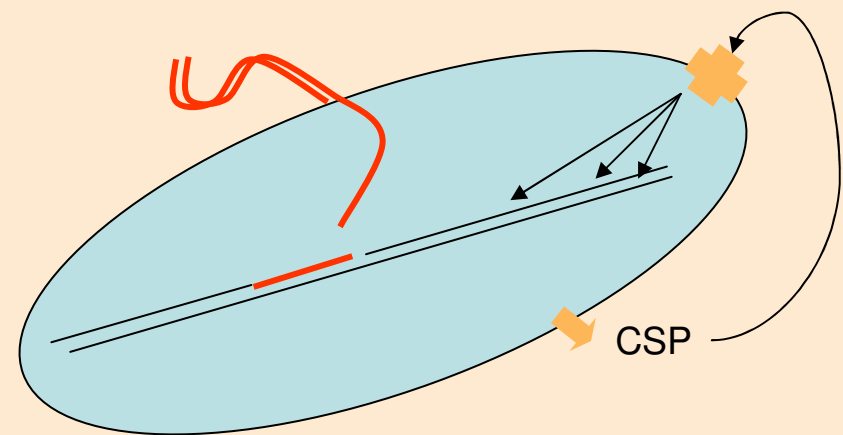


# Regulated Proteolysis in the Competence Pheromone Response of *Streptococcus pneumoniae*

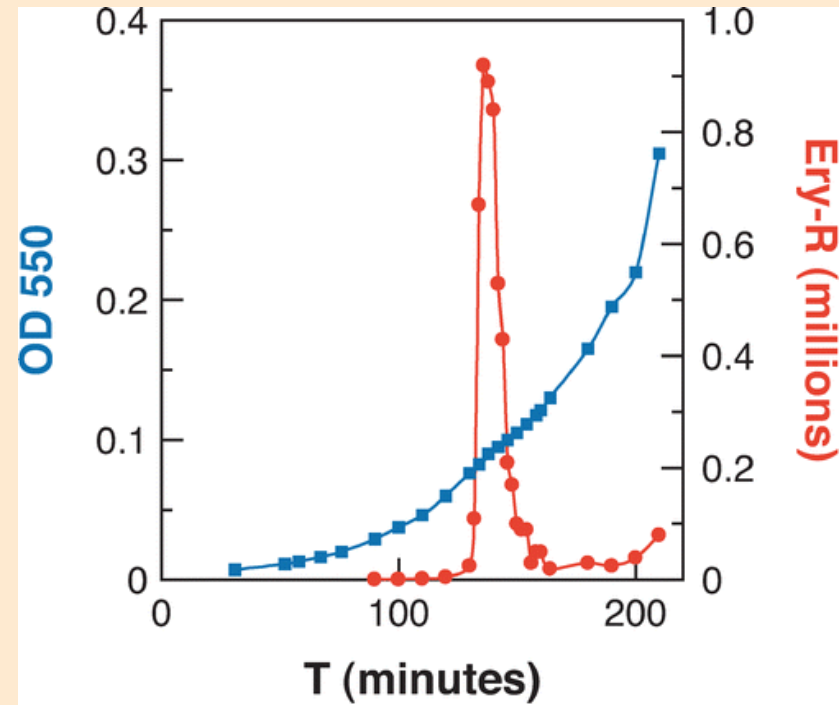
Don Morrison  
University of Illinois at Chicago

GMM3  
Oslo, Norway  
2008

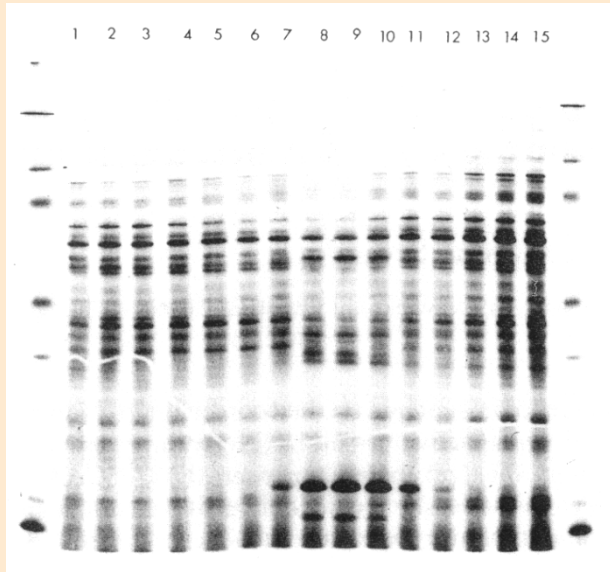


Thanks to:  
U.S. National Science Foundation

# Competence is Transient

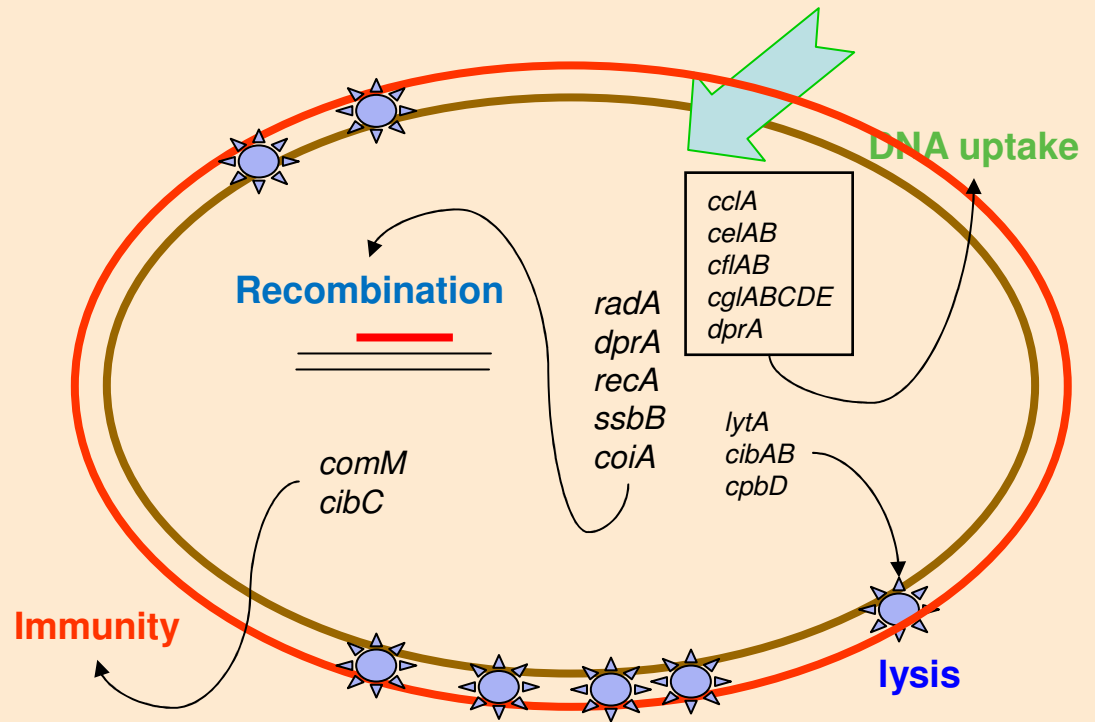
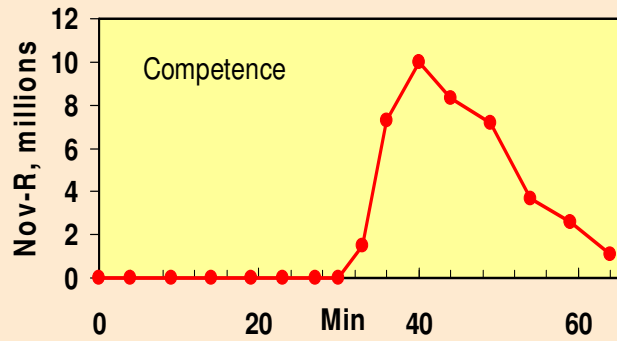


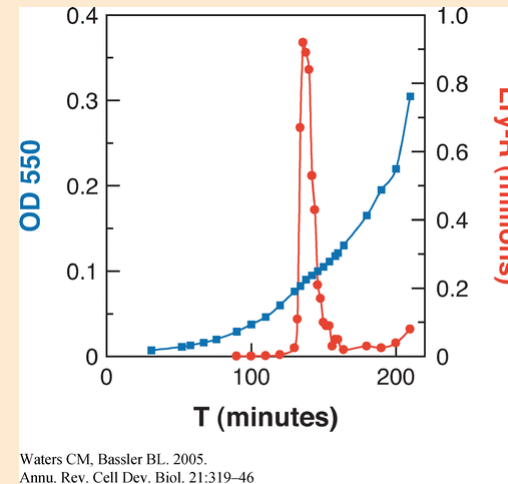
Waters CM, Bassler BL. 2005.  
Annu. Rev. Cell Dev. Biol. 21:319-46



Global developmental shift in protein synthesis.

DNA transport  
Genetic recombination  
Attacker cells.



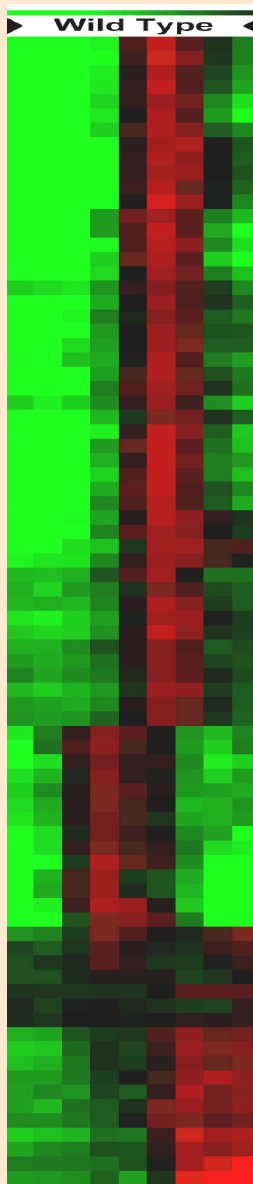


How is the switch started?

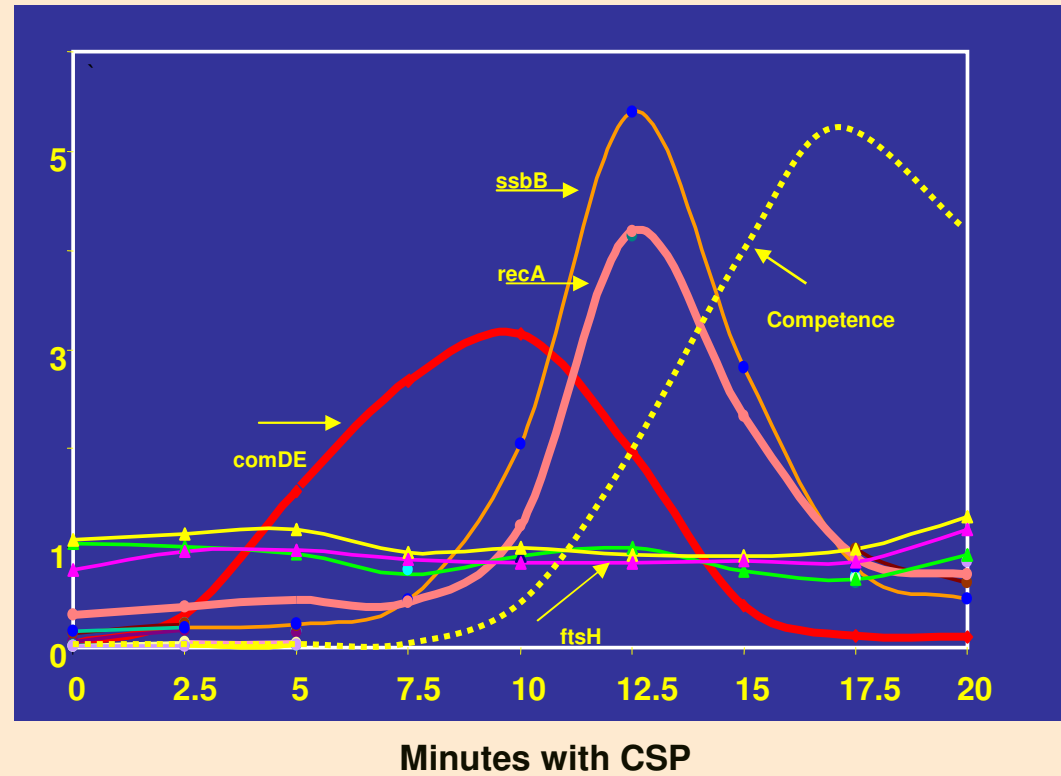
How is the switch coordinated?

How is a graceful exit from the X State accomplished?

Switch depends on a peptide pheromone,  
linked to effector genes by an alternative sigma factor,  $\sigma^X$ ,  
to create the "X state."



Transcription is rapidly modulated at competence.

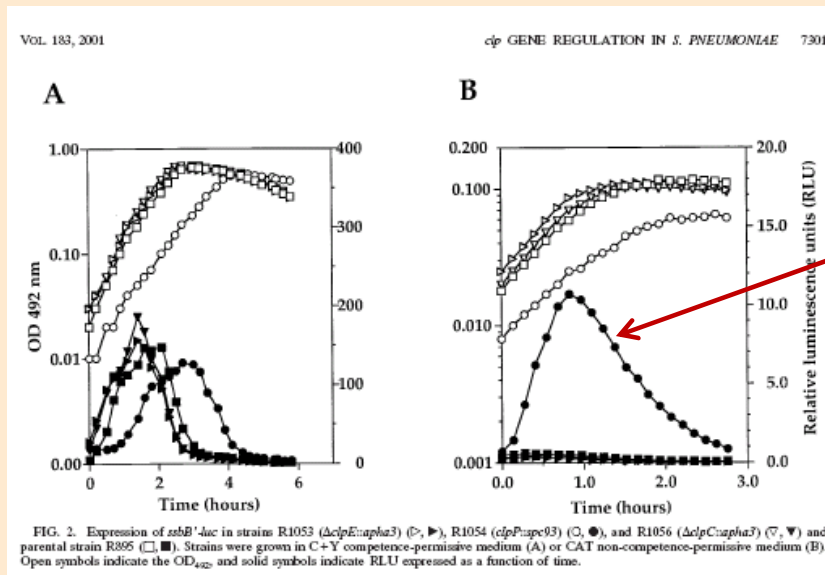


➤ 80 genes are under strict transcriptional regulation.

Is proteolysis superimposed to accomplish strict regulation?

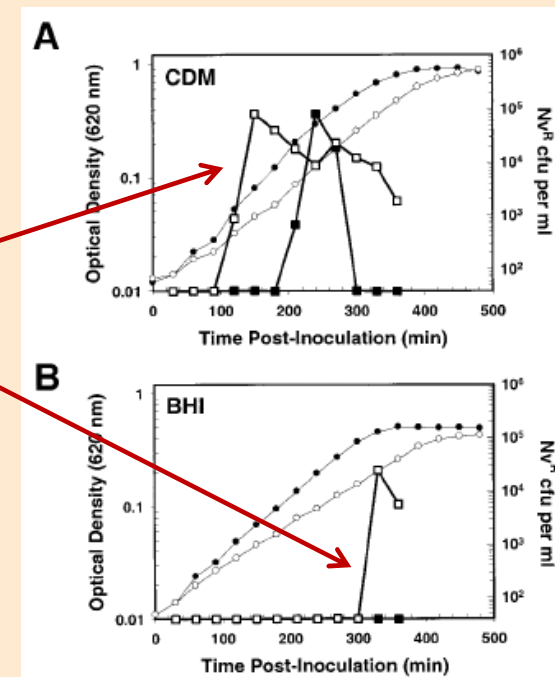
# First hints of proteolysis in competence regulation: *clpP* mutant develops competence precociously:

Chastenet, 2001



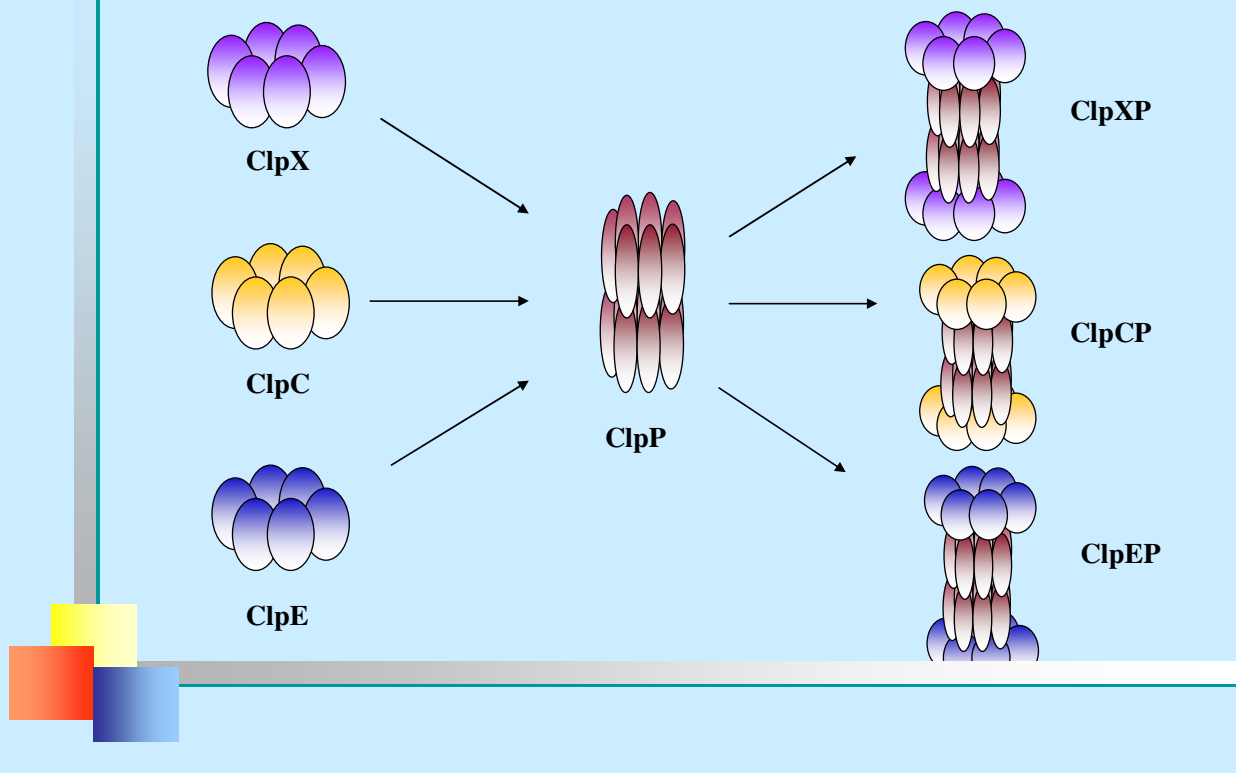
Chastanet A, et al. Regulation of *Streptococcus pneumoniae* *clp* genes and their role in competence development and stress survival. *J Bacteriol.* 2001. 183:7295.

Robertson, 2002



: Robertson GT, et al. 2002. Global transcriptional analysis of *clpP* mutations of type 2 *Streptococcus pneumoniae* and their effects on physiology and virulence. *J Bacteriol.* 184:3508.

ClpP can associate with various Clp ATPases to form functionally different Clp proteases

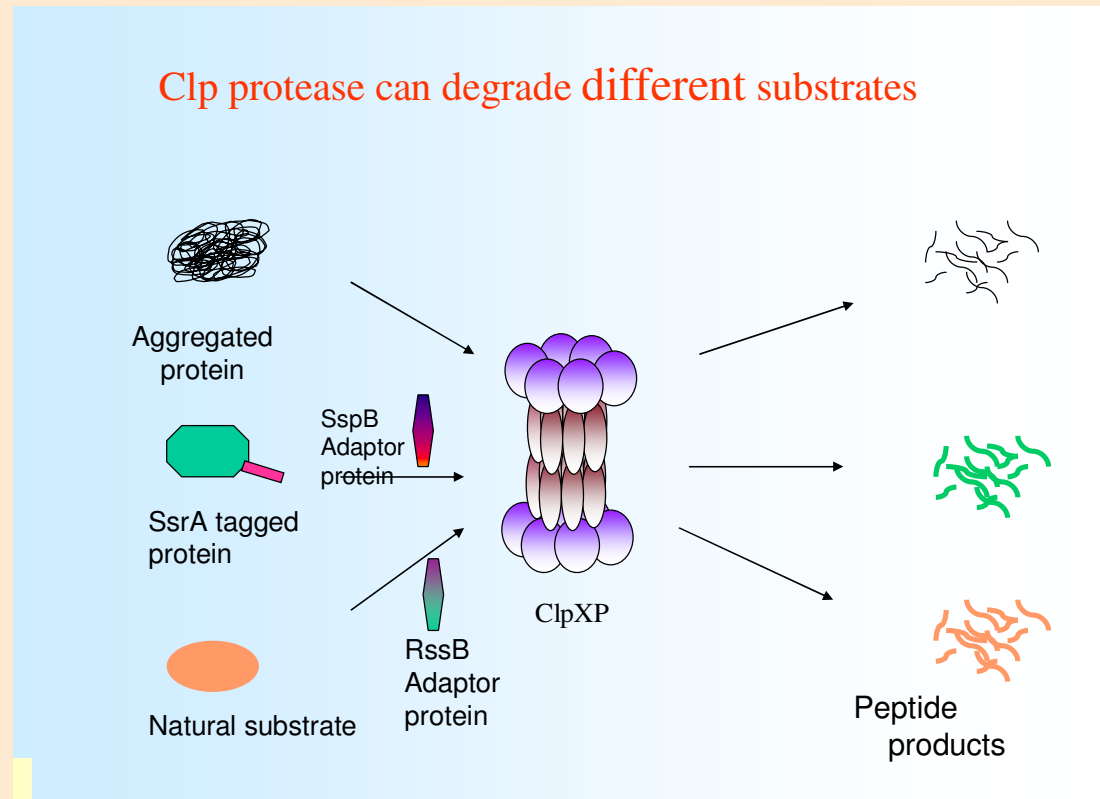


None have known pneumococcal substrates

## ATP-Dependent Protease Complements

<i>E. coli</i>	<i>B. subtilis</i>	<i>S. pneumoniae</i>
ClpQ/ClpY	ClpQ/ClpY	
Lon	LonA, LonB	
FtsH	FtsH	FtsH
ClpP	ClpP	ClpP
ClpC	ClpC	ClpC
ClpX	ClpX	ClpX
	ClpE	ClpE
ClpA		
ClpB		ClpL

## Clp protease can degrade different substrates



## Potential roles of ClpP in competence regulation:

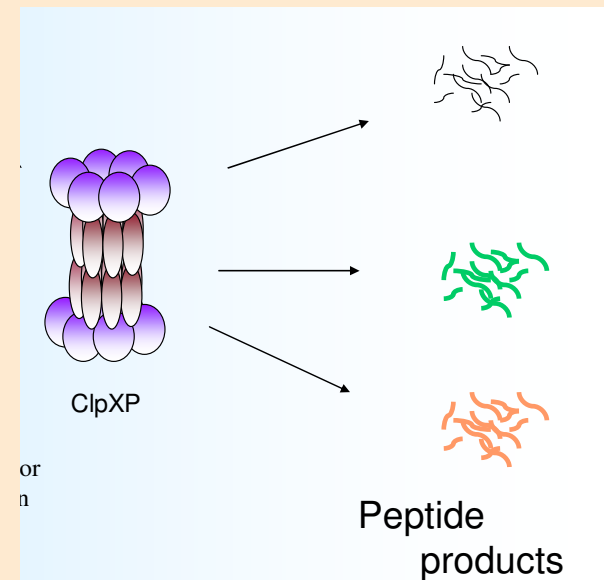
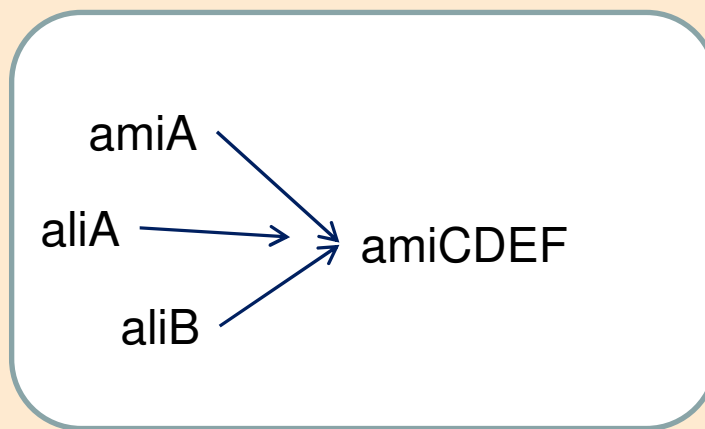
- Peptide products as negative signals
- Stress as positive signal
- Protein effectors or regulators as substrates for proteolytic regulation?

None are yet ruled out; each has precedent.

a. Restricted peptide uptake alters competence induction.

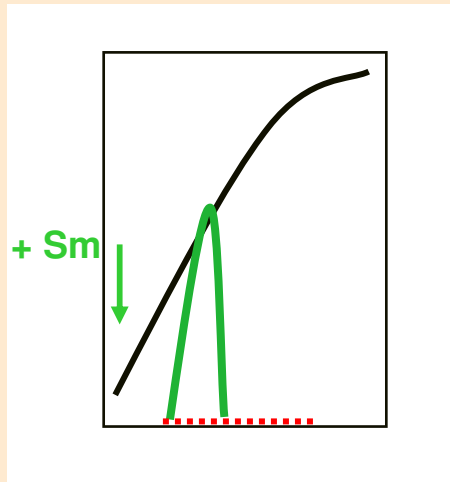
Loss of ClpP removes one (intracellular) source of peptides.

**Oligopeptide binding proteins:  
Loss of all 3 promotes competence**

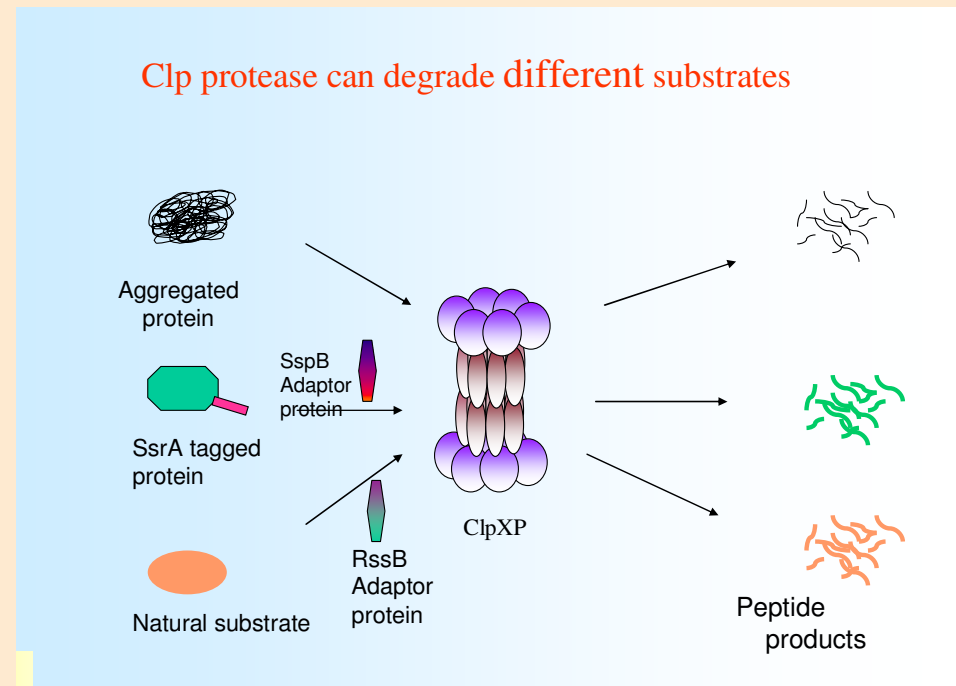


Alloing, G., et al., 1998. Development of competence in *Streptococcus pneumoniae*: pheromone autoinduction and control of quorum sensing by the oligopeptide permease. *Molec. Microbiol.* 29:75-83.

## b. Stress can lead to competence induction.



- **Prudhomme M, et al. . 2006.** Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*.
- Sci. 313:89-92.

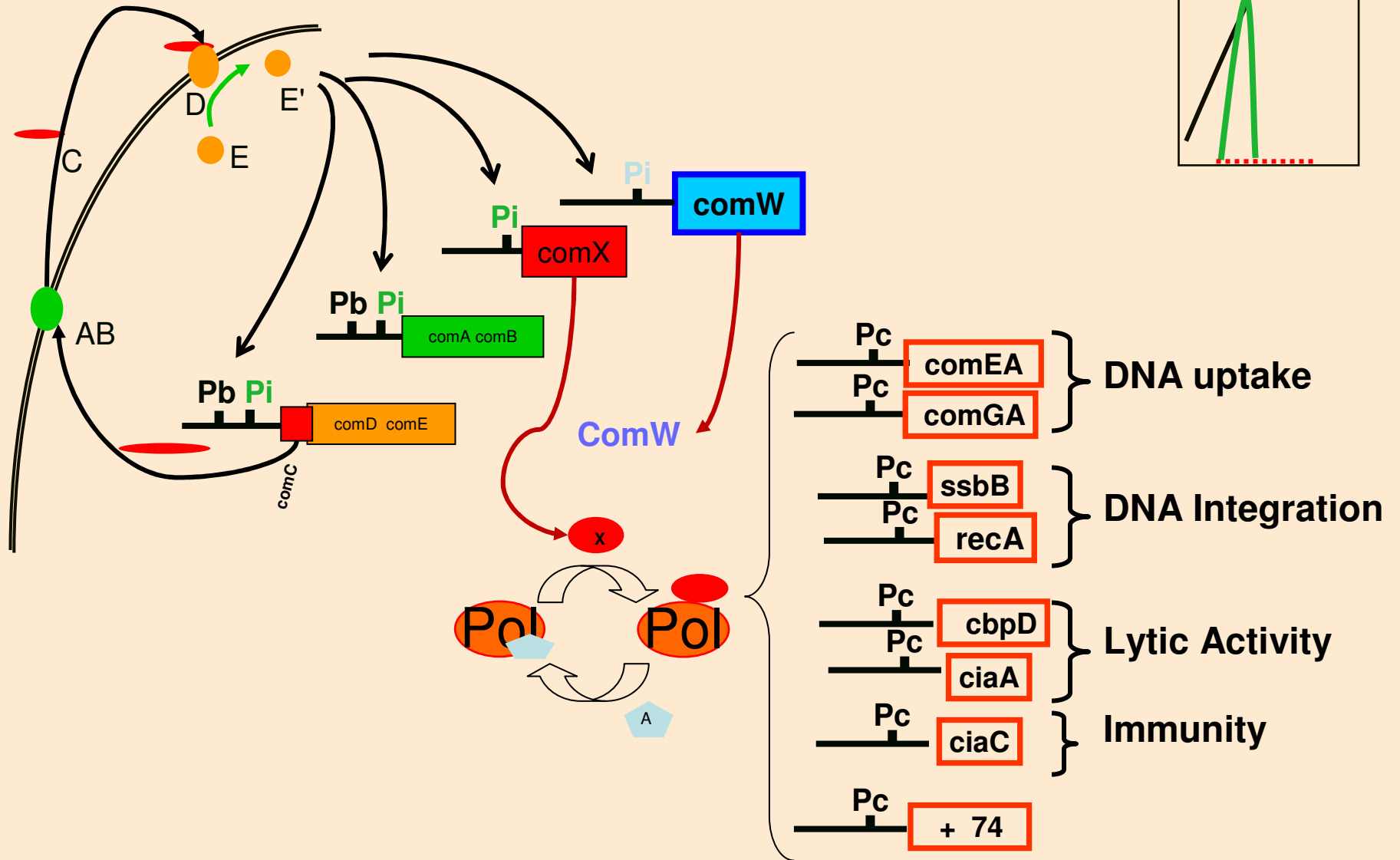


Loss of Clp activities could mimic some kinds of stress, as by accumulation of misfolded proteins.

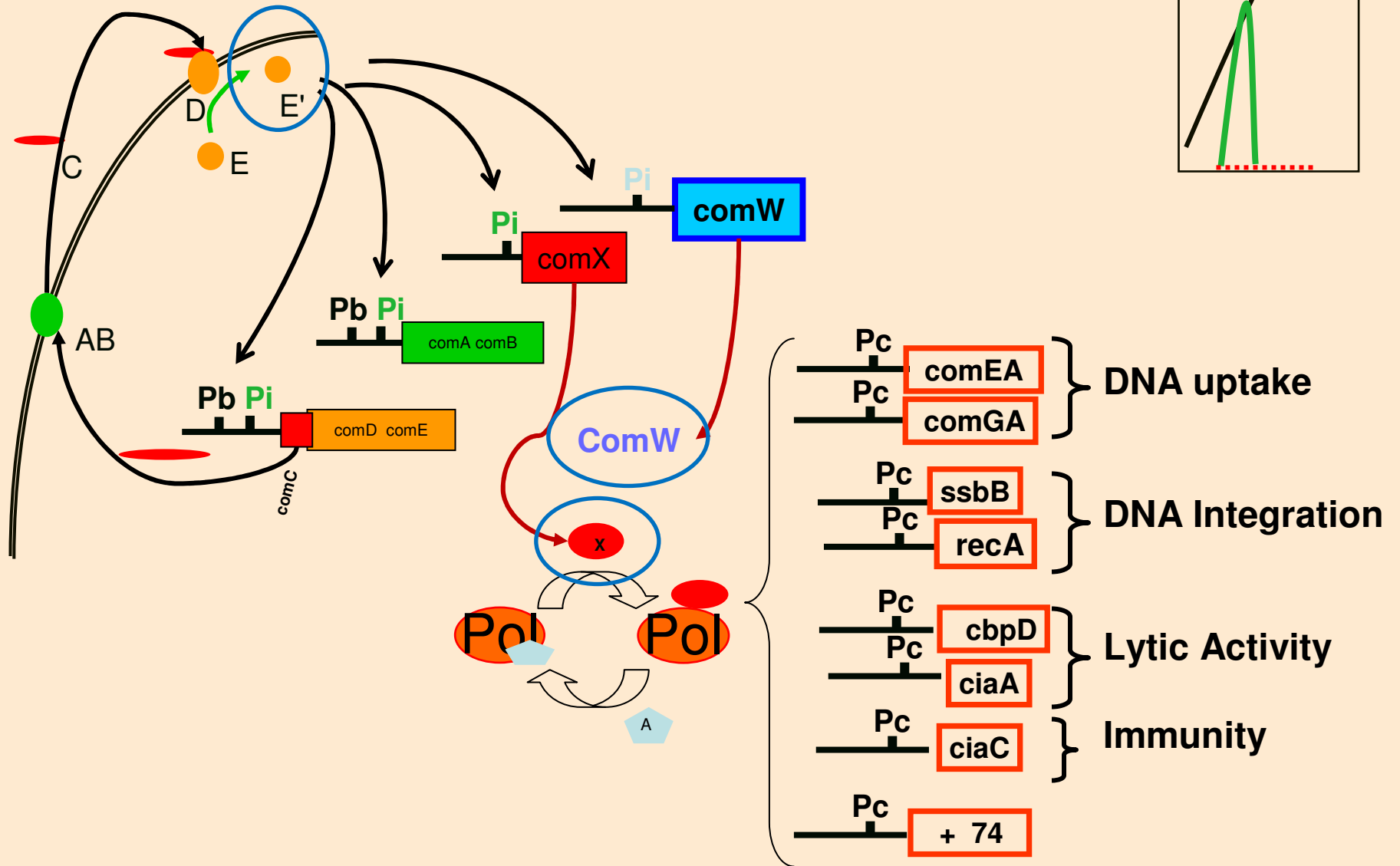
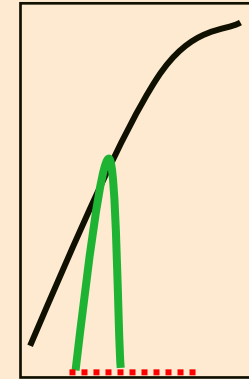
## Proteolysis of critical regulators in rapid bacterial responses:

System	Target	Modulator	Protease
ECF	Sigma E	RseA	DegS; YaeL
Sporulation	SpoAB	SigmaF	ClpC / ClpP
Stress	Sigma S	RssB	ClpX ClpP
Heat shock	Sigma 32	DnaK	FtsH
Defective proteins	ssrA tag	SspB	ClpX ClpP
Competence	ComK	ComS	ClpC / ClpP

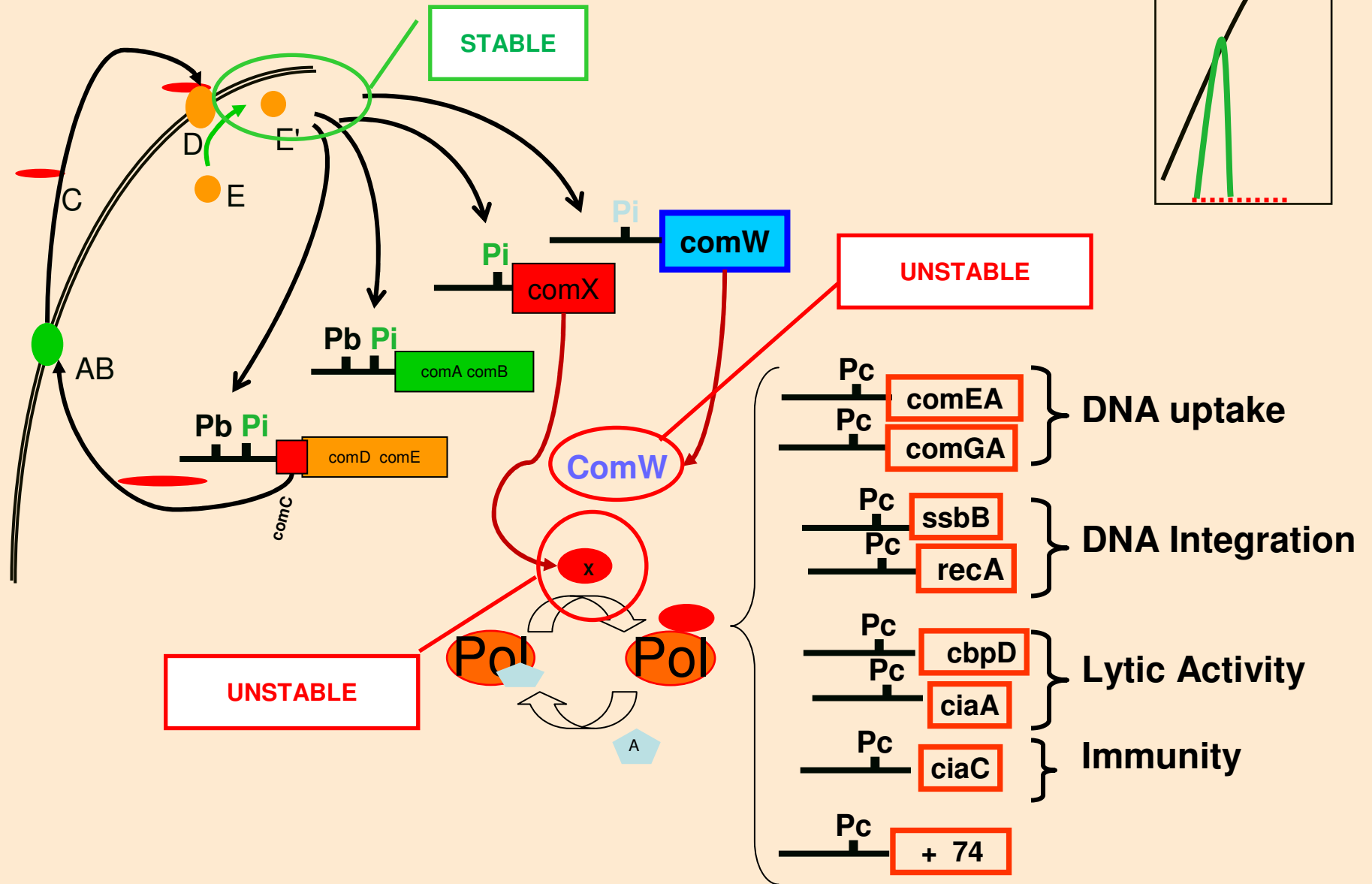
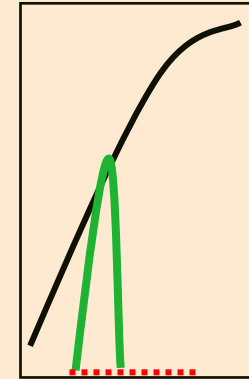
# Signal Transduction Pathway



# Critical competence regulators

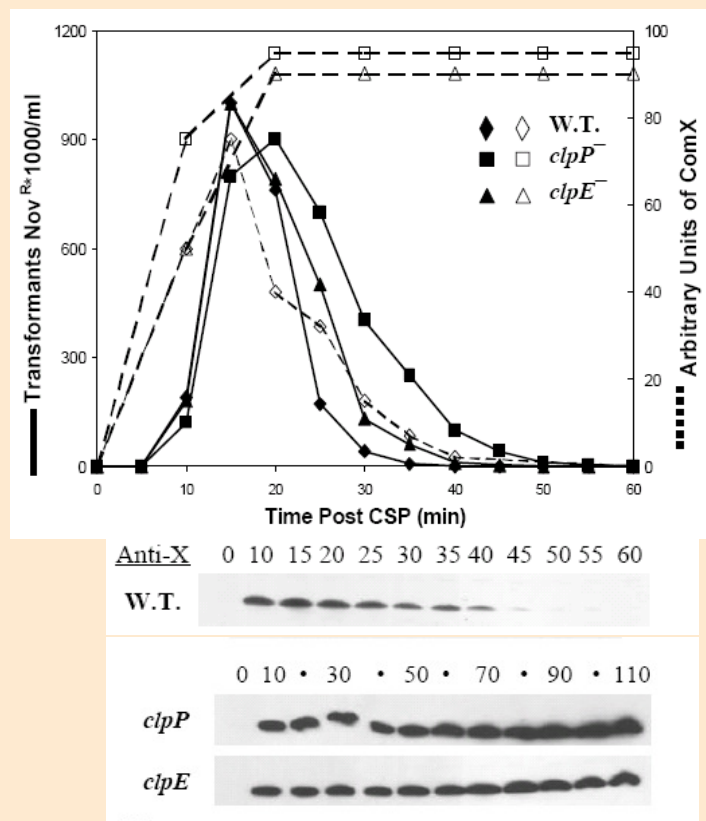


# Stability of regulators

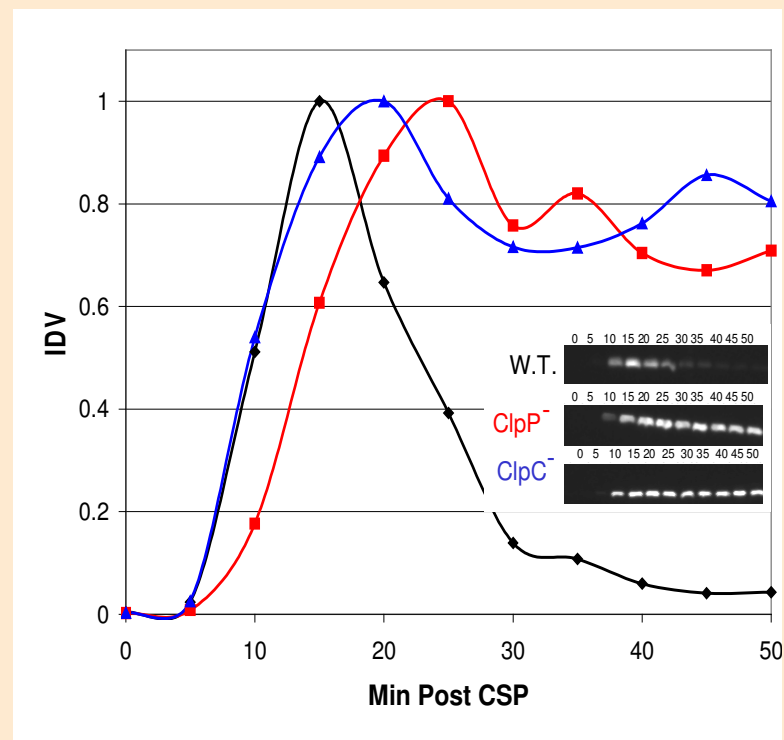


# Different AAA+ ATPase specificity factors are implicated genetically

## ComX vs. ClpEClpP

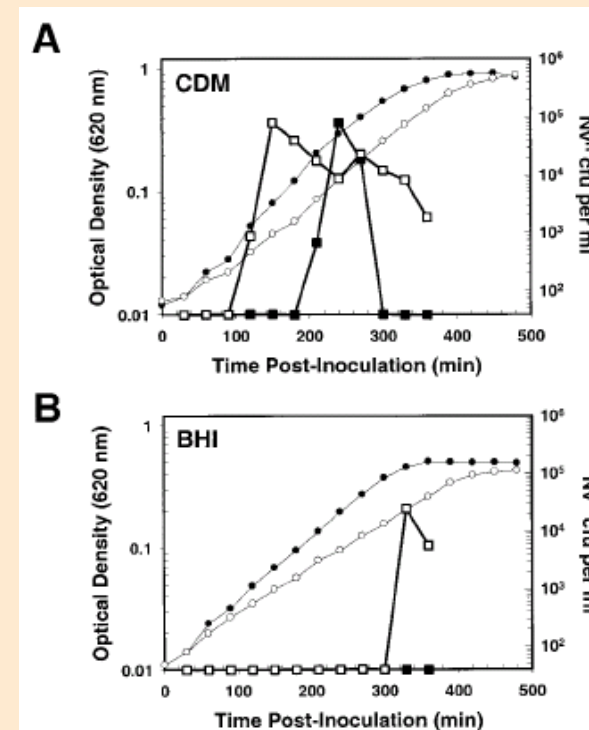


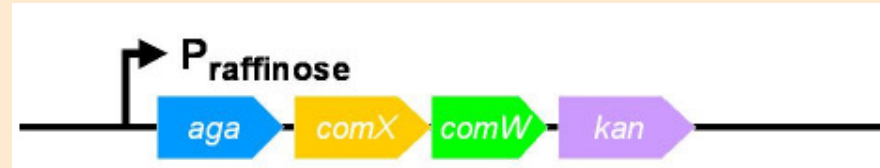
## ComW vs ClpC/ClpP



ComX & ComW can be stabilized by *clp* mutations, but does competence ever depend on blocking such proteolysis?

A. Spontaneous competence development can be suppressed by ClpP, but this is not yet directly linked to either a specific ATPase or a specific substrate.



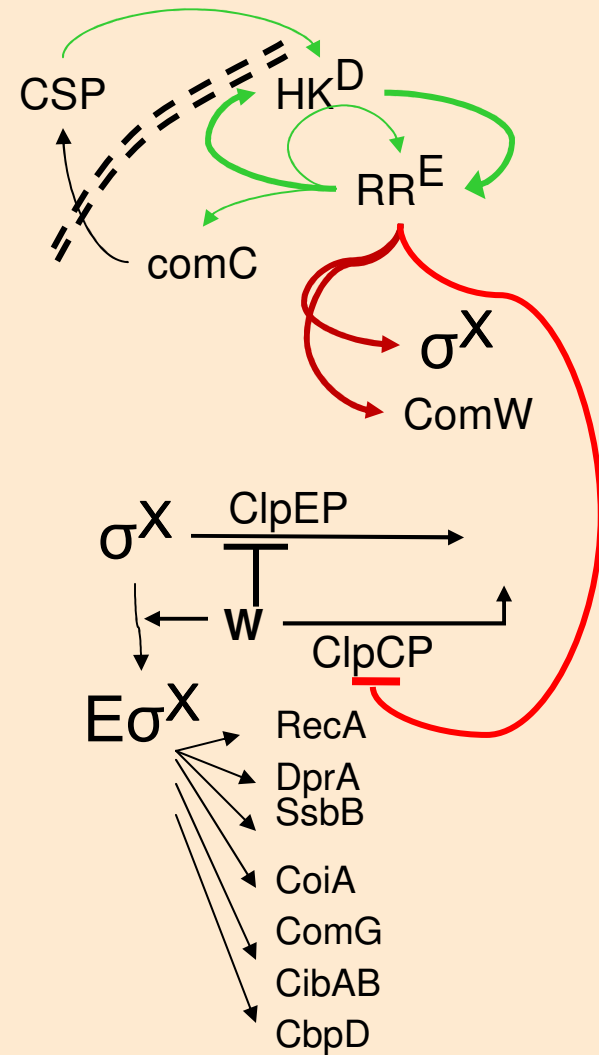
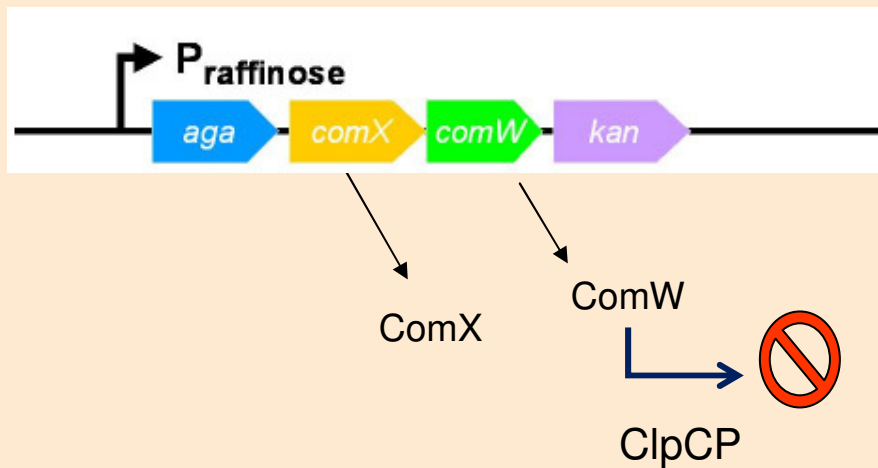


B. Ectopic expression of ComW and ComX by the raffinose promoter is sufficient to create the X state: but ClpC/ClpP must be inactivated.

Strain	Clp	CSP	Raffinose
WT	E+C+	100	0
PR ComComW	E+C+		.01
	E-		.2
	C-		100
	E-C-		100

Thus one path of control could be via ClpC

Yet, In WT, ClpP+ permits induction of competence.

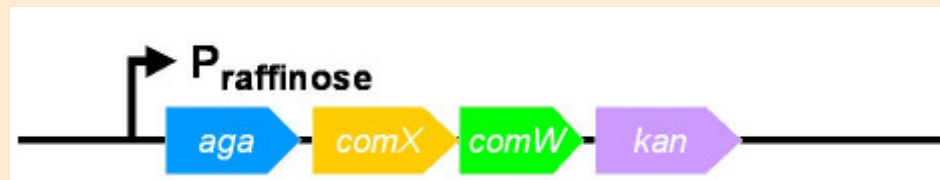
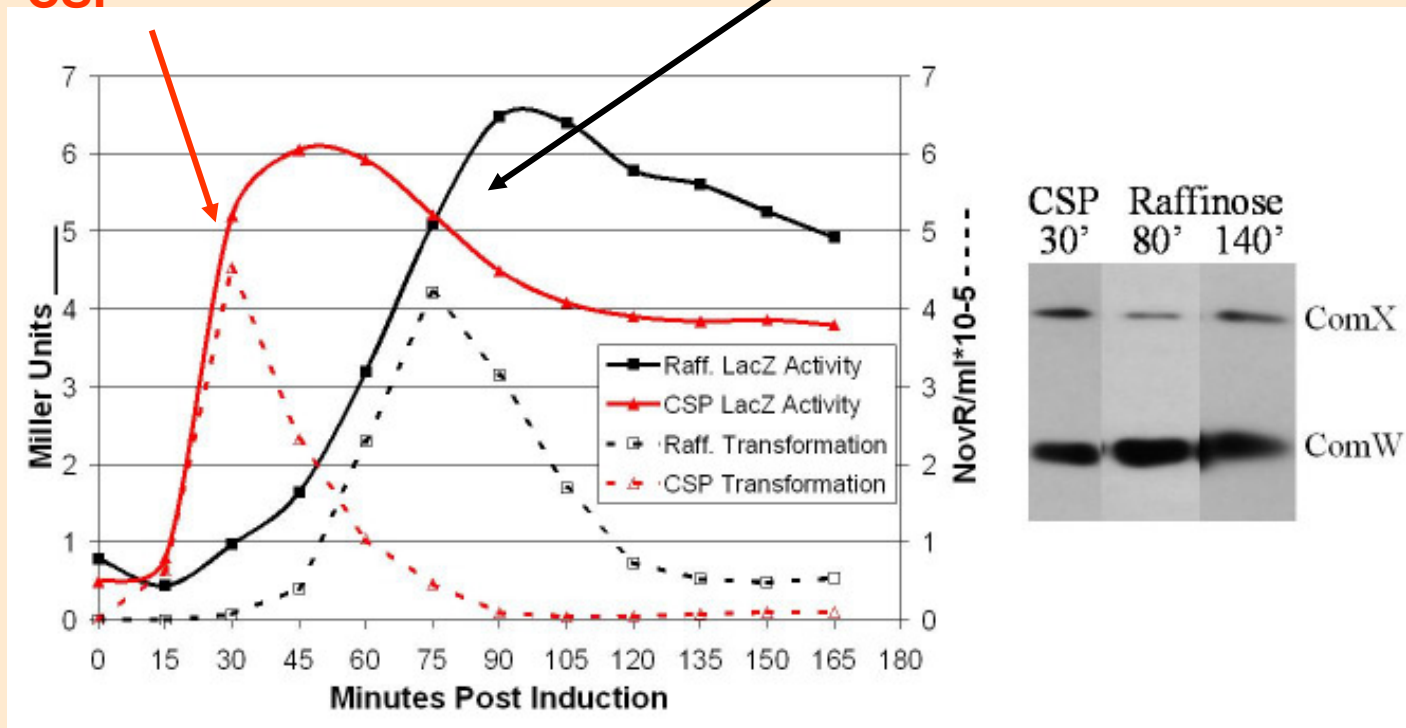


Hypotheses:  
 Stronger ComE promoters  
 Early gene inhibiting clpC?

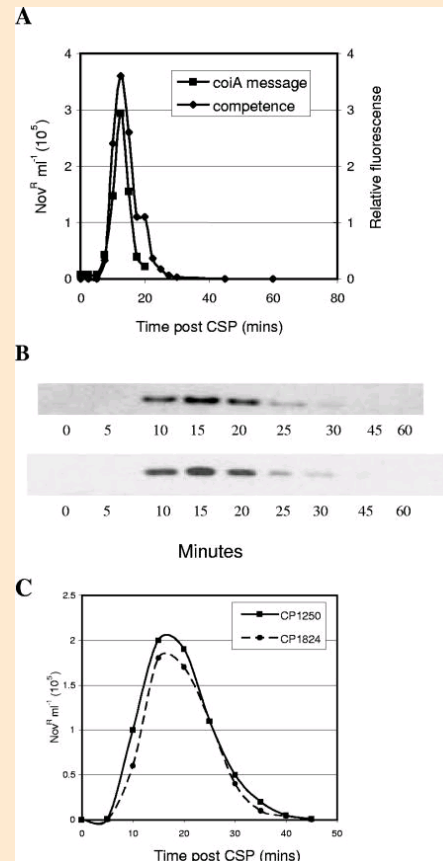
Shutoff depends not on protease but on a late gene product affecting activity of ComX or ComW.

WT + CSP

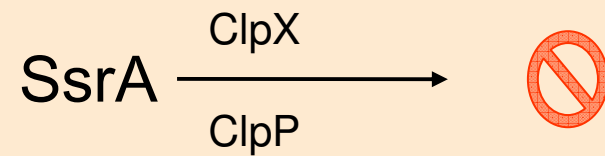
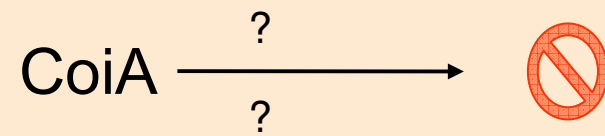
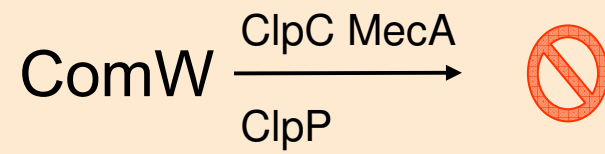
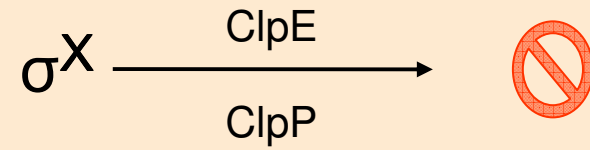
PR ComX ComW + RAFFINOSE

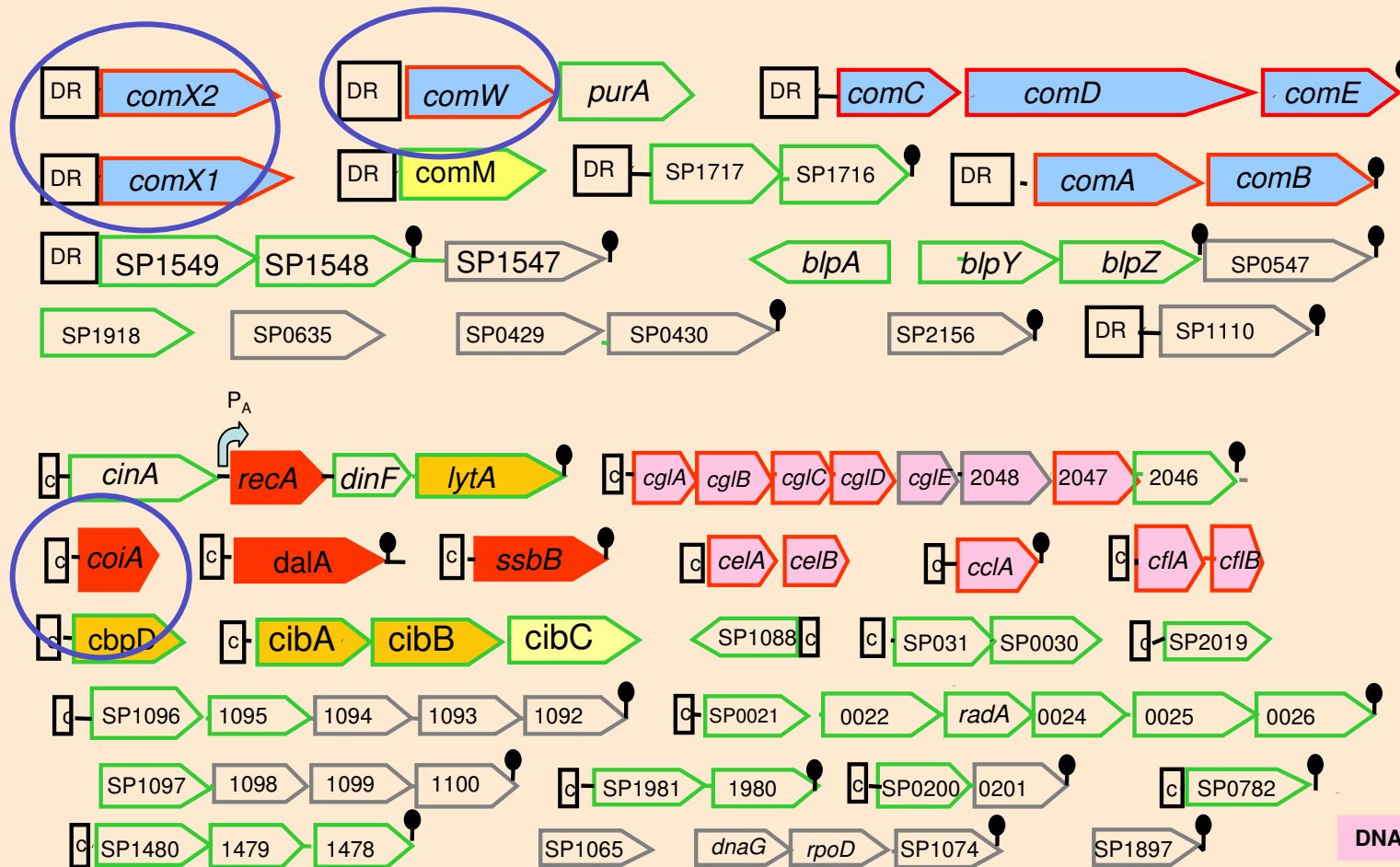


# One competence effector is unstable: CoiA



## Specific Targeted Proteolysis in Pneumococcus:





Roles of proteolysis in ?

- initiation
- coordination
- exit

- DNA TRANSPORT
- RECOMBINATION
- LYTIC ATTACK
- LYTIC IMMUNITY
- REGULATION

## Acknowledgments

Sarita Ahlawat  
Andrew Piotrowski  
Haiying Li  
Liming Weng

Mary Akhtar  
Sume Joseph  
Anup Reddy  
Daina Ringus  
Theresa Shen  
Tim Sommerville  
Vinay Soni

