Novel Regulatory Mechanisms Control Sporulation Initiation in *C. difficile*

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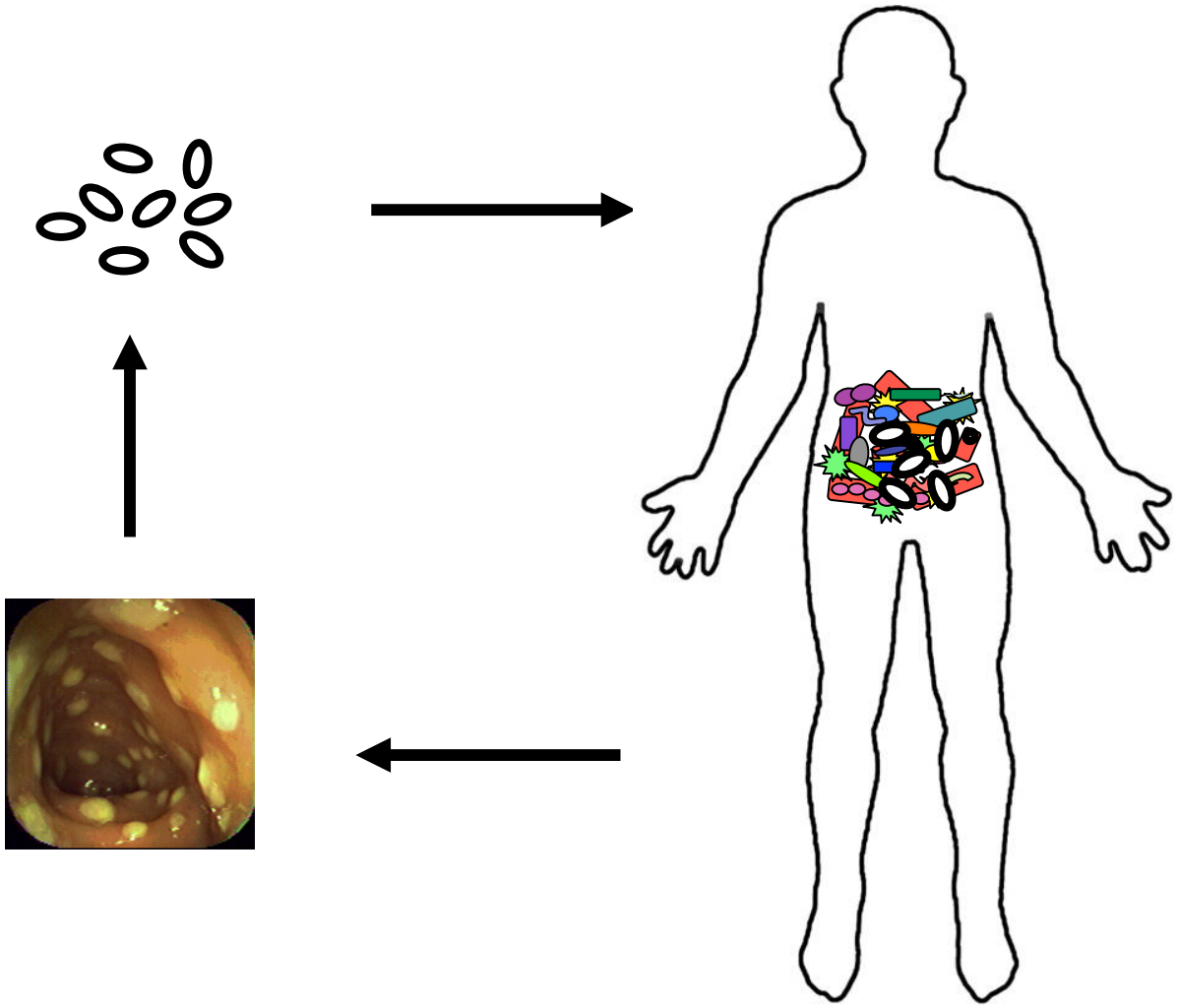
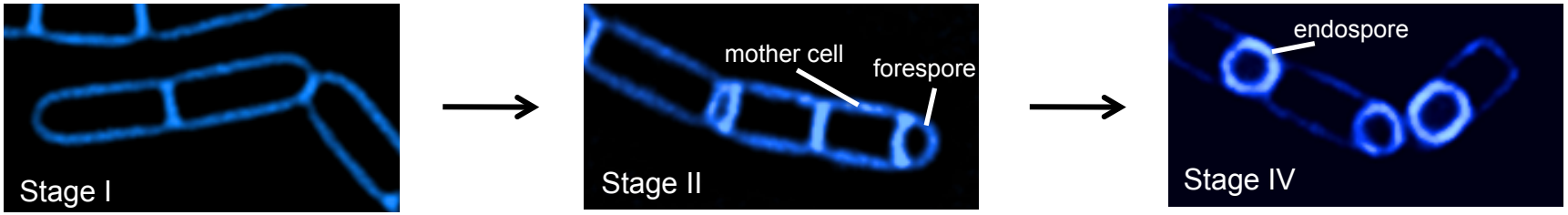
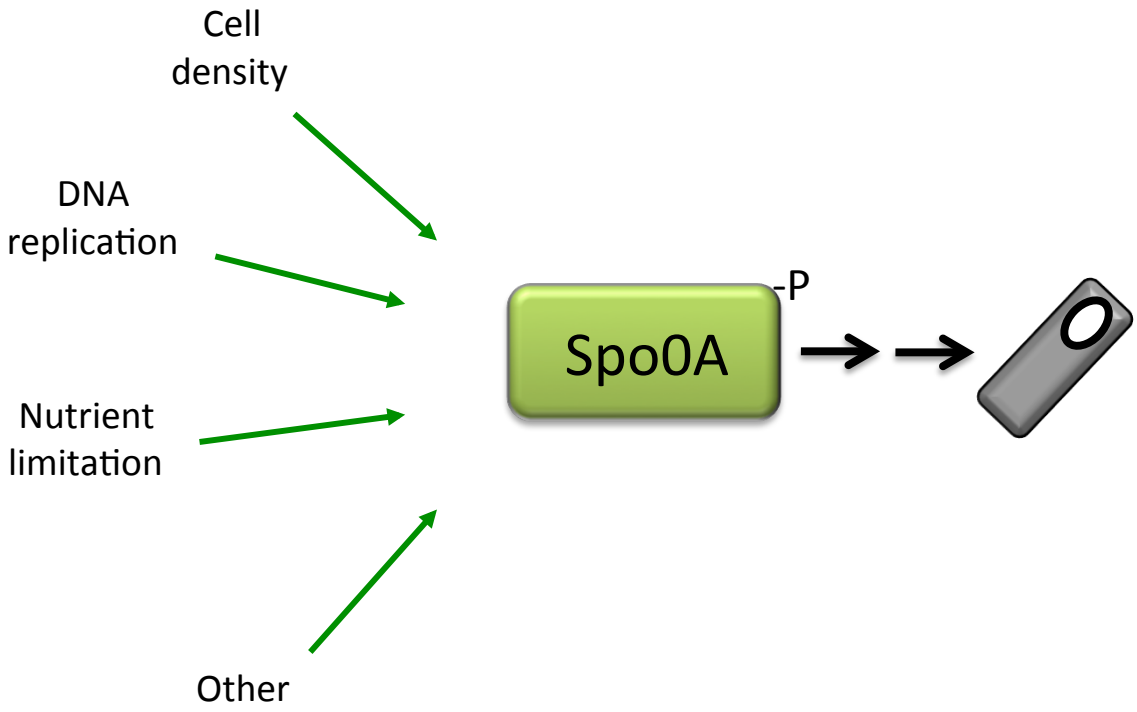
C. difficile Life cycle



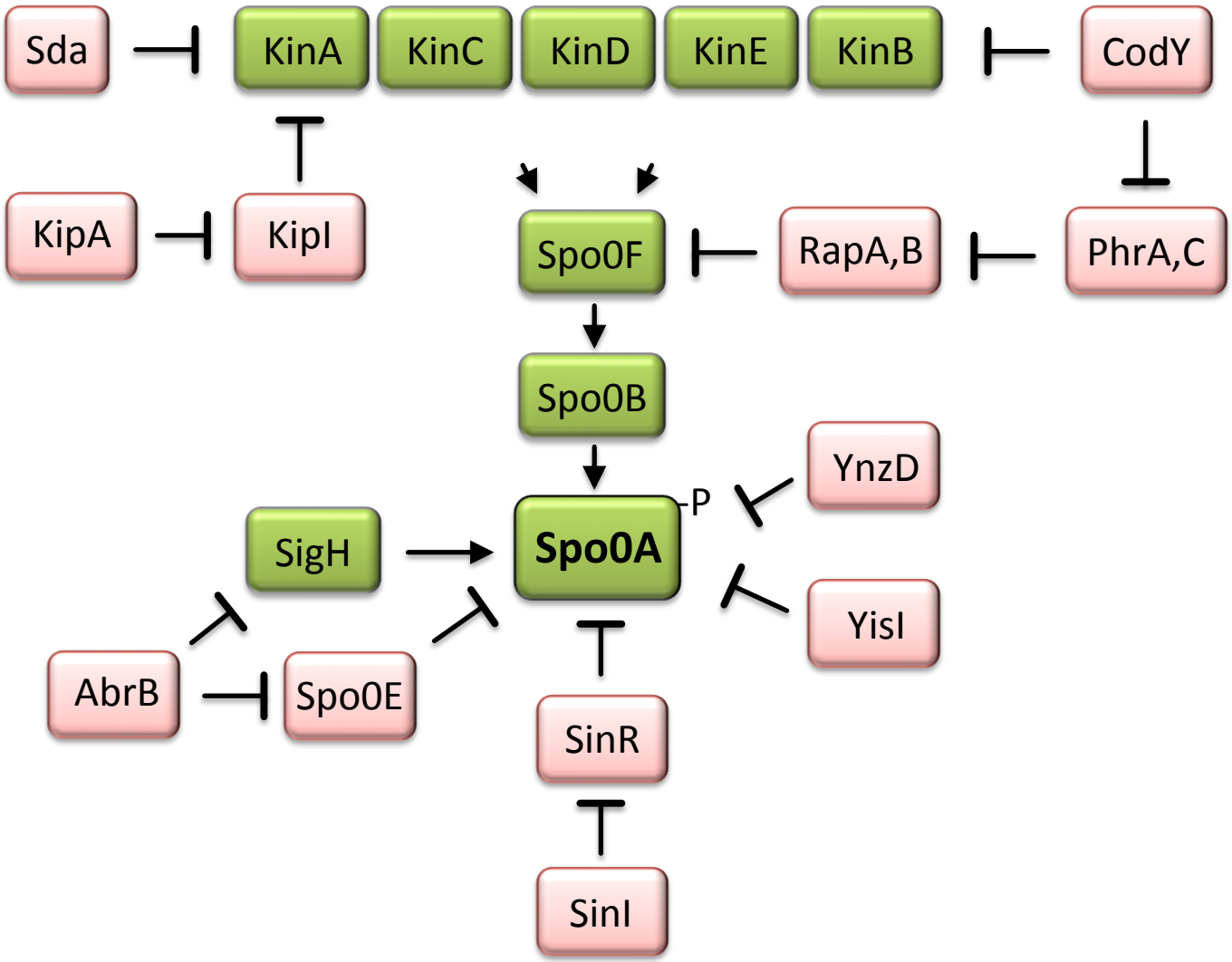
C. difficile Spore Development



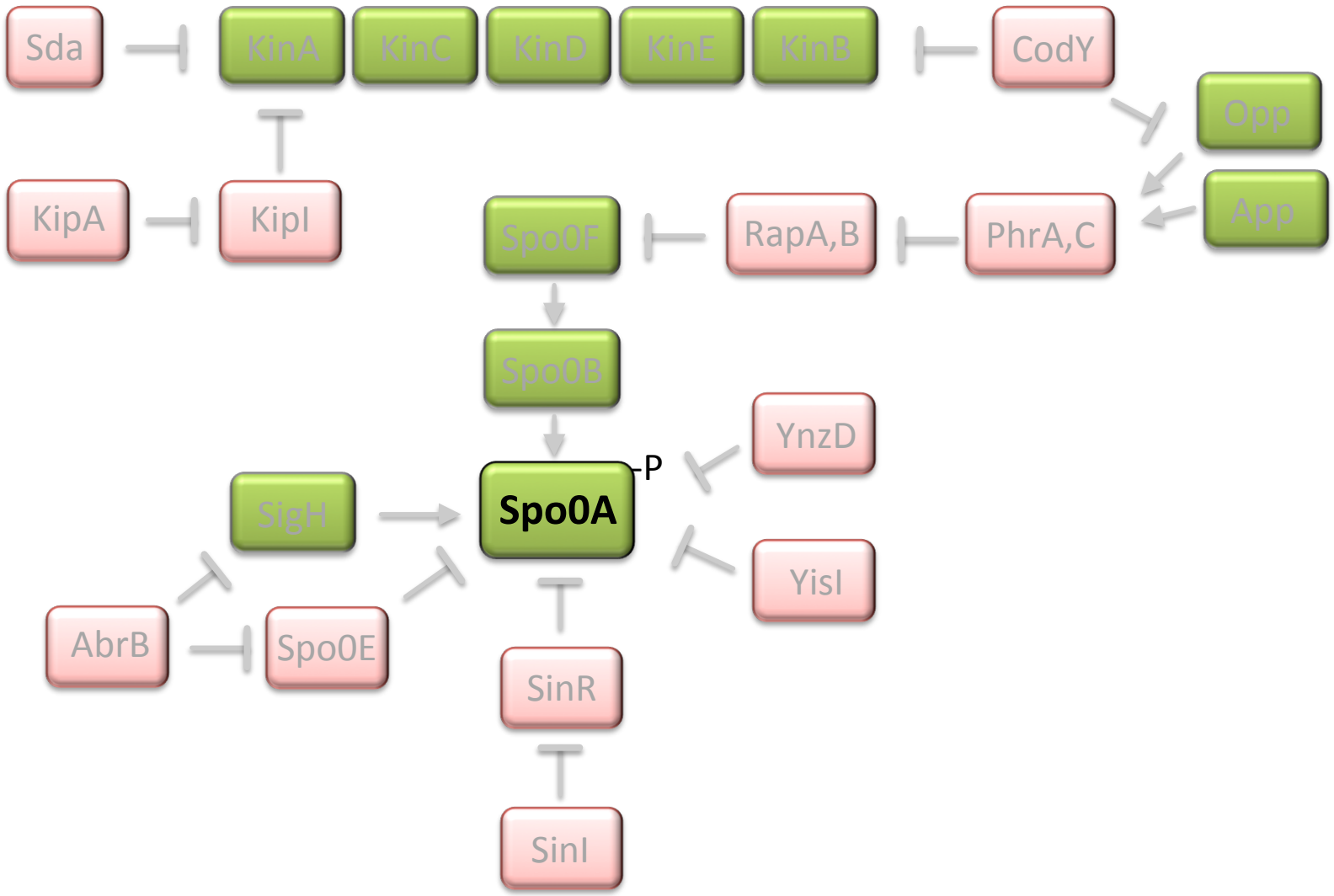
Spo0A is the master regulator of sporulation



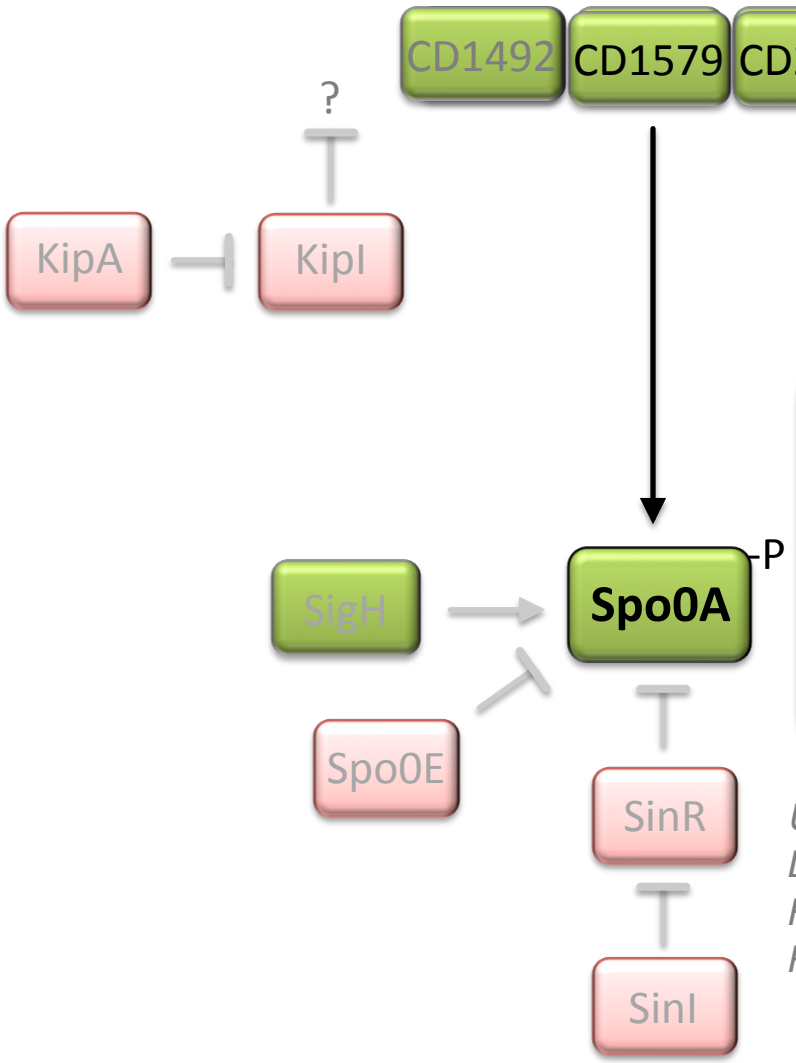
A Model for Initiation—*B. subtilis*



C. difficile Sporulation Initiation



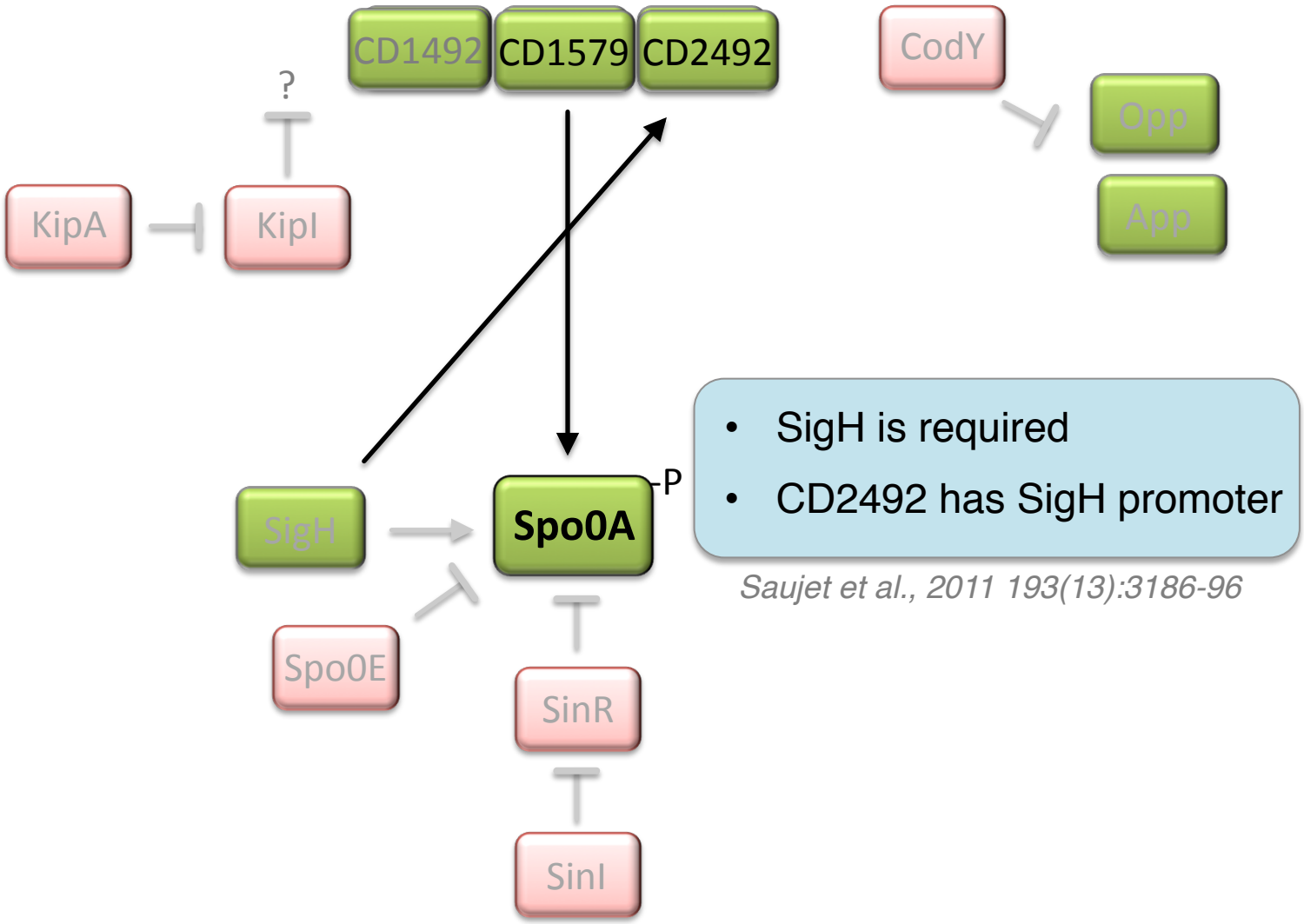
C. difficile Sporulation Initiation



- Spo0A is required
- Can be PO_4 by CD1579
- Spo0A is important for transmission
- Spo0A is needed for spo-specific gene expression

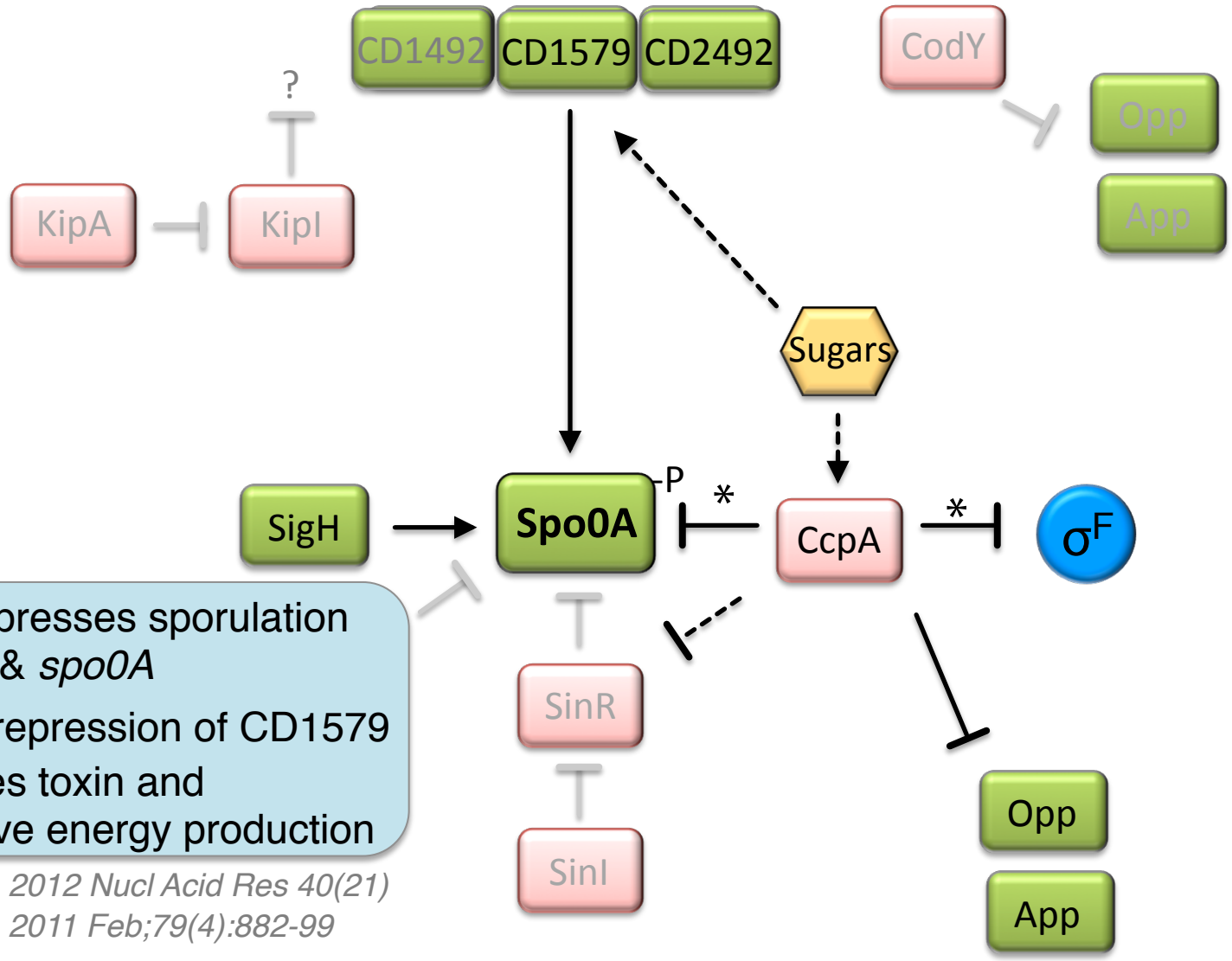
Underwood et al., 2009 J Bacteriol 191(23):7296
Deakin et al., 2012 Infect Immun 80(8):2704-11
Fimlaid et al., 2013 PLoS Genet 9(8):e1003660
Pettit et al., 2014, BMC Genomics 15:160

C. difficile Sporulation Initiation



Saujet et al., 2011 193(13):3186-96

C. difficile Sporulation Initiation



- CcpA represses sporulation via *sigF* & *spo0A*
- indirect repression of CD1579
- represses toxin and alternative energy production

Antunes et al., 2012 Nucl Acid Res 40(21)
Antunes et al., 2011 Feb;79(4):882-99

C. difficile Sporulation Initiation

Katie Nawrocki

- CodY represses sporulation
- repression of toxin & nutrient acquisition
- repression of *sinR*

Dineen, McBride et, 2010 J Bac 192(20)
Dineen et al., 2007 Mol Micro 66(1):206-19
Edwards et al., 2014 Infect Immun 82(10)

CD1492 CD1579 CD2492

?

KipA ⊣ KipI

Sugars

SigH → **Spo0A** ~P ⊣* CcpA ⊣* σ^F

Spo0E ⊣ Spo0A

Spo0A ⊣ SinR

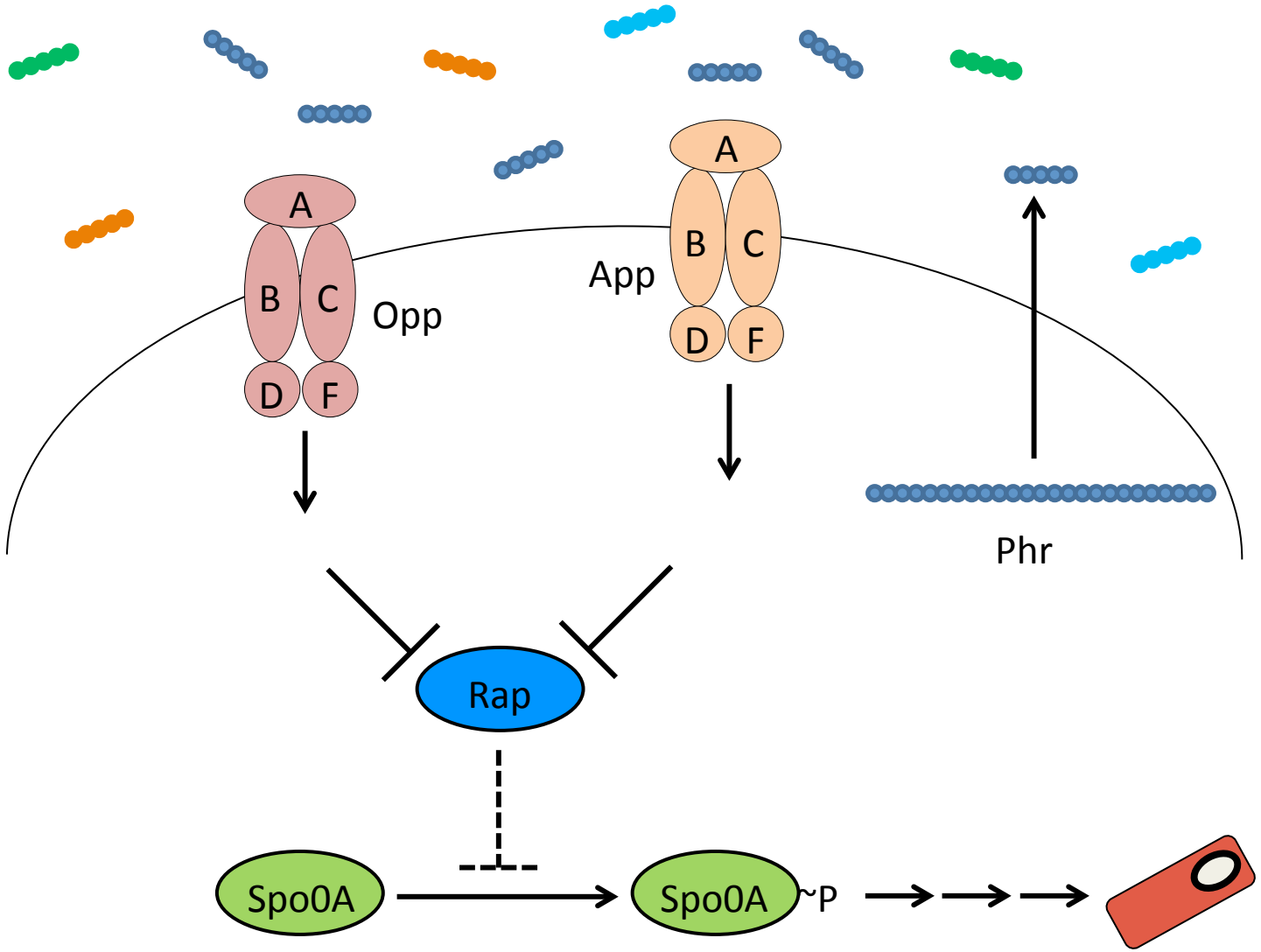
SinR ⊣ SinI

SinR ⊣ CodY

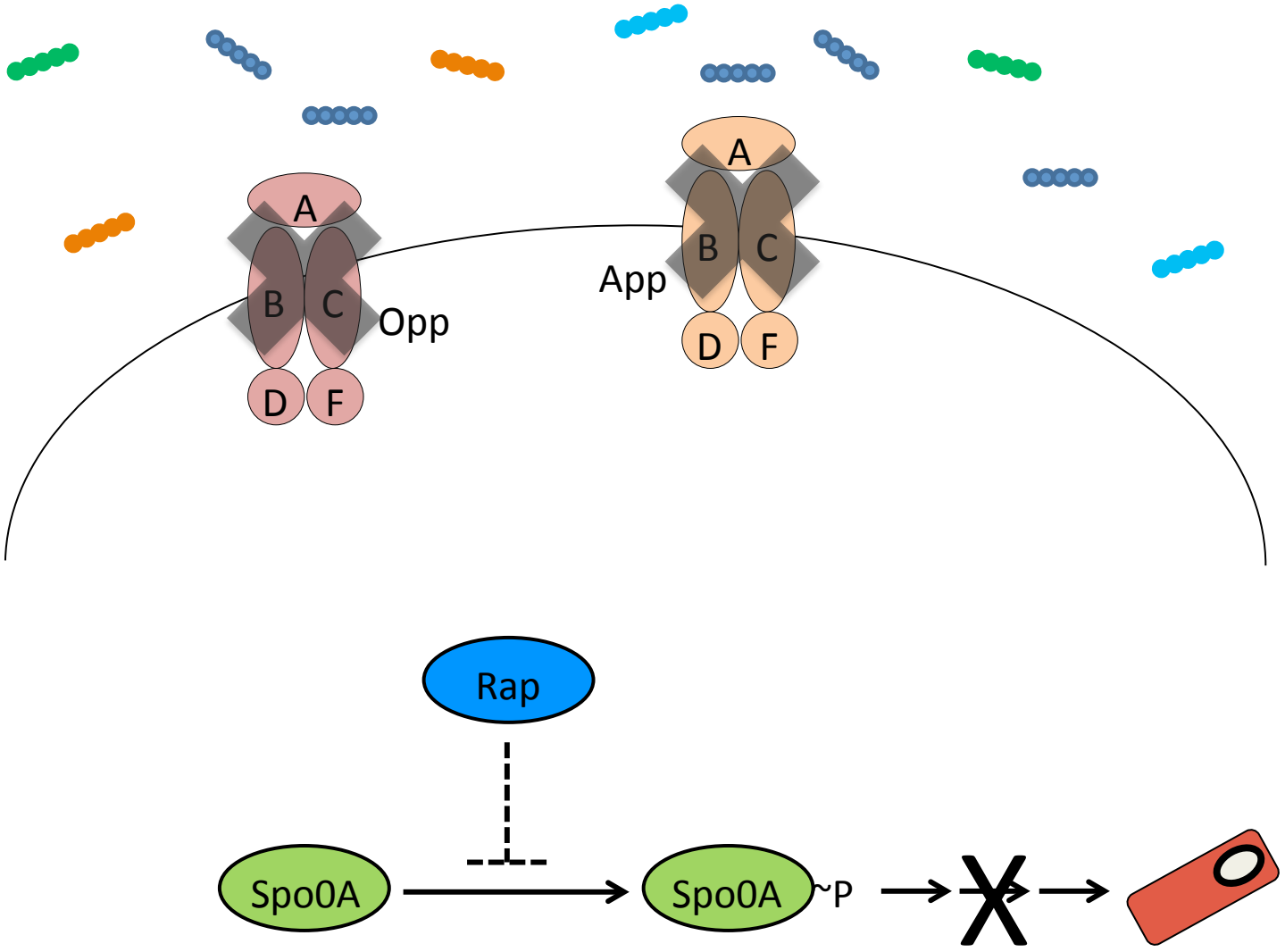
CodY ⊣ Opp

App

Opp and App oligopeptide permeases in *B. subtilis*

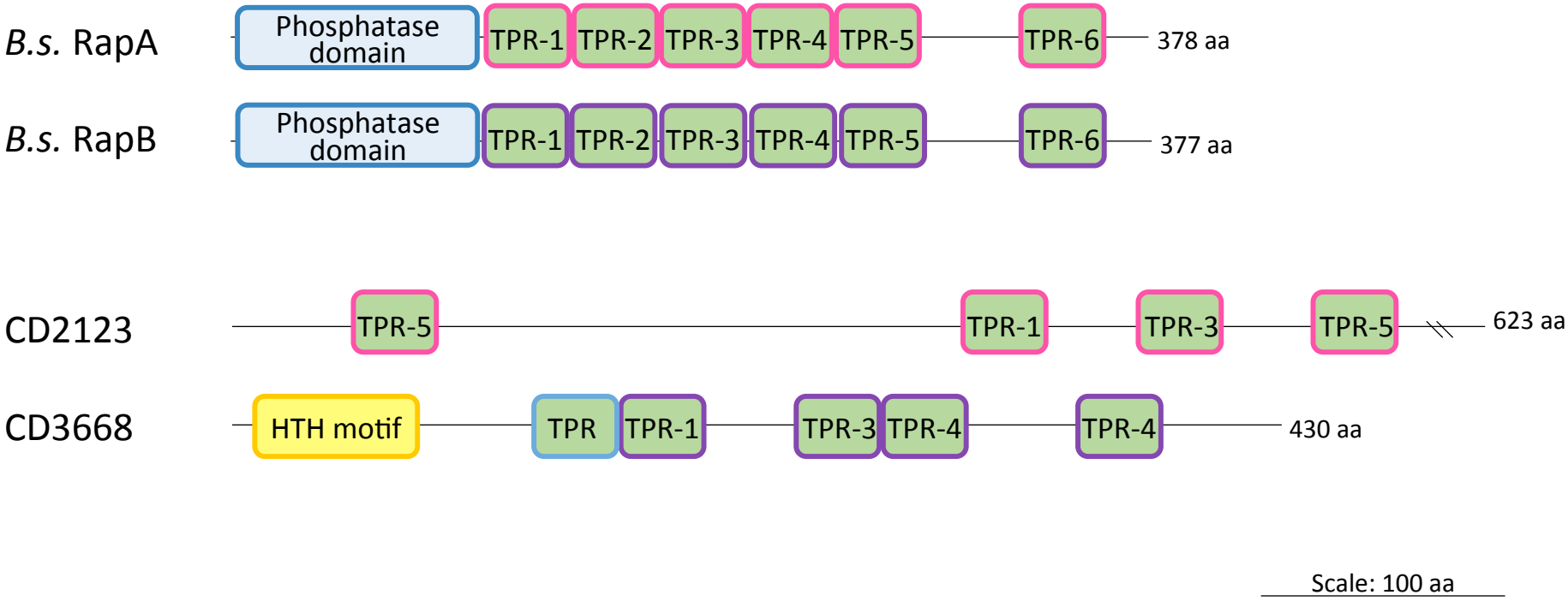


Opp and App permeases in *C. difficile*

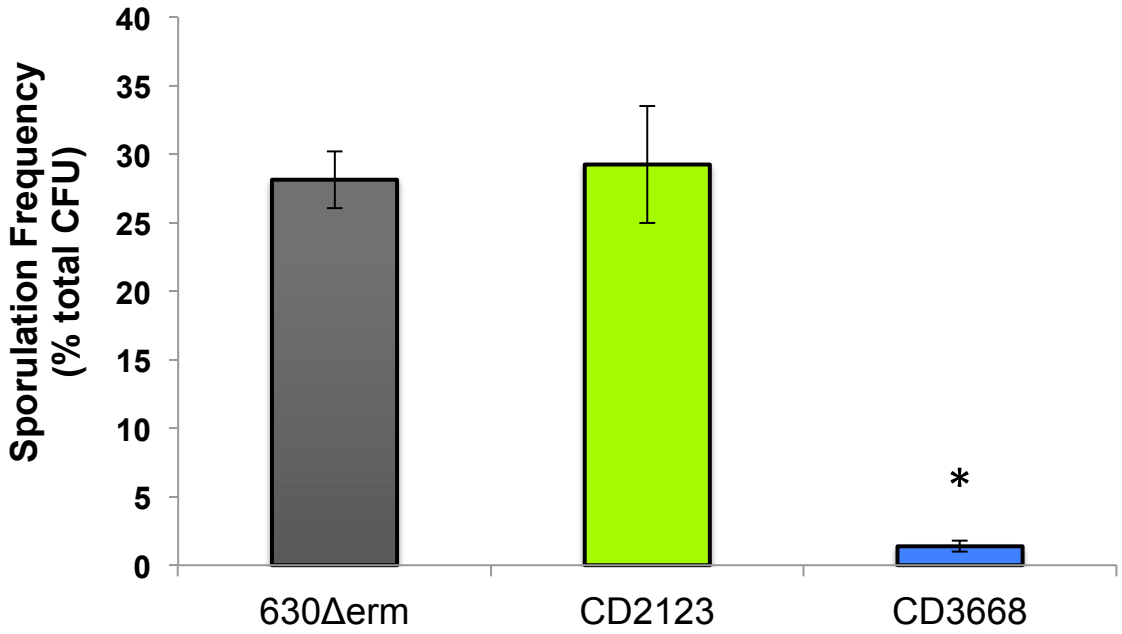


Rap Orthologs in *C. difficile*

Adrianne Edwards

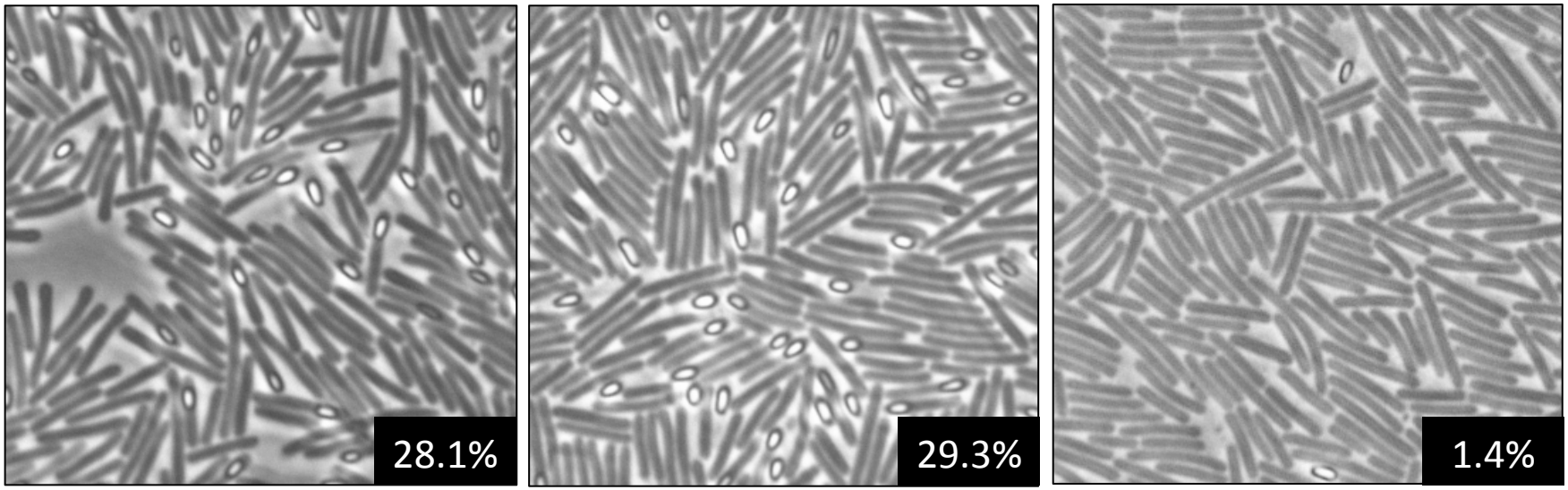


Sporulation of CD2123 & CD3668 mutants

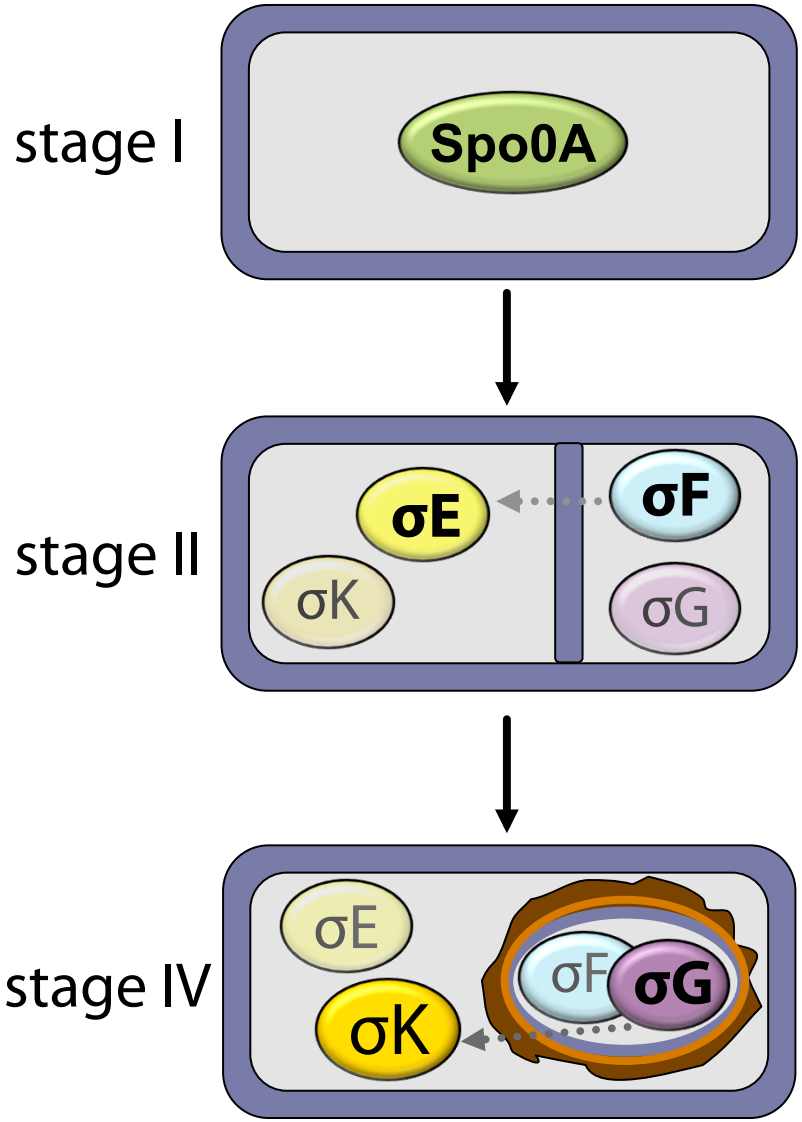


* P-value < 0.05

CD3668 mutant has a Sporulation Defect

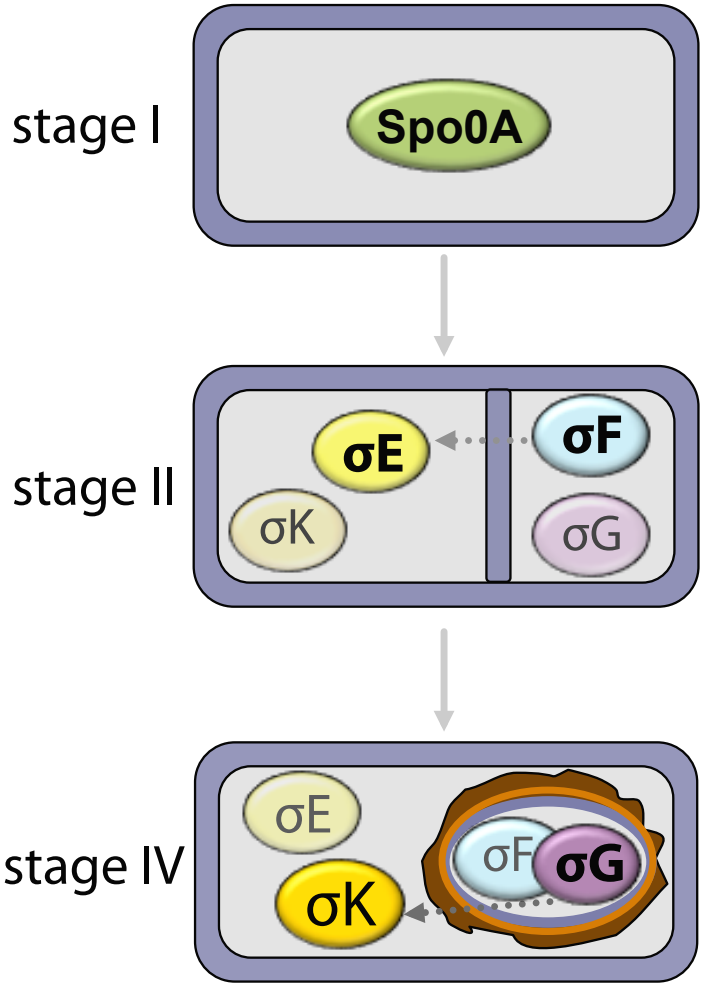


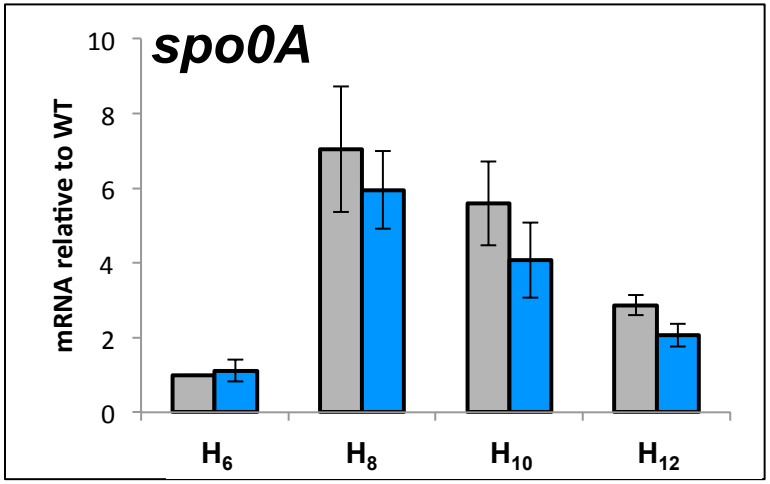
Orchestration of Sporulation Gene Expression

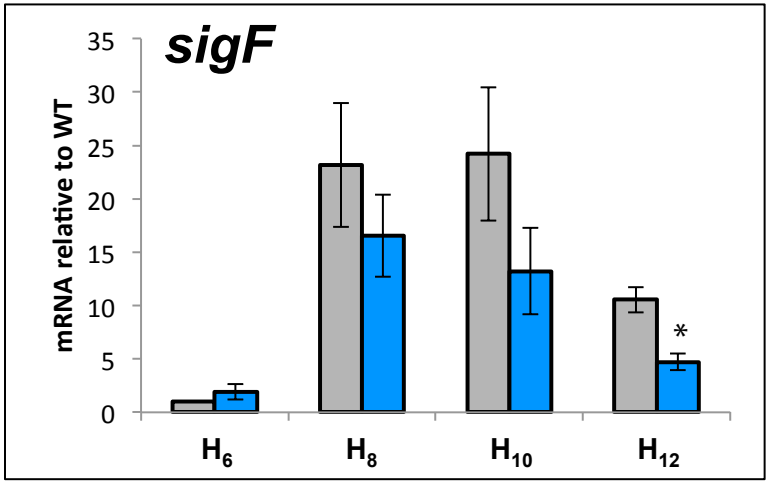


Adapted from: Fimlaid *et al.,* 2013 *PLoS Genetics*; Pereira *et al.,* 2013 *PLoS Genetics*; Saujet *et al., PLoS Genetics* 2013

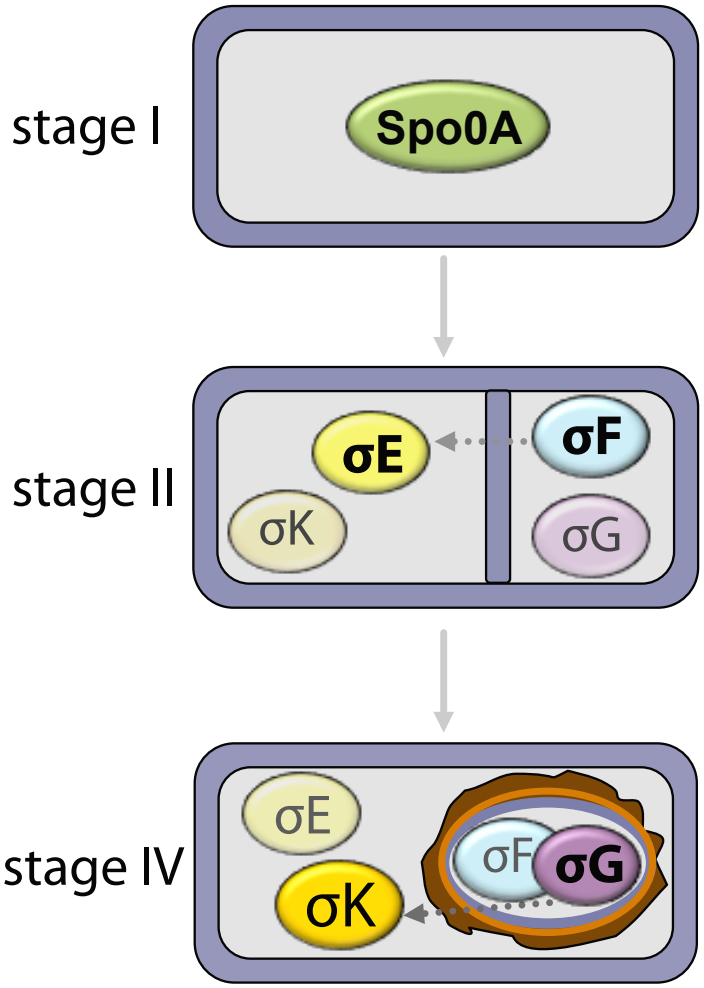
Orchestration of Sporulation Gene Expression

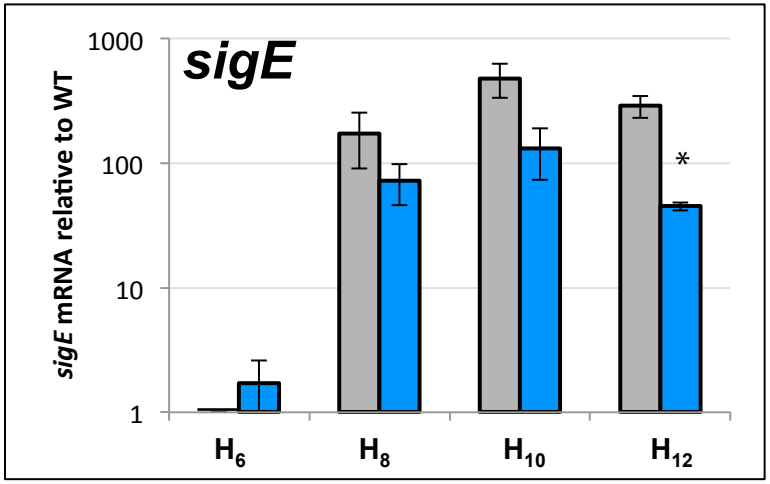


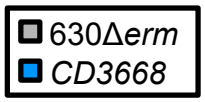


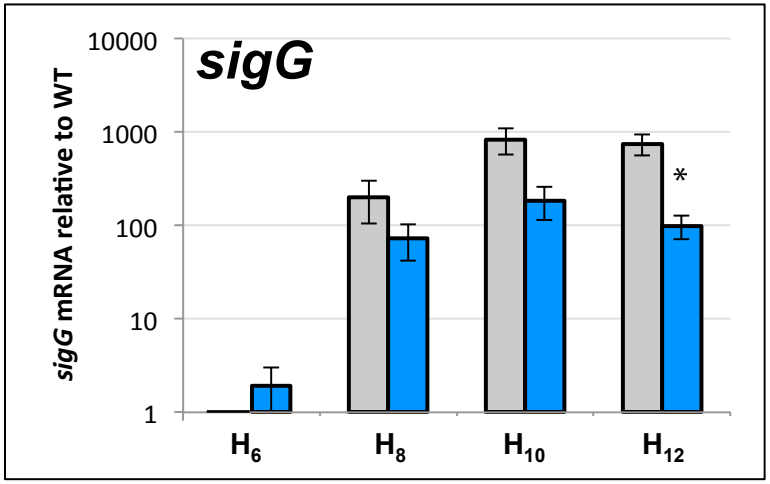


Sporulation Gene Expression is lower in the CD3668 Mutant

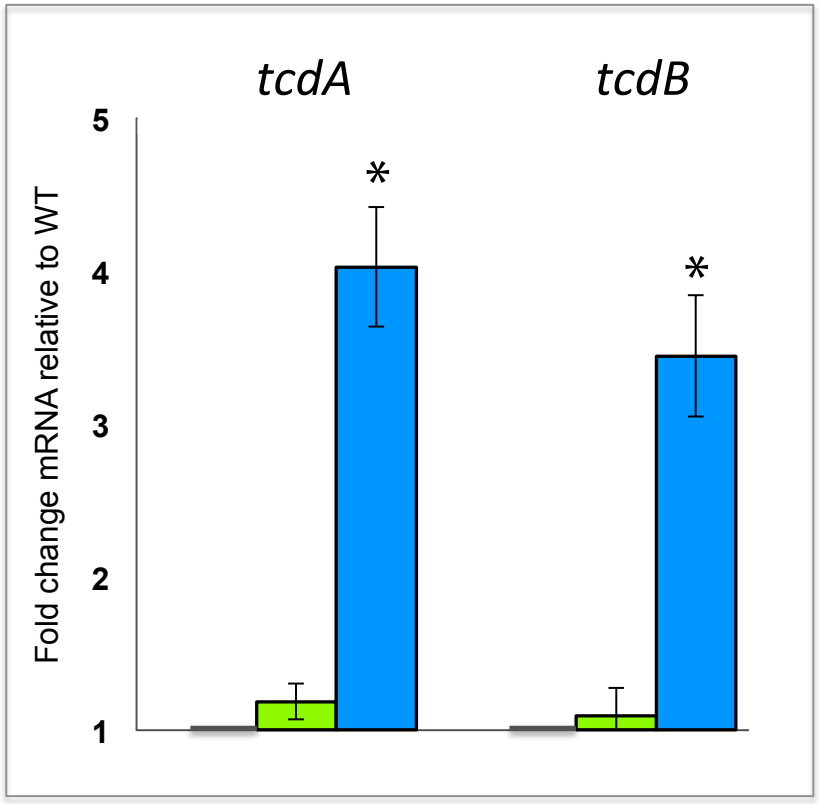


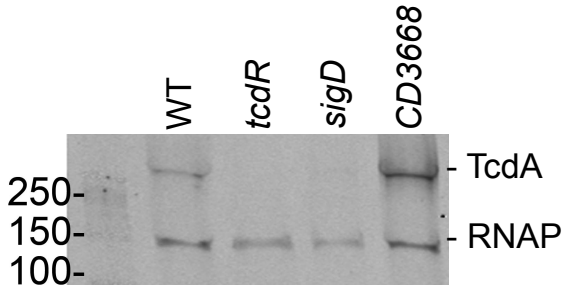






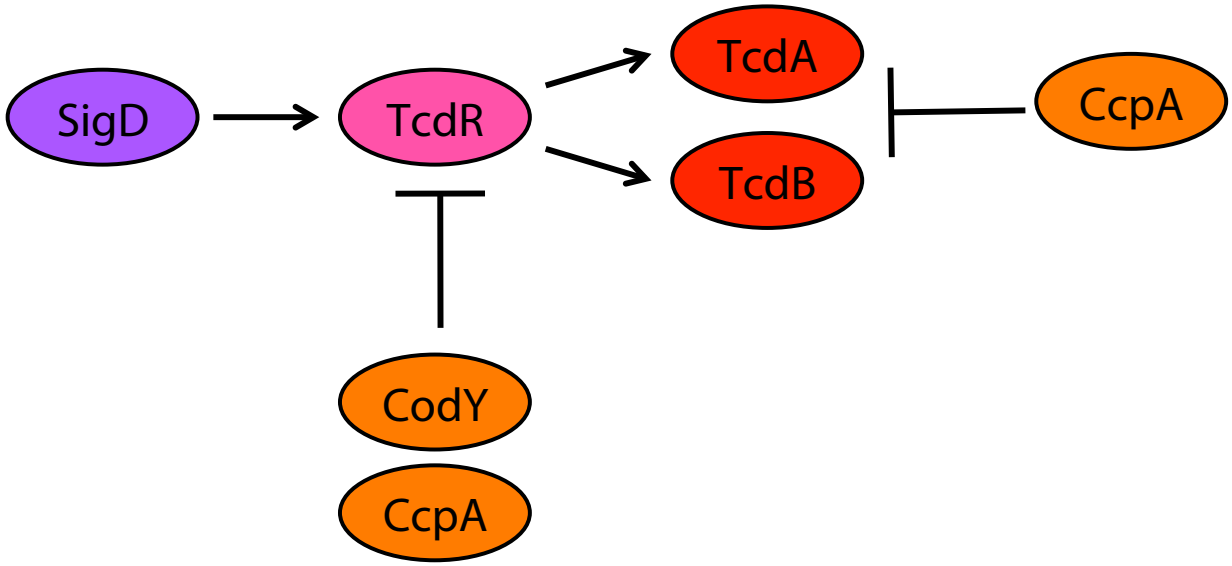
Toxin production is increased in the CD3668 mutant



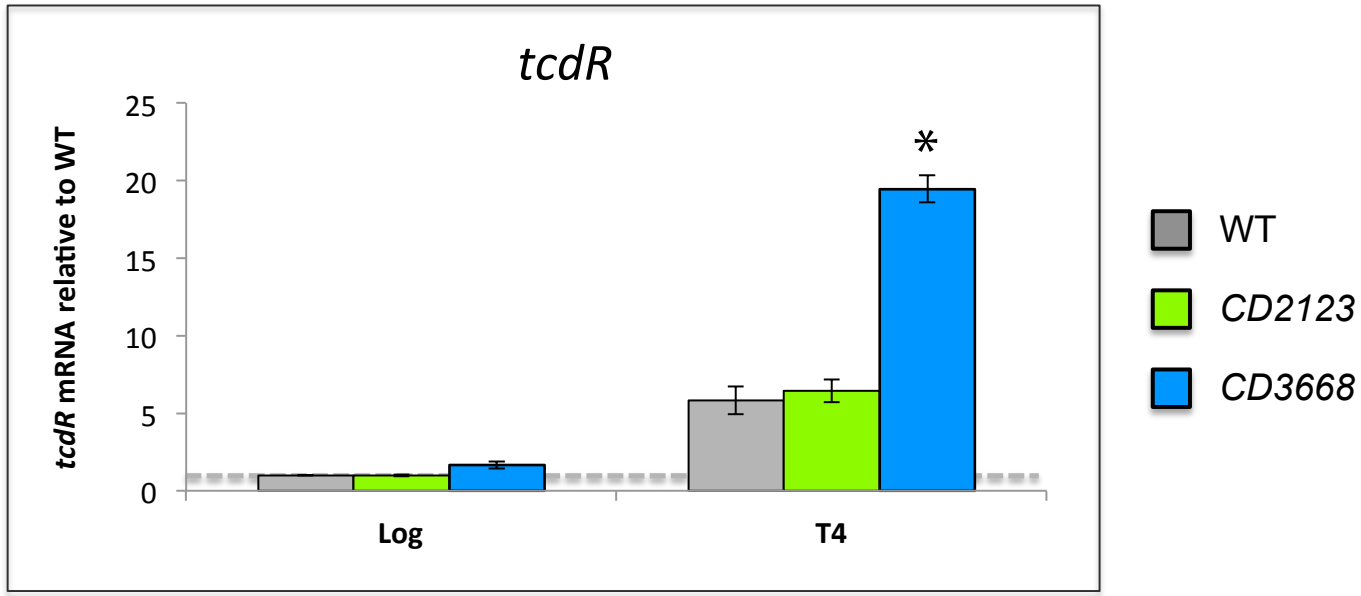


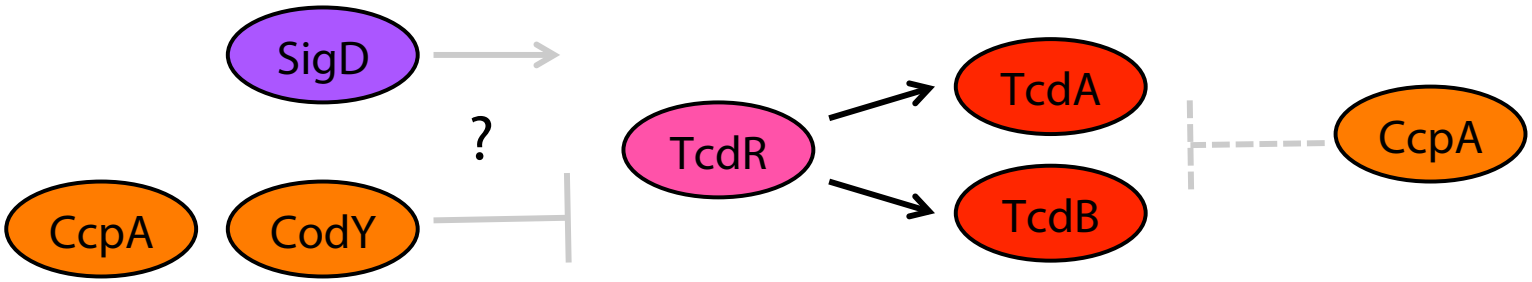
* P-value < 0.05

C. difficile Toxin Regulation

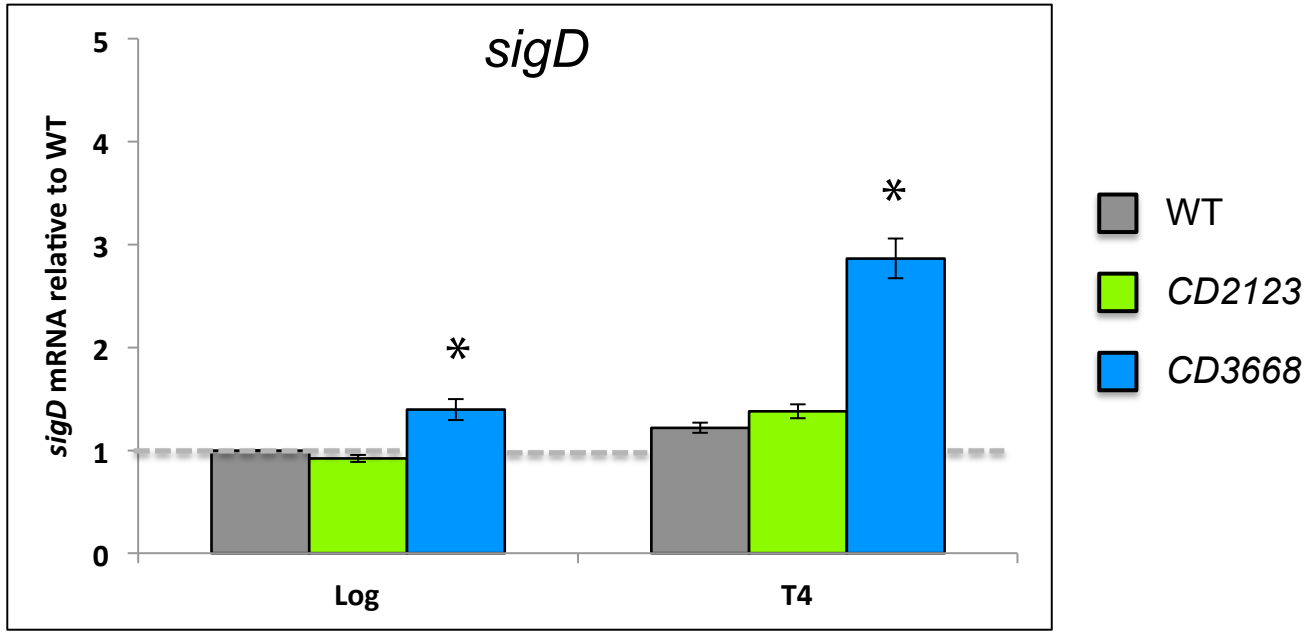


TcdR expression is higher in the CD3668 mutant





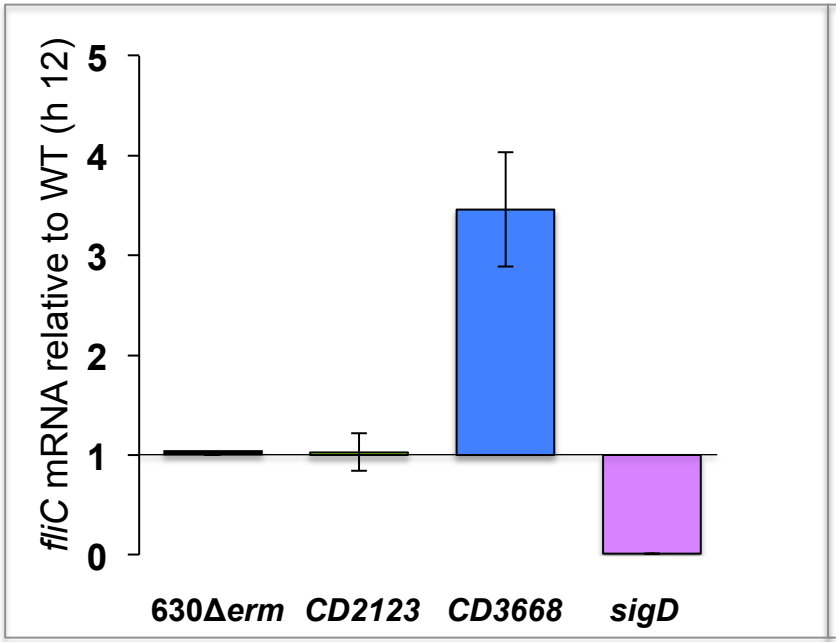
Transcription of *sigD* is increased in the CD3668 mutant



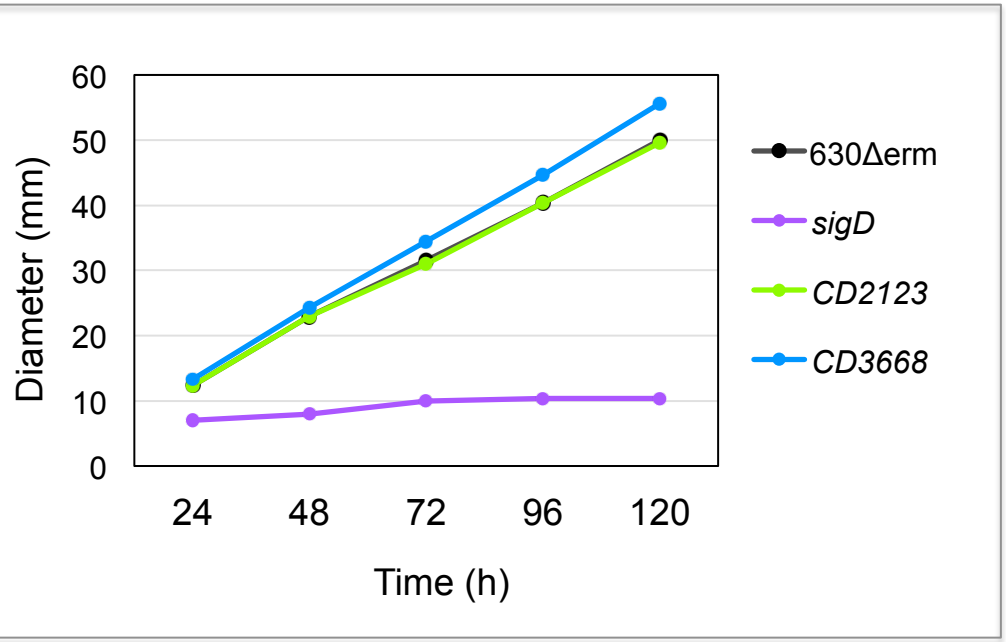
* P-value < 0.05

SigD-dependent Gene Expression and Motility are Greater in a CD3668 Mutant

fliC

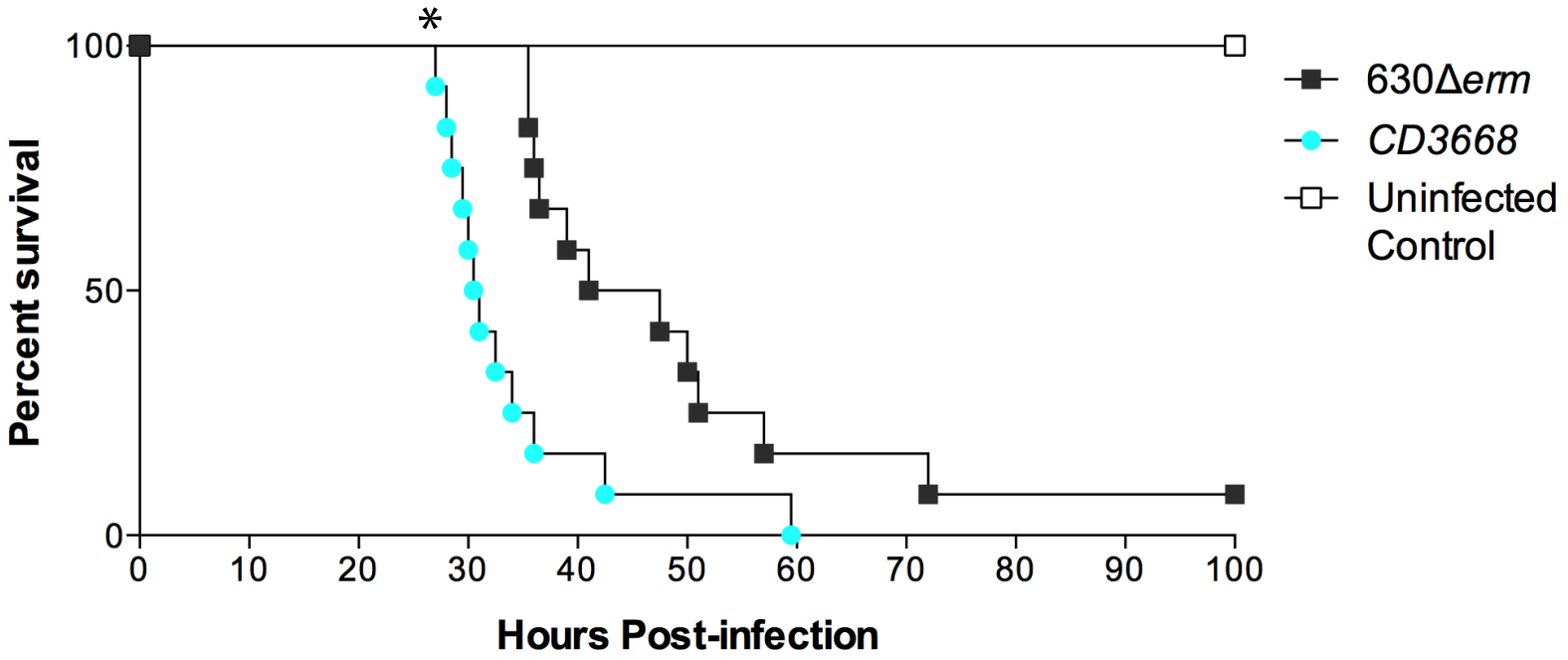


Motility



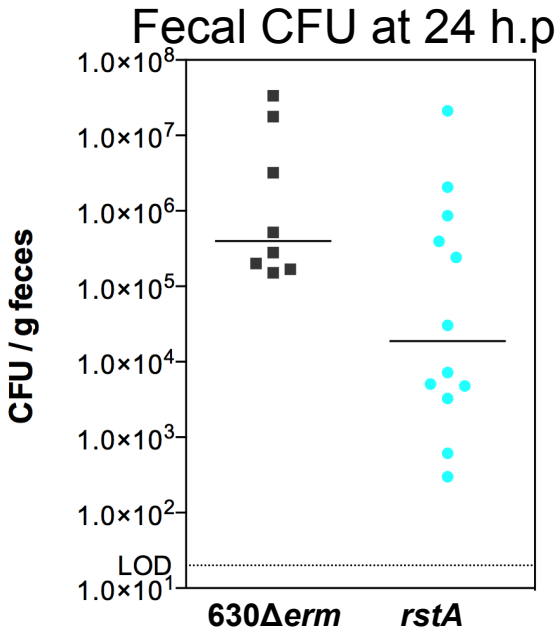
rstA: regulator of sporulation and toxin

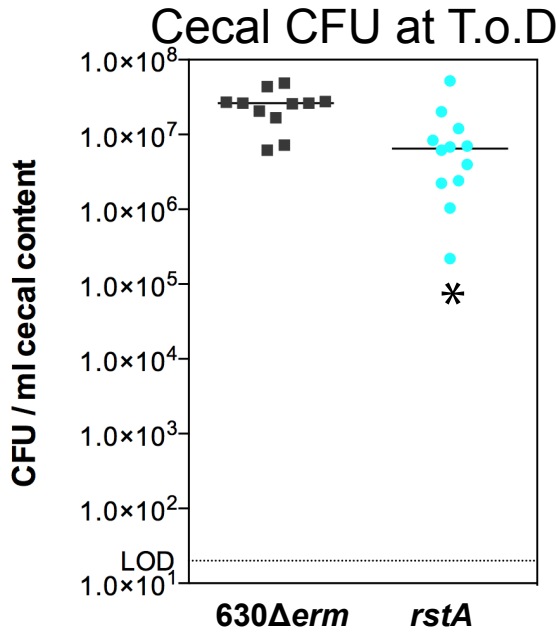
rstA Mutant Demonstrates Increased Virulence in the Hamster Model of CDI



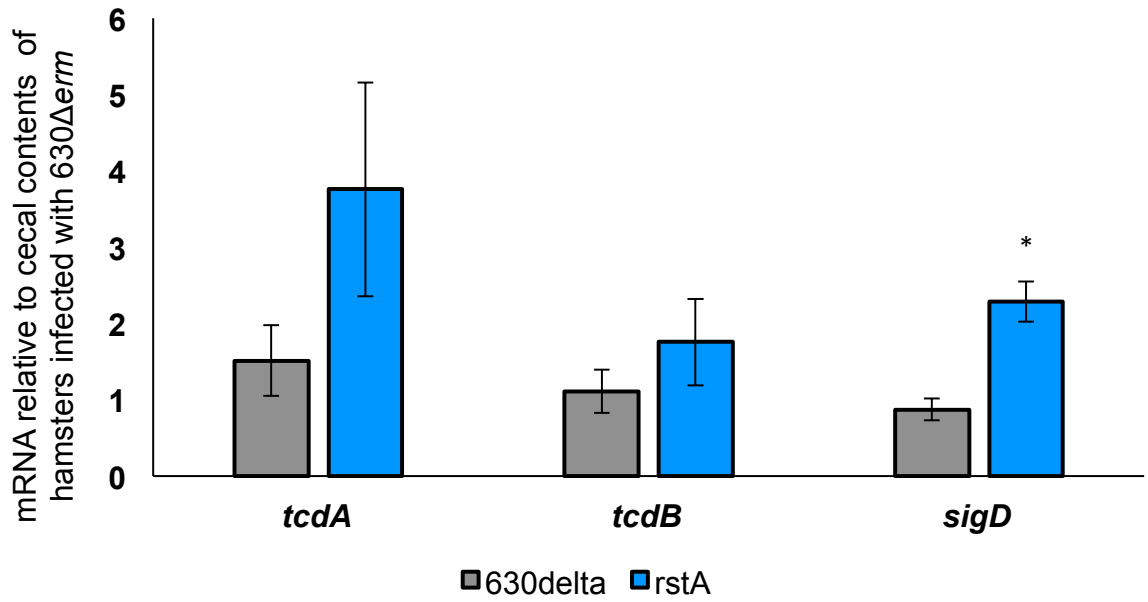
* P-value < 0.05

rstA Mutant Does Not Produce Higher CFU During Infection





Toxin Gene Expression is Higher in the *rstA* mutant *in vivo*



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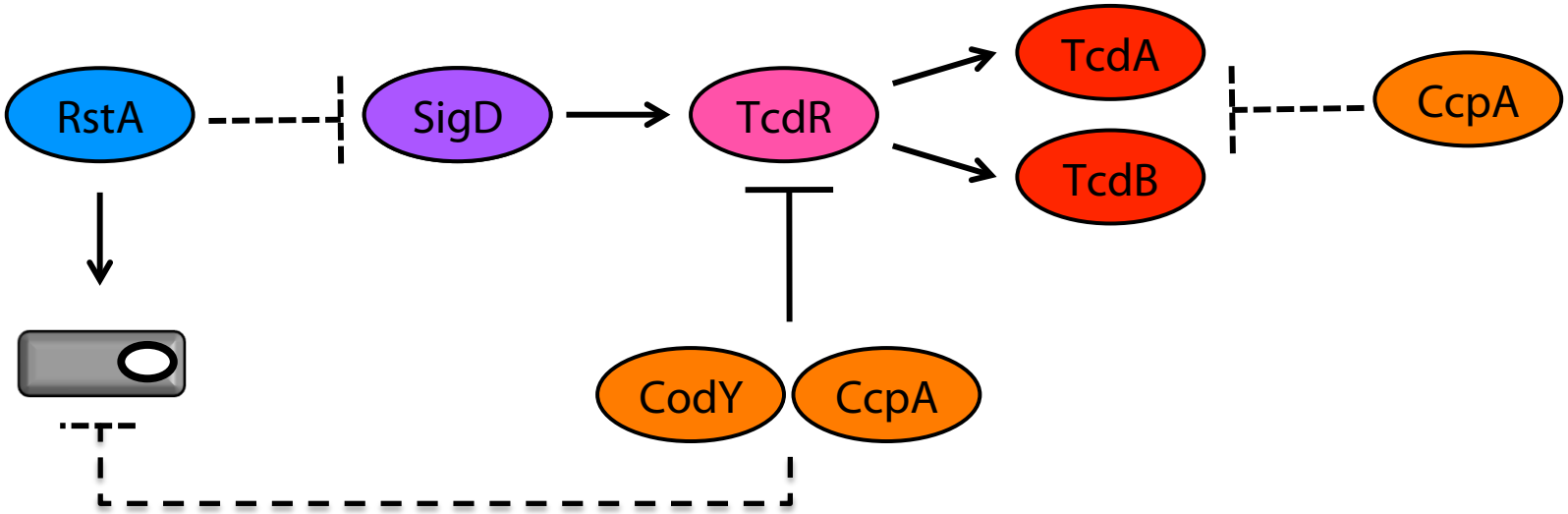
C. difficile Toxin & Sporulation Regulation



Future Directions

- Determine the function of *rstA* in sporulation & toxin inhibition
- Identify other potential regulatory targets for RstA
- Determine how RstA transcription and activity is regulated

Acknowledgments

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(UNC-Chapel Hill)

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