Pilin gene variation in *Neisseria gonorrhoeae*

Reassessing the old paradigms

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Infection by Neisseria

S. Gray-Owen
Human experimental infections

Swanson et al., Curr Op Genet 1992
Surface Changes in *Neisseria*

**Antigenic Variation**

*Homologous route – RecA dependent*

- pilin gene variation
- limited *opa* variation via horizontal transmission
Surface Changes in *Neisseria*

Phase Variation - *on/off switching*

*Illegitimate route – RecA independent*

- *opa*
- *los*
- *porin*
- *pilus expression*

*Transcriptional regulation*

- pilus expression?
Neisseria gonorrhoeae’s pil gene family

- pilE is the pilin expression locus and encodes PilE (pilin) polypeptide

- all pil genes contain constant regions interspersed with variable segments

- the pilS loci contain multiple truncated pil gene copies

- pilS gene copies not transcribed

- downstream of each pil locus resides a Sma/Cla repeat

Clal-digested chromosomal DNA probed with common pil probe
Primary *intra*-cellular route for PilE variation

Nonreciprocal RecA-mediated gene conversion
“Mini-cassette” model for pilin variation

Haas and Meyer, Cell 1986
Less frequent intra-cellular events

a) reciprocal exchange involving a double crossover

b) reciprocal exchange involving a single crossover
“Looping-out” model for pilin variation

A

unequal sister chromatid exchange

B

pilE::pilS fusion formation

C

excision of closed circle utilizing repeated sequences

Meyer and Hill, 2003
closed circle carrying \textit{pilE::pilS} fusion recombines with \textit{pilE} utilizing upstream homology
Predictions

1. Requires duplicated \textit{pilE}s to be in direct orientation

2. Inverted \textit{pilE}s will not “loop-out”

3. If \textit{pilE} antigenic variation proceeds via “looping-out” then an inverted \textit{pilE} locus should abrogate switching
pilE/pilS recombination proceeds normally with an inverted pilE locus

Hill et al., J Bacteriol 2007
Genetic Requirements for Homologous Recombination

*Escherichia coli*  
*Neisseria*

- three pathways
  - RecBCD  
  - RecF  
  - RecE

+++
++/-
not applicable

no exonuclease I  
no RecF
“RecF-like” Pathway Proposed for PilE variation in FA1090

- modest recombination defect in recB mutants
- modest defect in repair capabilities in recB mutants
- RT PCR assay indicates pilE/pilS exchanges comparable to wild type in recB, recC, and recD mutants
- recombination and repair defects demonstrated for various RecF pathway mutants

Mehr and Seifert, Mol Micro 1998
Pilin switching in MS11 *recD* mutants

A - wild type 37°C
B - wild type 33°C
C - *recD*
D - *dud-1*
E - *recD dud-1*
F - *comA*
G - *recD comA*
H - *recD opaE::recD+*

Chaussee et al, Microbiology 1999
Growth characteristics of strain MS11 recB mutants
MS11 recB mutants are extremely sensitive to DNA damaging reagents

- nalidixic acid survival

- MMS sensitivity
Wild type strains FA1090 and P9 are repair deficient

- Nalidixic acid survival
- MMS sensitivity
- UV survival
N. gonorrhoeae recB mutants

1. Qualitatively identical results for strains MS11, P9 and FA1090 in all assays

2. More pronounced phenotypes in strain MS11 for nalidixic acid and MMS treatment but not UV

3. Suppressor mutants arise at $1 \times 10^{-3}$

4. Suppressor mutants selected in all assays and following freeze/thawing at -80°C

5. Identical results for gonococcal recC mutants
Double chain break repair model for \textit{pilE/pilS} recombination

\textbf{A} \hspace{1cm} \textbf{B}\hspace{1cm} \textbf{C}\hspace{1cm} \textbf{D}\hspace{1cm} \textbf{E}

\textit{pilE} invades \textit{pilS}

\underline{broken \textit{pilE} copies \textit{pilS} template}

\underline{non-crossover resolution of Holiday junctions}

\underline{variant \textit{pilE}}

\textit{Hill et al.}, J Bacteriol 2007