Interplay of DprA, RecA, and SsbB in the processing of transforming DNA in *Streptococcus pneumoniae*
The Pneumococcus

- Gram+ coccus, human commensal (nasopharynx)
- Pathogen (pneumonia, otitis, meningitis...)
- Naturally transformable species
Steps in pneumococcal transformation

- Non-competent cell
  - CSP
  - dsDNA
  - ssDNA uptake
  - single strand exchange
  - Transformed cell
  - Physical integration

requires competence (CSP) & exogenous dsDNA
Competence-induced proteins: The transformation machinery

Non-competent cell $\xrightarrow{\text{CSP}}$ Competent cell

DNA processing proteins

DNA uptake machine 11 genes

dsDNA

single strand exchange

Transformed cell $\rightarrow$ Physical integration
Genetic transformation, a highly integrated strategy?

- **Non-competent cell**
  - 6 genes
  - DNA release "fratricide"
  - DNA processing proteins

- **Competent cell**
  - 11 genes
  - DNA uptake machine
  - dsDNA
  - single strand exchange
  - Physical integration
  - Transformed cell
A DNA-uptake machine similar to that of *Bacillus subtilis* (except EndA) internalizes ssDNA (from 3' → 5').

DNA-uptake produces ssDNA, a key recombination intermediate.

No RecBCD-like activity is required for pneumococcal transformation.

Halpern et al. (2004)
The DNA-uptake machine localizes at the poles in *B. subtilis*

Hahn *et al.* (2005); Kidane and Graumann (2005)
DNA-processing proteins also localize at the poles in *B. subtilis*

Hahn *et al.* (2005); Kidane and Graumann (2005)
DNA-processing proteins of *S. pneumoniae*

The most important defect is observed in the absence of RecA or DprA (black arrows)

*Martin et al.* (1995); *Bergé et al.* (2003); *Desai and Morrison* (2006); *Burghout et al.* (2007)
Interplay of DprA, RecA, and SsbB in the processing of ssDNA
Internalized ssDNA is bound to a competence-induced protein

Nucleoprotein complex (NPC)

Control ssDNA

Hydroxylapatite chromatography

NPC eluted with 0.10-0.13 M phosphate
ssDNA alone eluted with 0.2 M phosphate

Morrison (1977)
Internalized ssDNA is bound to a competence-induced protein

Which protein?

Morrison (1977)

Hydroxylapatite chromatography
SsbB is the major protein component of the eclipse complex

Morrison, Mortier-Barrière et al. (2007)  Laetitia Attaiech (unpublished; see Poster)
The finding that ssDNA is complexed with SsbB raises an important question:

How does RecA alleviate the SsbB barrier to access ssDNA?
Dedicated proteins called RMP (Recombination Mediator Proteins) overcome the SSB barrier in both Procaryotes and Eukaryotes

<table>
<thead>
<tr>
<th>Activity</th>
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<th>Escherichia coli</th>
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<td>RecA</td>
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Beernink and Morrical (1999)
What about SsbB and the processing of transforming DNA?

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*RecOR are not required for genetic transformation in S. pneumoniae* (unpublished)

Beernink and Morrical (1999)
DprA is a widely conserved bacterial protein

Spn-DprA  
282 aa

Bsu-DprA  
297 aa

46% identity

Mortier-Barrière#, Velten#, Dupaigne#, Mirouze# et al. (2007)
Studies of the biochemical properties of DprA

° Protein-DNA interactions

Isabelle Mortier-Barrière
Marion Velten (Patrice Polard)

Pauline Dupaigne
Olivier Piétrement (Eric Le Cam)

° Protein-protein interactions

Isabelle Mortier-Barrière
Nicolas Mirouze (Philippe Noirot)

Mortier-Barrière#, Velten#, Dupaigne#, Mirouze# et al. (2007)
DprA displays all activities that characterize RMPs

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DprA interacts with RecA

Co-capture in pneumococcal extracts (from competent cells harboring a C-ter His-tagged chromosomal copy of recA)

Interaction in yeast two-hybrid assays

Mortier-Barrière#, Velten#, Dupaigne#, Mirouze# et al. (2007)
DprA interacts with ssDNA and promotes the loading of RecA on ssDNA

Mortier-Barrière#, Velten#, Dupaigne#, Mirouze# et al. (2007)
These nucleofilaments catalyze the formation of joint molecules.
DprA also displaces SSB to permit the loading of RecA on SSB-precoated ssDNA

SSB/nt, 1/5
RecA/nt, 1/3

Mortier-Barrière#, Velten#, Dupaigne#, Mirouze# et al. (2007)
We propose that DprA is a transformation-dedicated RMP
Open questions - Perspectives:

- *In vitro* experiments carried out with \textit{E. coli} RecA and \textit{E. coli} SSB:
  Need to document the homospecific situation

- Internalized ssDNA immediately degraded in \textit{dprA} or \textit{recA} mutant cells:
  Why is SsbB unable to access ssDNA in this genetic context?
Open questions - Perspectives:

- *In vitro* experiments carried out with $E_c^{\text{RecA}}$ and $E_c^{\text{SSB}}$:
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- Internalized ssDNA immediately degraded in $dprA$ or recA mutant cells:
  Why is SsbB unable to access ssDNA in this genetic context?

- Investigate localization dependencies

- Capture of protein and nucleoprotein complexes *in vivo*
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