sagittula stellata</i>	2			1	
<i>Flavobacterium</i> sp. MED217				1		<i>Shewanella baltica</i>		2			

Possible applications

Photo-sequenced protein synthesis

- Use a pre-existing light-triggered mechanism (in *E. Coli* or *B. Subtilis*) to act on the synthesis of a protein (e.g. the YcgF mechanism).
- A second light receptor could be added from another bacterium (if possible at the other end of the light spectrum e.g. bacteriophytochrome) that would be linked to another pathway (leading to the formation of another product/enzyme)
-
- Industrial Use: having a feedback on the bacteria's production. A sensor in the bioreactor could calculate the concentration of a product (or toxic byproduct) and report back to the bacteria (inhibit or activate the production) through light control
- « Easy » and fast way to « communicate » with the cell
- There would also be the possibility to have a sequenced production. A informatic system would switch from one light to another inducing one or expression or another

Advantages :

- Non invasive process, no risk to harm the cells (in opposite to chemical inductors)
- Faster process than chemicals (no need to wait for diffusion in the reactor for example)
- Light is easily produced

Disadvantages :

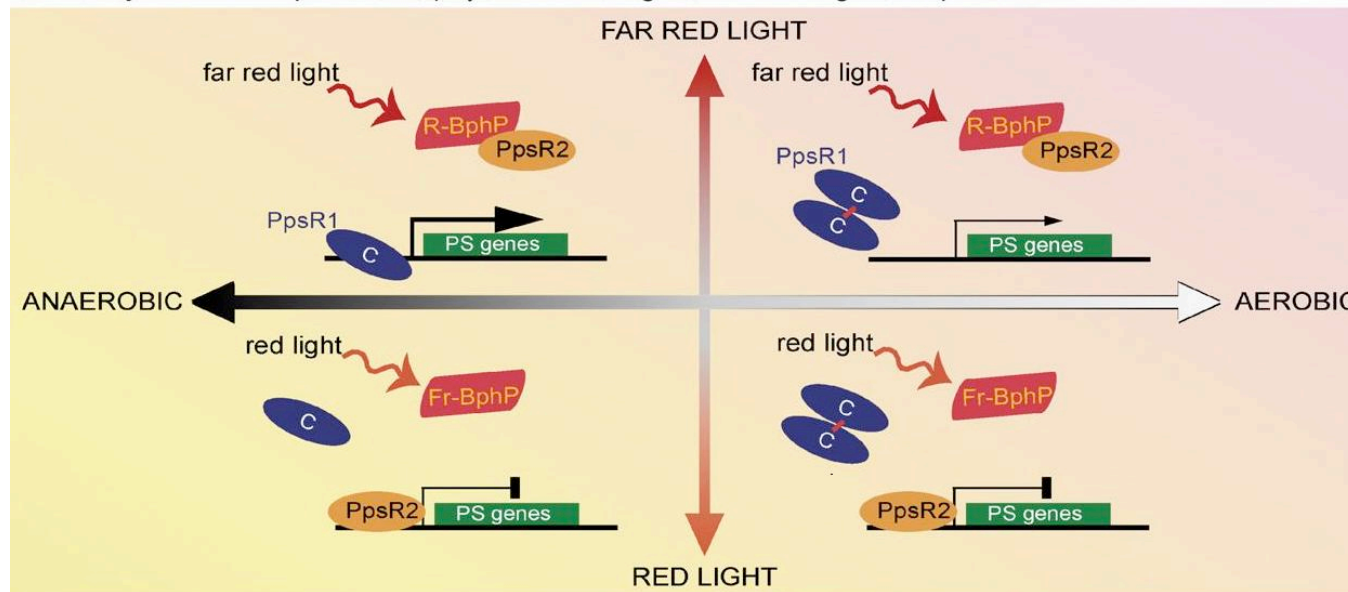
- The two spectrum domains of the different photoreceptor must be as far away as possible (to reduce the risk of interferences)
- Some mechanisms are anaerobic-dependant. The concentration of oxygen must be kept low

Combination w/ magnetotactic bacteria

- For diagnostics, or sample screening, we don't always need the whole set of receptors --> Subset of receptors expressed depending on light.
- Regulation by light could also permits a low-budget project. Eg: using LED or translucent paper (permit utilization of standard bulb).
- Oxygen level is very critical for vesicles' formation in magnetotactic bacteria --> We need a gene regulation system which is related to the light differences rather than the aerobic condition.

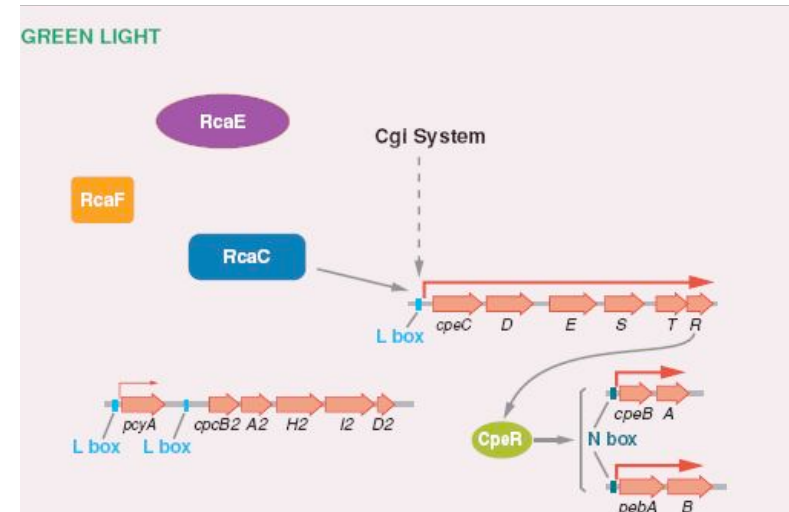
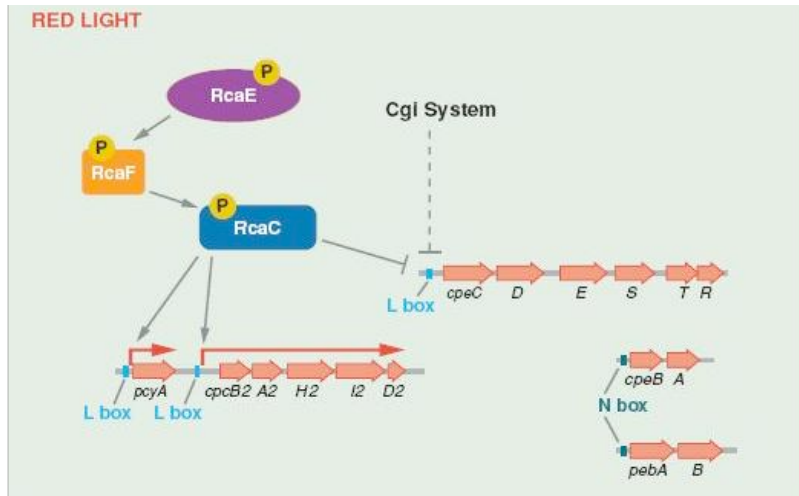
C.Bradyrhizobium

C. *Bradyrhizobium* sp. bacteriophytochrome regulation of PS gene expression



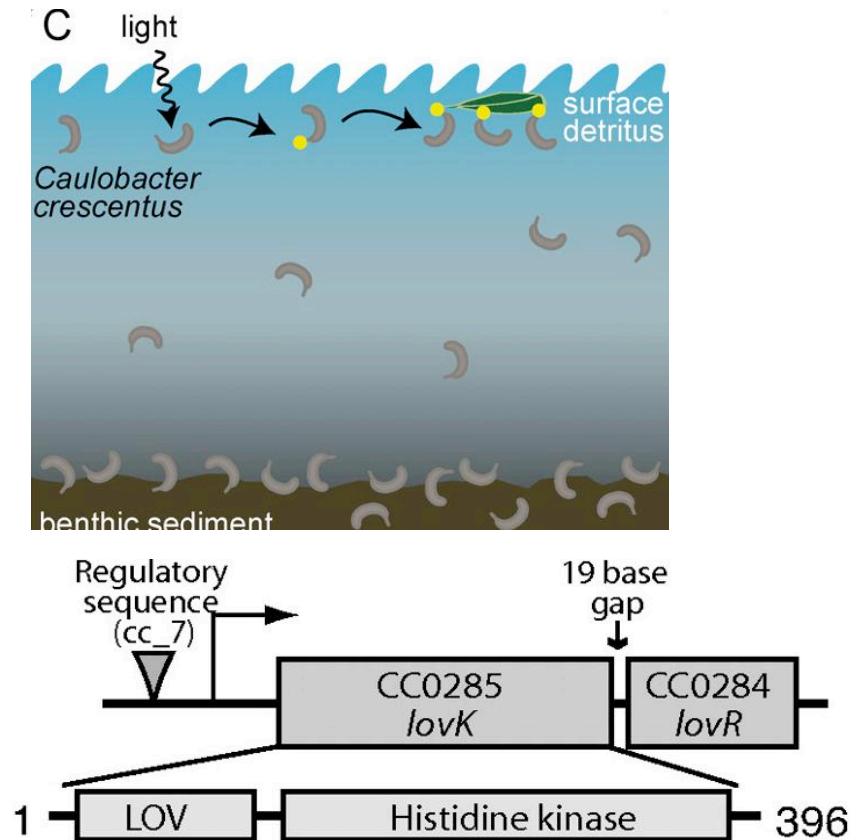
- The result is more correlated to the light differences than the oxygen level: Far-red --> Expression; Red --> Repression.

Fremyella diplosiphon



- Pigmented proteins depend on the color of ambient light --> Insert receptor genes downstream of the pigmented proteins.

Caulobacter crescentus



- Exposing cells that are coordinately overexpressing LovK and LovR to blue light enhances the attachment capacity of these cells.
- Light regulates LovK kinase activity.
- LovR regulates cell-surface attachment.

The BLUF-EAL protein YcgF in *E. coli*

A few fast facts:

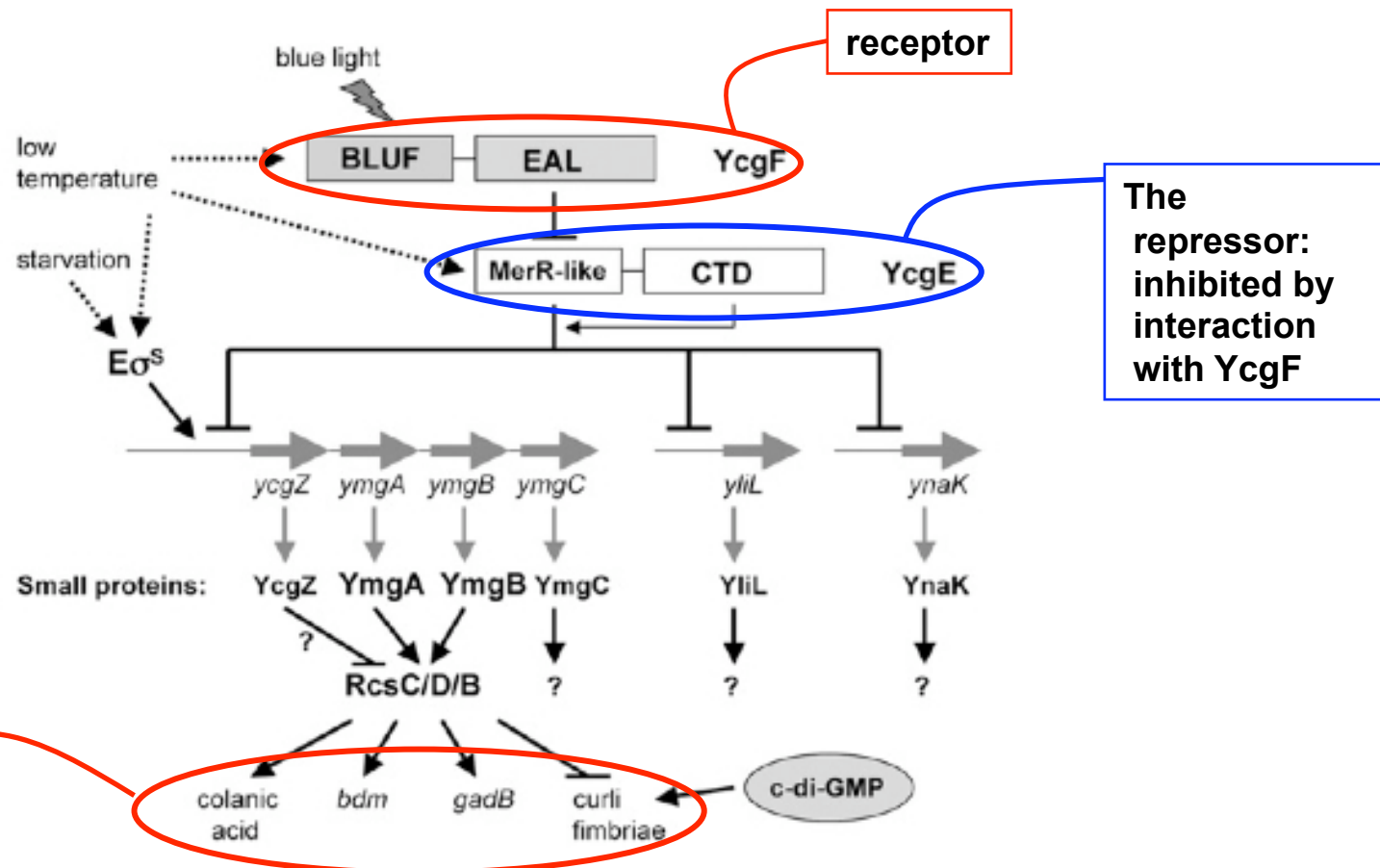
- YcgF: blue-light sensor of *Escherichia coli*
- BLUF: receptors that sense **Blue Light** by **Utilizing FAD** as a chromophore
- EAL: domain involved in c-di-GMP (cyclic di-guanosine monophosphate) hydrolysis
- c-di-GMP: global second messenger used by bacteria to control multicellular behavior



The receptor in reality doesn't interact with c-di-GMP, but instead functions as an anti-repressor in a pathway involved in biofilm formation/maturation.

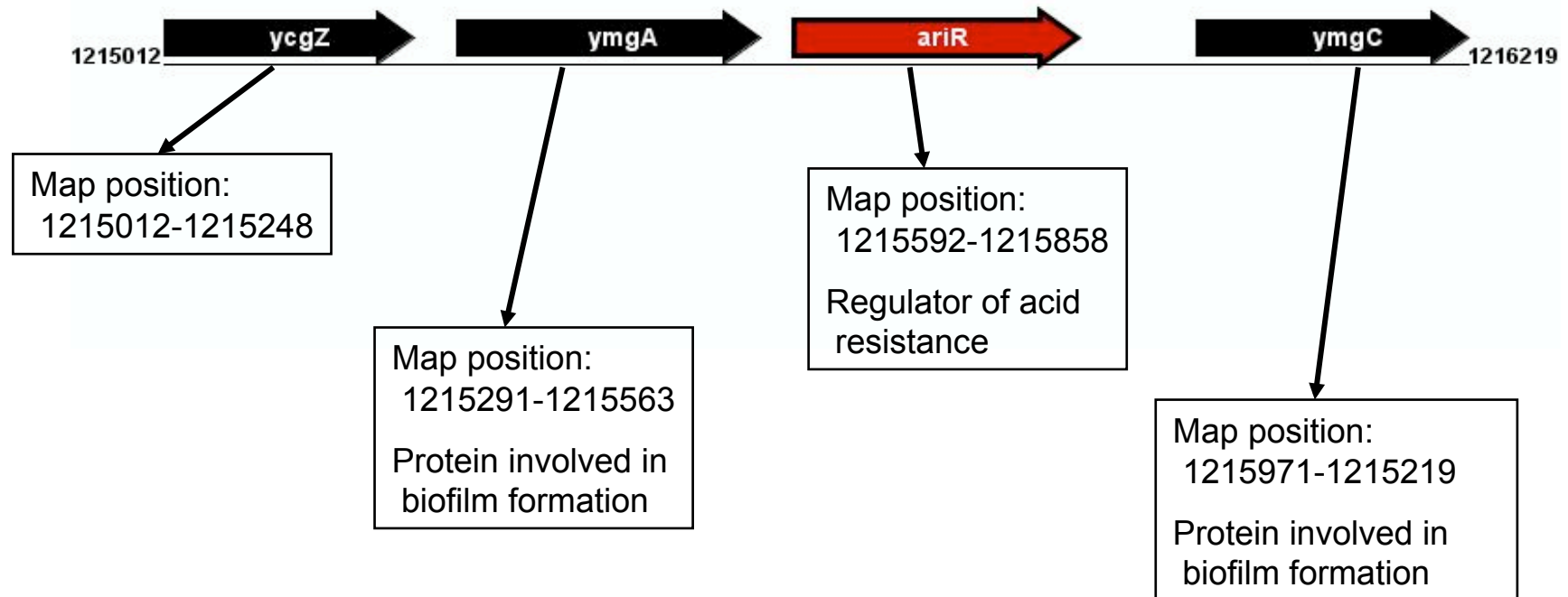
The BLUF-EAL protein YcgF in *E. coli*

The YcgF pathway:



The BLUF-EAL protein YcgF in *E. coli*

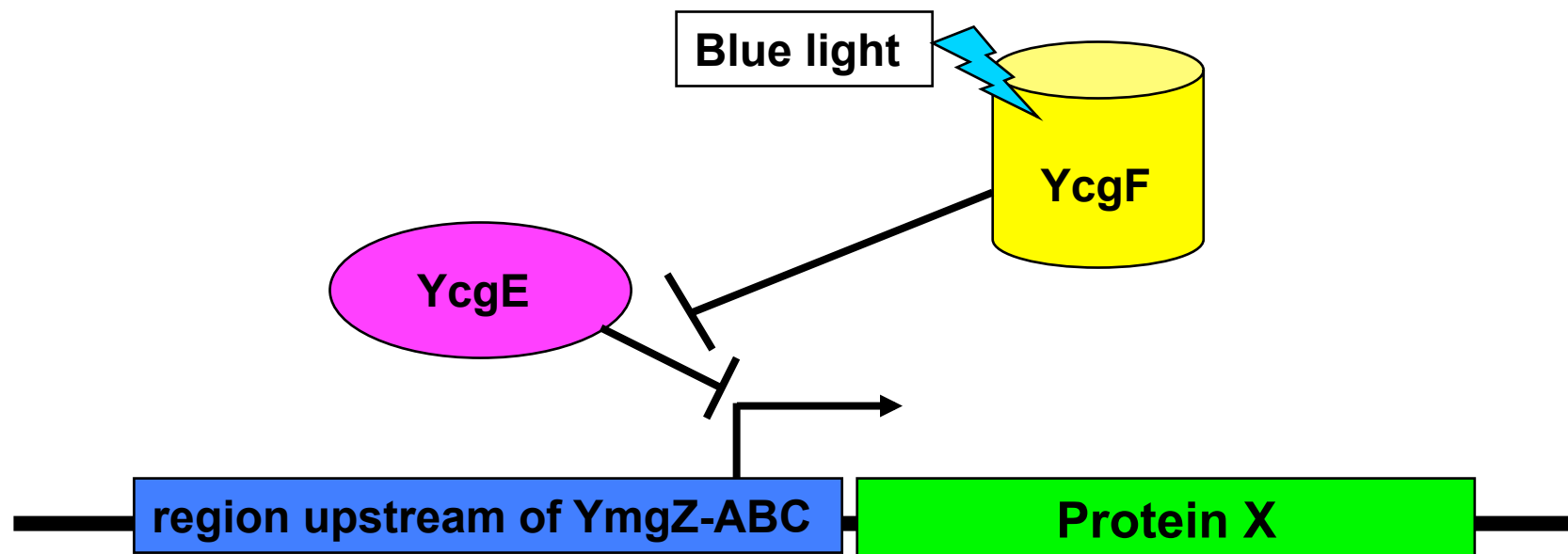
The YcgZ-ABC operon in *Escherichia coli*:



The BLUF-EAL protein YcgF in *E. coli*

The project idea:

Link the YcgF pathway with the biofilm subject by promoting the secretion of a protein that could give interesting properties to the film to the YcgF pathway: use a promoter repressed by YcgE.



The BLUF-EAL protein YcgF in *E. coli*

Advantages:

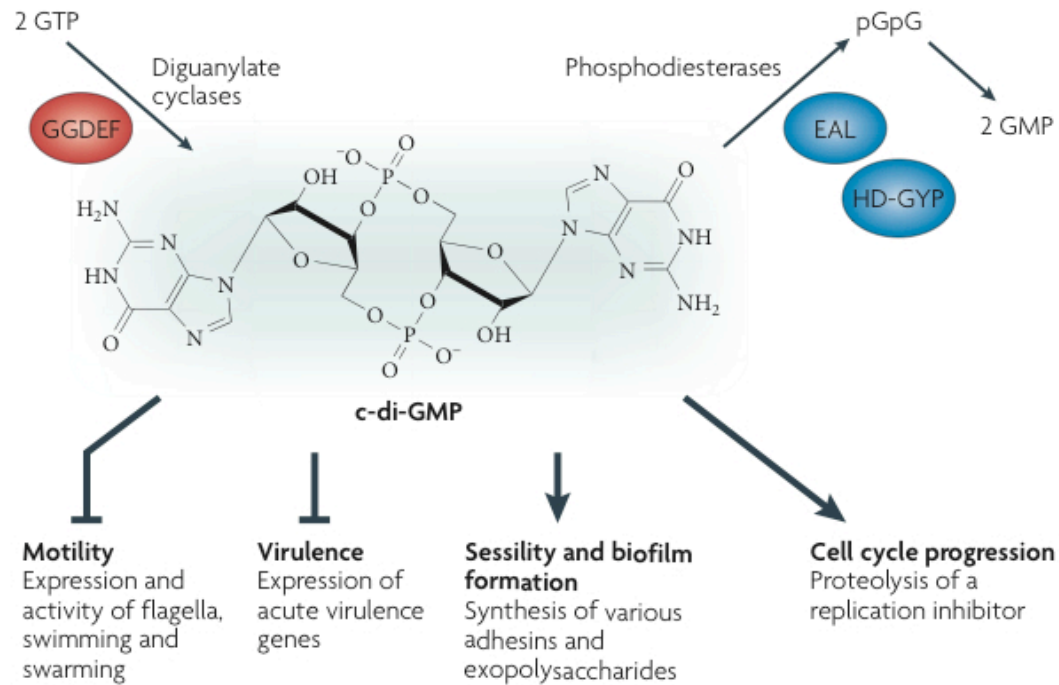
- It is a native pathway in *E. coli* (which is easy to manipulate)
- This type of mucosal biofilm is “naturally” induced by blue light: we could think of applications for an aquatic environment

Possible problems:

- Articles very recent (January & April 2009) so not much info about the sequences and proteins
- We would need to “characterize” the type of biofilm the bacteria form when the YcgF pathway is active
- The pathway seems to be part of a stress-response circuit: there could be interferences with other pathways

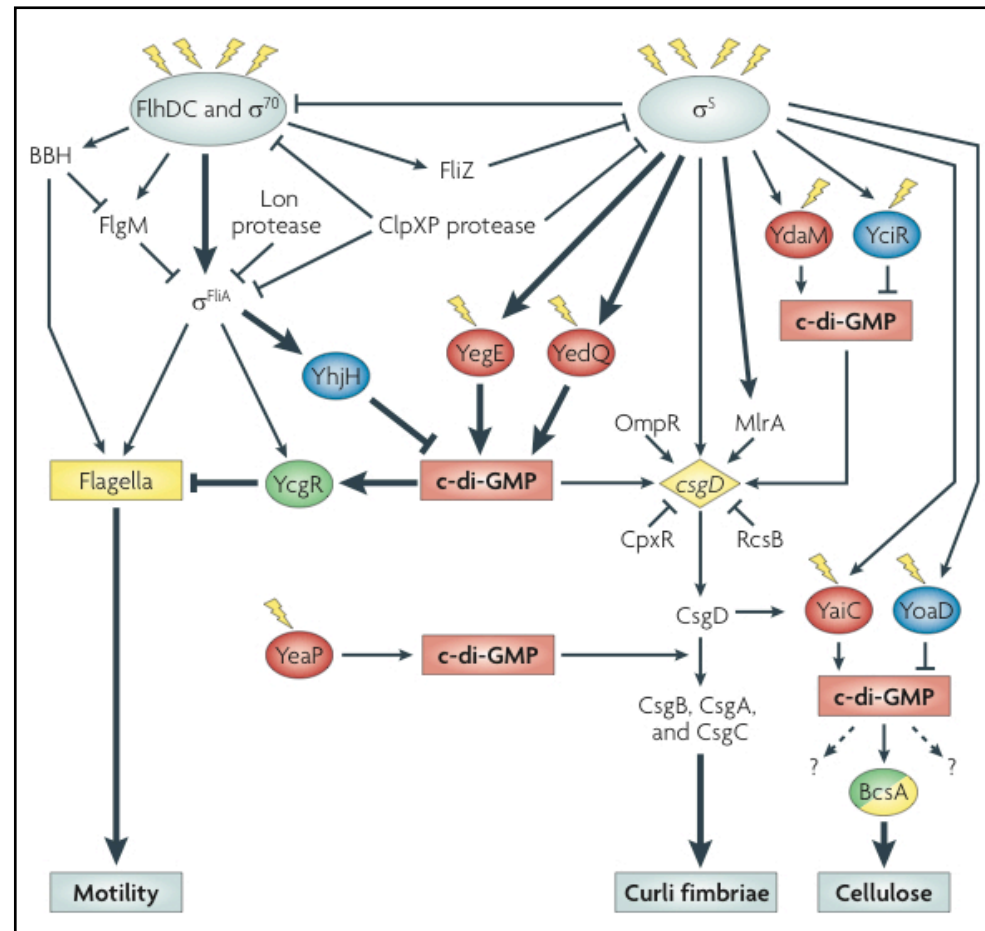
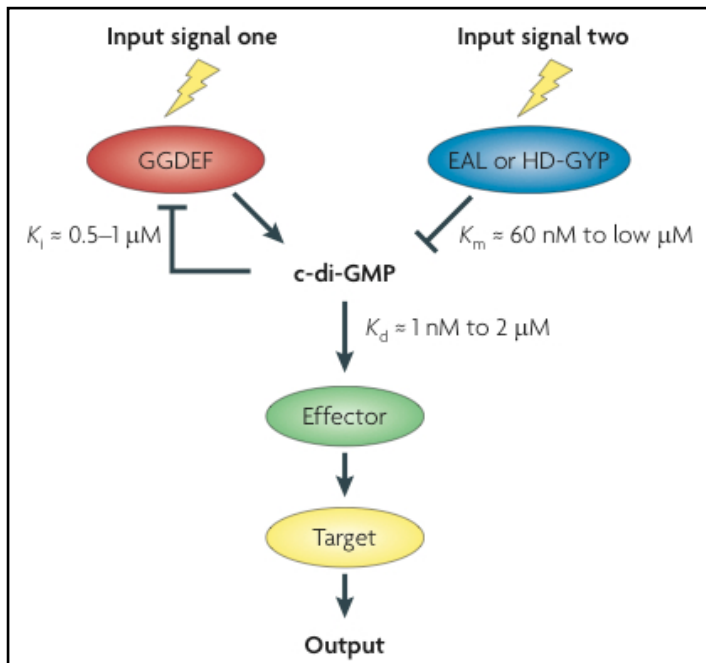
An interesting molecule for biofilms: c-di-GMP

The molecule and its roles:



An interesting molecule for biofilms: c-di-GMP

Basic (left) and more complex pathways (right, in *E. coli*):



An interesting molecule for biofilms: c-di-GMP

Examples of effects on c-di-GMP in various organisms:

Table 1 | c-di-GMP-binding effector components

Effector family*	Example	Species	Functions controlled [‡]	Refs
<i>Protein effectors</i>				
PilZ (+)	Alg44	<i>Pseudomonas aeruginosa</i>	Alginate synthesis (+)	55
PilZ (+)	BcsA	Various Gram-negative bacteria	Cellulose synthesis (+)	54,66
PilZ (+)	DgrA	<i>Caulobacter crescentus</i>	Flagellar activity (-)	67
PilZ (+)	PilZ	<i>P. aeruginosa</i>	Twitching motility (-)	115
PilZ (+)	Plz proteins	<i>Vibrio cholerae</i>	Virulence gene expression	68
PilZ (+)	YcgR	<i>Escherichia coli</i> and <i>Salmonella</i> spp.	Flagella activity (-)	69
FleQ (-)	FleQ	<i>P. aeruginosa</i>	Flagella expression (+) and Pel (part of the EPS) synthesis (-)	58
PelD (+)	PelE	<i>P. aeruginosa</i>	Pel (part of the EPS) synthesis (+)	59
I site effectors (+)	PopA	<i>C. crescentus</i>	Cell cycle progression (+)	13
<i>RNA effectors</i>				
GEMM (+ and -)	Vc1 (encoded by <i>gbpA</i>)	<i>V. cholerae</i>	Intestinal adhesion	61
GEMM (+ and -)	Vc2 (encoded by VC1722)	<i>V. cholerae</i>	Biofilm formation and rugosity	61
GEMM (+ and -)	Cd1	<i>Clostridium difficile</i>	Flagella synthesis	61

*A + indicates that the effector is activated by c-di-GMP and a - indicates that the activity of the effector is reduced by c-di-GMP. †A + indicates that the function is positively controlled by the effector and a - indicates that the function is negatively controlled by the effector. ‡ See the main text for information on the direct molecular targets contacted by the effector c-di-GMP bis (2',5') cyclic

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