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.

Competence stimulating peptide (CSP)

ComAB

Pre-CSP

DNA uptake and transport

CiaRH, Stress, Unknown environmental factors

Early com genes

Late com genes e.g., *ssbB, recA*

Recombination intermediates

Genome diversification

Exogenous DNA

ComD

P

ComE
S. pneumoniae

S. oralis

S. infantis

S. mitis

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<th>Species</th>
<th>Strain</th>
<th>Ref.</th>
<th>Amino acid sequence of ComC</th>
<th>Leader sequence</th>
<th>Mature signal peptide</th>
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Note: The sequence of the mature CSP is given in bold capital letters.
### Amino acid sequence of ComC

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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

Co-cultivation

Competent cells

Non-competent cells

Lysis of non-competent cells

Co-cultivation

Competent cells

Competent cells

Competent cells are resistant to lysis due to the ComM immunity protein

Guiral et al. (2005) PNAS 102: 8710
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.
DNA binding, Processing, uptake And recombination

LytA
CbpD
DNA release

CSP regulated expression of proteins involved in fratricide

ComAB
ComM

14 proteins involved in DNA uptake and recombination

~80 proteins, ~ 40 of totally unknown function

outside cell

7 proteins needed for competence

11 proteins of unknown function

Gene products involved in natural transformation

Gene products involved in lysis of non-competent cells

Gene products involved in unknown processes

ComX

ComE

ComD

Outside cell

Immunity

DNA release

LytA
CbpD
LytC

ComM
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

Involved in fratricide

CbpD: CHAP-domain, CBR

LytA: Amidase domain, CBR

LytB: CBR

LytC: CBR, Lysozyme

CBR: Choline binding repeats
Cleavage sites of LytA, LytC and CbpD

Lopez and Garcia, 2004
CbpD is required for efficient gene transfer in mixed cultures of competent and non-competent pneumococci

Non-competent cells (Nov\textsuperscript{R})

Competent Cells (Spc\textsuperscript{R})

Transformants (Nov\textsuperscript{R}, Spc\textsuperscript{R})

270 000 Nov\textsuperscript{R} transformants per ml

175 Nov\textsuperscript{R} transformants per ml
Efficient transfer of a Nov$^R$ marker from $S. \textit{mitis} / S. \textit{oralis}$ to $S. \textit{pneumoniae}$ depends on CbpD.

![Efficient transfer of a Nov$^R$ marker from $S. \textit{mitis} / S. \textit{oralis}$ to $S. \textit{pneumoniae}$ depends on CbpD.](image)
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: EIRQTHONIFFNFKRR
CSP–1: EMRLSKFFRDFILQRKK
CSP–612: ESRLSRLLRDFIFOIKQ
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.

Jonsborg et al. (2008)
Mol. Microbiol. 69: 245
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* \textit{in vitro}.

- It is likely that this mechanism is important for efficient dissemination of antibiotic resistance determinants and exchange of capsular genes \textit{in vivo}.
Acknowledgements

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