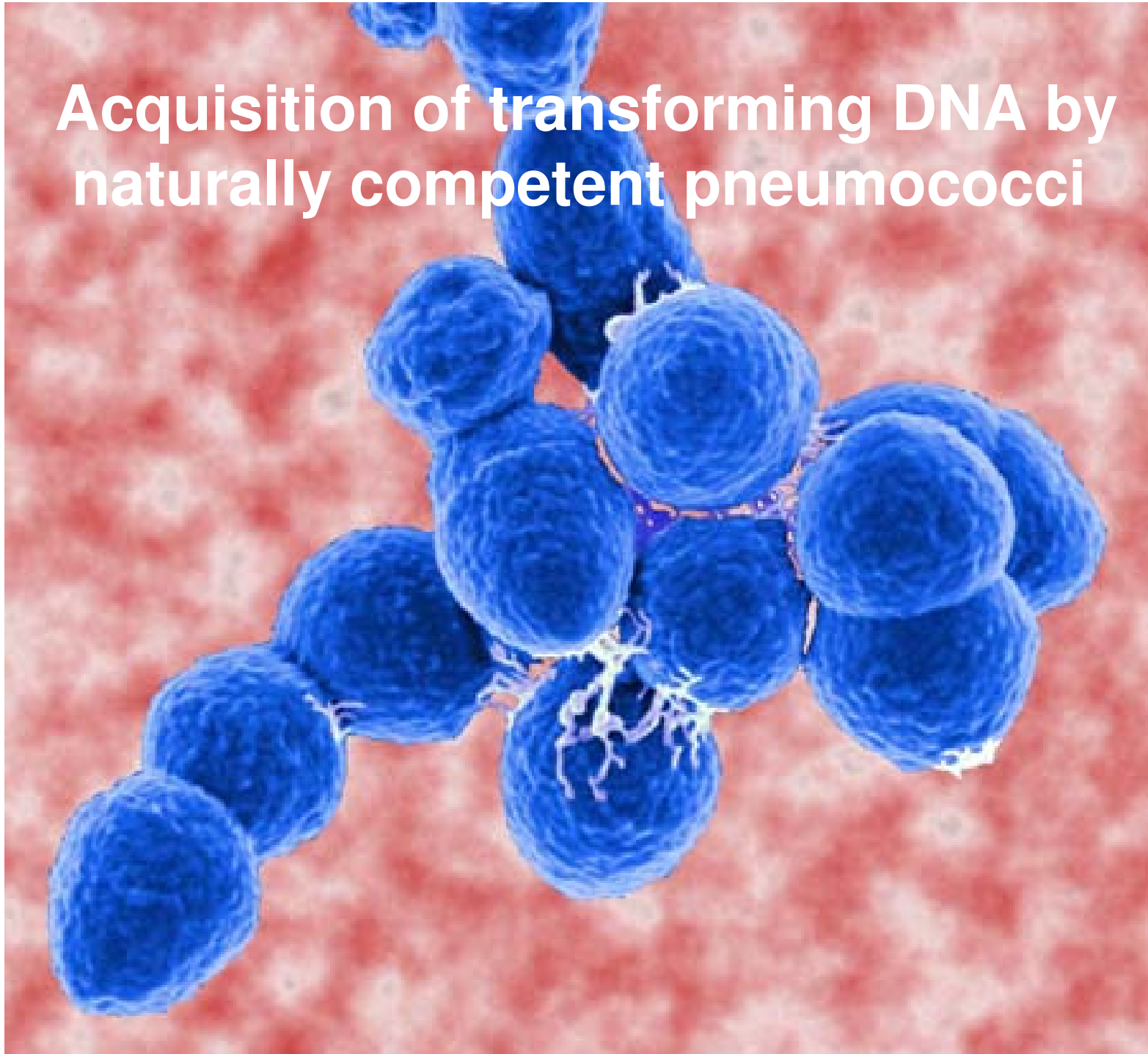
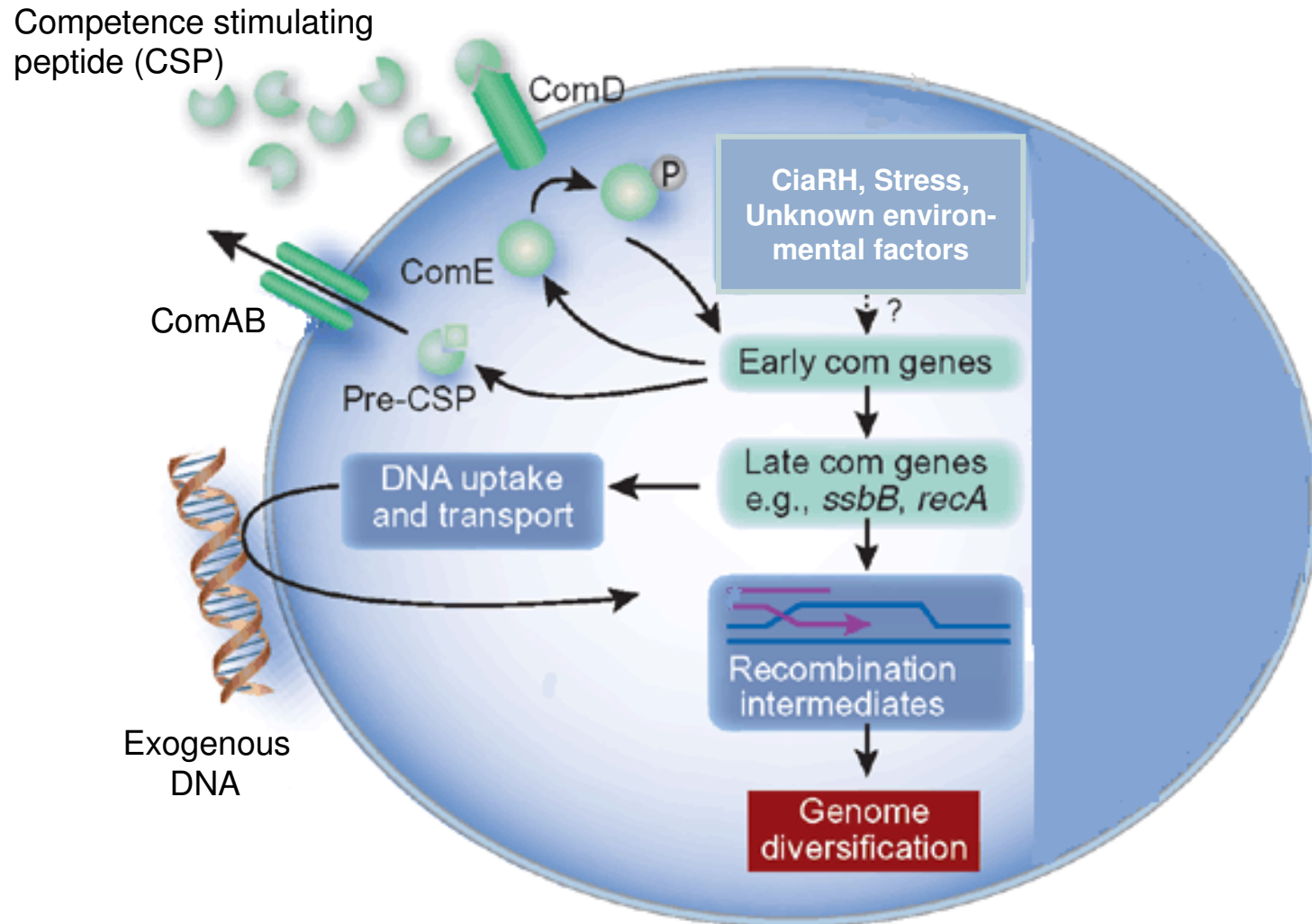


# Acquisition of transforming DNA by naturally competent pneumococci

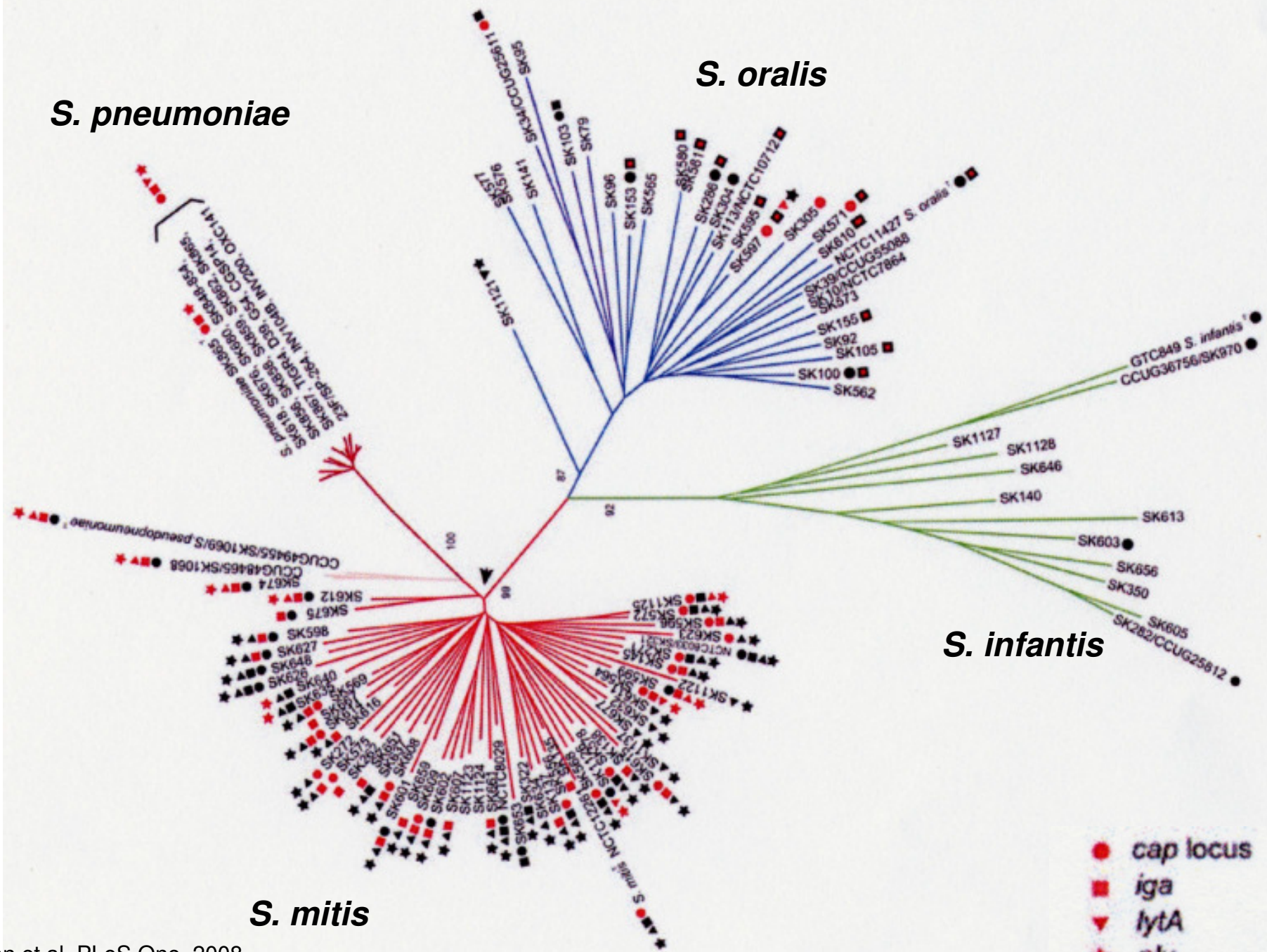


## Induction of the competent state in *Streptococcus pneumoniae* and its close relatives in the mitis phylogenetic group.



*S. pneumoniae*

*S. oralis*



*S. infantis*

*S. mitis*

- cap locus
- iga
- ▼ lytA
- ★ ply

Species Strain	Ref.	Amino acid sequence of ComC Sequence of mature CSP is given in bold capital letters
<i>S. pneumoniae</i>		
Rx	[29]	
NCTC 7465 <sup>T</sup> /SK673		
SK 848		
SK 852		
SK 853		
SK 856		
SK 858		
SK 862		
SK 865		
SK 867		
A66	[30]	
SK 618		
SK 680		
SK 851		
SK 854		
CSP-2.2	[31]	
SK 676		
CSP-3	[31]	
CSP-4	[31]	
CSP-5	[31]	
CSP-6.1	[31]	

Leader sequence

Mature signal peptide

mkntvkleqfvalkekdlqkikgg**EMRLSKFFRDFILQRKK**

mkntvkleqfvalkekdlqkikgg**EMRISRIILDFLFLRKK**

mkntvkleqfvalkekdlqnikgg**EMRISRIILDFLFLRKK**

mkntvkleqf**kl**te**ke**lqeiqgg**ERRIPDVIRSLLFQKRK**

mkntvkleqfvalkekdlqniqgg**EMRKMNEKSFNIFNFFNFFNFFRRR**

mkntvkleqfvalkekdlqniqgg**EMRKMNEKSFNIFNFFNFFRRR**

mkntvkleqf**vd**kekdlqeiqgg**ESRLPKILLDFLFLRKK**

mkntvkleqfvalkekdlqkikgg**EMRLPKILRDFIFPRKK**

## Amino acid sequence of ComC

**Species**  
Strain

Leader sequence

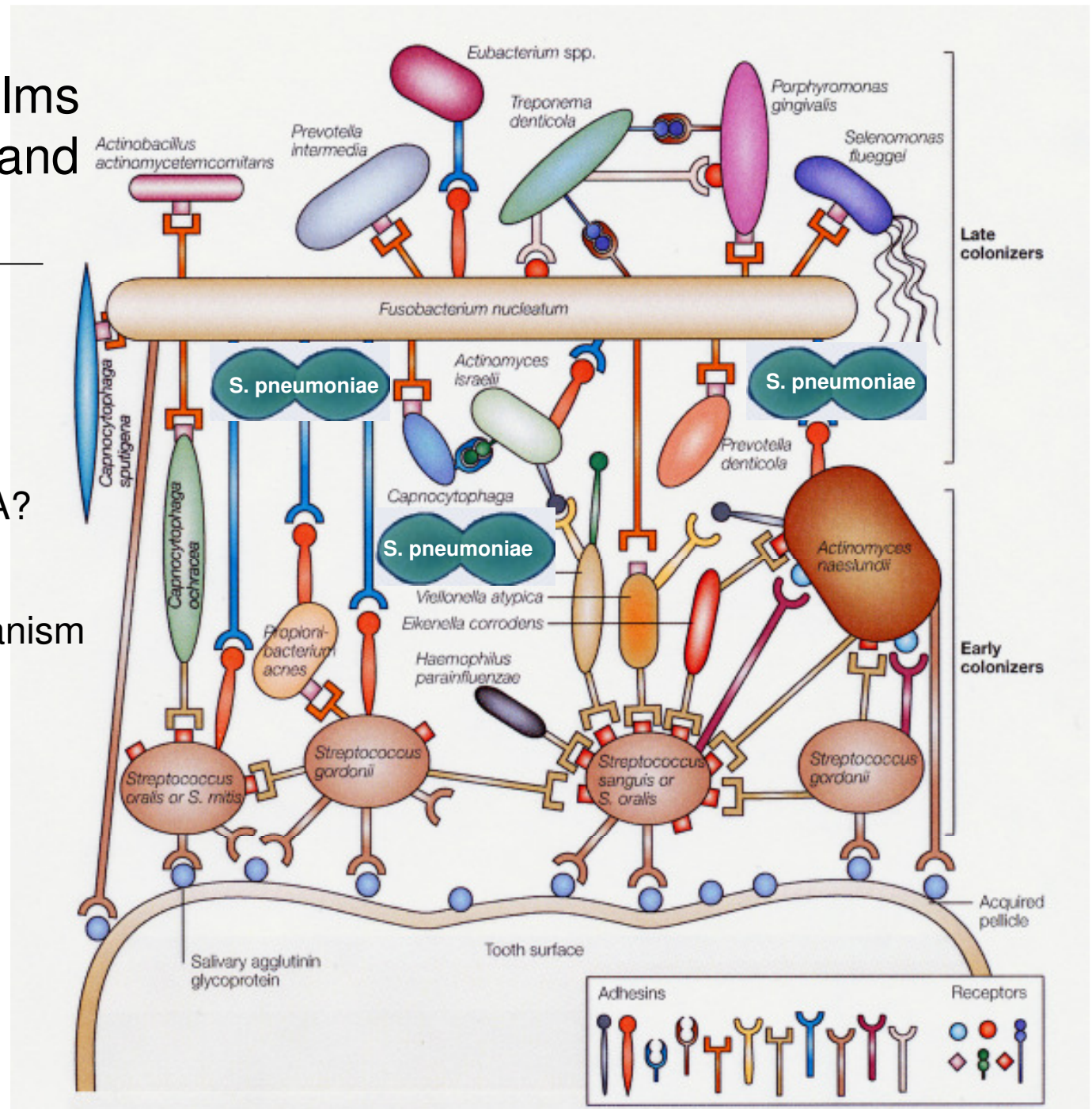
Mature signal peptide

<i>S. mitis</i>			
B5	[32]	mkntvkleqfvalkekdlqkikgg	ESRLPKIRFDFIFPRKK
B6	[32]	mkntvkleqfvalkekdlqkikgg	EMRKP DGALFNLFRRR
Hu8	[32]	mkntvkleqfvalkekdlqkiqgg	EMRKSNNNFFHFLRRI
SK 262		mkntvkleqfvalkekdlqkiqgg	EMRRSNNNFFNFLRRI
CSP-7	[31]	mkntvkleqfvalkekdlqeirgg	ESRVSRIILDFLFQRKK
CSP-8	[31]	mkntvkleqfvalkekdlqnikgg	ESRISDILLDFLFQRKK
SK 675		mkntvkleqfvalkekdlqeirgg	ESRVSRIILDFLFQRKK
SK 612		mkntvkleqfvalkekdlqkikgg	ESRLSRLLRDFIFQIKQ
SK 611		mkntvkleqfvalkekdlqeikgg	EMRLPKILRDFIFPRKK
SK 564		mknivkleqfvvlkekdlqkiqgg	ESRVSDILLDFLFRRKK
SK 137		mkntvkleqfvalkekdlqeiqgg	ESRLPKIRFDFIFPRKK
SK 615		mkntvkleqfvalkekdlqkiqgg	EMRKSNNNTFFNFLRRI
SK 598		mkntvkleqfvalkekdlknikgg	EMRRIDKIFINFLKRR
SK272		mkntvkleqfvalkekdlgniqgg	EMRRIDKIFINFLKRR
SK616			
SK 599		mkntvkleqfvalkekdlqeikgg	EMRRIGSVLLNFFKRR
NCTC 8029/CSP-9	[32]	mkntvkleqfvalkekdlqkiqgg	EMRKP DGALFNLFRRR
SK 596		mkntvkleqfvslkekdlqkiqgg	EMRKP DGALFILFRRR
SK 601		mkntvqleqfvalkekdlqkikgg	EMRKMNEKSFNIFNIFSI FRRR
SK 614		mkntvkleqfvalkekdlqkikgg	EMRKMNEKSFNFFNIFRRR
SK 667		mkntvkleqfvslkekdlqkiqgg	EIRKSNSALVNFFKRR

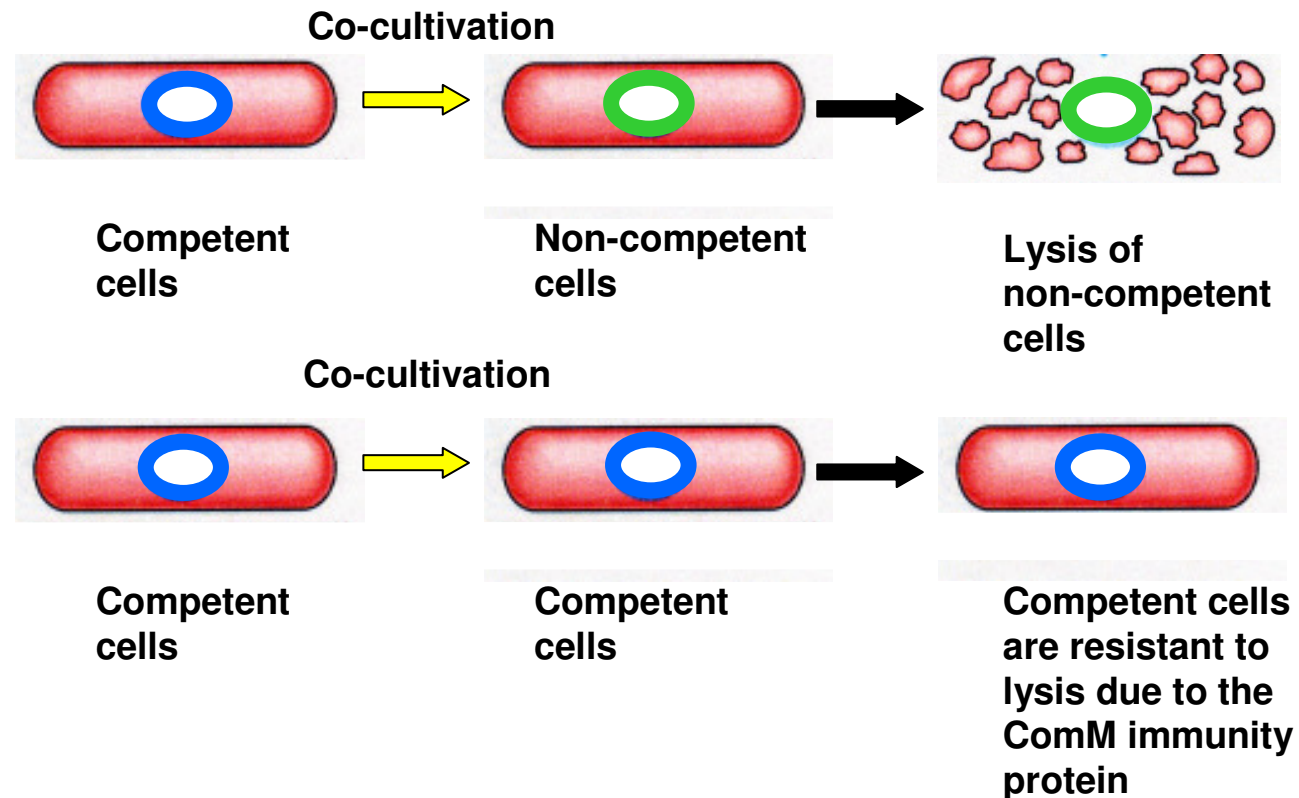
# Multispecies biofilms in the oral cavity and nasopharynx

How does competent streptococci avoid taking up foreign DNA?

Do they have a mechanism that facilitates uptake of homologous DNA?

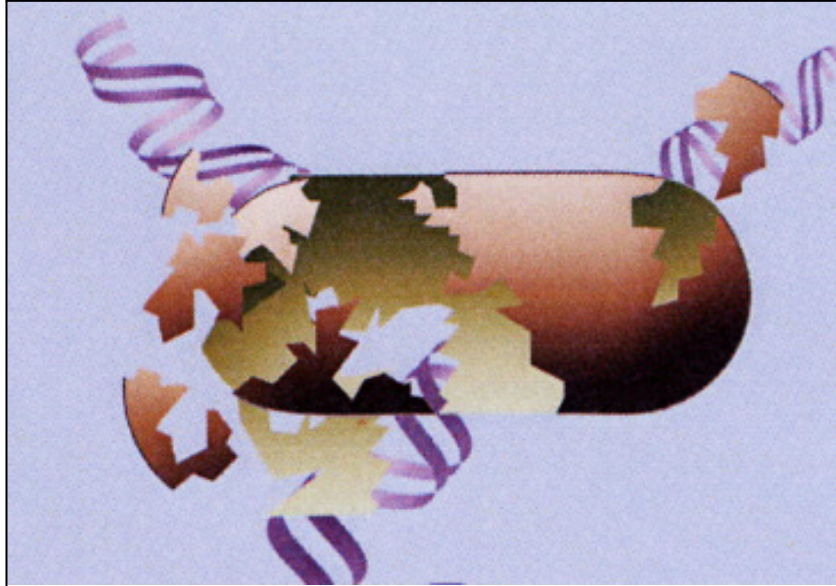


# Competent pneumococci lyse their non-competent siblings



Steinmoen *et al.* (2003) *J. Bacteriol.* 185: 7176  
Guiral *et al.* (2005) *PNAS* 102: 8710  
Håvarstein *et al.* (2006) *Mol. Microbiol.* 59: 1297

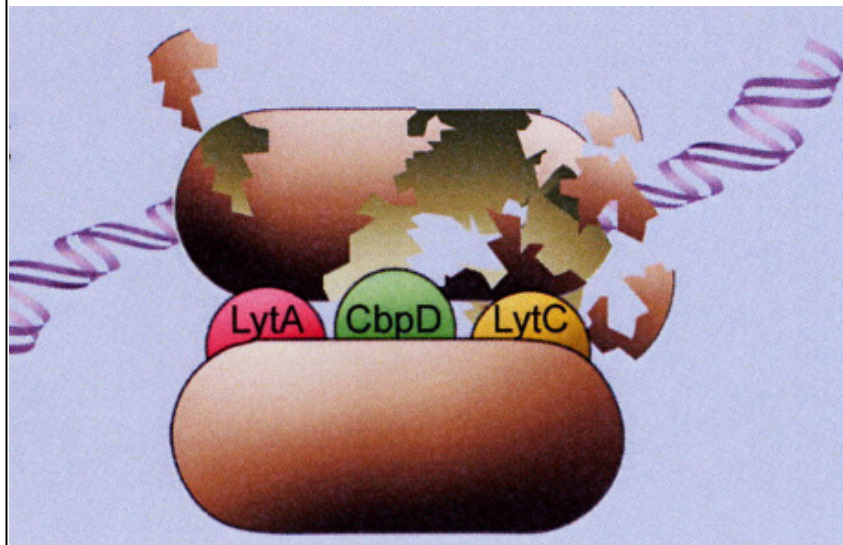
## Two different sources of transforming DNA are available for competent mitis-group streptococci.



### A: Natural cell death

Non-specific mechanism.

DNA acquired by this mechanism is potentially harmful to the recipient and in most cases not beneficial.

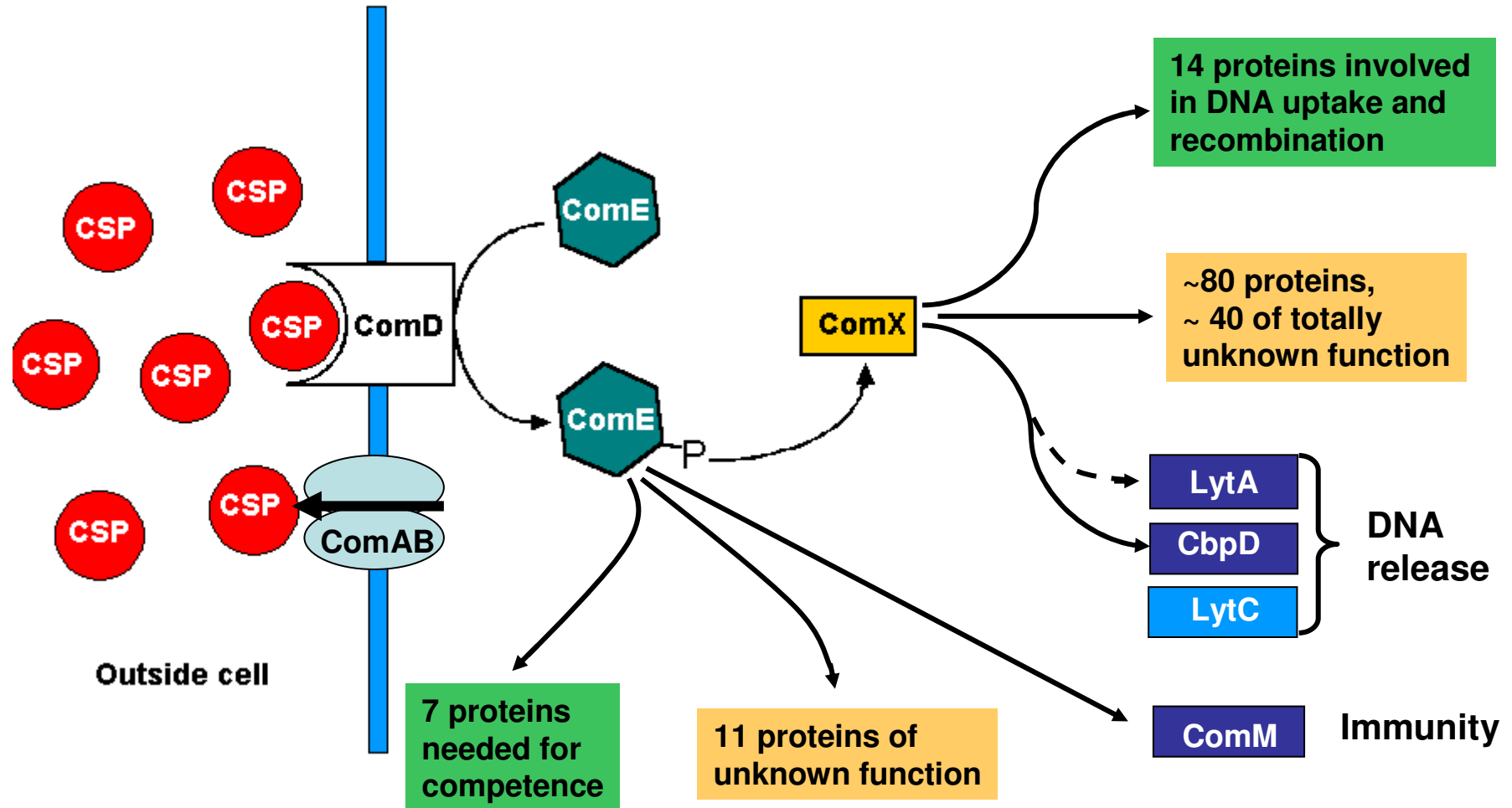


### B: Predation (fratricide).

Competent streptococci kill and lyse related non-competent streptococci and steal their DNA.

Mechanism believed to ensure that homologous DNA becomes available to competent streptococci.

# CSP regulated expression of proteins involved in fratricide



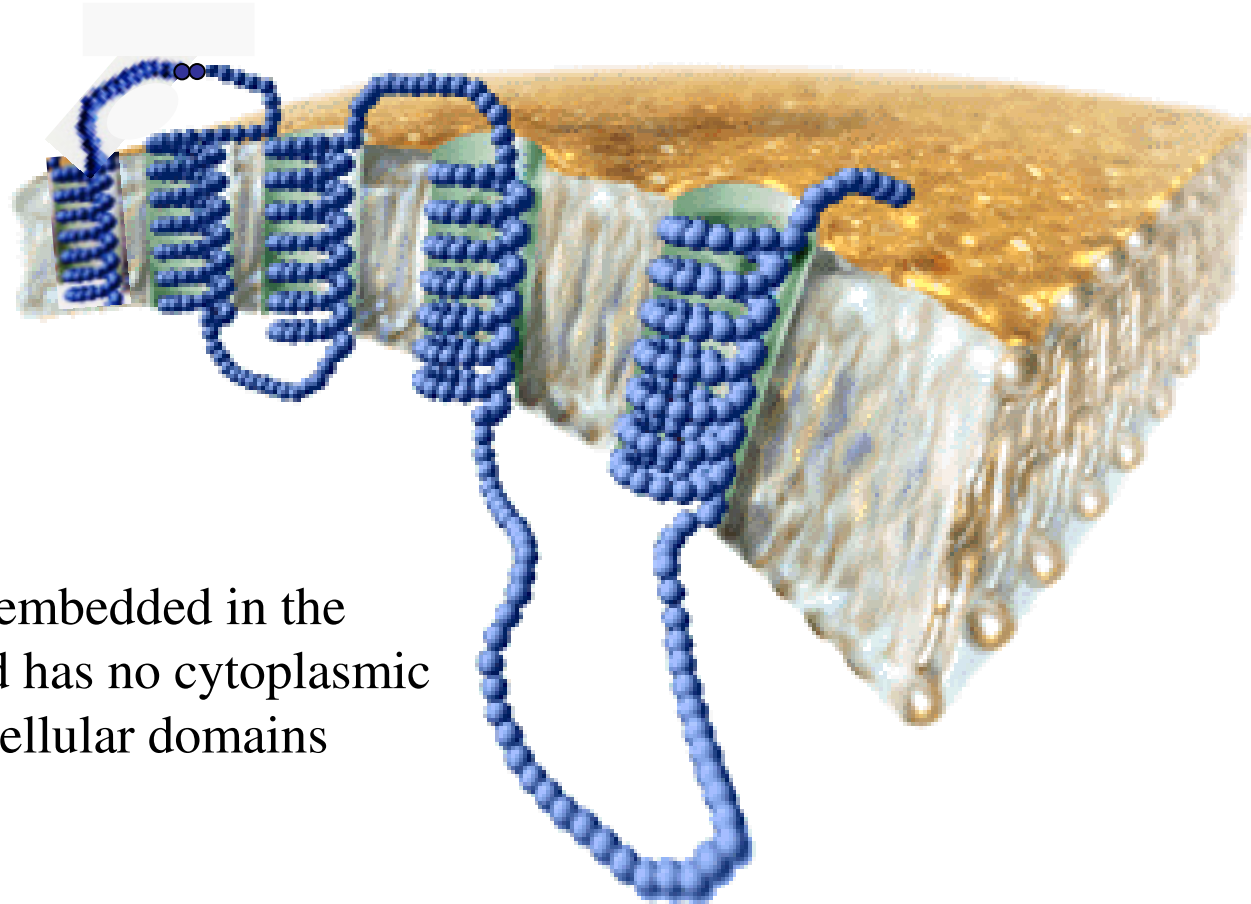
■ Gene products involved in natural transformation

■ Gene products involved in lysis of non-competent cells

■ Gene products involved in unknown processes

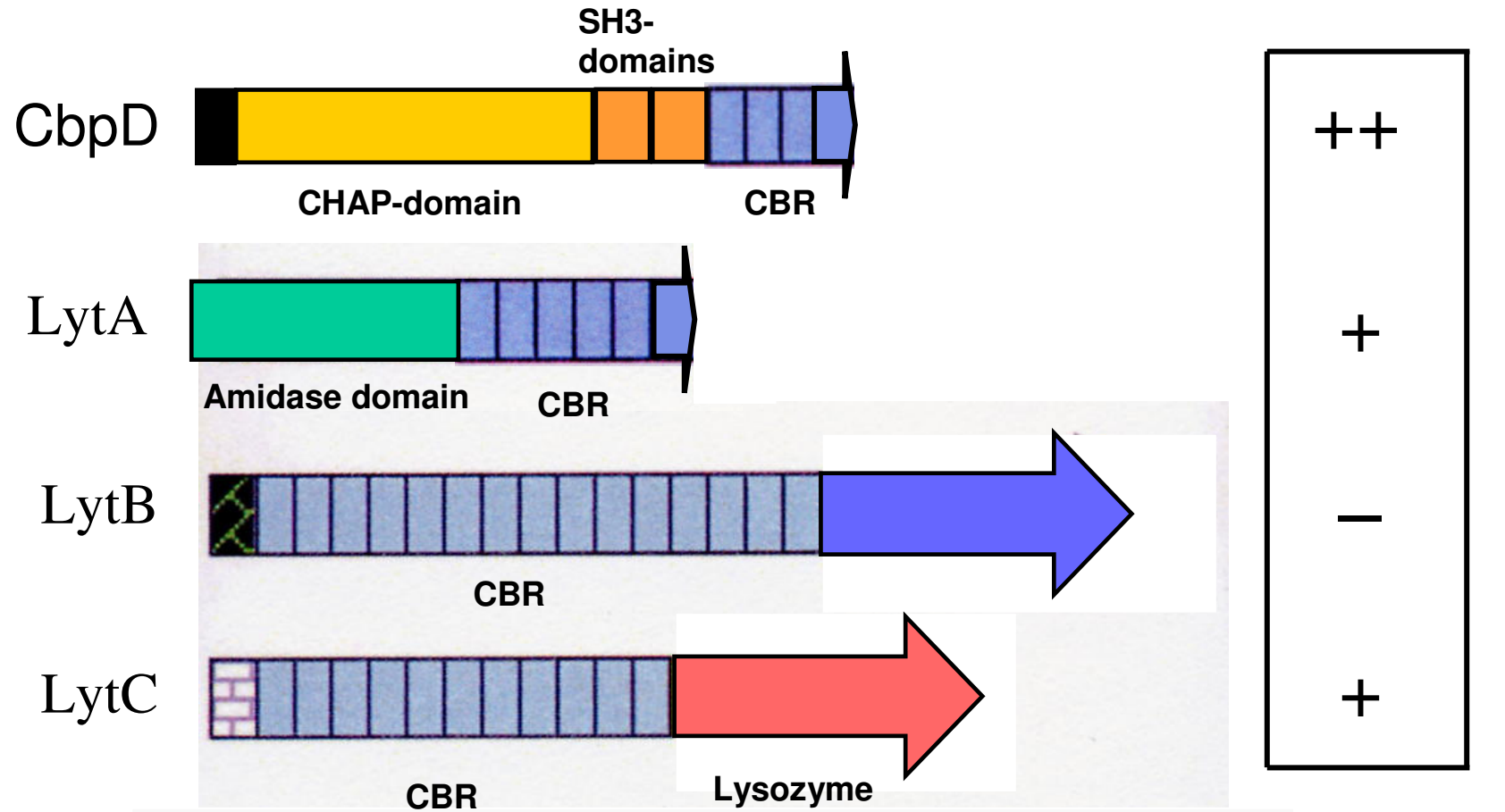
## The ComM immunity protein

ComM is an integral membrane protein with 5-6 transmembrane helices.



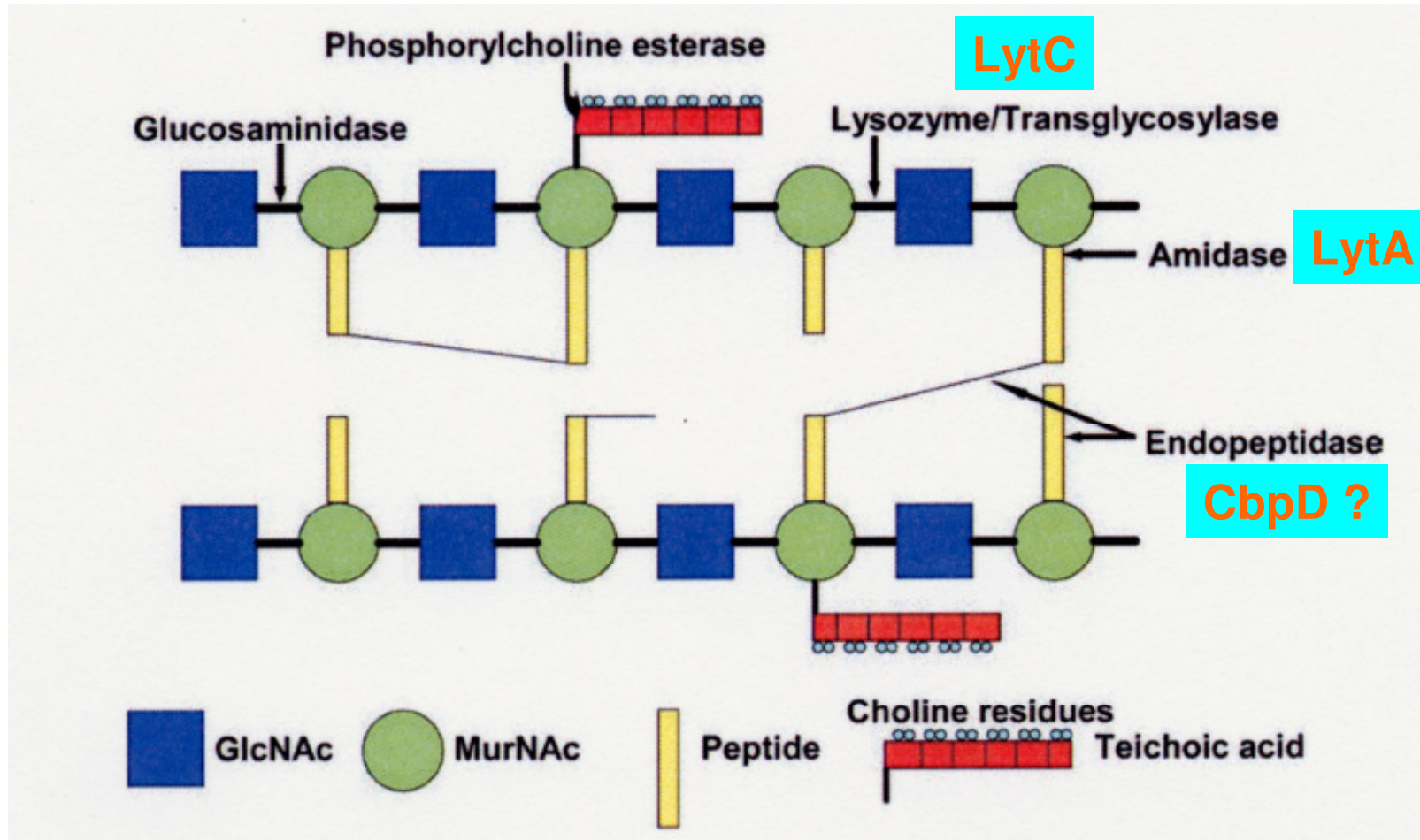
ComM is embedded in the membrane and has no cytoplasmic or extracellular domains

# CbpD, LytA and LytC are involved in competence induced cell lysis

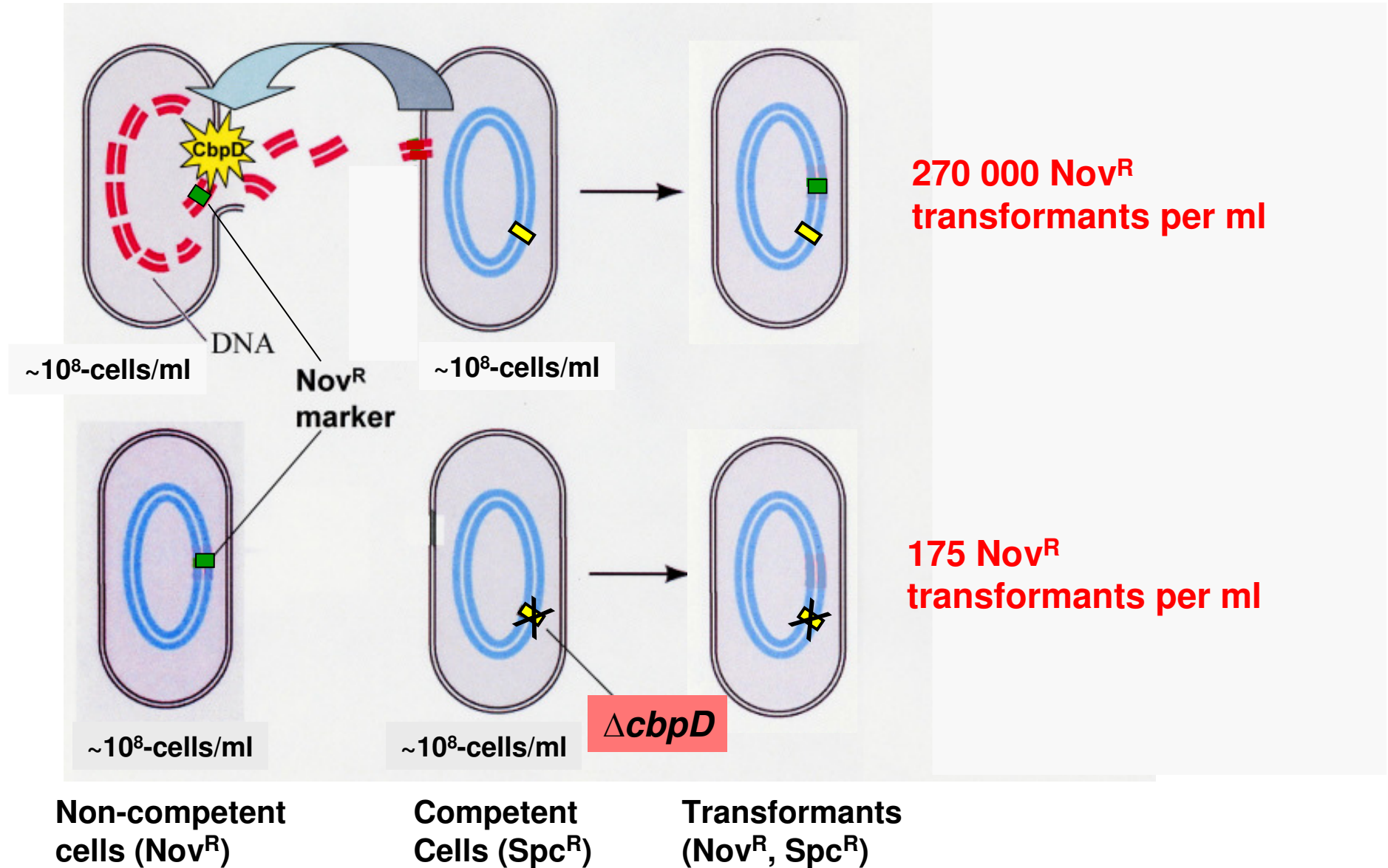


**CBR:** Choline binding repeats

# Cleavage sites of LytA, LytC and CbpD



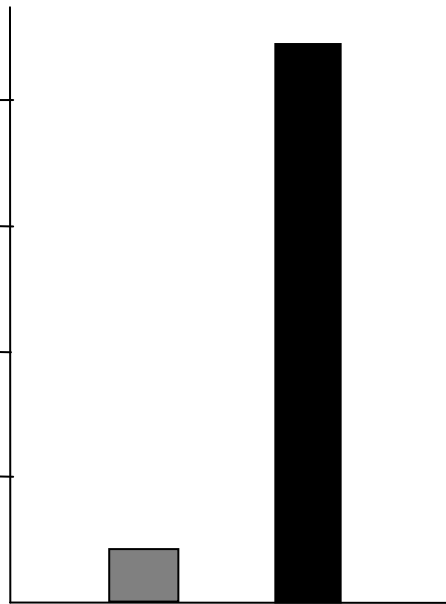
# CbpD is required for efficient gene transfer in mixed cultures of competent and non-competent pneumococci



# Efficient transfer of a Nov<sup>R</sup> marker from *S. mitis* / *S. oralis* to *S. pneumoniae* depends on CbpD.

Transformants  
per ml

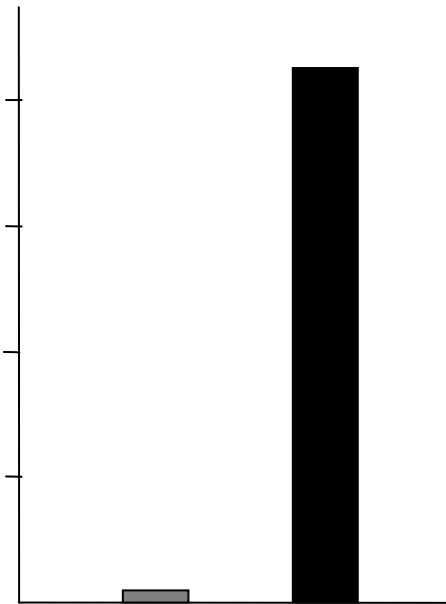
800 000  
600 000  
400 000  
200 000



Attacker: *S. pneumoniae*  
Target: *S. mitis*

Transformants  
per ml

80 000  
60 000  
40 000  
20 000



Attacker: *S. pneumoniae*  
Target: *S. oralis*

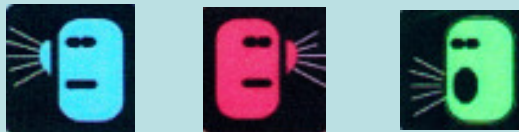
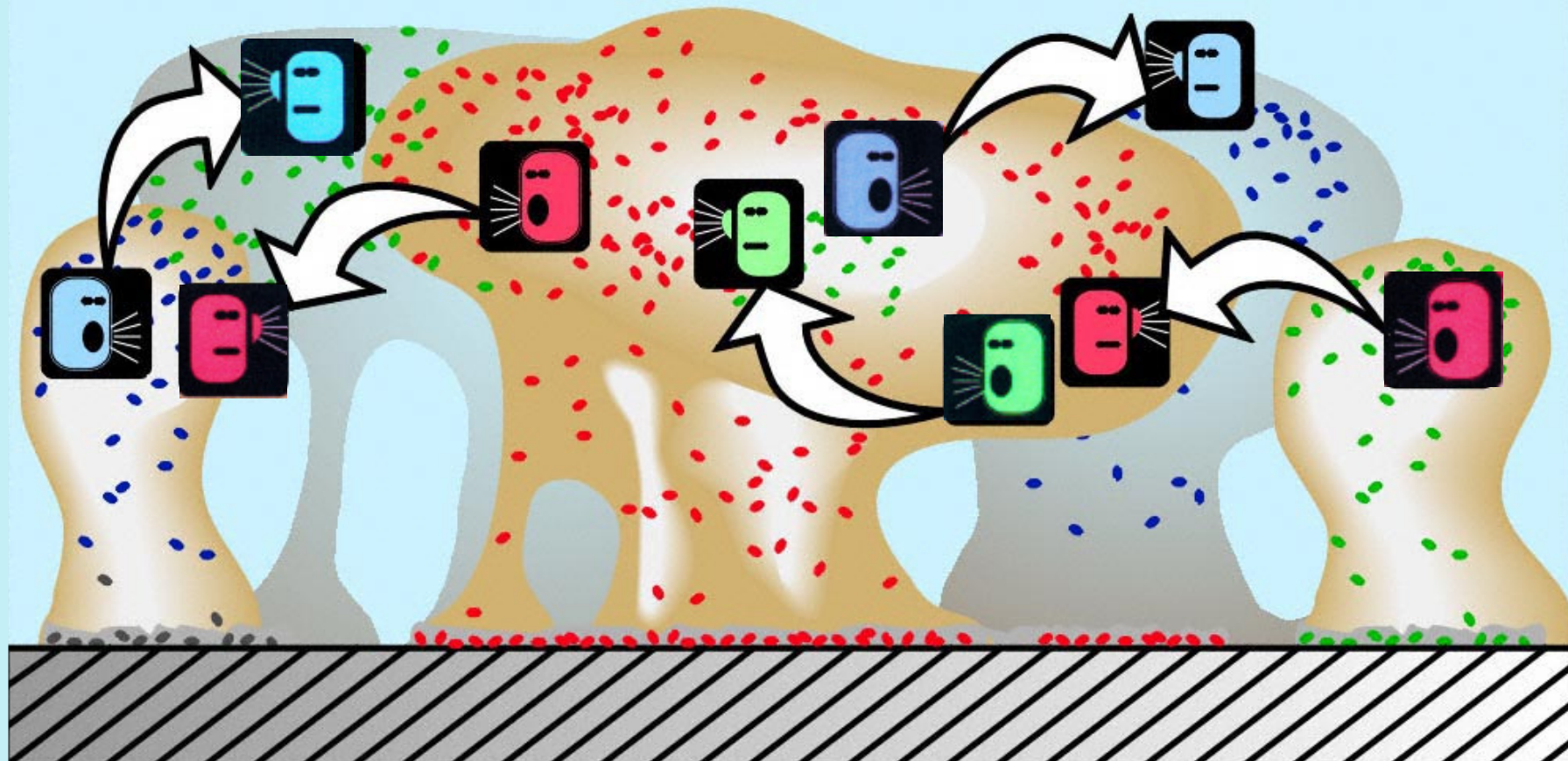


*S. pneumoniae* attacker  
with deleted CbpD



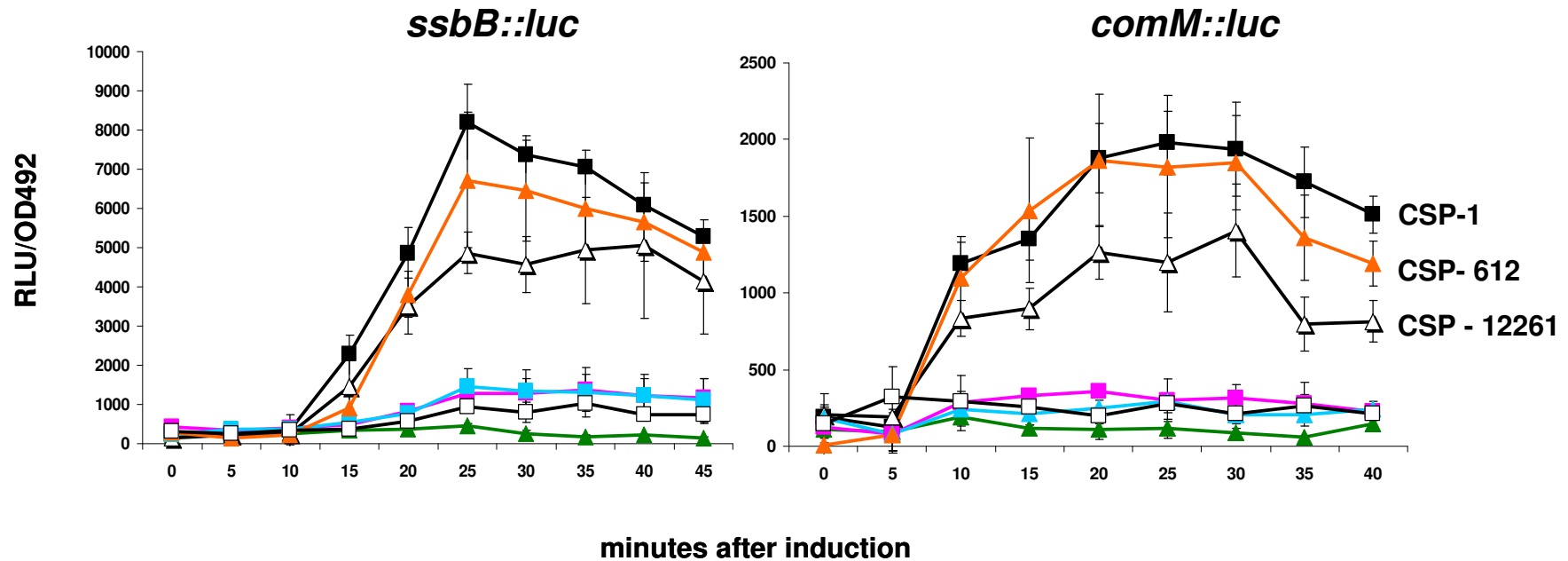
*S. pneumoniae* attacker  
with functional CbpD

# How do mixed populations of competent and non-competent streptococci arise in nature?



Strains producing different pheromone types

Cross-induction of competence in *S. pneumoniae* by CSPs produced by the *S. mitis* NCTC 12261 and *S. mitis* SK612 strains.

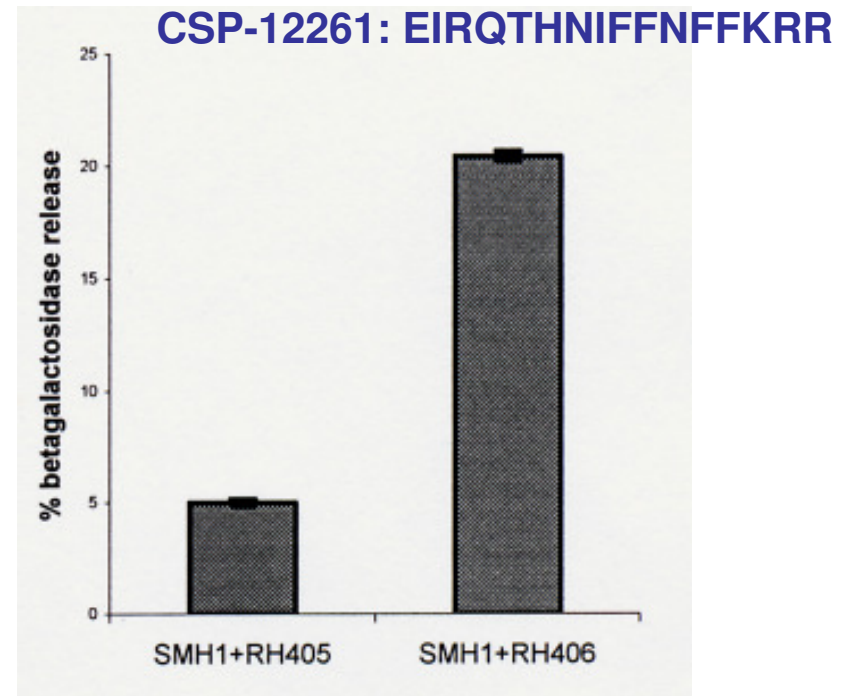
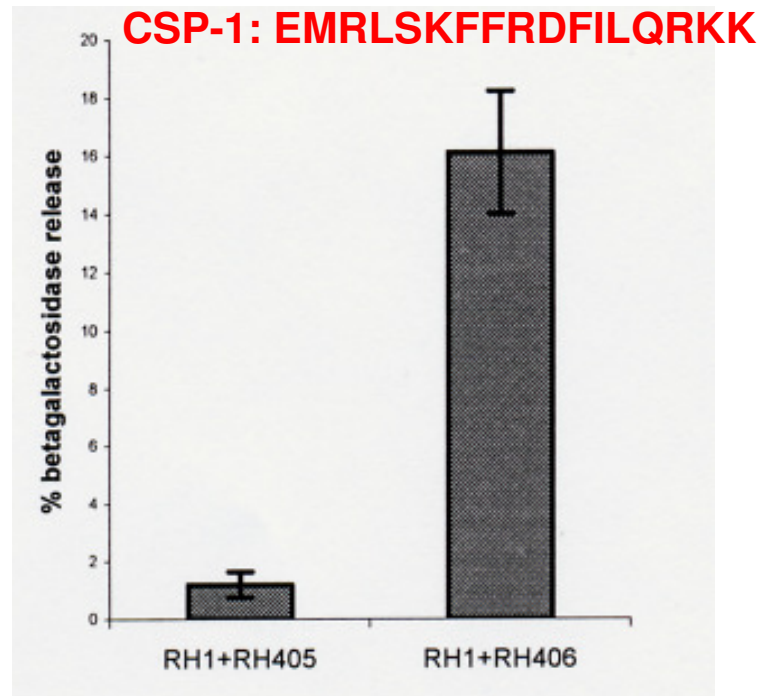


CSP-12261: **E**IRQTHNIF**F**N**F**K**R**R-

CSP-1: **E**M**R**L**S**K**F**F**R**D**F**I**L**Q**R**K**K**

CSP-612: **E**S**R**L**S**R**L**L**R**D**F**I**F**Q**I**K**Q**

# The ability to detect non-cognate CSPs provide protection against attack



## Attacker strains:

*S. pneumoniae*: RH1

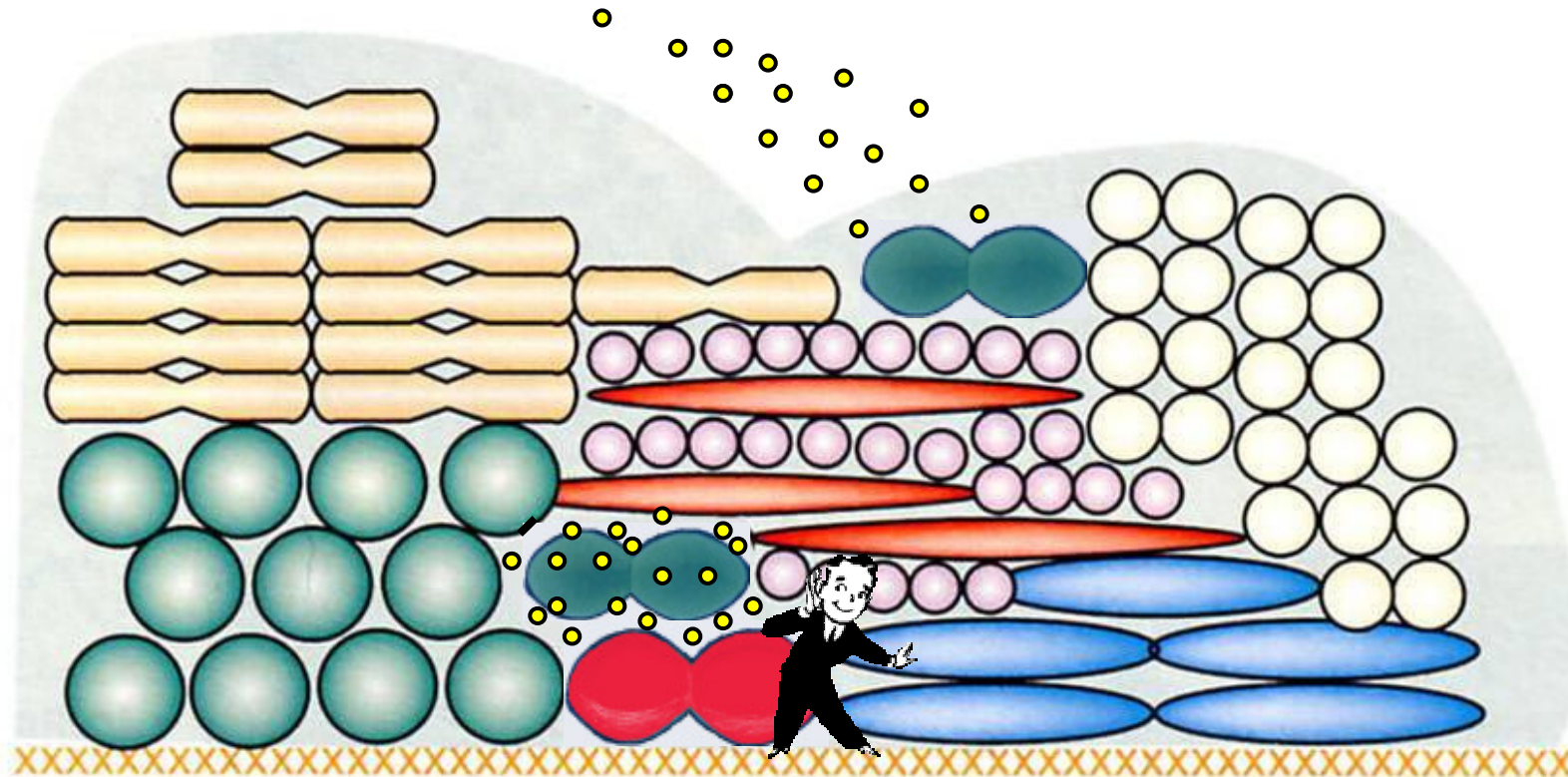
*S. mitis* 12261: SMH1

## Target strains:

*S. pneumoniae*: RH405 (*cbpD*, *hirL::lacZ*)

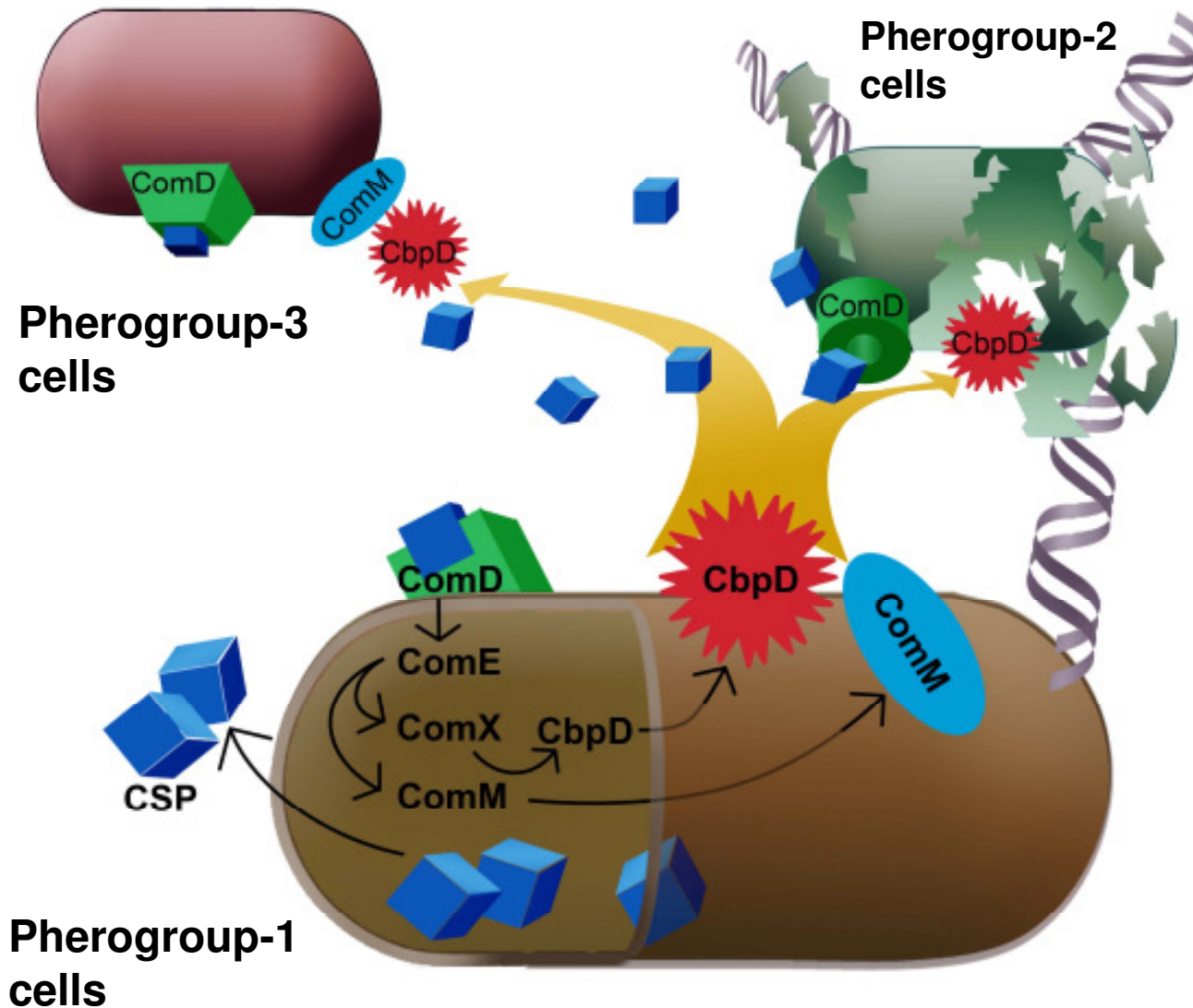
*S. pneumoniae*: RH406 (*cbpD*, *comM*, *hirL::lacZ*)

# Competence development in multi-species biofilm



The benefit of eavesdropping

# Model depicting CbpD mediated cell lysis and DNA transfer between streptococci belonging to different pherogroups.



## What drives the evolution of pheromone diversity?

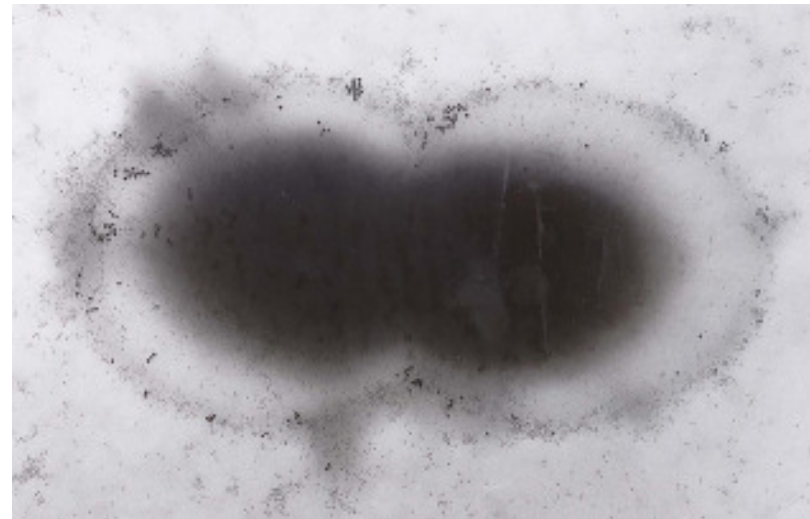
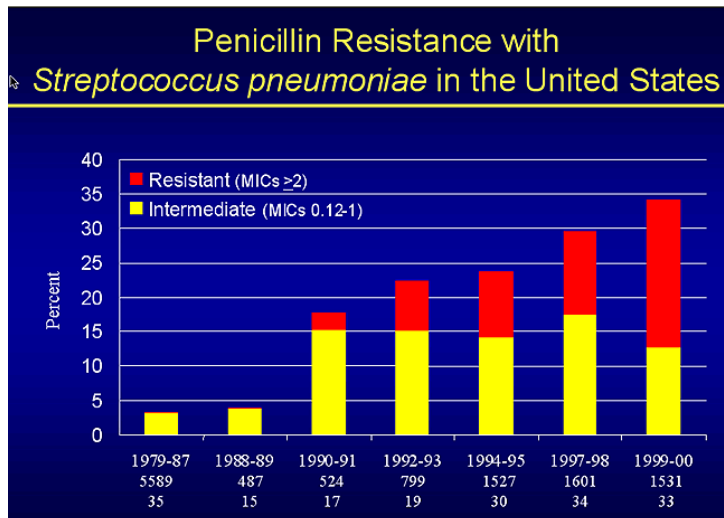
A selection pressure exists that favors the evolution of promiscuous ComD receptors that are able to detect CSPs from pherogroups often encountered in a particular habitat.

From the perspective of the attacker this will elicit a selection pressure that drives the evolution of novel pheromone types that will escape detection by competing strains.

It is likely that over time these opposing selection pressures have created the large diversity of pheromone types observed today.

# Conclusions:

- We have shown that a competence induced lysis mechanism dramatically increases the efficiency of gene exchange within and between the species *S. pneumoniae*, *S. mitis* and *S. oralis* *in vitro*.
- It is likely that this mechanism is important for efficient dissemination of antibiotic resistance determinants and exchange of capsular genes *in vivo*.



# Acknowledgements

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