METABOLIC CONTROL OF VIRULENCE IN *CLOSTRIDIUM DIFFICILE*

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Saul Tzipori, William Self and
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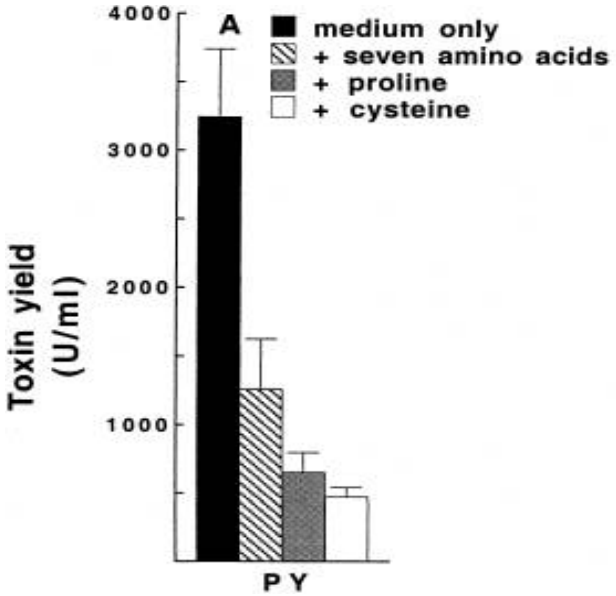
Regulation of toxin production

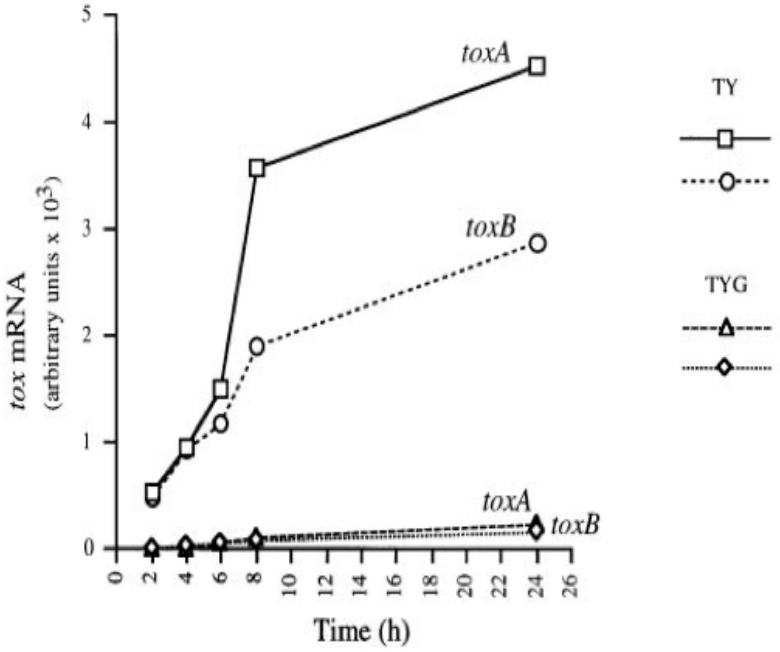
Amino acid response



7AA: Gly, **Iso, Leu**, Met, Thr, Trp, **Val**

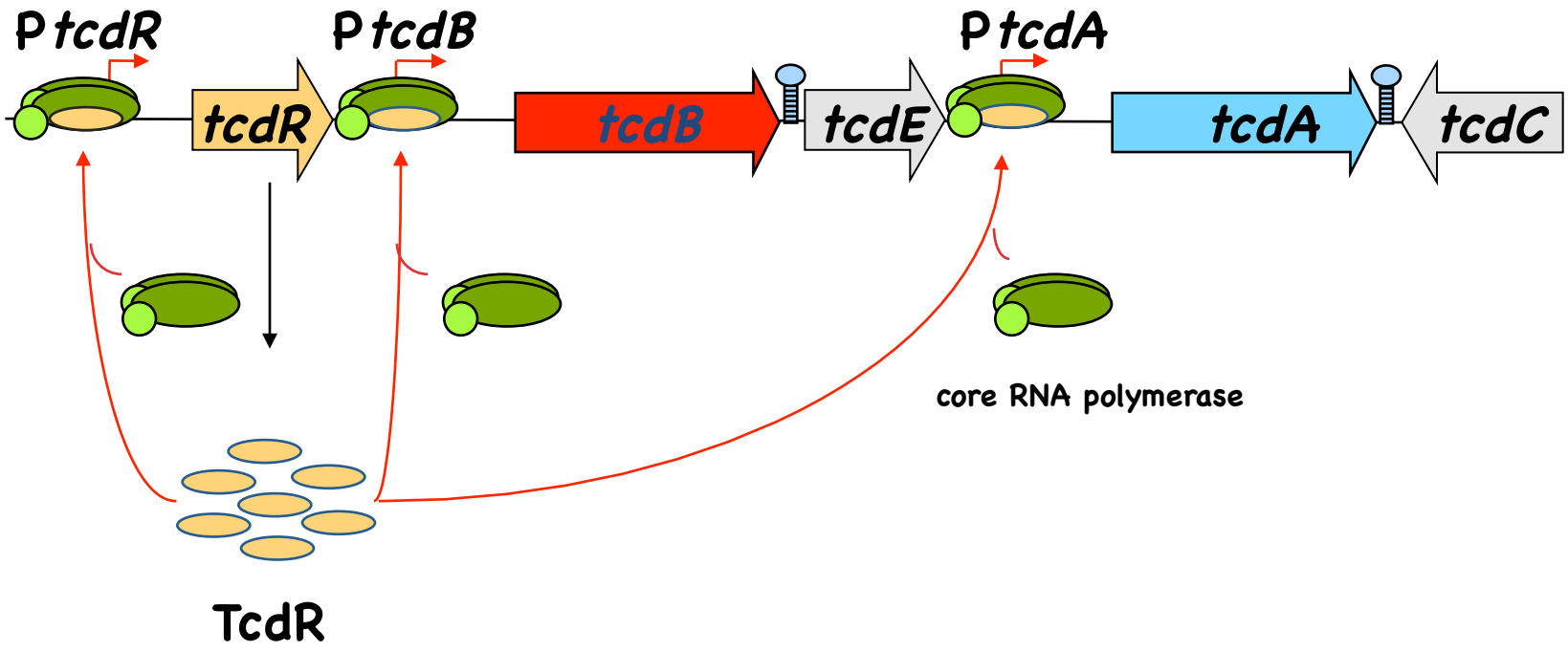
Karlsson S, et al. Infect Immun. 2000 Oct;68(10):5881-8.

Glucose response

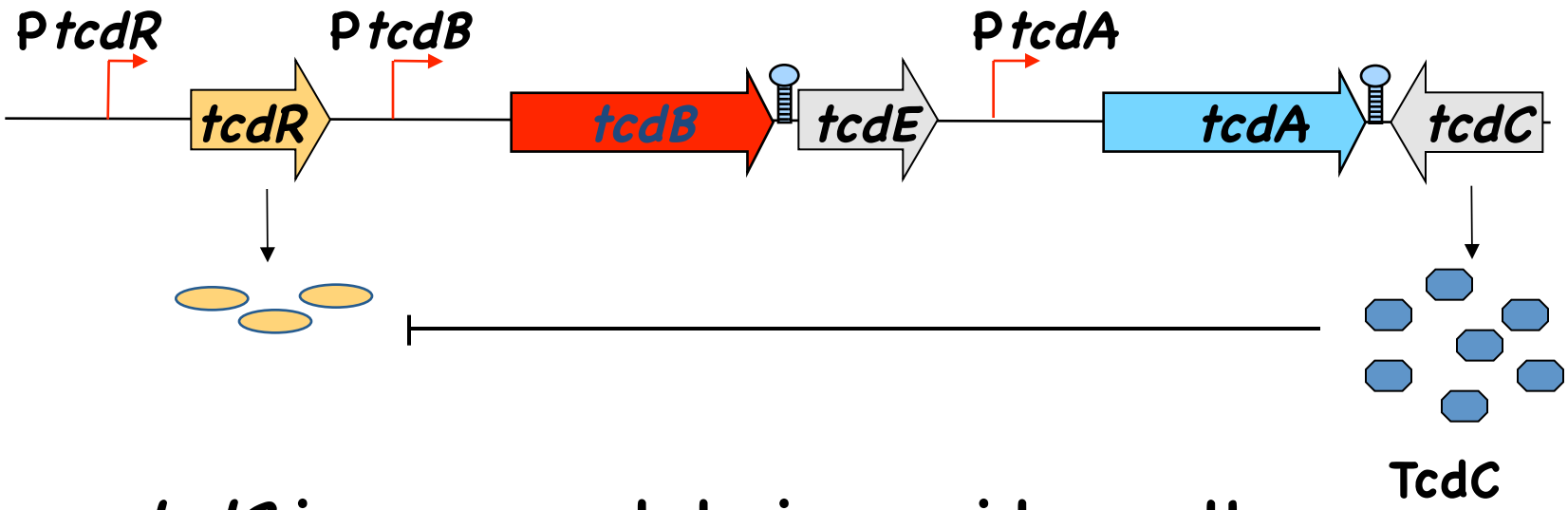


Hundsberger et al. Eur J Biochem. 1997 **244:** 735–742.
Dupuy and Sonenshein. Mol Microbiol. 1998 27:107–120.

TcdR is a sigma factor for toxin gene transcription (Mani & Dupuy)



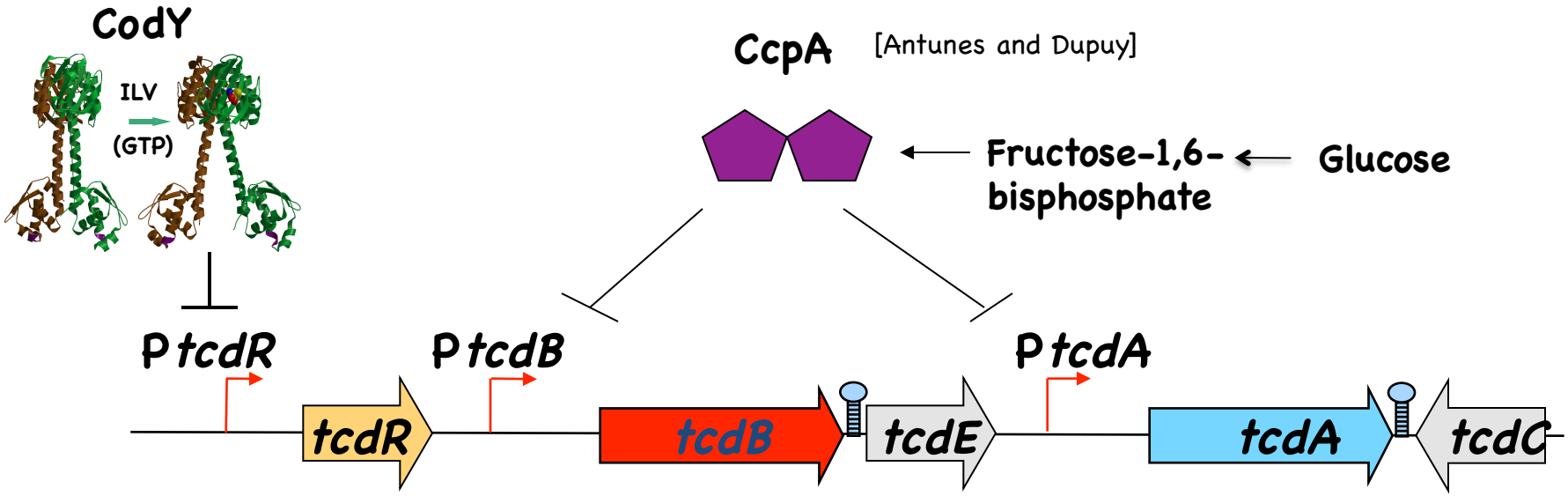
TcdC is an antagonist of TcdR (Matamouros & Dupuy)



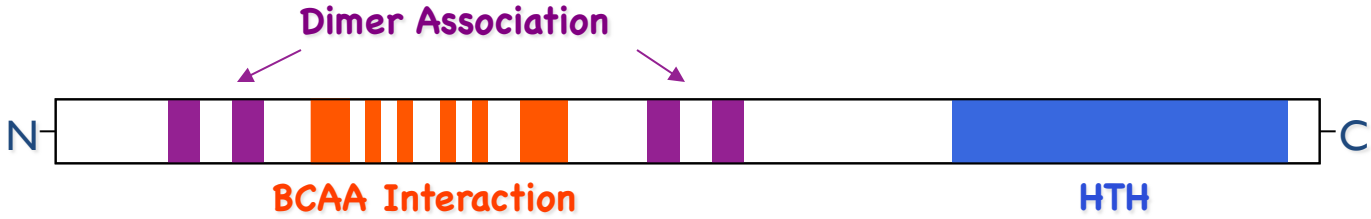
tcdC is expressed during rapid growth, but *tcdR* and the toxin genes are induced as cells enter stationary phase.

CodY and CcpA repress toxin synthesis *under conditions of nutrient excess*

[Dineen, McBride and Sonenshein]



CodY protein

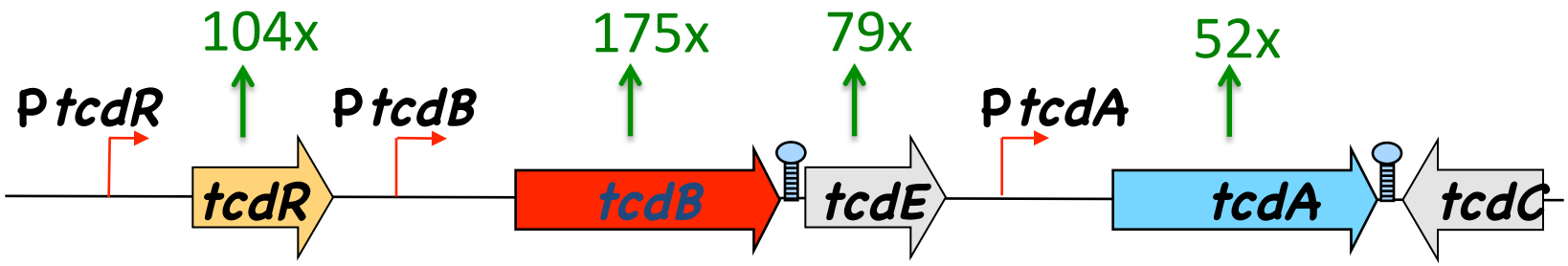


Global regulator of metabolism and virulence in Gram-positive bacteria

Activated as a DNA-binding protein
by isoleucine, leucine and valine (BCAAs)
and GTP

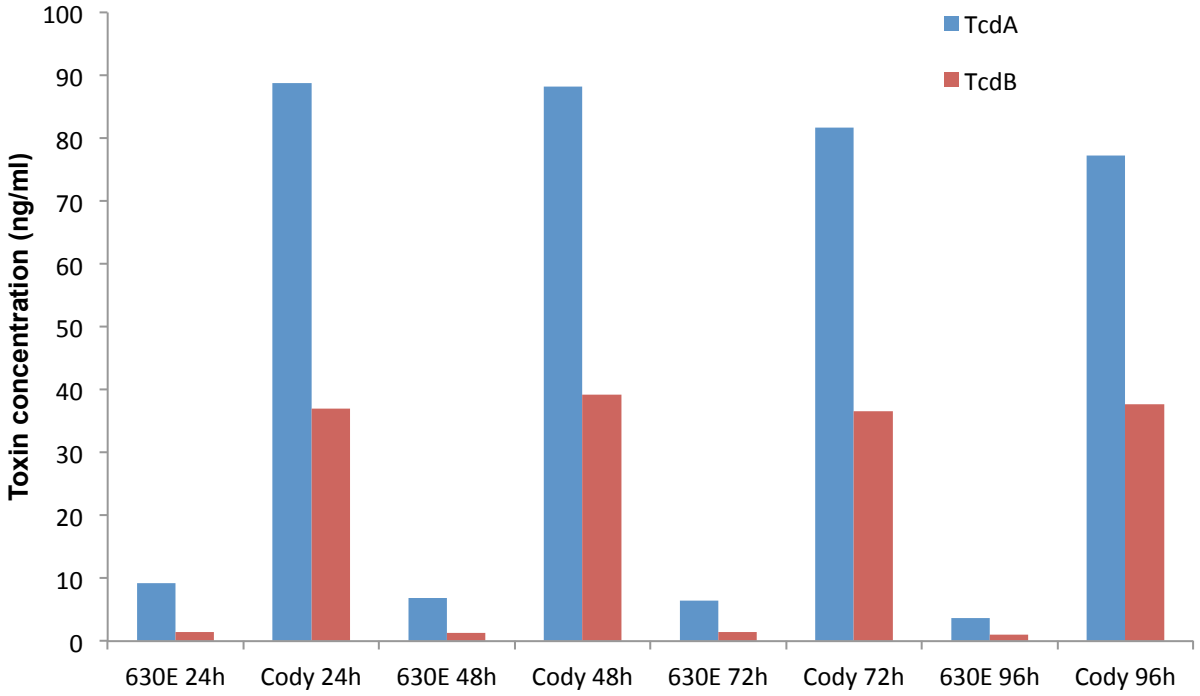
Regulates dozens of *C. difficile* metabolic genes
and the PaLoc genes during rapid exponential growth

Overexpression of the pathogenicity locus during growth of a *codY* mutant of 630E



(Dineen, McBride and Sonenshein, 2010)

Does derepression of toxin genes matter?



TcdA and TcdB concentrations in culture fluid of *C. difficile* 630E and its *codY* TargeTron mutant, as determined by ELISA.

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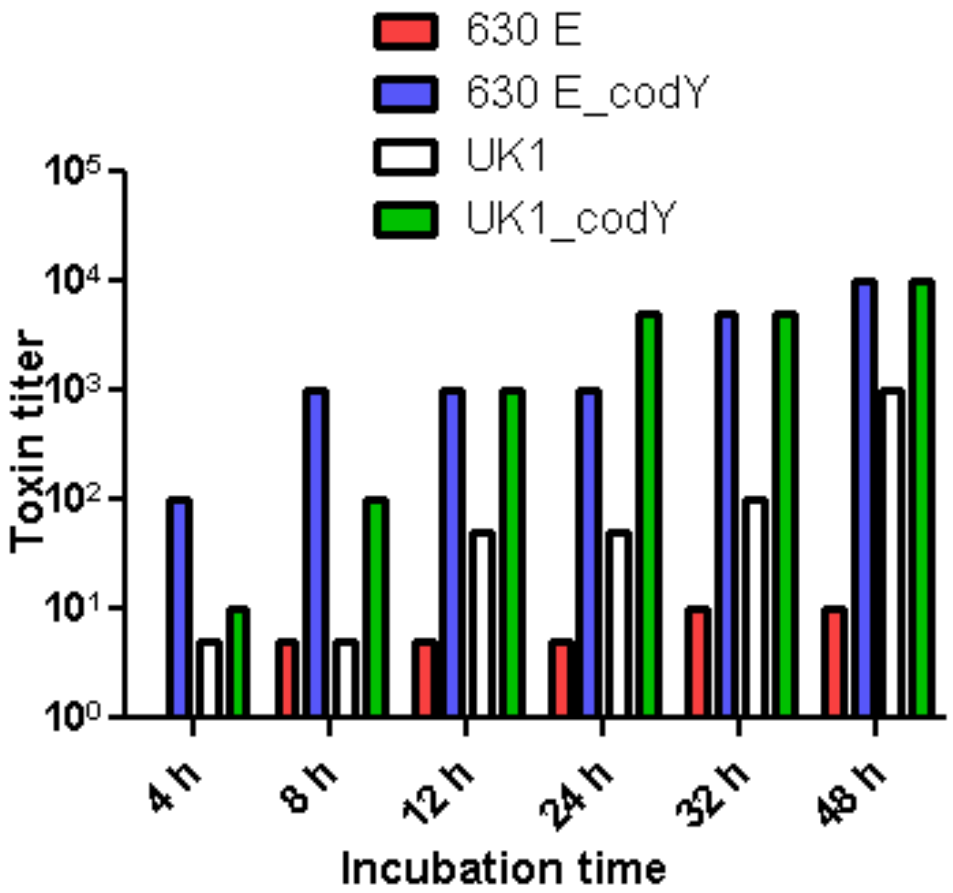

Cytoxicity (cell rounding) assays of filtered culture supernatants harvested at 4 - 48 hrs of growth in TY medium. Test cells: CT26 monolayers.

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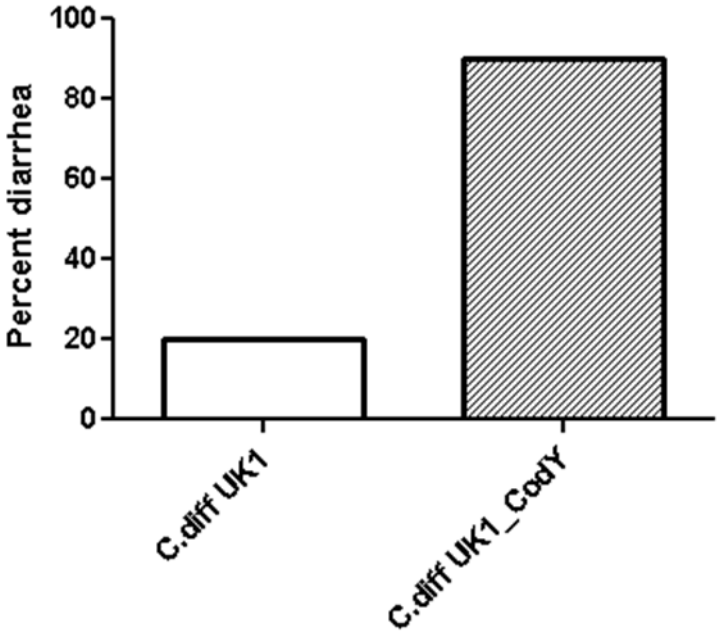
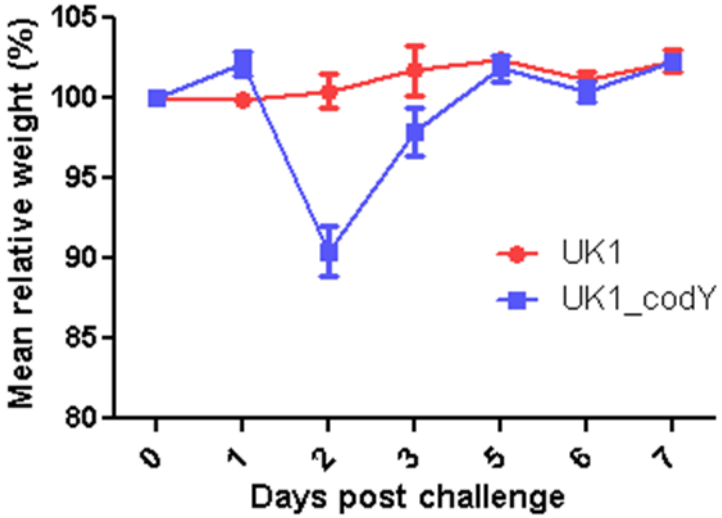
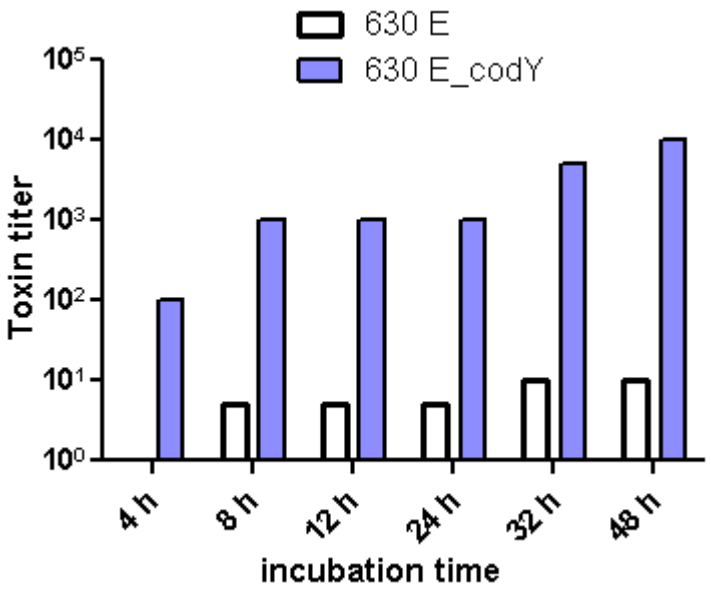
Is a *codY* null mutant more virulent than its *codY*$^+$ parent?

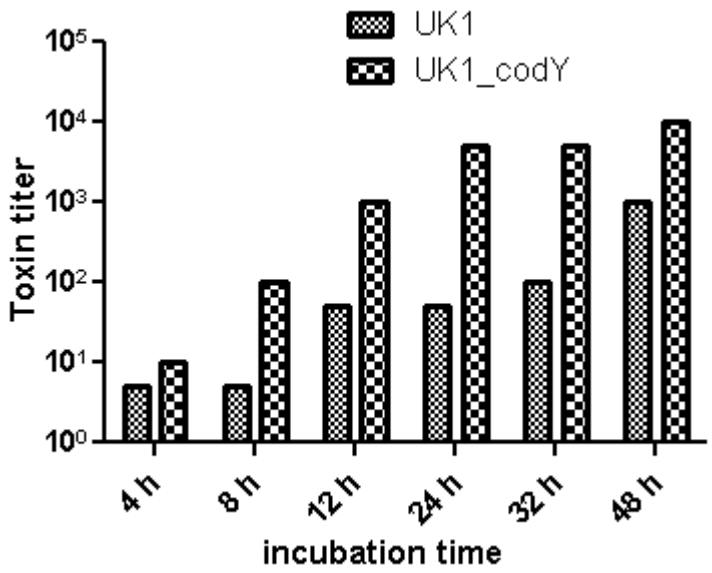


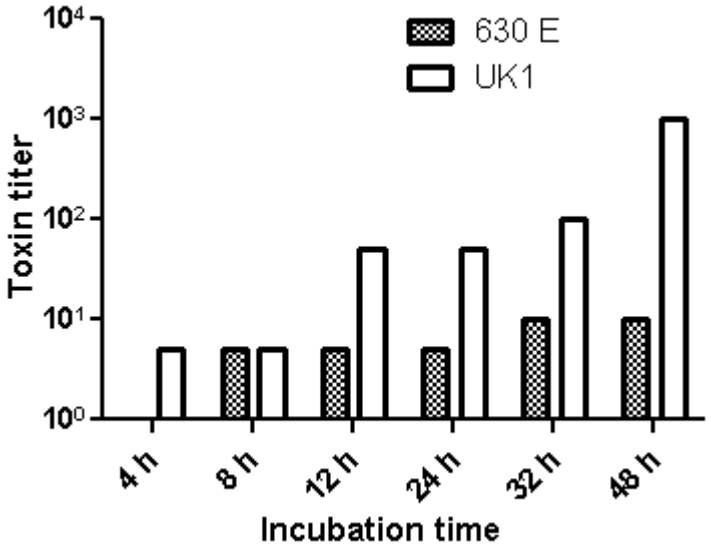


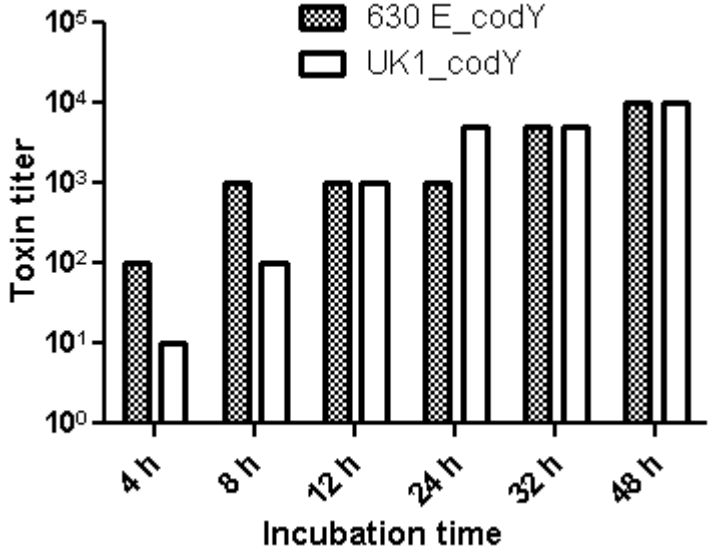
Sub-lethal challenge dose: 10^4 heat-resistant CFUs per mouse.
All mice (10 per group) survived.

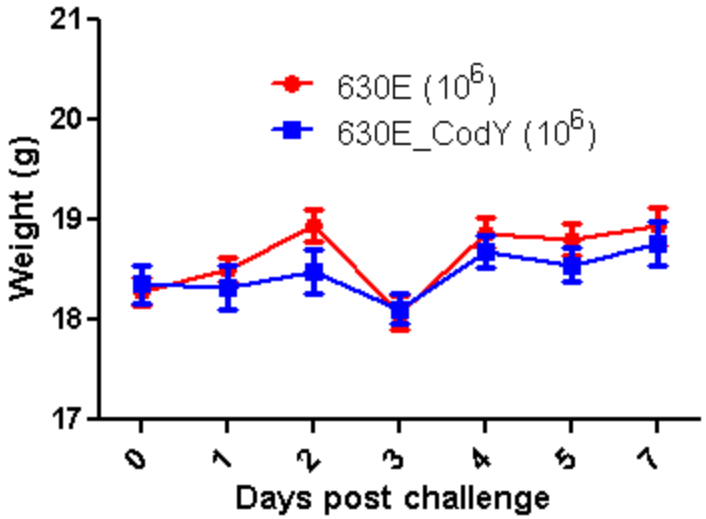
Xingmin Sun


630 E
630 E_codY
Toxin titer
10^5
10^4
10^3
10^2
10^1
10^0
4 h
8 h
12 h
24 h
32 h
48 h
incubation time


UK1
UK1_codY
Toxin titer
10^5
10^4
10^3
10^2
10^1
10^0
4 h
8 h
12 h
24 h
32 h
48 h
incubation time


630 E
UK1
Toxin titer
10^4
10^3
10^2
10^1
10^0
4 h
8 h
12 h
24 h
32 h
48 h
Incubation time


630 E_codY
UK1_codY
Toxin titer
10^5
10^4
10^3
10^2
10^1
10^0
4 h
8 h
12 h
24 h
32 h
48 h
Incubation time



Challenge dose: 10^6 heat-resistant CFUs;
all mice (10 per group) survived.

Xingmin Sun

Why are 630 *codY* and UK1 *codY* not equally virulent?

1) UK1 produces additional virulence factors (either CodY-regulated or CodY-independent) that are missing in 630.

2) UK1 produces the same CodY-regulated virulence factors as 630, but at a higher level.

Other CodY-regulated virulence factors?

Cell surface proteins – CD0440, CD1803

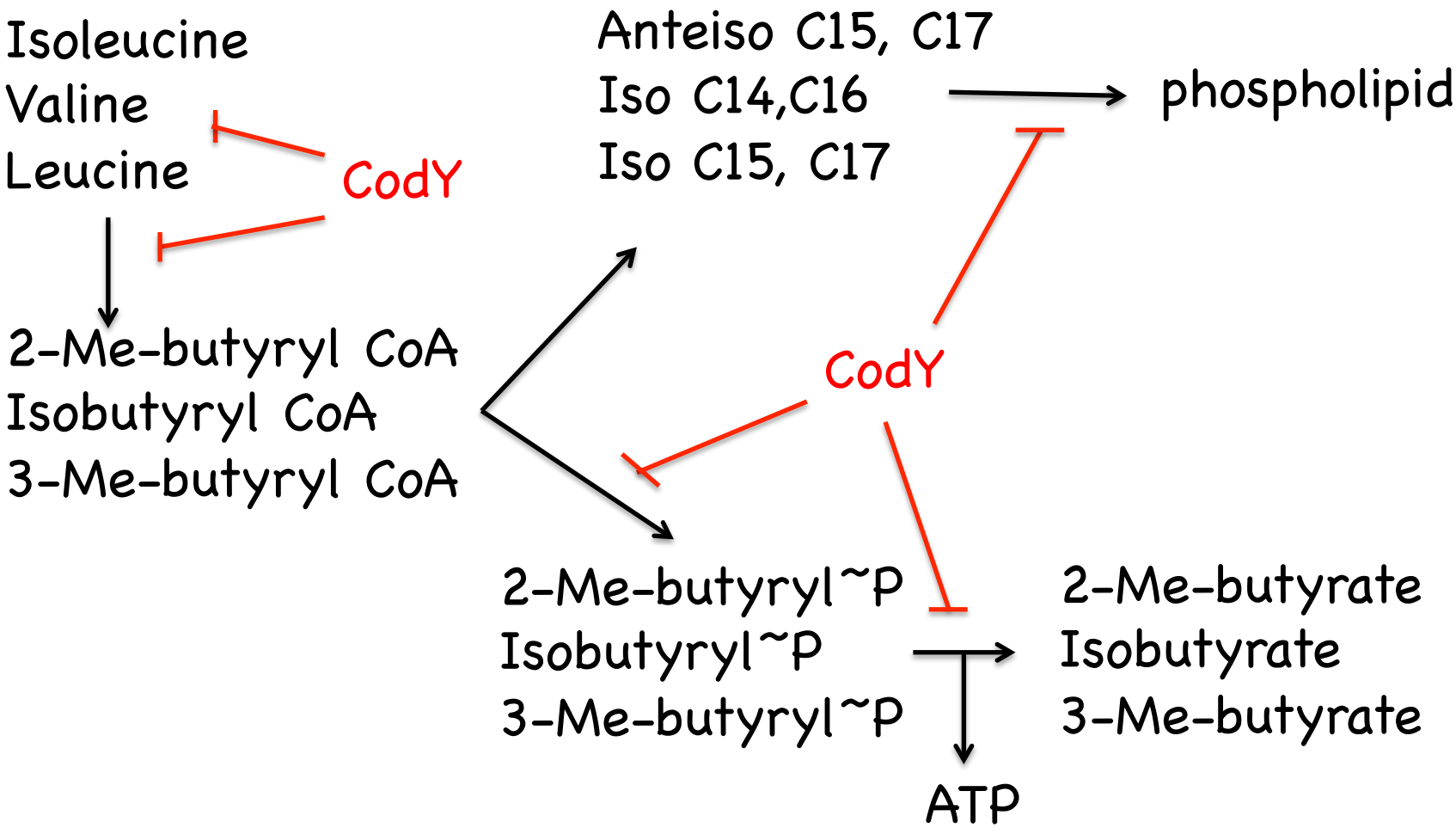
c-di-GMP signaling proteins – CD0757, CD1476

Metabolic pathways?

Amino acid biosynthesis and uptake

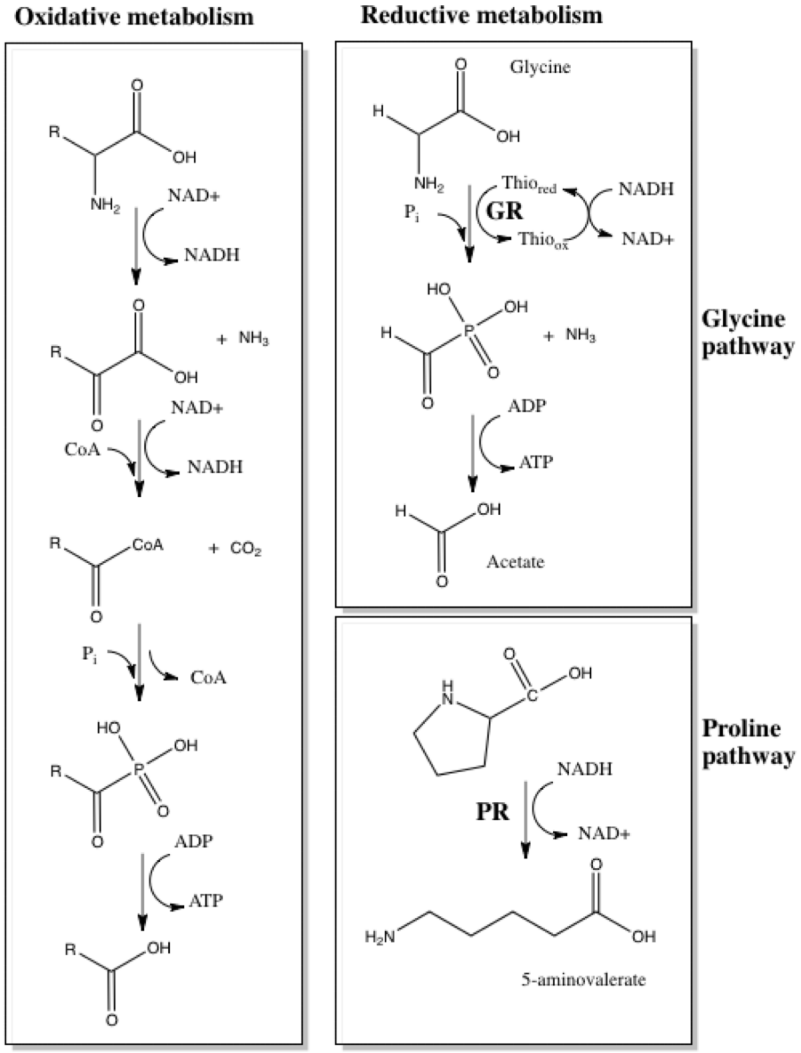
Phospholipid biosynthesis

Fatty acid biosynthesis in *C. difficile*



Stickland metabolism

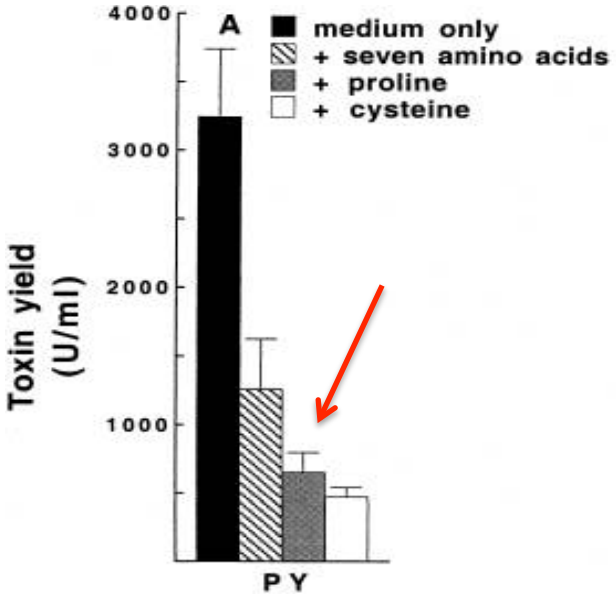
Electron donors:
Alanine
Isoleucine
Leucine
Valine



Electron acceptors:
Proline
Glycine

Regulation of toxin production

Amino acid response



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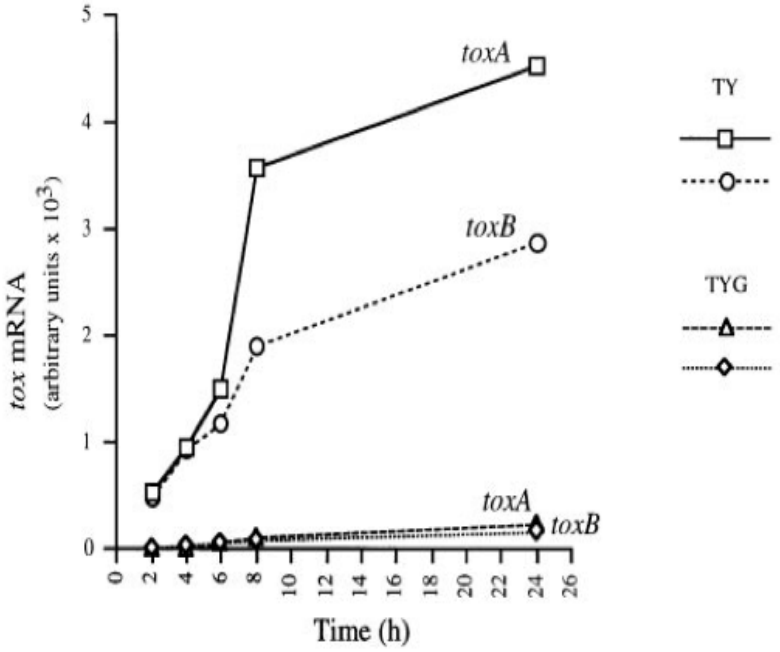
Glucose response



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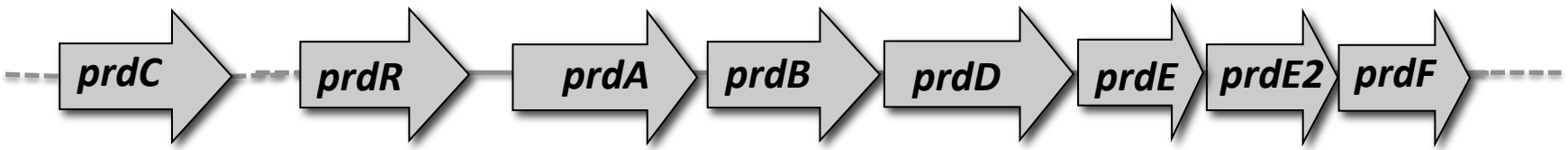
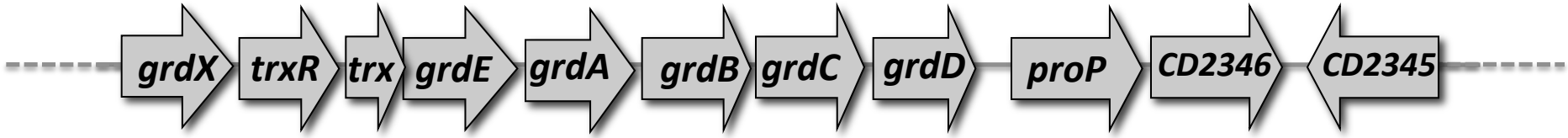
Dupuy and Sonenshein. Mol Microbiol. 1998 27:107-120.

D-Proline reductase (*prd*) operons



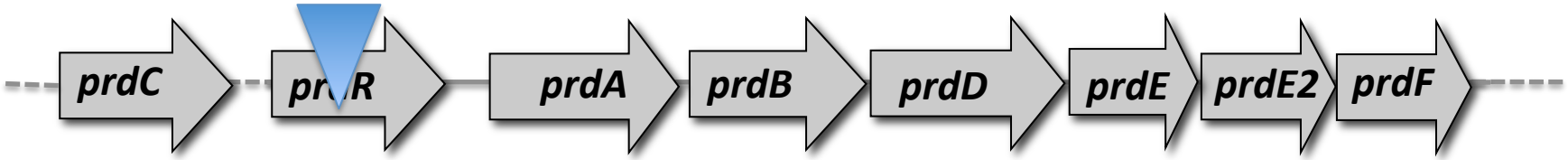
Glycine reductase (*grd*) operon



PrdR

A putative ATP-dependent regulatory protein of the σ^{54}-activating family.

D-Proline reductase (*prd*) operons



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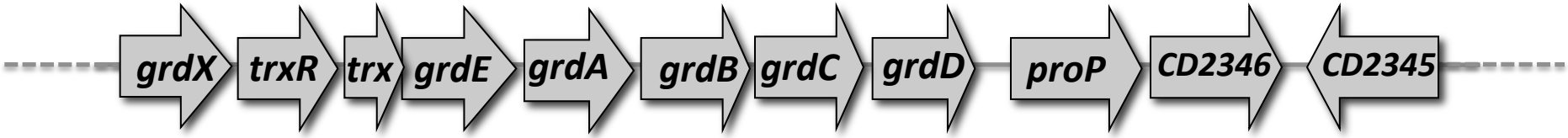

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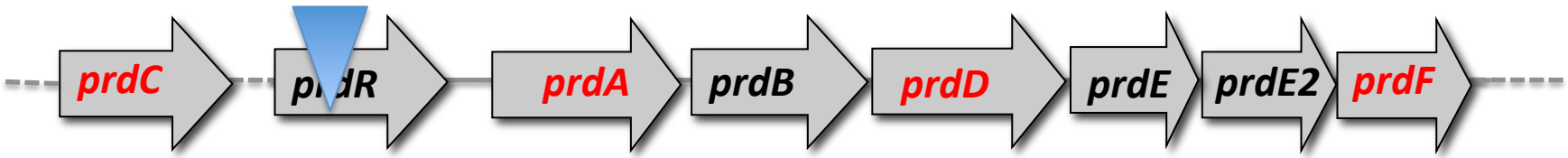
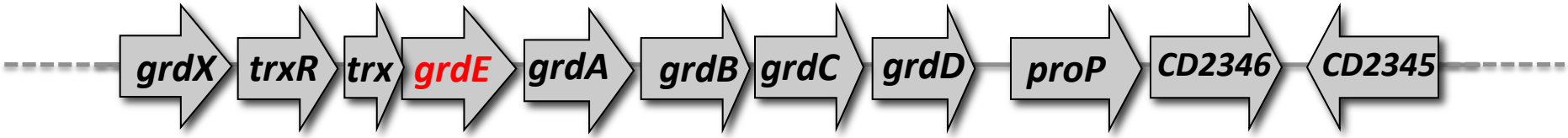
D-Proline reductase (*prd*) operons



Glycine reductase (*grd*) operon



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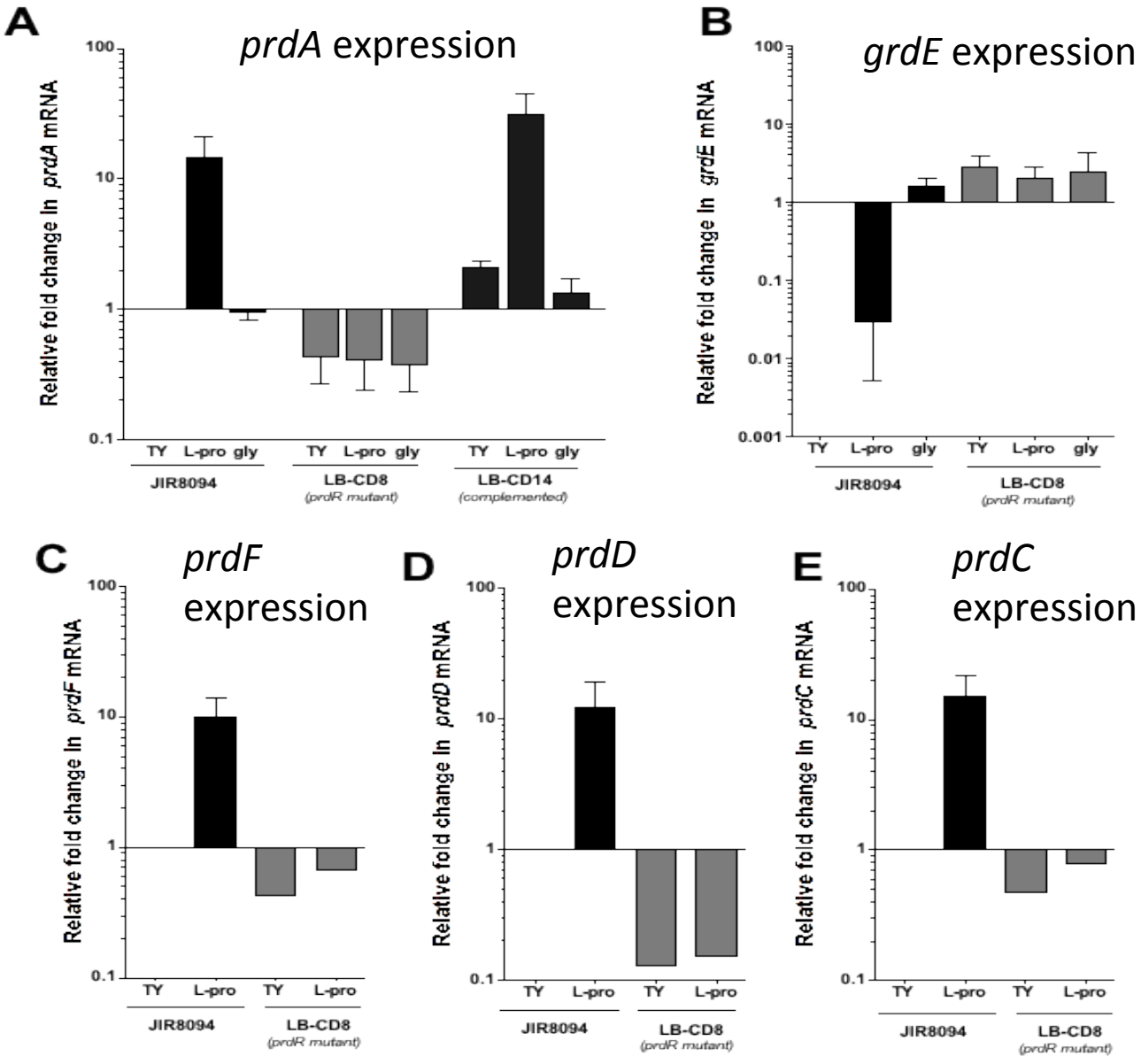
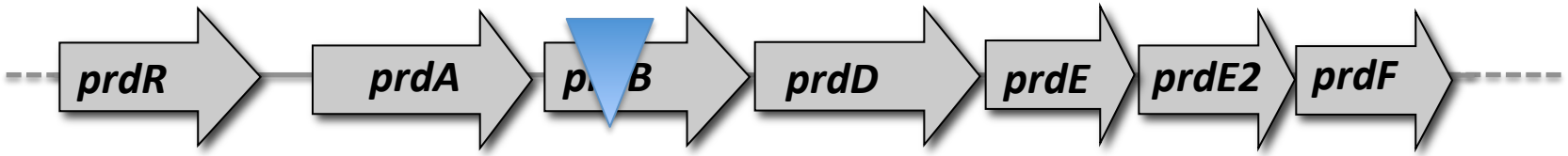

A
prdA expression
Relative fold change in prdA mRNA
100
10
1
0.1
TY L-pro gly TY L-pro gly TY L-pro gly
JIR8094
LB-CD8
(prdR mutant)
LB-CD14
(complemented)
B
grdE expression
Relative fold change in grdE mRNA
100
10
1
0.1
0.01
0.001
TY L-pro gly TY L-pro gly
JIR8094
LB-CD8
(prdR mutant)
C
prdF
expression
Relative fold change in prdF mRNA
100
10
1
0.1
TY L-pro TY L-pro
JIR8094
LB-CD8
(prdR mutant)
D
prdD
expression
Relative fold change in prdD mRNA
100
10
1
0.1
TY L-pro TY L-pro
JIR8094
LB-CD8
(prdR mutant)
E
prdC
expression
Relative fold change in prdC mRNA
100
10
1
0.1
TY L-pro TY L-pro
JIR8094
LB-CD8
(prdR mutant)

Sigma-54-type promoters

	-24	-12
prdA promoter region	TTTGTT$_{A}$A$_{T}$AAGTT**GG**CATAG$_{G}$AATT**GC**TT	
prdC promoter region	TTTGTT$_{T}$A$_{A}$AAGTT**GG**CATAG$_{A}$AATT**GC**TT	

D-Proline reductase (*prd*) operon



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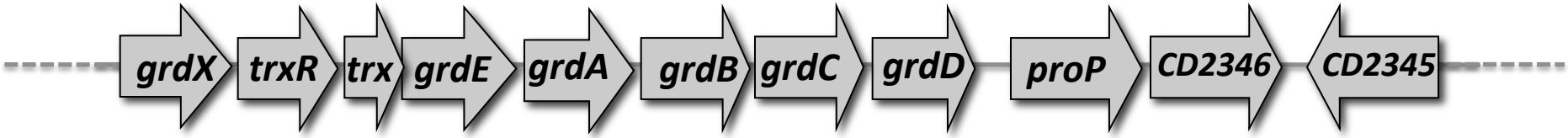

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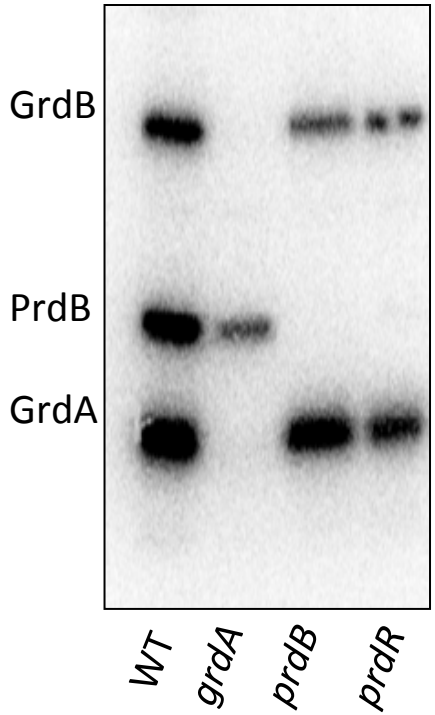
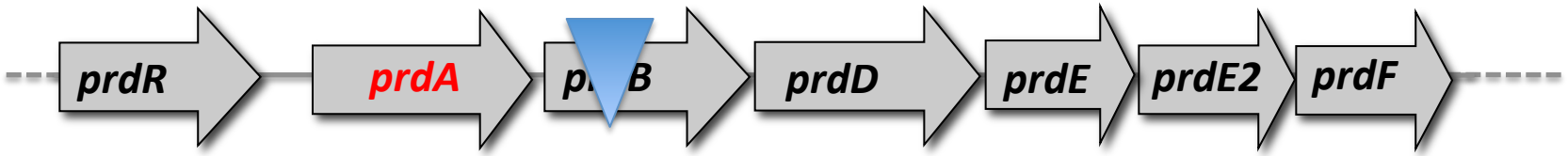


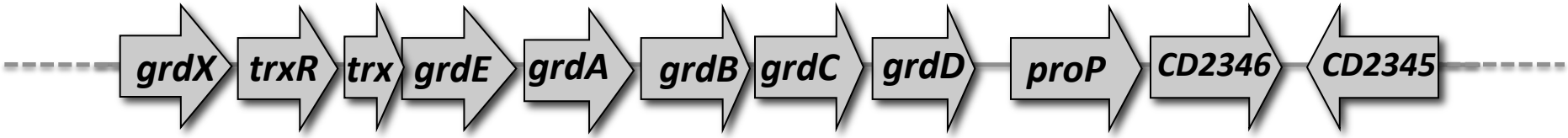
^{75}Se labeling of
Grd and Prd subunits
in *grd* and *prd* mutants.

William Self

D-Proline reductase (*prd*) operon



Glycine reductase (*grd*) operon



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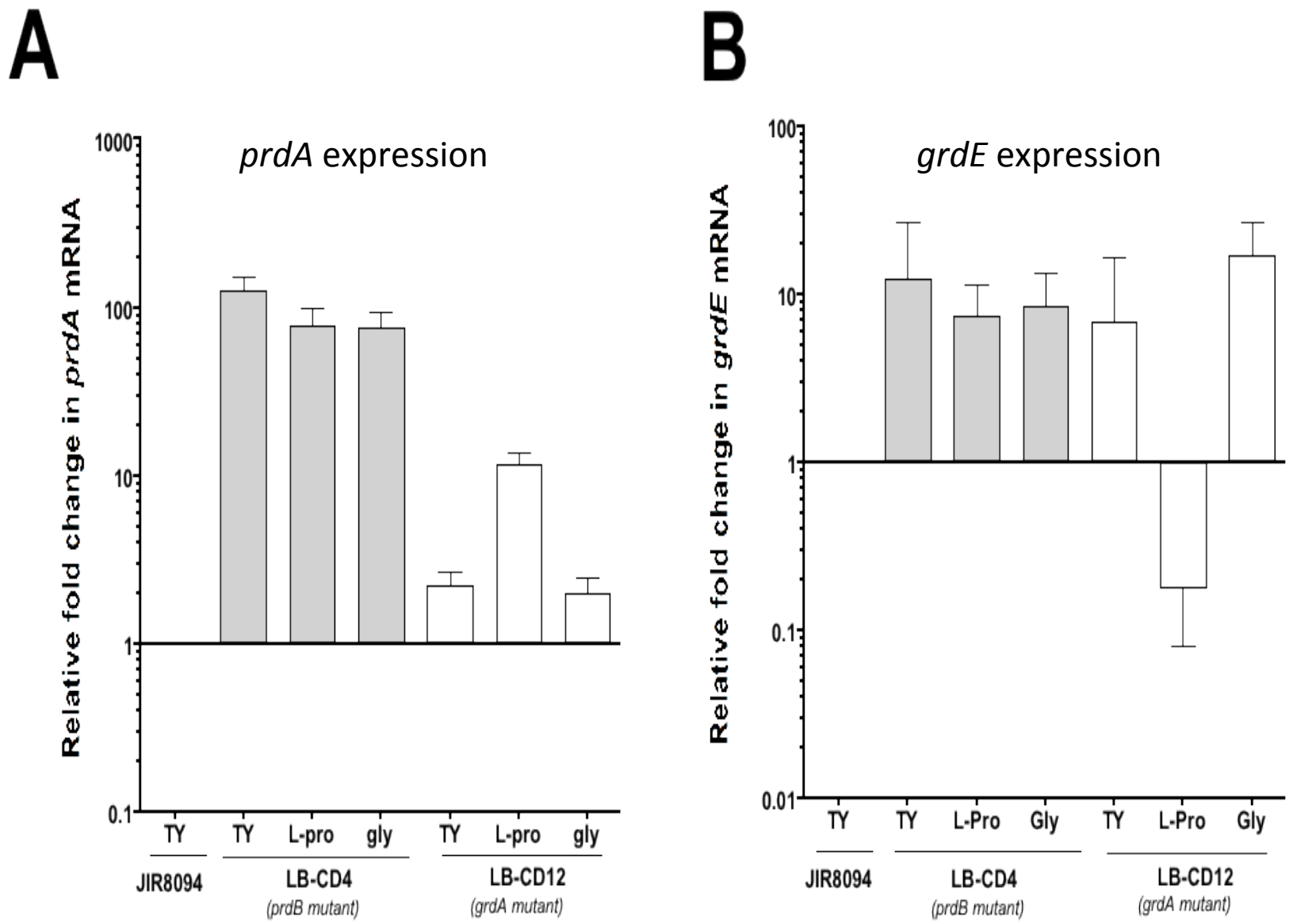
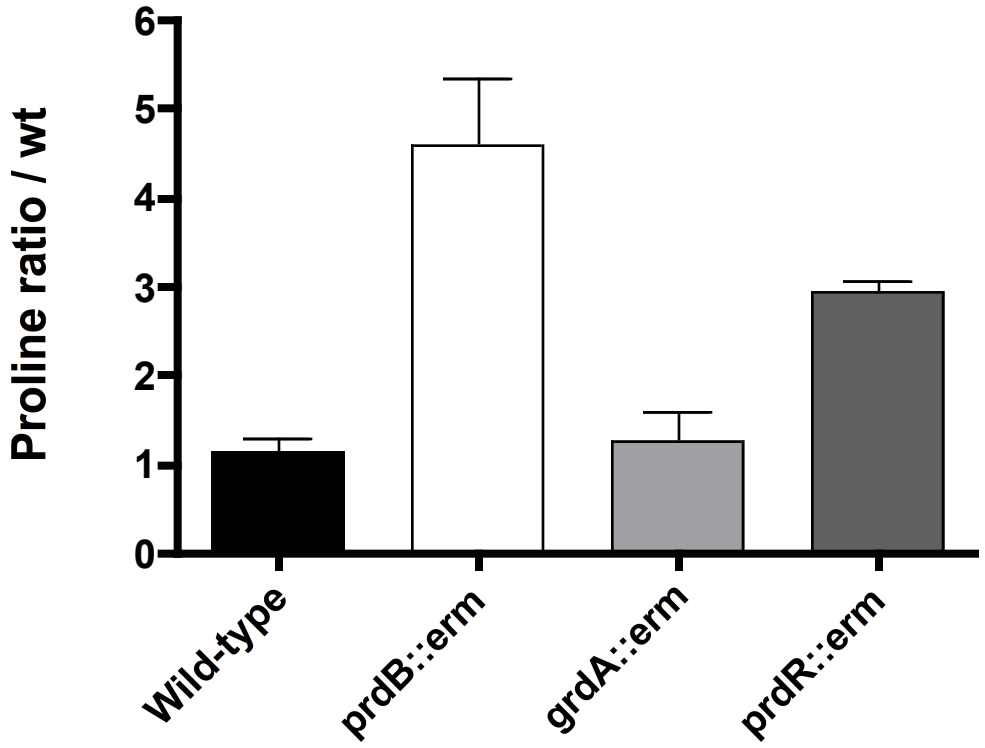

A
prdA expression
Relative fold change in prdA mRNA
1000
100
10
1
0.1
TY
TY
L-pro
gly
TY
L-pro
gly
JIR8094
LB-CD4
(prdB mutant)
LB-CD12
(grdA mutant)
B
grdE expression
Relative fold change in grdE mRNA
100
10
1
0.1
0.01
TY
TY
L-Pro
Gly
TY
L-Pro
Gly
JIR8094
LB-CD4
(prdB mutant)
LB-CD12
(grdA mutant)

Proline accumulation in a *prdB* mutant



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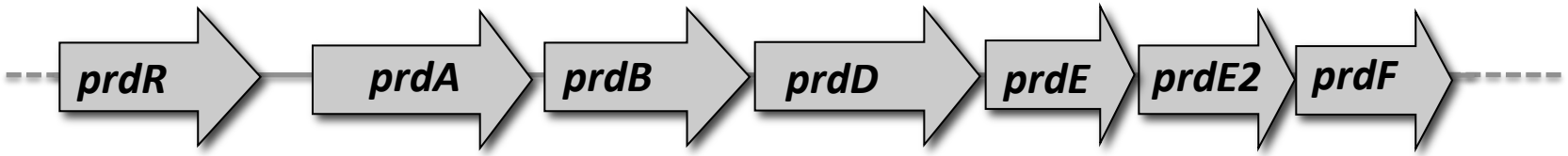
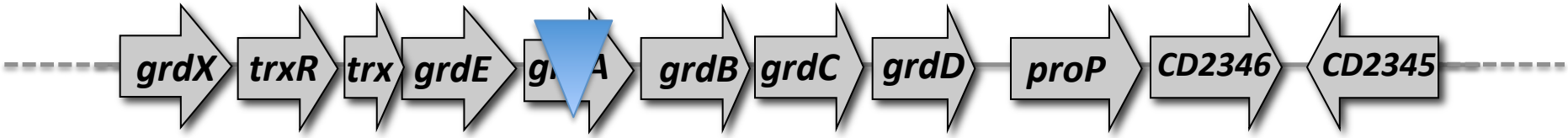
D-Proline reductase (*prd*) operon



Glycine reductase (*grd*) operon



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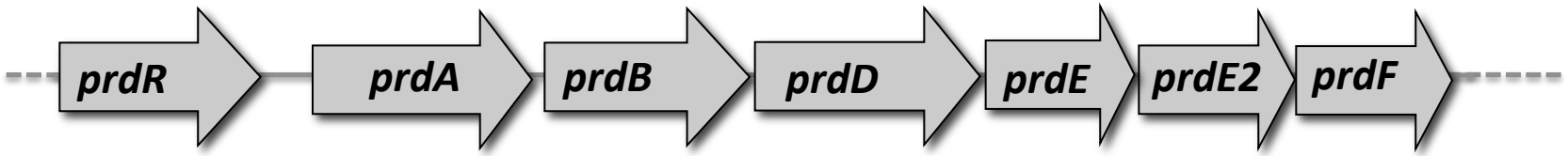
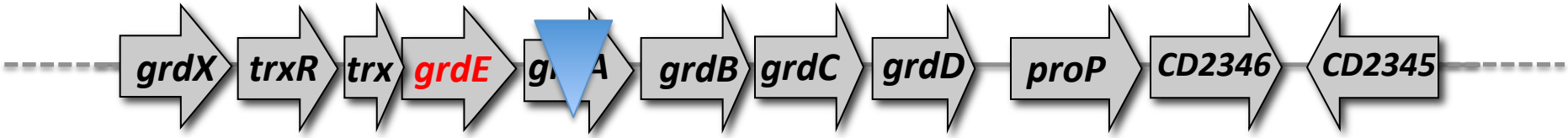
D-Proline reductase (*prd*) operon



Glycine reductase (*grd*) operon



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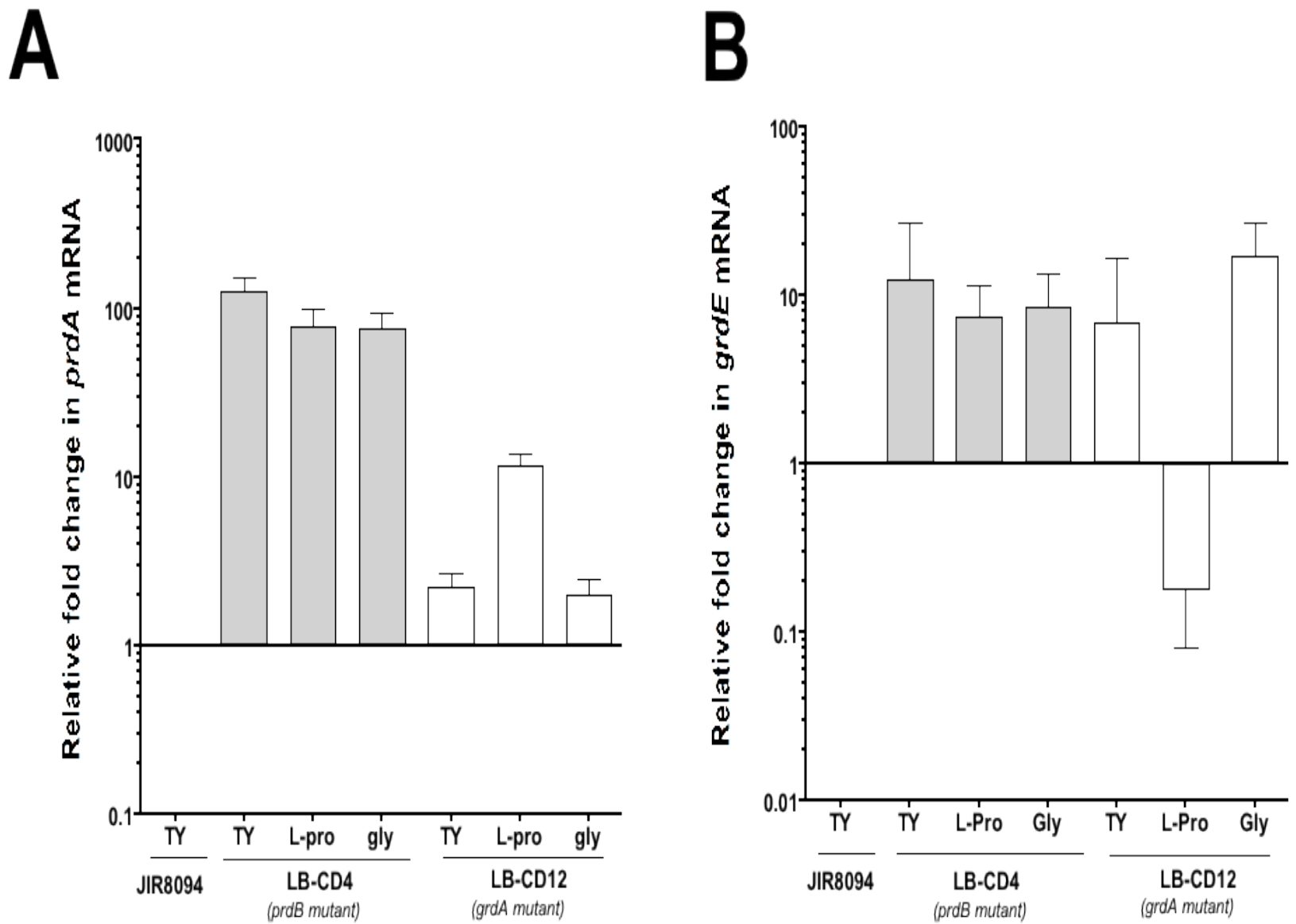
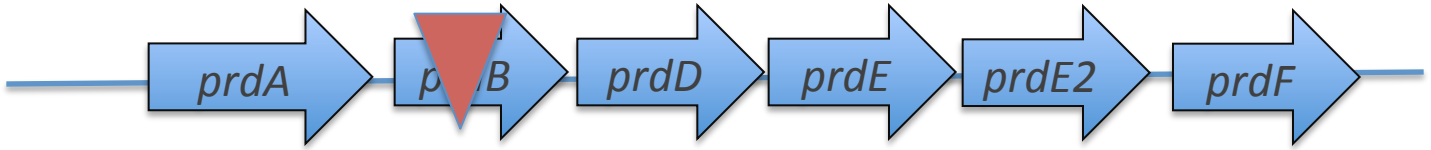
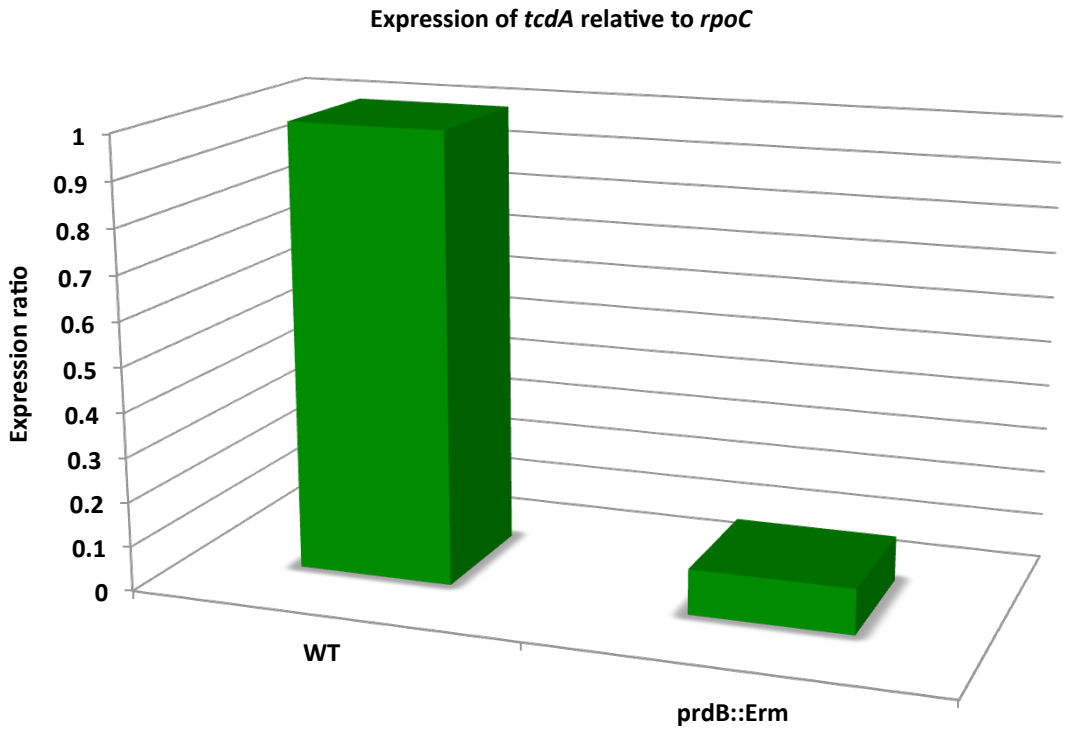

A
Relative fold change in prdA mRNA
1000
100
10
1
0.1
TY
TY
L-pro
gly
TY
L-pro
gly
JIR8094
LB-CD4
(prdB mutant)
LB-CD12
(grdA mutant)
B
Relative fold change in grdE mRNA
100
10
1
0.1
0.01
TY
TY
L-Pro
Gly
TY
L-Pro
Gly
JIR8094
LB-CD4
(prdB mutant)
LB-CD12
(grdA mutant)

Does Stickland metabolism affect toxin expression ?





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Summary: Regulation of Stickland Metabolism

1) The *prd* genes are induced by proline.

2) PrdR is a proline-responsive, direct (?), positive regulator of the *prd* gene clusters.

3) The *grd* locus is induced by glycine and repressed by proline.

4) Addition of proline to the medium or accumulation of proline intracellularly inhibits toxin gene expression.

Unanswered questions

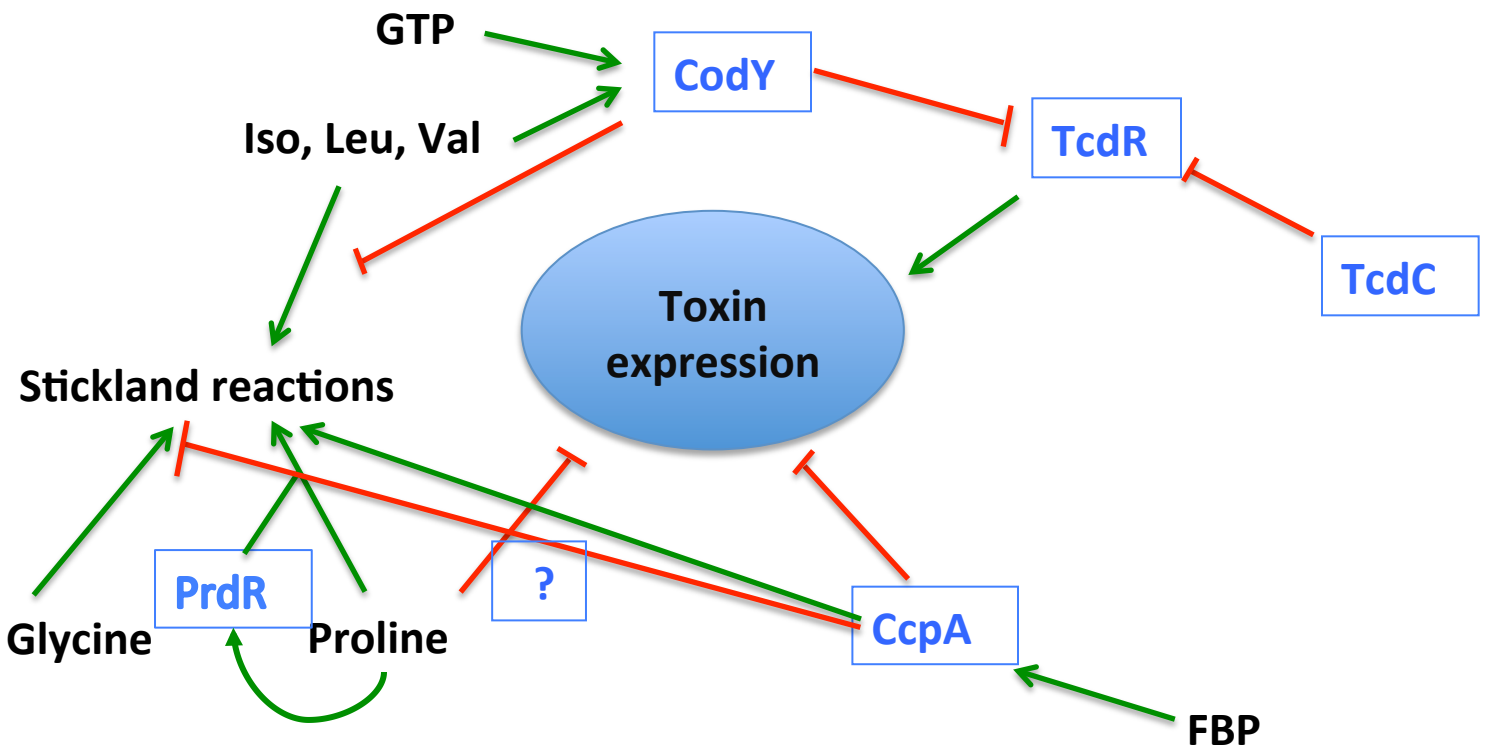
1) Is binding of PrdR to the *prd* promoter regions stimulated by proline?

2) Is the PrdR the mediator of the proline effect on toxin gene expression?

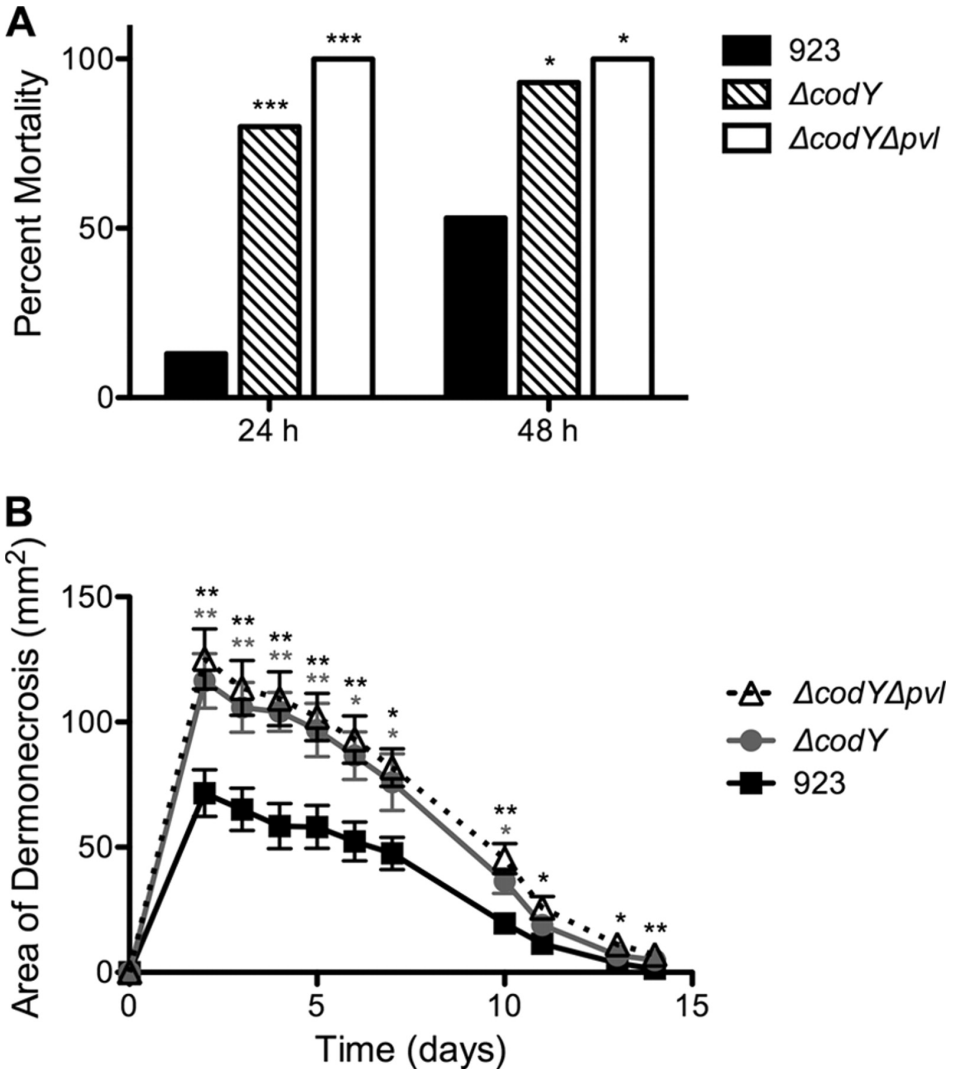
3) Which regulatory protein controls *grd* expression?

4) To what extent does Stickland metabolism contribute to pathogenesis in animal models of CDI?

Toxin regulation overview



Deletion of *codY* increased the virulence of USA300 (MRSA) in mouse models of *S. aureus* necrotizing pneumonia and dermonecrosis.



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