Pneumopath: 'Pneumococcal physiology'
Insight into the importance and interconnection of regulators of central metabolism

Objectives
Identification of the connection(s) between carbon and nitrogen metabolism, and of the interplay between carbon and nitrogen regulators

Groups involved:
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Published so far

Sugar Transporters

CelR (sugar; virulence)

CcpA (sugars; virulence)

CiaR (β-lactam susceptibility; virulence)


AdrR (zinc homeostasis; virulence)

CopY (copper homeostasis; virulence)

CodY (BCAAs & peptides, iron homeostasis; virulence)

CodY is an essential regulator in S. pneumoniae

CodY roles
Metabolic pathways
Virulence

Metabolic pathways
In Bae, the CodY regulon encompasses nearly 200 genes (repressed during exponential growth, induced upon starvation)

In Spn, CodY was shown to function mainly as a repressor (43 of 47 genes upregulated in a codY mutant)
Attempt at inactivating $\text{codY}$ using mariner mutagenesis (transposon insertion). 

This strongly suggested that $\text{codY}$ is essential.
Inactivation of \textit{codY} in the diploid strain

These results were consistent with the hypothesis that \textit{codY} is an essential gene.

Transfer of \textit{codY}::\textit{spc}

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Essentiality of \textit{codY} in different capsulated strains?

\textit{codY} is an essential gene in R6/R800
Essentiality of codY in different capsulated strains?

- D39 background (serotype 2)
- TIGR4 background (serotype 4)
- 6B background (serotype 6B)

What about the codY::trim used for transcriptome studies?

Hendriksen et al., J Bacteriol, 2008

Characterization of codY::trim transfer

- DONOR
- 'reference marker'
- wt
- diploid
- RECIPIENTS

Characterization of codY::trim transfer

- DONOR
- RECIPIENTS
- 300x
- 20x

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Transfer of *codY:trim* by transformation requires the co-transfer of two unlinked suppressor mutations.

Genome sequence of *codY:trim* mutant to identify the suppressors.

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**Transfer of *codY:trim* by transformation requires the co-transfer of two unlinked suppressor mutations.**

**Genome sequence of codY:trim mutant to identify the suppressors.**

**Genome sequence of codY:trim mutant**

Mutation X: Inactivation of a major iron transporter (100% of sequences)

Mutation Y: Inactivation of *amiC* by three different mutations (each present in about 30% of sequences)

Genome sequence of *codY:trim* mutant

Construction of a *fatC* *amiC* recipient then used to demonstrate that the double mutant readily accepts *codY* inactivation.

We suggest *CodY* is essential as inactivation results in increased iron uptake and oxidative stress.
Another published D39 codY::trim mutant

A glnR-codY double mutant was used during analysis of the GlnR regulon

- Repression of glutamine synthesis and uptake (glnA and glnPQ),
- Repression of glutamate synthesis (gdhA), ...

Expression of gdhA is also repressed by the pleiotropic regulator CodY

Kloosterman et al., JBC, 2006

Transfer analysis indicated that
- a glnR mutant does not tolerate codY inactivation
- the glnR-codY double mutant was fatC+ and amIC+

Further transfer analysis suggested the presence of two suppressors.

Does the glnR- mutation allow tolerance of codY inactivation? Or does this strain possess further suppressing mutations?

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Expression of gdhA is also repressed by the pleiotropic regulator CodY

Genome sequencing of the glnR-codY double mutant then revealed the presence of a mutation in fecE

- fecE is truncated after AA13

This confirmed derepression of iron uptake is a major problem for codY mutant cells

The glnR-codY strain possesses suppressing mutations

but genome sequencing of the glnR-codY double mutant failed to identify the 2nd suppressor...

Conclusions

- Take home messages re gene inactivation in S. pneumoniae
  - Evaluation of KO by 'quantitative back transformation', a safe strategy...
  - Genome sequencing, a possible way to identify suppressor mutations but...
  - ... not necessarily successful if 'suppressor' is not a point mutation
  - Merodiploids (partial chromosome duplications) can also occur (not easy to detect)
Conclusions

- Take home messages re gene inactivation in *S. pneumoniae*
  - Evaluation of KO by ‘quantitative back transformation’, a safe strategy...
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- CodY roles and essentiality
  - CodY essentiality clearly indicates that it fulfills important function(s)
  - Essentiality likely results from alteration of iron homeostasis
  - Hitherto ignored regulatory connections between aminoacid and oligopeptide transport, and iron metabolism
  - Additional evidence that iron is important for *S. pneumoniae*
  - CodY (and regulators) are potentially interesting therapeutic targets