



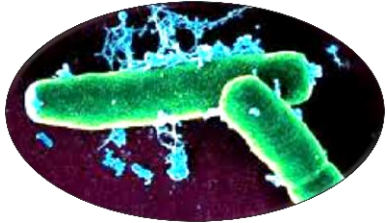
Interaction mechanism of GTP with CodY of *Bacillus anthracis*



Presented by
Shikha Joon
(Grad student)

School of Biotechnology
Jawaharlal Nehru University
New Delhi, India

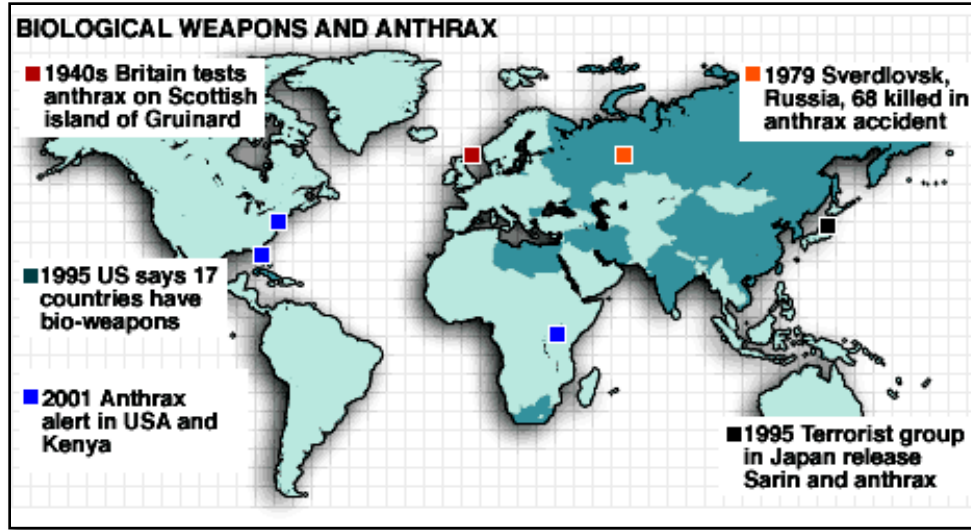
Bacillus anthracis- a bioterrorism weapon



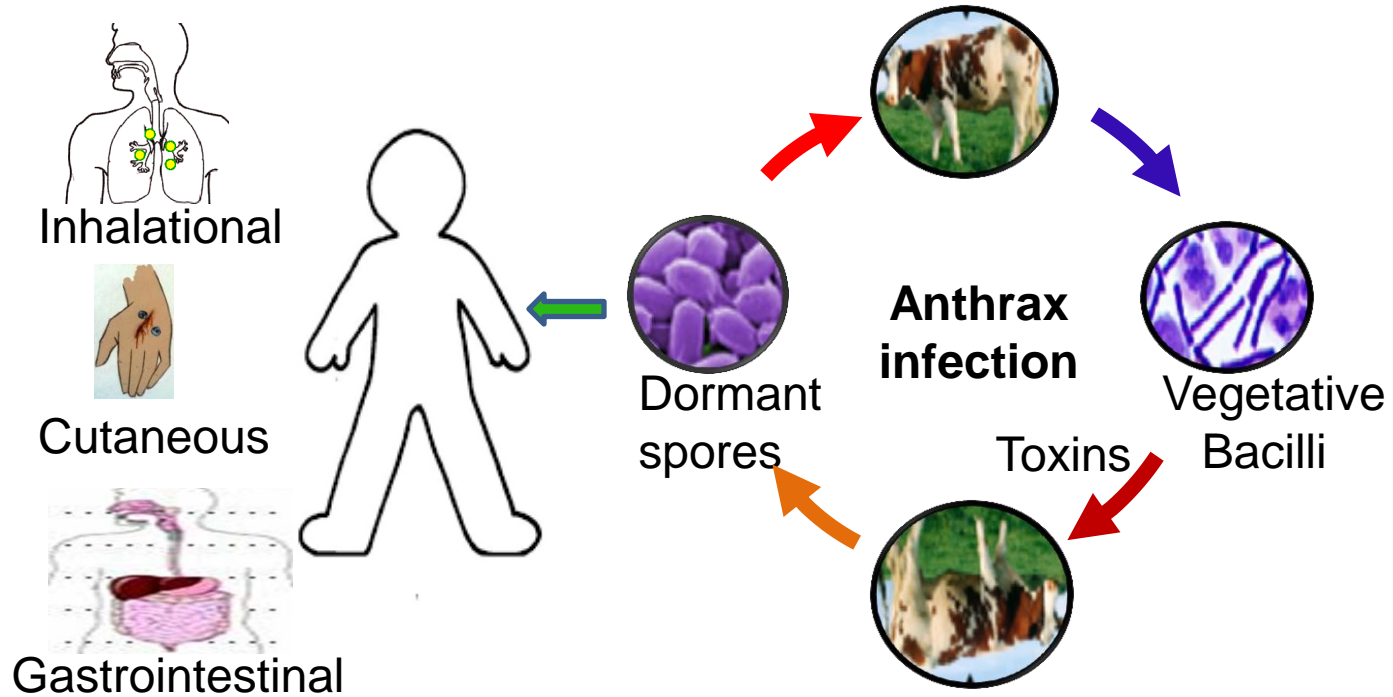
- Gram-positive, forms spores
- Non-motile, facultatively anaerobic
- Anthrax

Category 'A'
agent

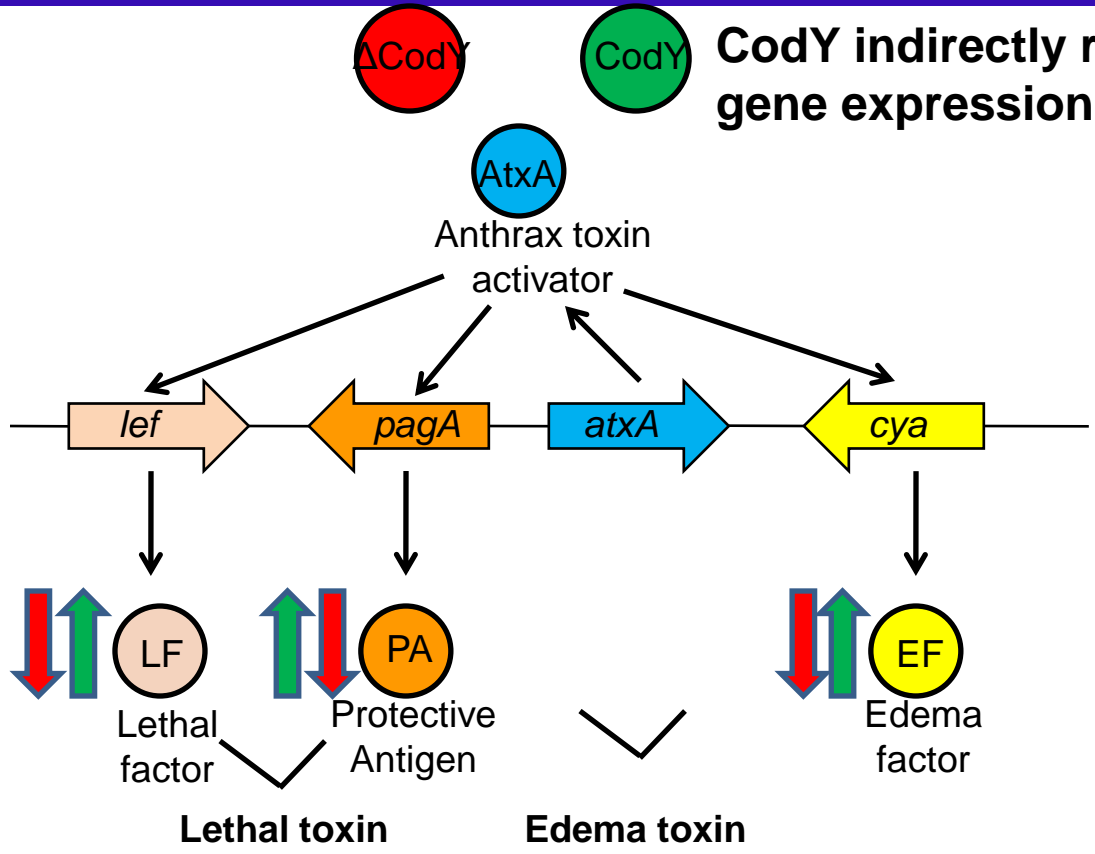
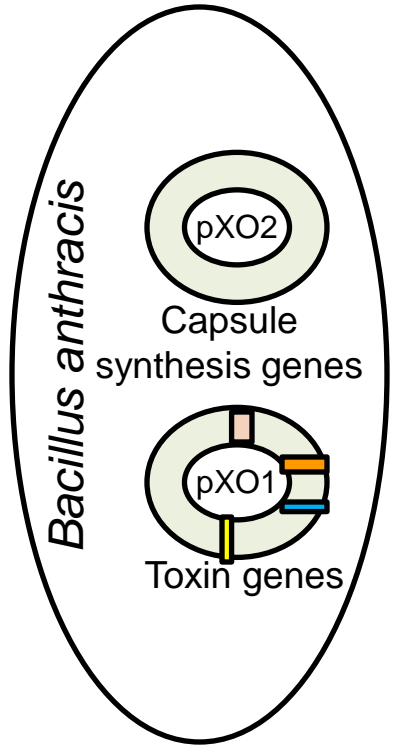
- Ease of dissemination
- High mortality rates
- Major public health impact



Anthrax- mode of transmission



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



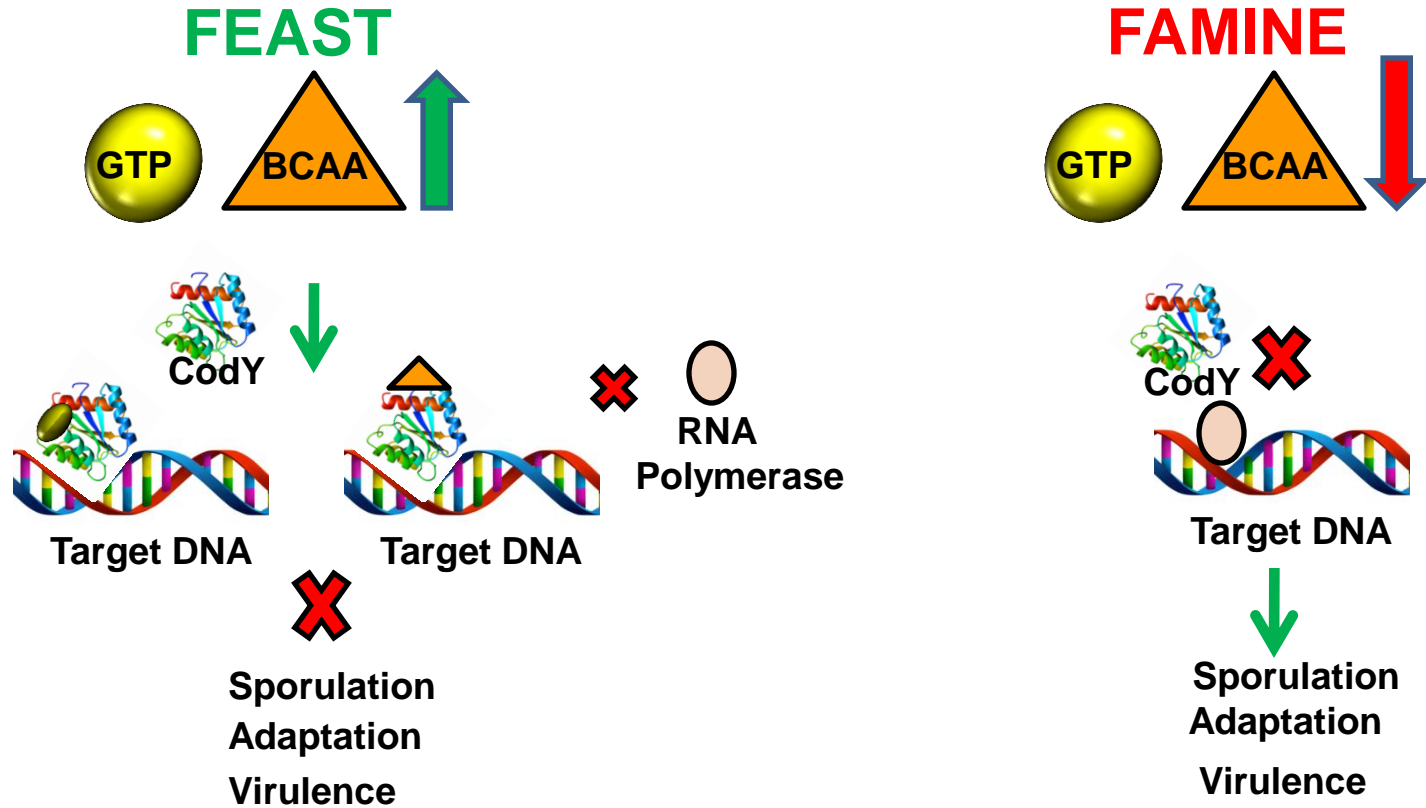
CodY indirectly regulates toxin gene expression in *B. anthracis*

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).

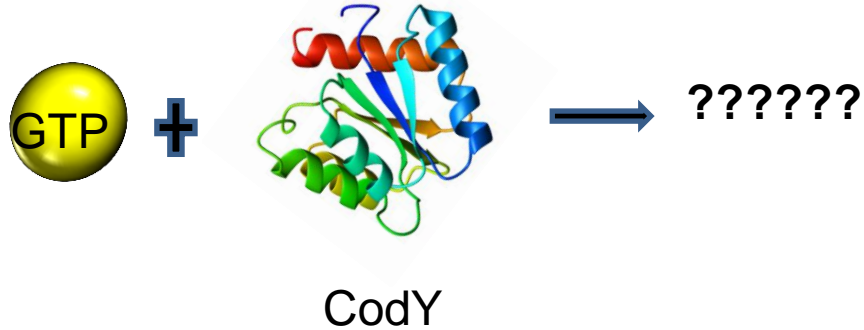
CodY – the mechanism of action



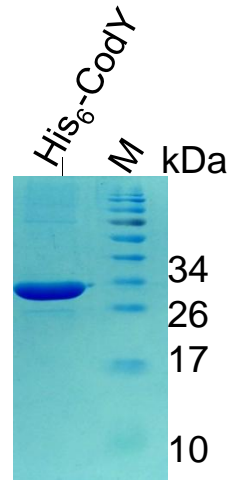
Purpose of the Study



- Propose a model of interaction of CodY and GTP



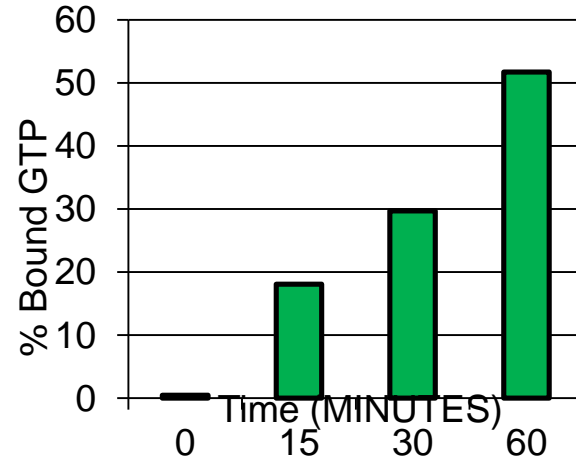
Purification of His₆-CodY



GTP binding activity of CodY



His ₆ -CodY	+	+		+	-	Negative control
[α - ³² P]-GTP	+	+	+	+	+	BAS0540
Time (MIN)	60	30	15	0	60	60



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



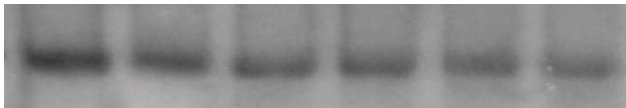
His ₆ -CodY	+	+	+	+	+	+
Cold GTP (μM)	0	0.1	0.5	1	2	5
[α- ³² P]-GTP	+	+	+	+	+	+
Time (MIN)	60	60	60	60	60	60



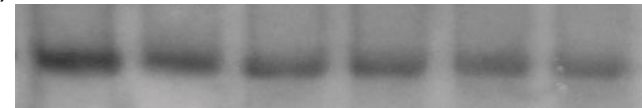
His ₆ -CodY	+	+	+	+	+	+
Cold ATP (μM)	0	0.1	0.5	1	2	5
[α- ³² P]-GTP	+	+	+	+	+	+
Time (MIN)	60	60	60	60	60	60



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

23

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ban  -----P---NE REMSDTMC EVI EANVF VV R G L L G Y A I H Q Q I E N E R M K Q M L A - E R F P F E E
lmo  -----T---NE KEMADT L T D V I E A N T Y I V R G L L G Y S E A L P I E N D R M K Q M L T - E R F P F E E
bsu  -----F---NE KEMAET L R D V I D S N I E V V R G L L G Y S I N Q Q I E N D R M K K M L E - D R O F P F E E
sau  -----A---DE KDVAQT I S S V T V T N V F I V R G L L G S S L N E L L K S Q R I I O M L E - E R L I P S E E
cdf  ---GSS---S E D L L A G A L G D V L S S N V Y V V A G G V L G L H L N D V Q D S S V I E D E Y T K Q K F S D E
spy  -SLE TEL F Y N T M A S R L A D I I D C N A C I I G G G L L G Y A M K Y K T N T D R V E E F F E - A K G F P D T
sag  -SLD A E F Y N T M A A Q L A D I I D C N A C I I G G G L L G Y A M K Y K T N T D R V E E F F E - T K G F P D Y
sth  -SLE G D F Y N N M A A Q L A D I I D C N A A I V G G G A L L G F A M K Y K T N N D R V E K E F F K - A K G L P E E
spn  -QLQ D E L F Y N A I T R Q L A D I I H C N A C I I S K G L L G Y F M R Y K T N T D R V E Q E F F Q - T K G F P D D
lla  -DLQ Q E L F Y N S M T E R L A N V I D C N A C V I T K G L L G Y S L P Y N T N N D R V Q E F F Y - D R K L P D E
efa  F D V Q A E T F Y N K M M I L G D I L E S N A Y I I S G L L G Y T E K L D V N N A R I K N M F K - E K G F P Q G

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75

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ban  Y T Q S L F N I T E T S S N L D V N S A Y T A F P V E N K E L F G Q G L T T I V P I V G G G E R L G T L V L A R L G O E
lmo  Y T Q S L F N V G E T S S N L E V S S O Y T A F P I E N S E L F T K G L T T I V P I V G G G E R L G T L I L S R L E S N
bsu  Y T K N L F N V P E T S S N L D I N S E Y T A F P V E N R D L F Q A G L T T I V P I I G G G E R L G T L I L S R L Q D Q
sau  Y T E R L M E V K Q T E S N I D I D N V L T V F P P E N R E L F I D S R T T I F P I L G G G E R L G T L V L G R V H D D
cdf  Y T Q N V L K I D E T L E N L N G E K I L E I F P E E H G - - R L Q K Y T T V V P I L G S G Q R L G T L V L S R Y S N S
spy  Y V K A A S R V Y D T E A N L S V E N E L T I F P V E S K D T Y P G G L T T I A P I Y G G G M R L G S L I I W R N D N E
sag  Y V K S A S R V Y D T E A N L S V D N D L S I F P V E T K E N F Q D G I T T I A P I Y G G G M R L G T F I I W R N D K E
sth  Y I R G I S R V Y D T O E N I G I D S D L T I F P V E L K D D F P D G L T T I A P I Y G G G M R L G S F I I W R N D H D
spn  Y V Q A N M I Y E T E A N L P V E H D M S I F P I E S R D D F P D G L T T I A P I H V S G I R L G S L I I W R N D K K
lla  Y V R A A V R I Y D T M A N V P V D R P L A I F P E E S L G D F P K G V T T L A P I Y G S G M R L G T F I M W R E D G E
efa  Y T E A V D M L K V T E A N I P I D S D L T A F P F E S R E L Y P F G L T T I V P L Y G A G K R L G T I I L A R V E K S

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135

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ban  F L D D D L I L A E Y S S T V V G M I L R E K A E E I E E E A R S K A V V Q M A I S S L S Y S E L E A I E H I F E E L
lmo  F T D D D L L L A E Y G G T V V G M I L H E K A E E I E E E A R S R A V V Q M A I S S L S Y S E L E A I E H I F D E L
bsu  F N D D D L I L A E Y G A T V V G M I L R E K A E E I E E E A R S K A V V Q M A I S S L S Y S E L E A I E H I F E E L
sau  F N E N D L V L G E Y A A T V I G M I L R E H S E V E K E A R D K A A I T M A I N S L S Y S E K E A I E H I F E E L
cdf  F N D D D L V I A E Y S A T V V G L I L R A T G E E L E E M R K K A V V Q M A I G T L S Y S E L E A V E H I F A E L
spy  F S D D D L I L V E I S S T V V G I L L N L O T E N L E D T I R K Q T A V N M A I N T L S Y S E M K A V A A I L G E L
sag  F S D D D L I L V E I A S T V V G I L L N L O T E N L E N I R K Q T A V T M A I N T L S Y S E M K A V A A I L G E L
sth  F V D D D L I L V E I A S T V V G L I L L H L O T E N L E E T I R K Q T A I N M A I N T L S Y S E I K A V S A I L N E L
spn  F E D E D L V L V E I A S T V V G I L L N F O R E E D E K N I R R R T A V T M A V N T L S Y S E L R A V S A I L G E L
lla  F T D D D L V L V E L A T T V I G V L S N L L E Q M E N I R K D T M A T M A V N T L S Y S E M K A V K A I I E E L
efa  F N E D D L V L A E Y S A T V V G M I L Y H C S R T I E A E V R S A T A V Q M A I N T L S Y S E L K A V H A I F E A L

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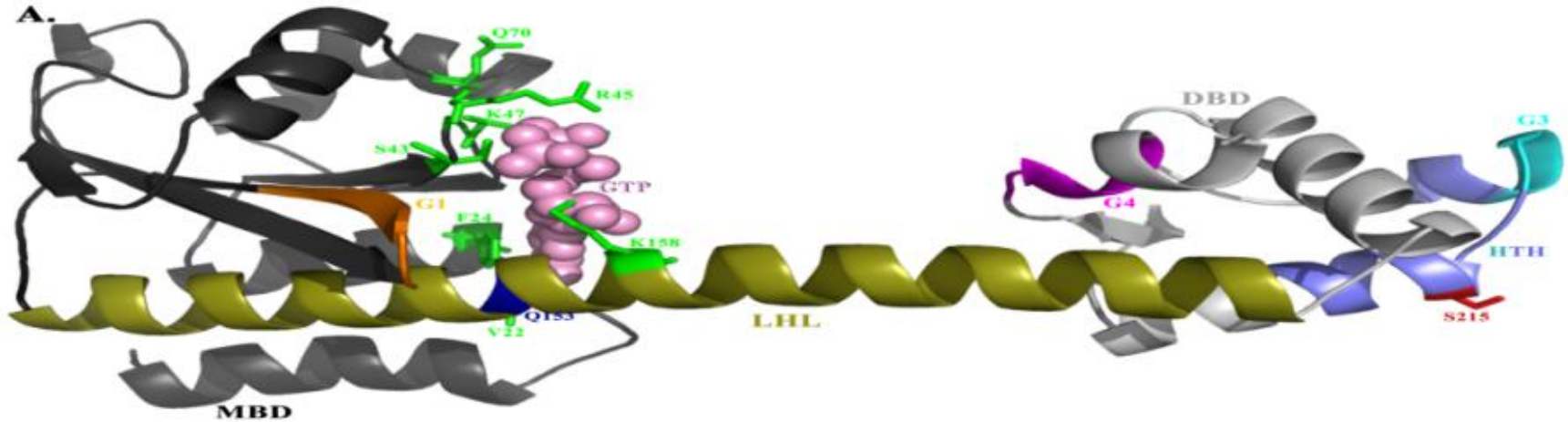
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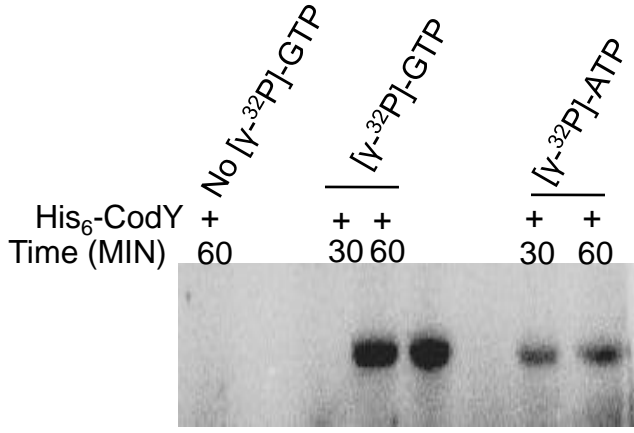
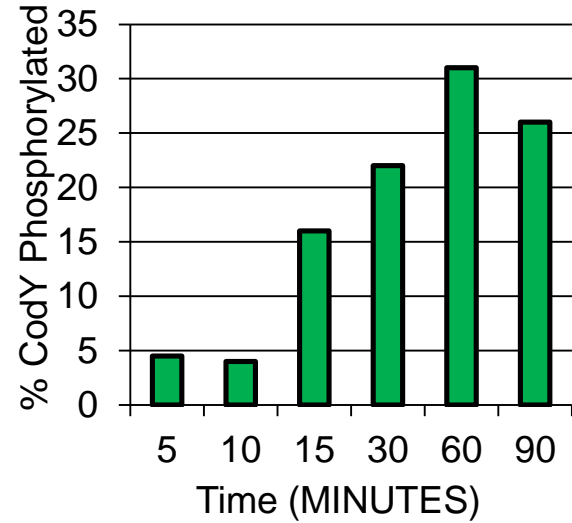
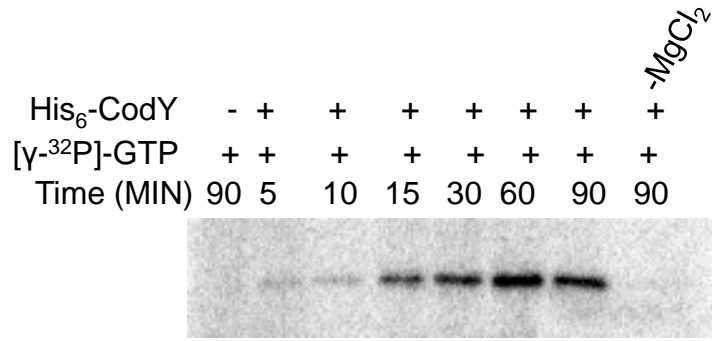
ban  N G T E G L L V A S K I A D R V G I T R V I V N A L R K L E S A G V I E S R S L G M K G T Y I K V L N D K P I H E L A
lmo  N G K E G L L V A S K I A D R V G I T R V I V N A L R K L E S A G V I D S R S L G M K G T F I R V L N D K P I V E L E
bsu  D G N E G L L V A S K I A D R V G I T R V I V N A L R K L E S A G V I E S R S L G M K G T Y I K V L N N K P I I E L E
sau  G G T E G L L V A S K V A D R V G I T R V I V N A L R K L E S A G V I E S R S L G M K G T F I K V K K E K P I D E L E
cdf  D G K E G L L V A S K I A D R V G I T R V I V N A L R K F E S A G V I E S R S L G M K G T H I R I L N D K L T D E L K
spy  D G N E G R L T A S V I A D R I G I T R V I V N A L R K L E S A G I I E S R S L G M K G T Y L K V I N E G I F A K L Y
sag  D G L E G R L T A S V I A D R I G I T R V I V N A L R K L E S A G I I E S R S L G M K G T Y L K V I N E G I F D K L -
sth  D G L E G R L T A S V I A D R I G I T R V I V N A L R K L E S A G I I E S R S L G M K G T Y L K V I N E G I V D K L -
spn  N G N E G K L T A S V I A D R I G I T R V I V N A L R K L E S A G I I E S R S L G M K G T Y L K V I S D I F E E V K -
lla  D G E E G H V I A S V I A D K I G I T R V I V N A L R K L E S A G V I E S R S L G M K G T Y L K V I N T G L F D K L -
efa  D G E E G R L T A S S I A D E I G I T R V I V N A L R K L E S A G I I E S R S L G M K G T Y L K V I N Q O F I K E -

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Homology model of CodY bound to GTP.



CodY autophosphorylate itself with a higher affinity for GTP.



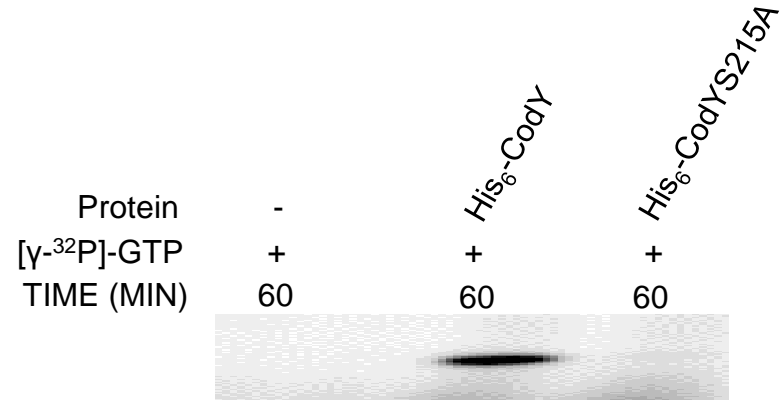
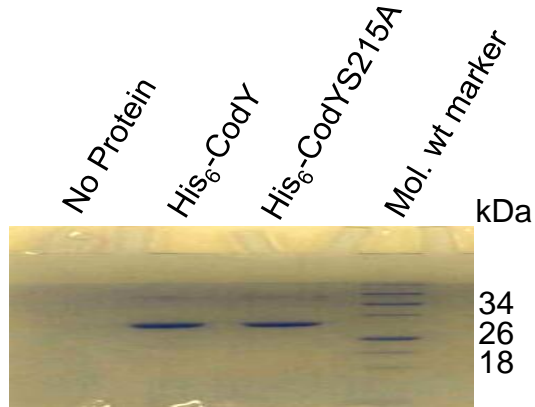
CodY undergoes serine/ threonine phosphorylation.

His ₆ -CodY	+	+	+
[γ - ³² P]-GTP	+	+	+
HCl	+	+	-
NaOH	+	-	+
TIME (MIN)	60	60	60

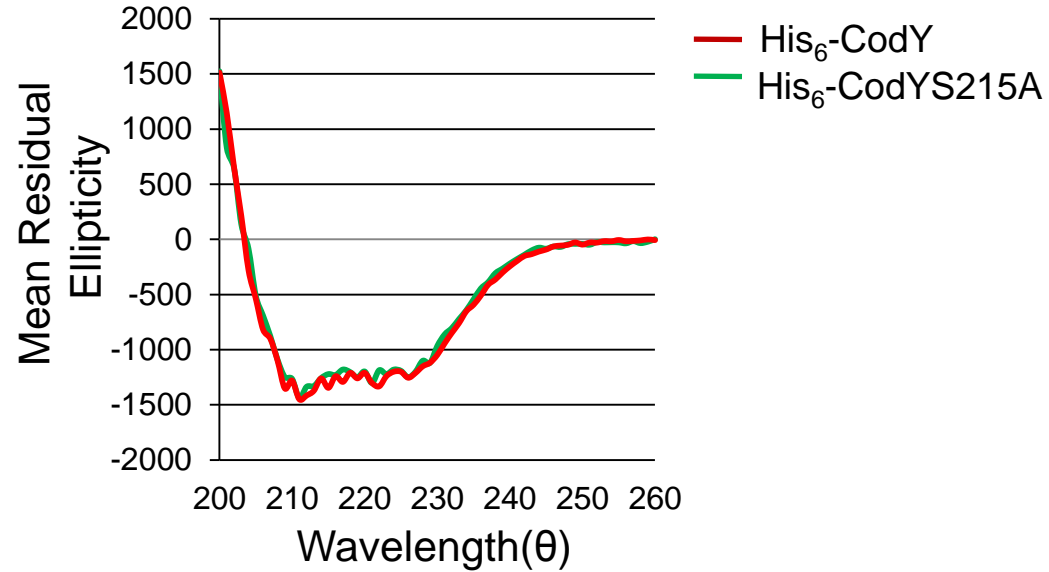


- Phosphoproteome analysis of CodY of *B. subtilis* showed phosphorylation at a conserved **Serine-215** residue.

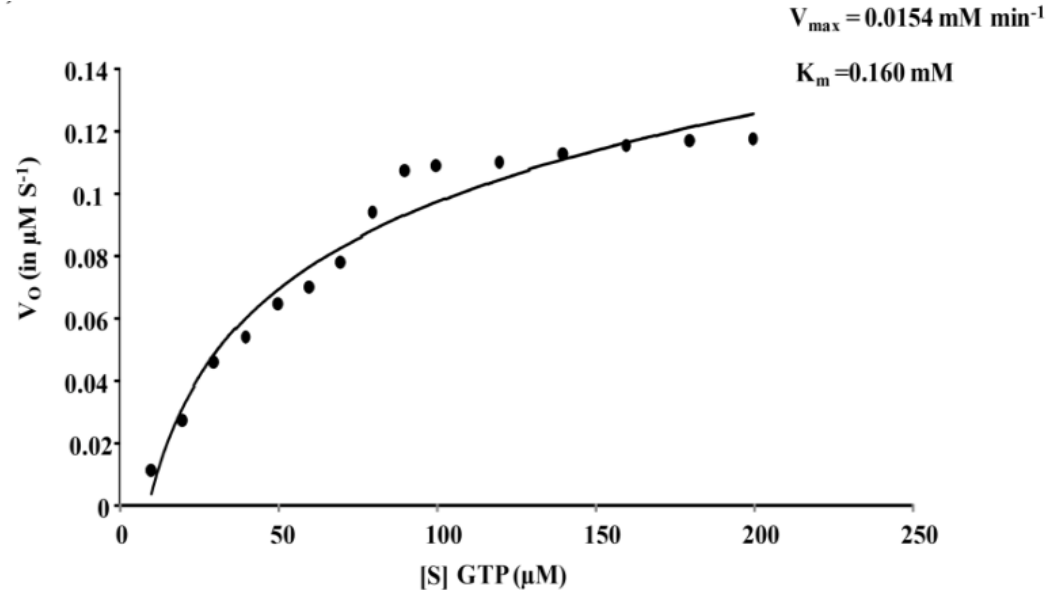
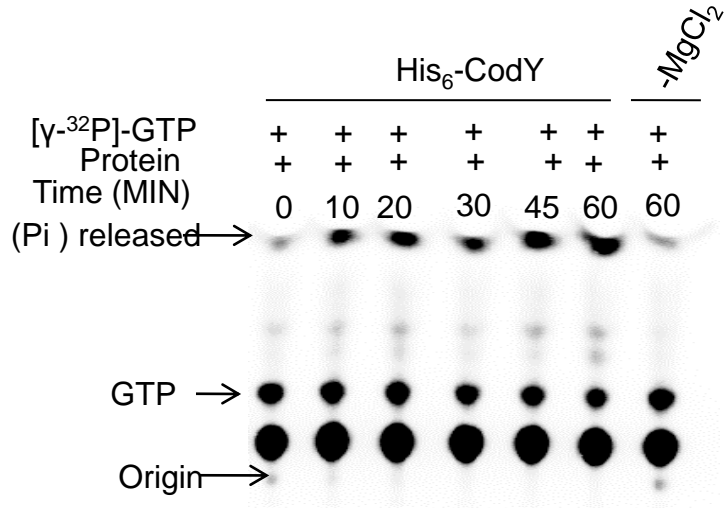
CodYS215A lacks autophosphorylation activity.



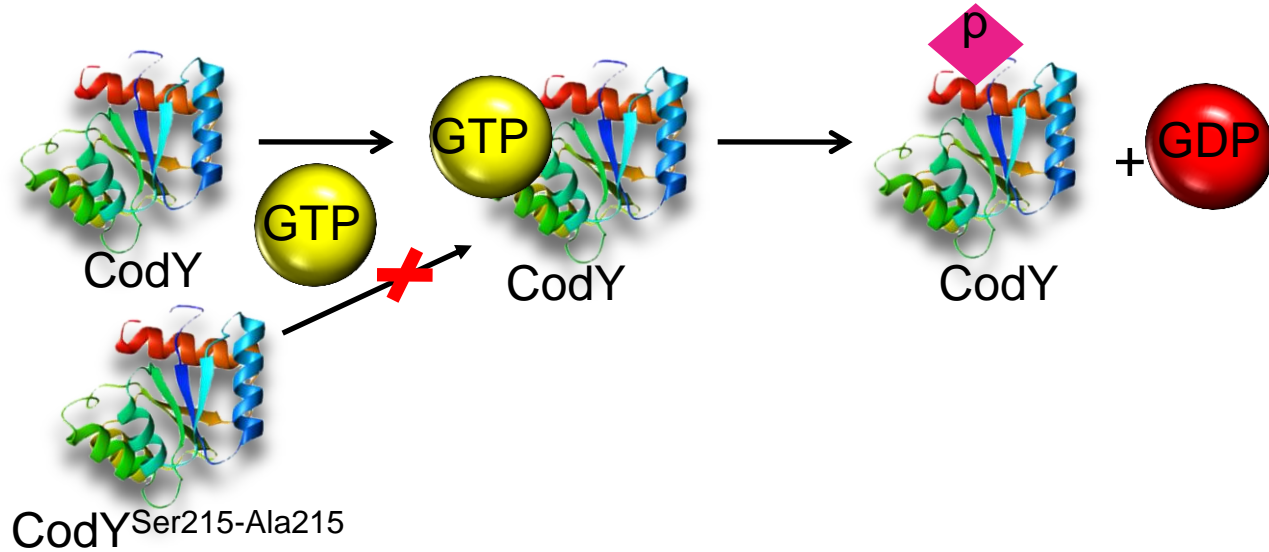
Comparison of the CD spectra of CodY and CodYS215A.



GTPase activity of CodY



A Proposed Model of CodY and GTP interaction

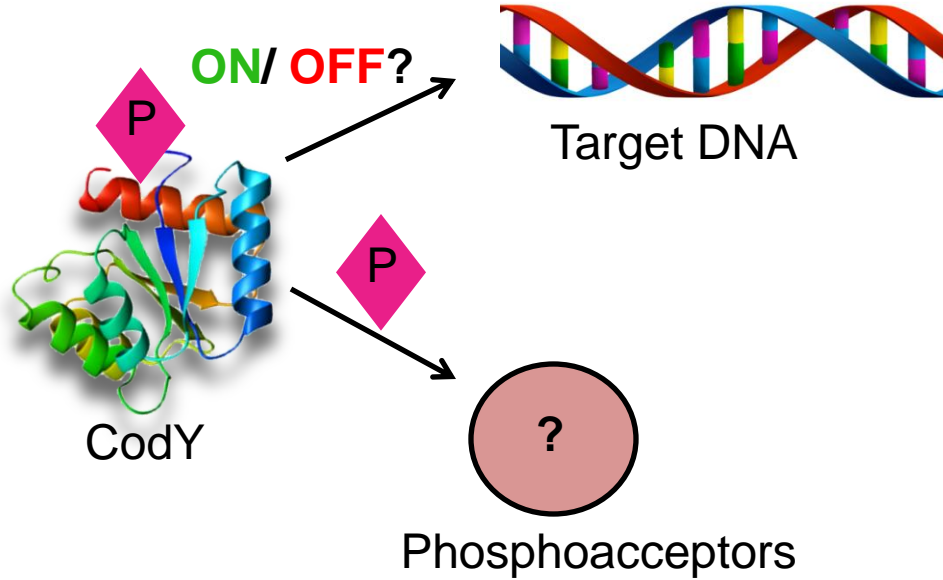


Conclusion

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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Thank you...
...for your kind attention