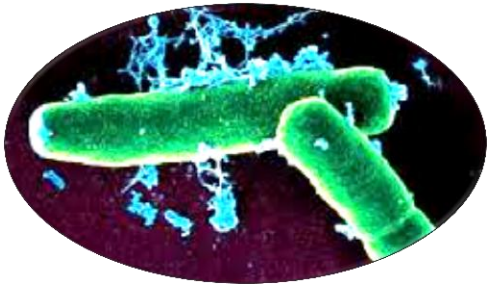
Interaction mechanism of GTP with CodY of *Bacillus anthracis*



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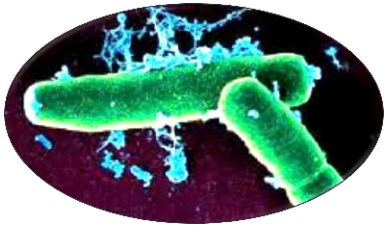
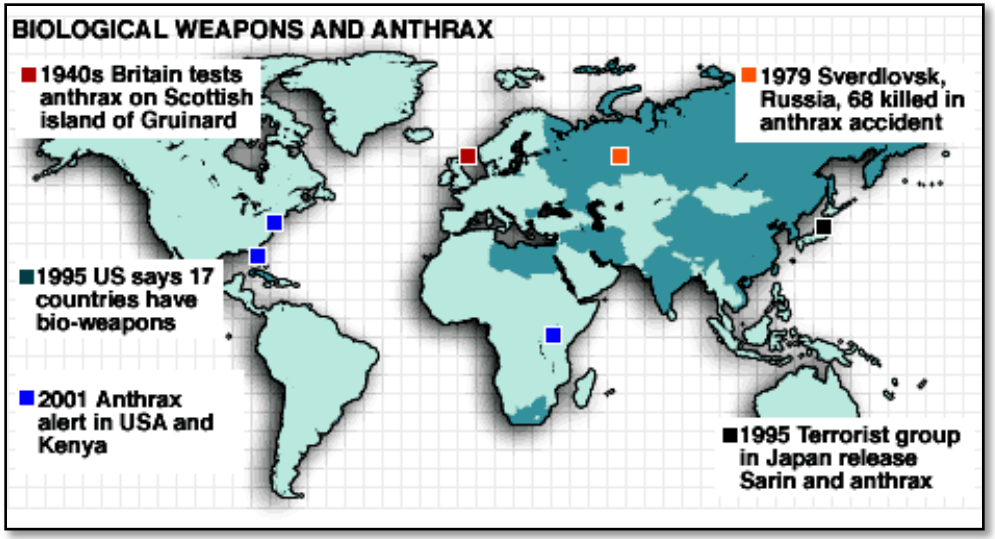
Bacillus anthracis- a bioterrorism weapon



- Gram-positive, forms spores
- Non-motile, facultatively anaerobic
- Anthrax

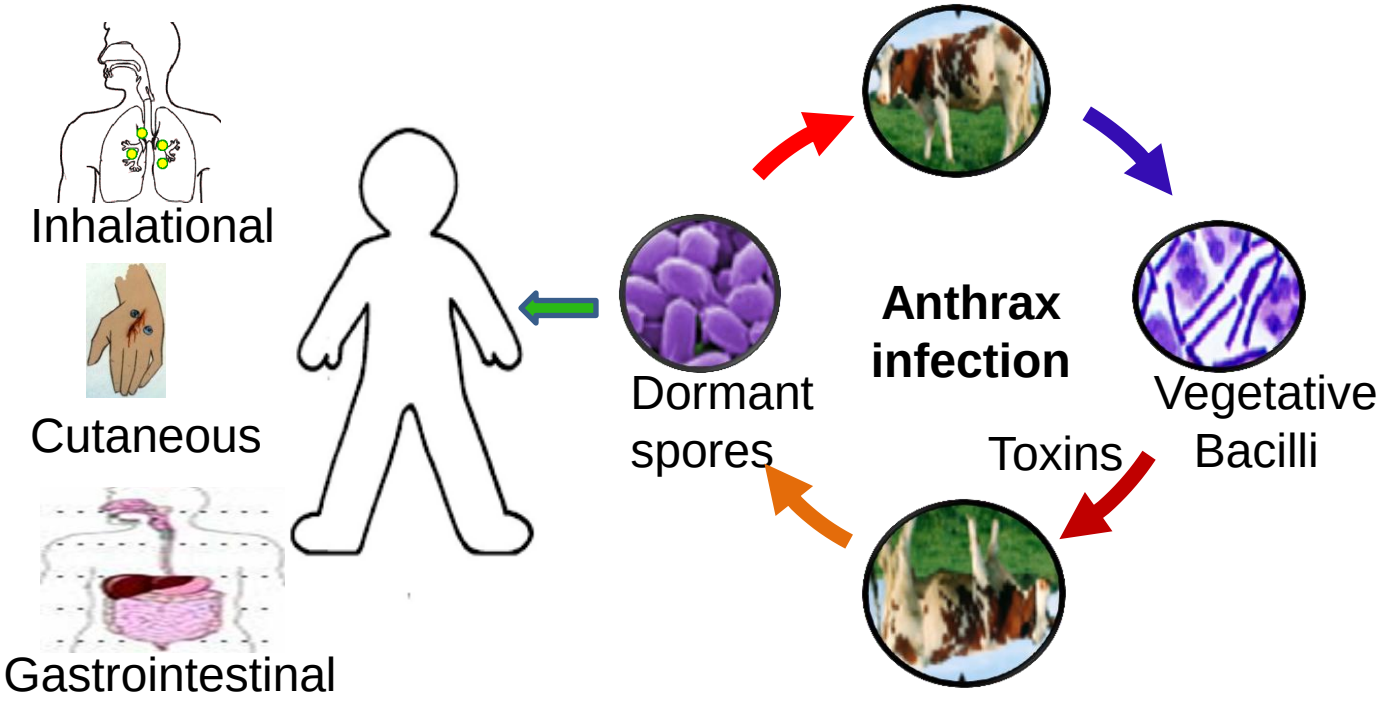
Category 'A' agent

- Ease of dissemination
- High mortality rates
- Major public health impact

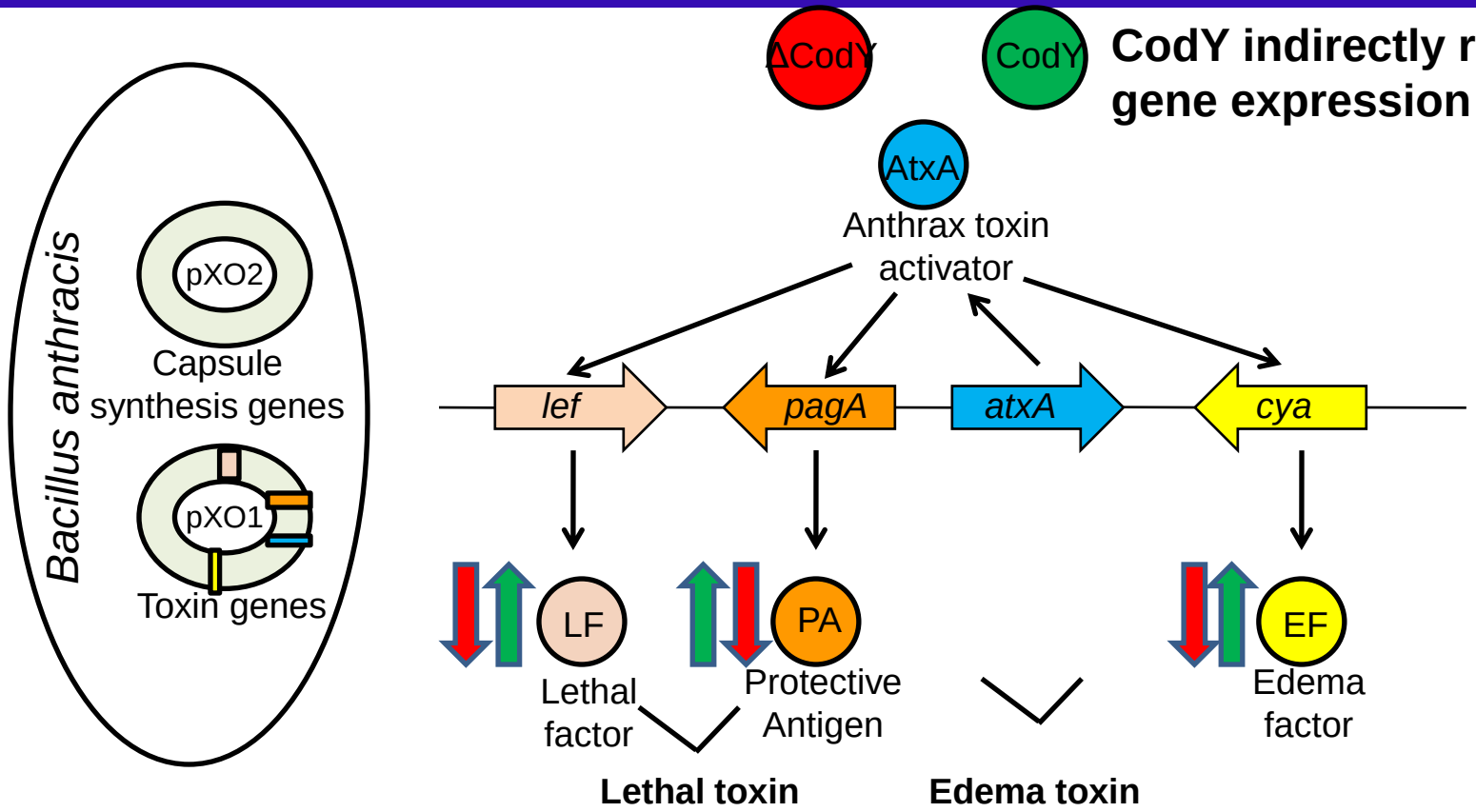


http://publpol122msamy.blogspot.in/, http://www.infectionlandscapes.org/2013/08/anthrax.html.

Anthrax- mode of transmission



Expression of toxin genes in *B. anthracis* and CodY

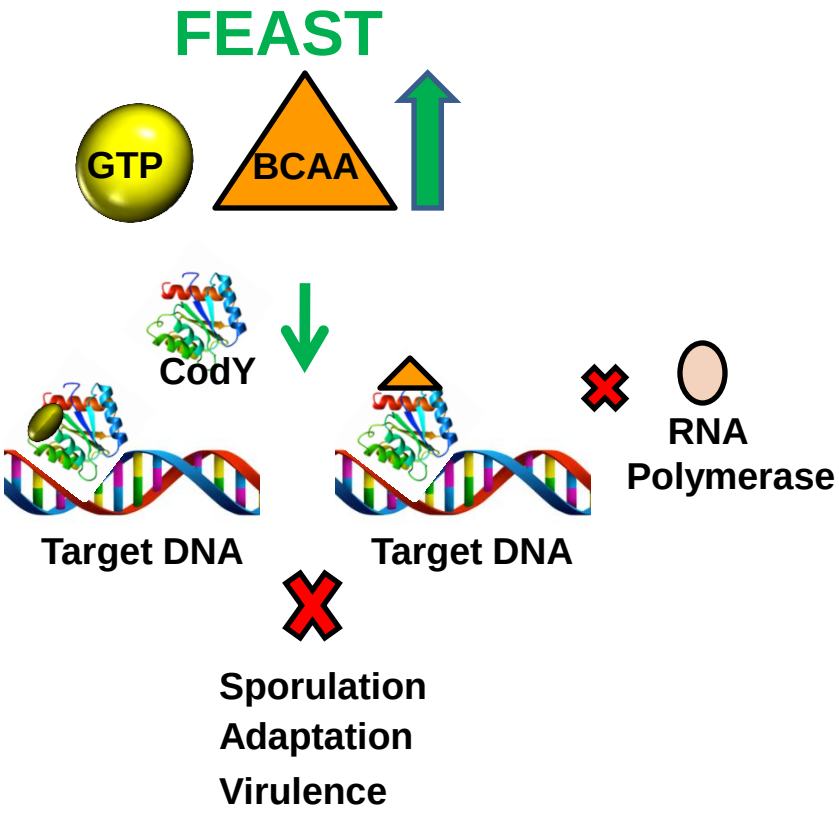


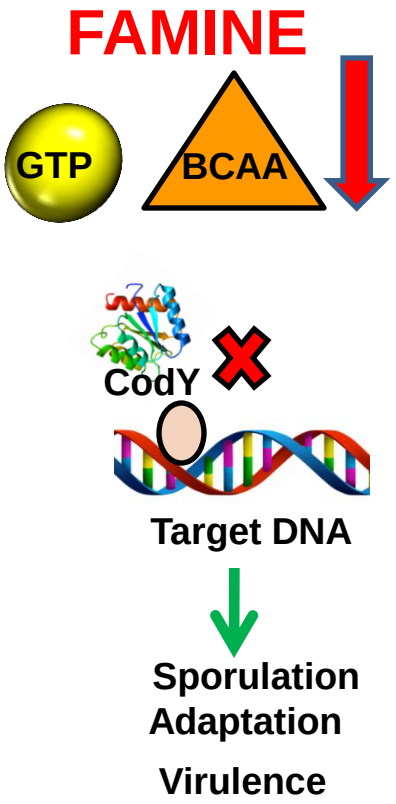
Schaik *et al.* 2009

CodY – background

- The pleiotropic transcriptional regulator found in low G+C, gram-positive bacteria.
- Regulates genes involved in metabolism, sporulation, virulence, biofilm formation.
- *B. anthracis* - ~500 target genes, including master regulator, AtxA.
- Metabolic effectors: GTP and Branched Chain Amino Acids (BCAAs).

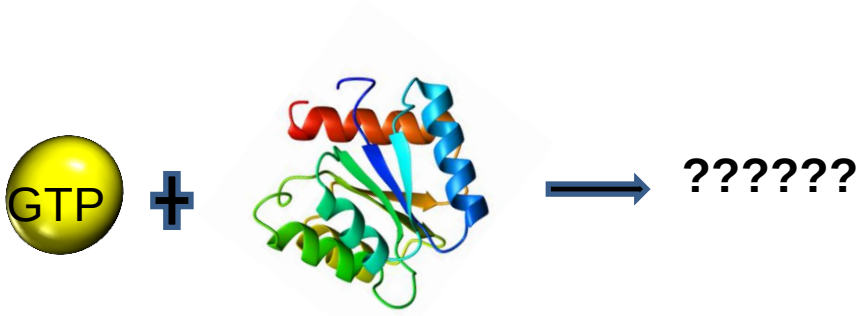
CodY – the mechanism of action



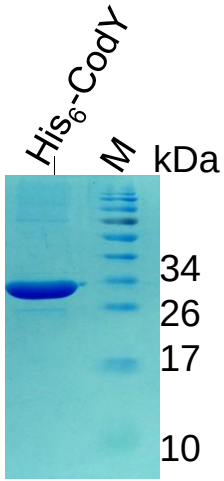


Purpose of the Study

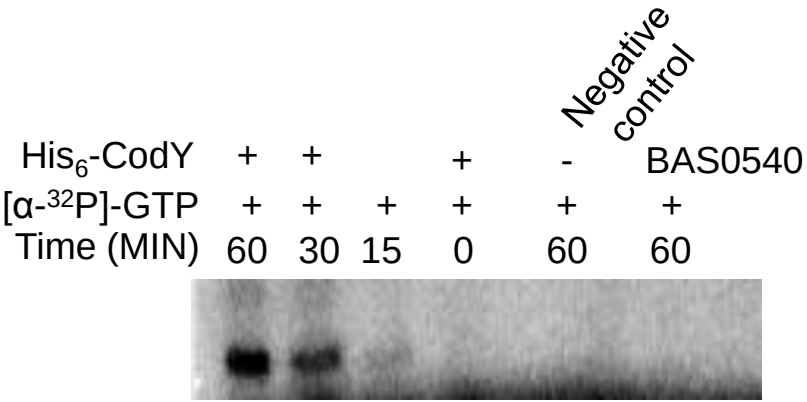
- Propose a model of interaction of CodY and GTP

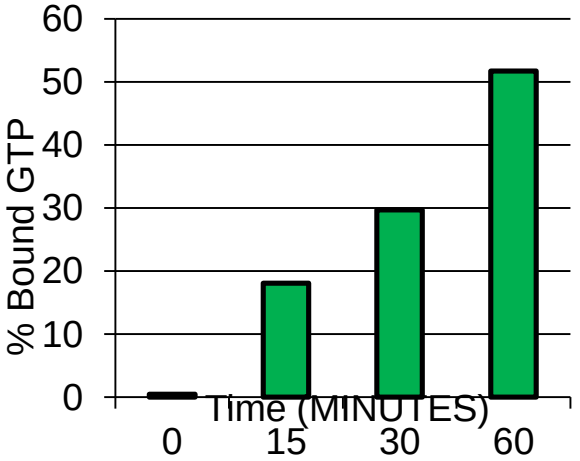


Purification of His_6-CodY



GTP binding activity of CodY



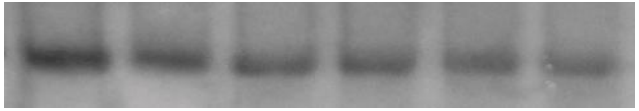


Affect of non- radiolabeled nucleotides on GTP binding activity of CodY.

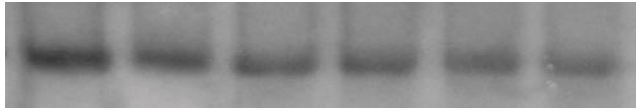
His_6-CodY	+	+	+	+	+	+
Cold GTP (μM)	0	0.1	0.5	1	2	5
[α-^{32}P]-GTP	+	+	+	+	+	+
Time (MIN)	60	60	60	60	60	60

His_6-CodY	+	+	+	+	+	+
Cold ATP (μM)	0	0.1	0.5	1	2	5
[α-^{32}P]-GTP	+	+	+	+	+	+
Time (MIN)	60	60	60	60	60	60

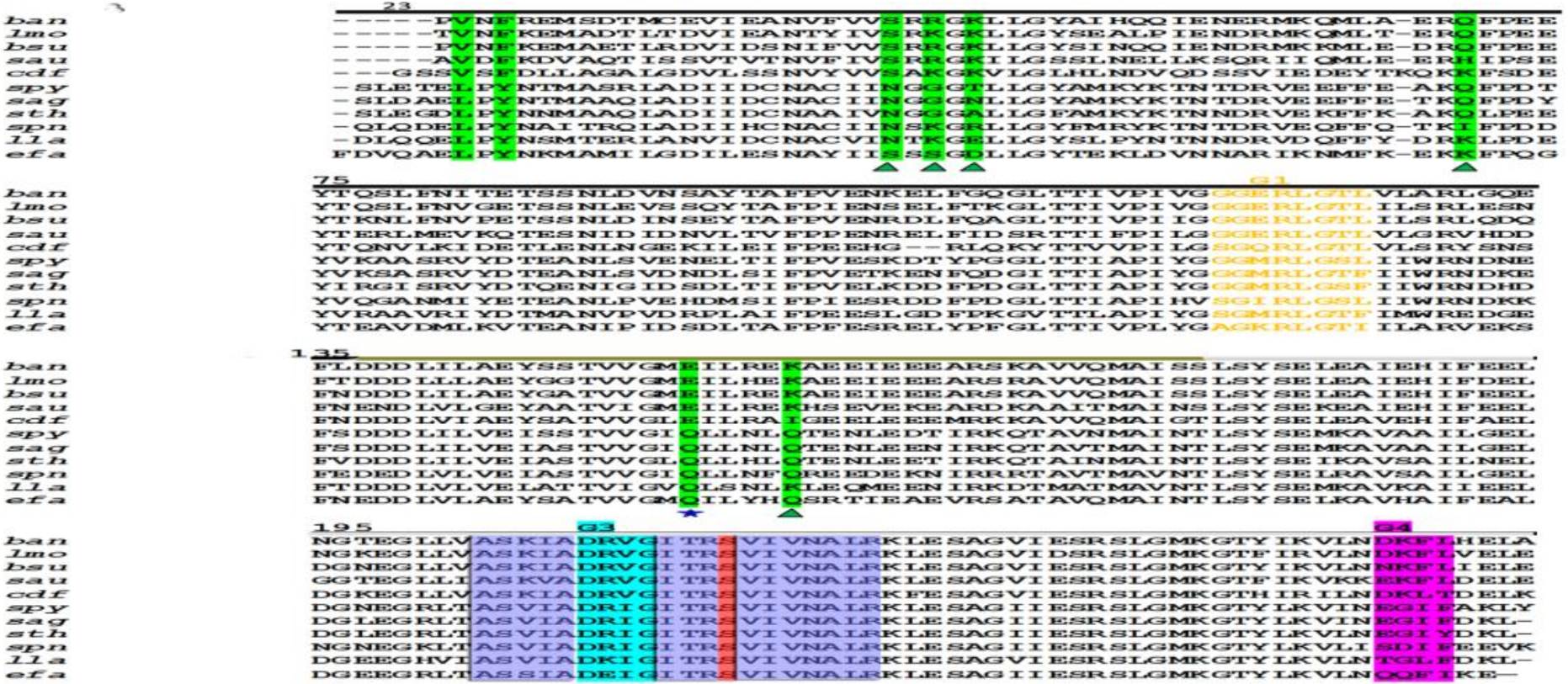
His_6-CodY	+	+	+	+	+	+
Cold TTP (μM)	0	0.1	0.5	1	2	5
[α-^{32}P]-GTP	+	+	+	+	+	+
Time (MIN)	60	60	60	60	60	60



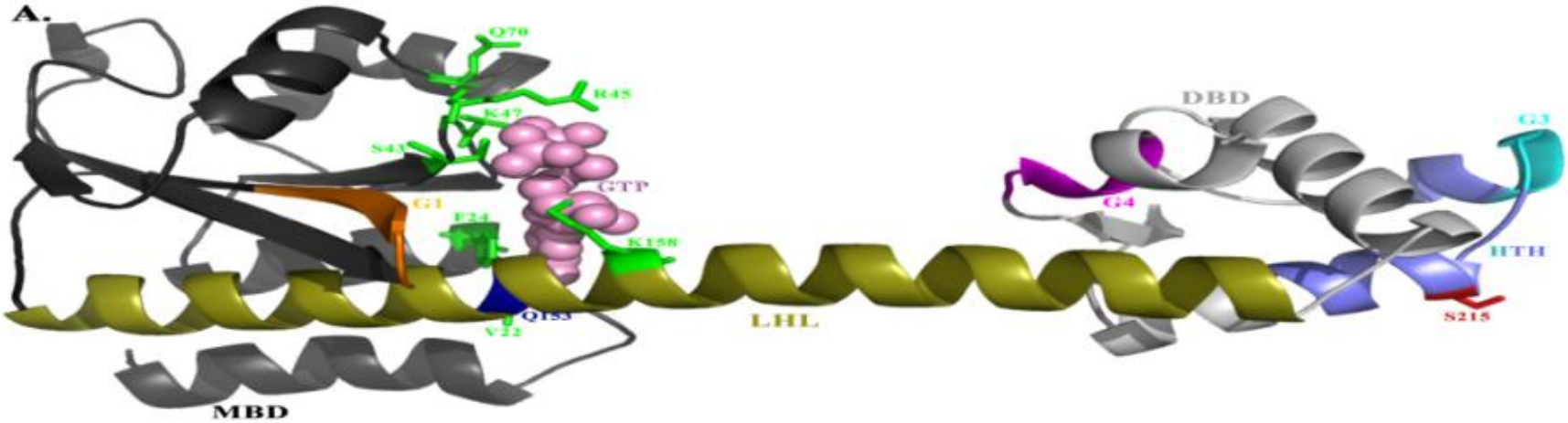
His_6-CodY	+	+	+	+	+	+
Cold CTP (μM)	0	0.1	0.5	1	2	5
[α-^{32}P]-GTP	+	+	+	+	+	+
Time (MIN)	60	60	60	60	60	60



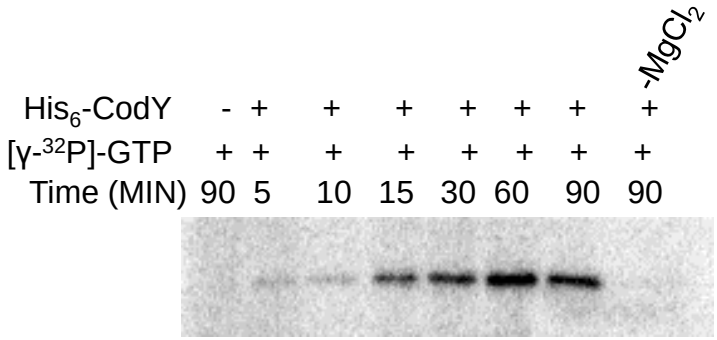
Sequence alignment of CodY with its homologs

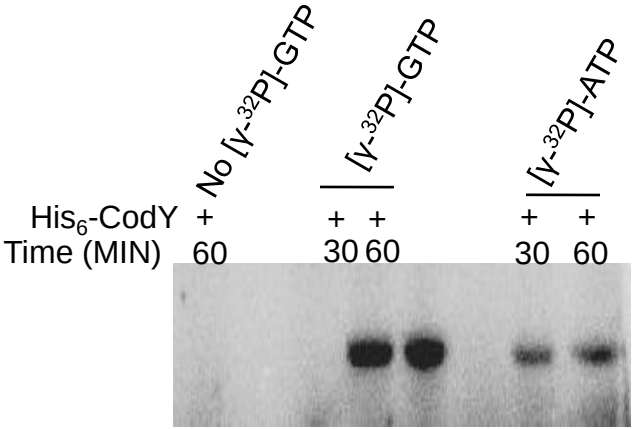


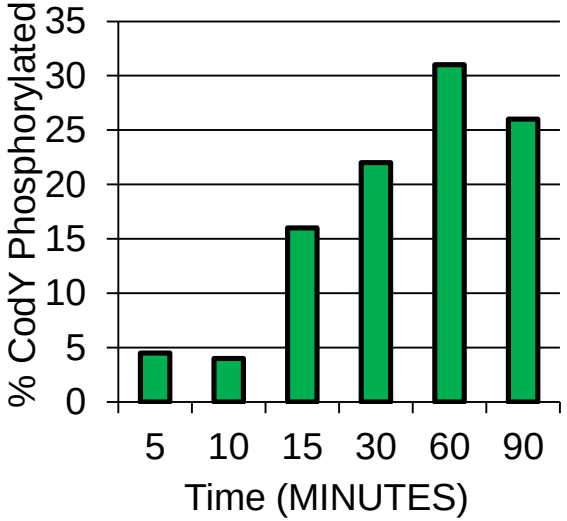
Homology model of CodY bound to GTP.



CodY autophosphorylate itself with a higher affinity for GTP.

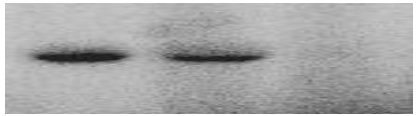






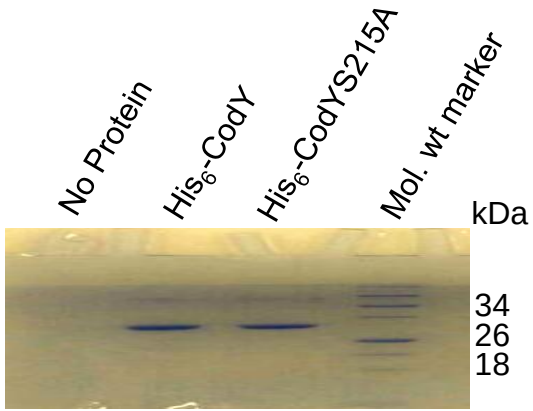
CodY undergoes serine/ threonine phosphorylation.

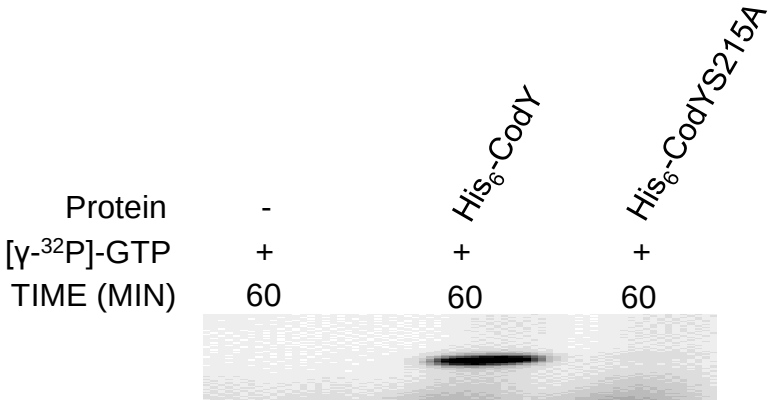
His_6-CodY	+	+	+
[γ-^{32}P]-GTP	+	+	+
HCl	+	+	-
NaOH	+	-	+
TIME (MIN)	60	60	60



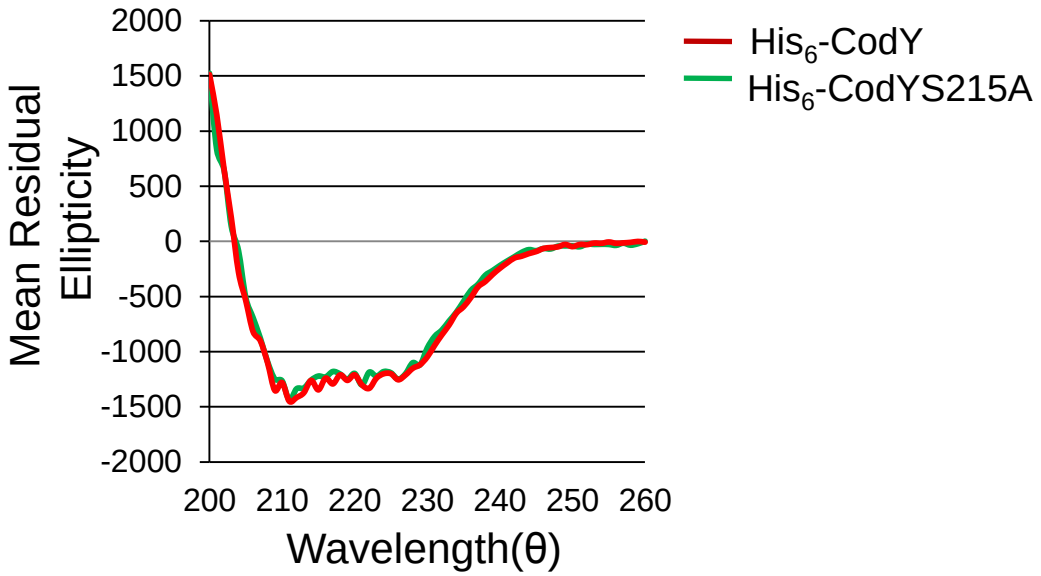
- Phosphoproteome analysis of CodY of *B. subtilis* showed phosphorylation at a conserved Serine-215 residue.

CodYS215A lacks autophosphorylation activity.

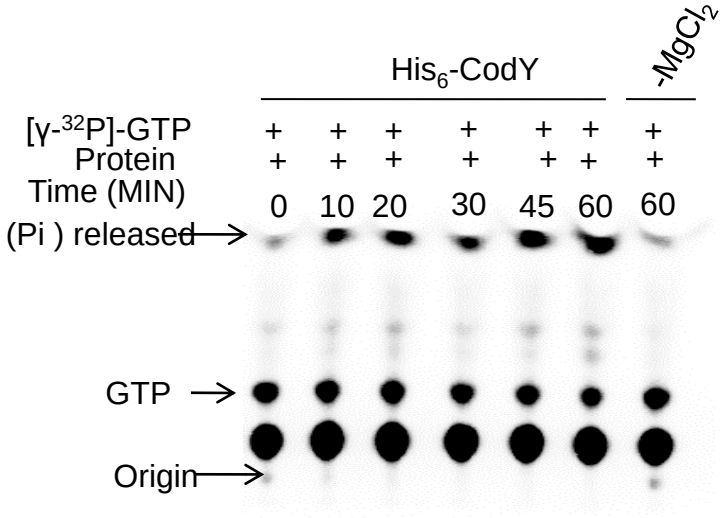


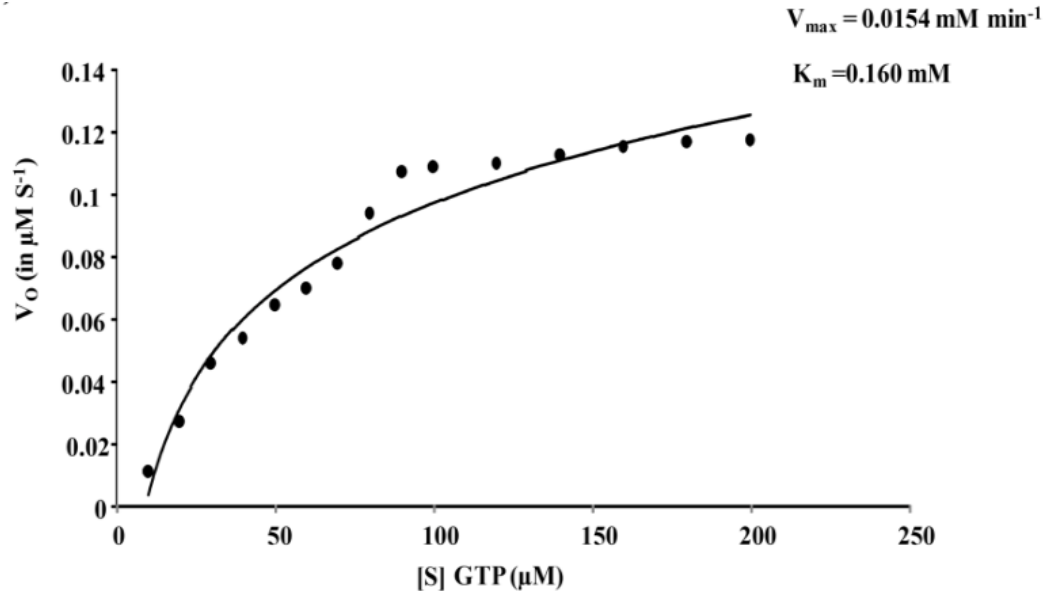


Comparison of the CD spectra of CodY and CodYS215A.

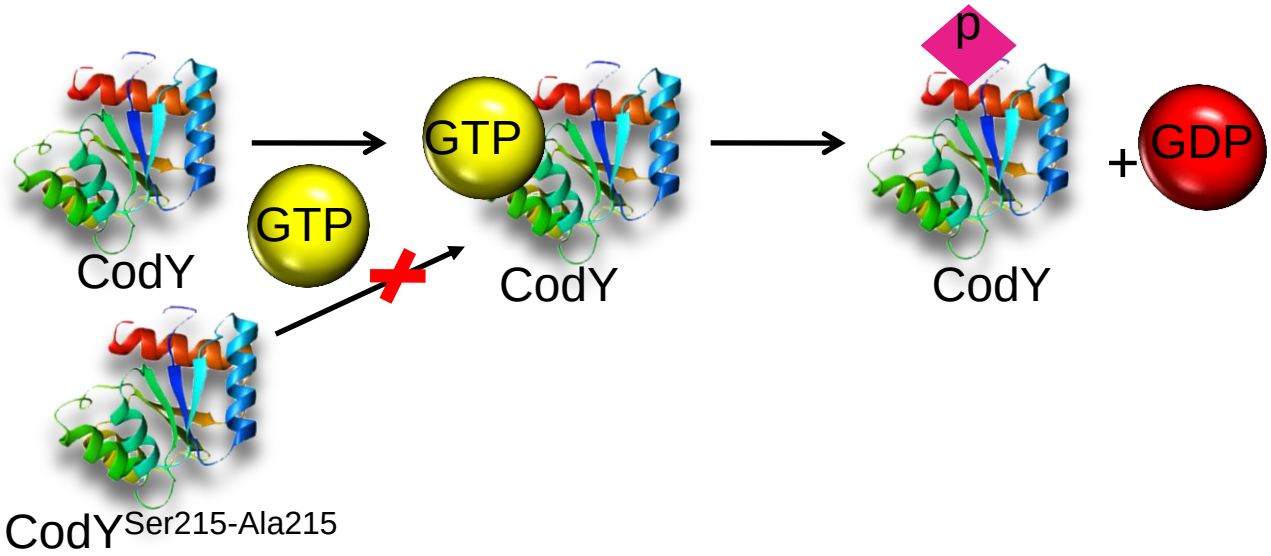


GTPase activity of CodY





A Proposed Model of CodY and GTP interaction

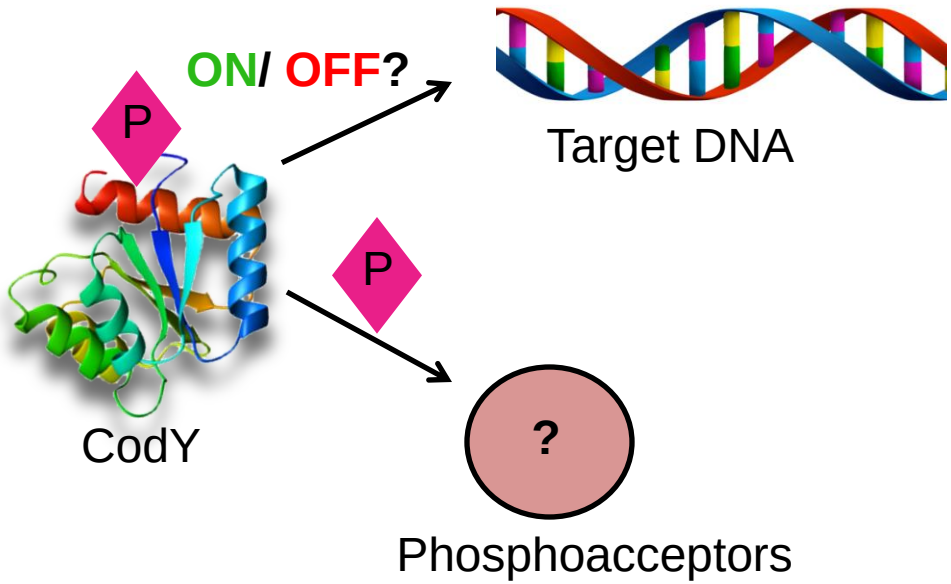


Conclusion

CodY of *B. anthracis*

- ✓ Binds to GTP and cleaves it releasing inorganic phosphate(Pi) + GDP.
- ✓ Possess conserved GTP binding residues.
- ✓ Autophosphorylate itself on a conserved Serine-215.

Future endeavors



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Mr. Himanshu Gogoi

Thank you…

…for your kind attention