Pneumopath:
'Pneumococcal physiology'

Insight into the importance and interconnection of regulators of central metabolism

Pneumococcal physiology

Objectives

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

Groups involved:

Jean-Pierre Claverys
Regine Hakenbeck
Oscar Kuipers
Nic Lindley
Marco Oggioni

Published so far

Sugar Transporters

Bidossi, A., Mulas, L., Decorosi, F., Colomba, L., Ricci, S., Pozzi, G. *et al.* (2012) A functional genomics approach to establish the complement of carbohydrate transporters in *Streptococcus pneumoniae*. *PLoS ONE* **7**: e33320.

CelR (sugar; virulence)

Shafeeq, S., Kloosterman, T.G., and Kuipers, O.P. (2011a) CelR-mediated activation of the cellobiose-utilization gene cluster in *Streptococcus pneumoniae*. *Microbiology* **157**: 2854-2861.

CcpA (sugars; virulence)

Carvalho, S.M., Kloosterman, T.G., Kuipers, O.P., and Neves, A.R. (2011) CcpA ensures optimal metabolic fitness of *Streptococcus pneumoniae*. *PLoS ONE* **6**: e26707.

CiaR (β-lactam susceptibility; virulence)

Halfmann, A., Schnorpfeil, A., Muller, M., Marx, P., Gunzler, U., Hakenbeck, R., and Bruckner, R. (2011) Activity of the two-component regulatory system CiaRH in *Streptococcus pneumoniae* R6. *J Mol Microbiol Biotechnol* **20**: 96-104.

Muller, M., Marx, P., Hakenbeck, R., and Bruckner, R. (2011) Effect of new alleles of the histidine kinase gene *ciaH* on the activity of the response regulator CiaR in *Streptococcus pneumoniae* R6. *Microbiology* **157**: 3104-3112.

Published so far

AdcR (zinc homeostasis; virulence)

Shafeeq, S., Kloosterman, T.G., and Kuipers, O.P. (2011b) Transcriptional response of *Streptococcus pneumoniae* to Zn2+ limitation and the repressor/activator function of AdcR. *Metallomics* **3**: 609-618.

CopY (copper homeostasis; virulence)

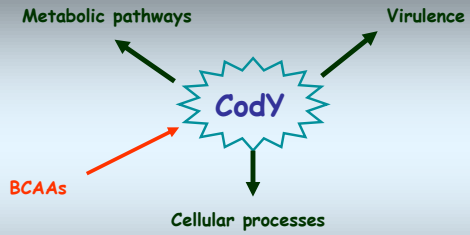
Shafeeq, S., Yesilkaya, H., Kloosterman, T.G., Narayanan, G., Wandel, M., Andrew, P.W. *et al.* (2011c) The *cop* operon is required for copper homeostasis and contributes to virulence in *Streptococcus pneumoniae*. *Mol Microbiol* **81**: 1255-1270.

CodY (BCAAs & peptides, iron homeostasis; virulence)

Caymaris, S., Bootsma, H.J., Martin, B., Hermans, P.W.M., Prudhomme, M., and Claverys, J.P. (2010) The global nutritional regulator CodY is an essential protein in the human pathogen *Streptococcus pneumoniae*. *Mol Microbiol* **78**: 344-360.

CodY is an essential regulator in *S. pneumoniae*

CodY roles



Motility, sporulation, competence (*Bsu*)…

Sonenshein, *Curr Opin Microbiol*, 2005

CodY roles

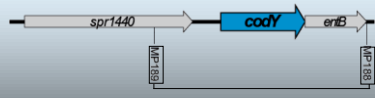
Metabolic pathways

In *Bsu*, the CodY regulon encompasses nearly 200 genes (repressed during exponential g., induced upon starvation)

In *Spn*, CodY was shown to function mainly as a repressor (43 of 47 genes upregulated in a *codY* mutant)

Hendriksen *et al.*, *J Bacteriol*, 2008 Sonenshein, *Curr Opin Microbiol*, 2005

Attempt at inactivating *codY* using *mariner* mutagenesis (transposon insertion)



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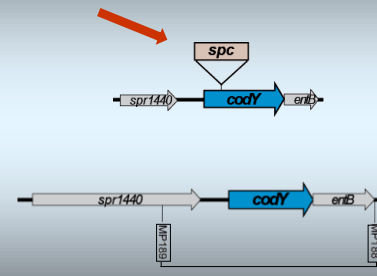
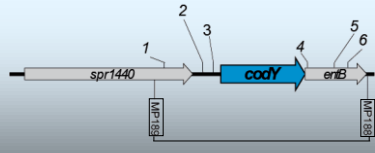
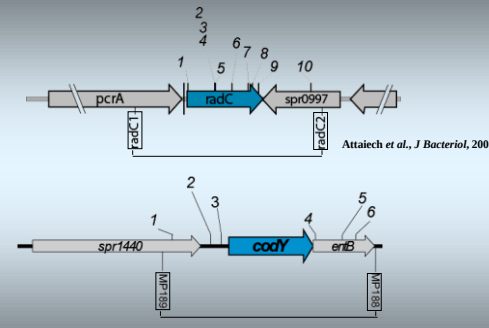


Attempt at inactivating *codY*

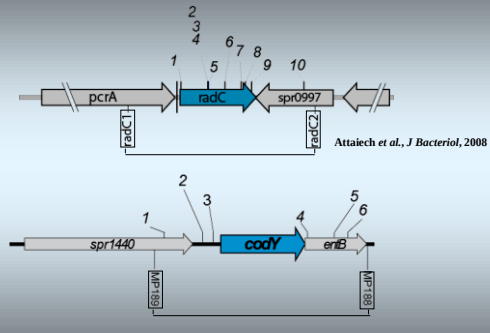


Attempt at inactivating *codY*



Attaiech *et al., J Bacteriol*, 2008

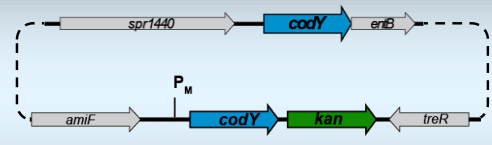
Attempt at inactivating *codY*



Attaiech *et al., J Bacteriol*, 2008

This strongly suggested that *codY* is essential

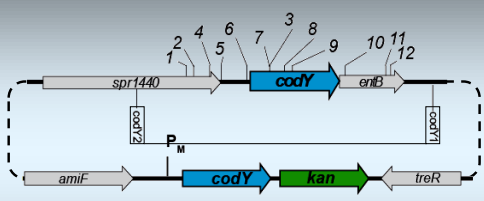
Construction of a *codY* diploid strain



CEP-*codY*

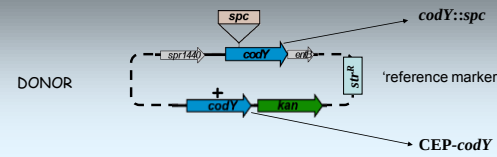
Guiral *et al., Microbiology (Special Issue on Pneumococcus)* 2006

Inactivation of *codY* in the diploid strain



These results were consistent with the hypothesis that *codY* is an essential gene

Transfer of *codY*::*spc*



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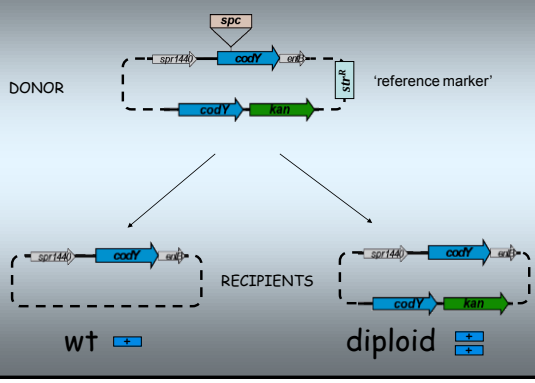
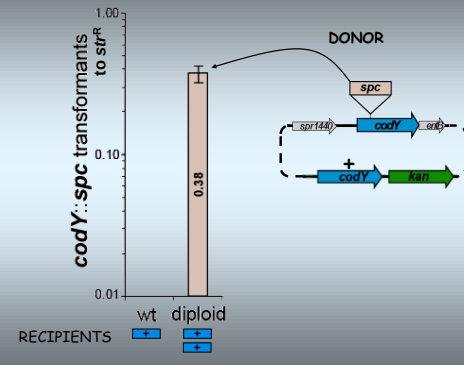


Transfer of *codY*::*spc*



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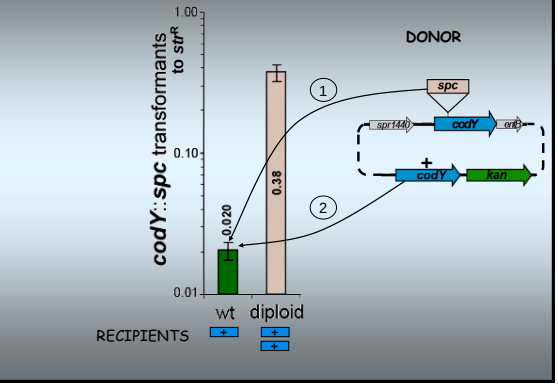


Essiality of *codY* in different capsulated strains?

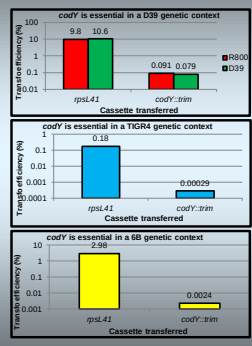
***codY* is an essential gene in R6/R800**

Essentiality of *codY* in different capsulated strains?

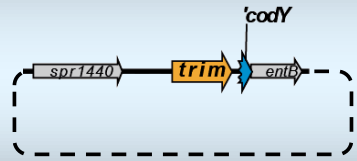
D39 background (serotype 2)

TIGR4 background (serotype 4)

6B background (serotype 6B)

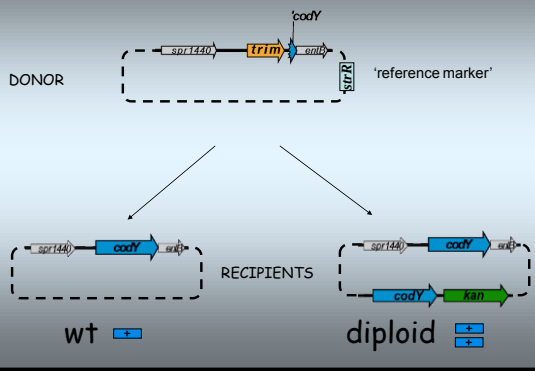


What about the *codY*::*trim* used for transcriptome studies?



Hendriksen *et al., J Bacteriol*, 2008

Characterization of *codY*::*trim* transfer



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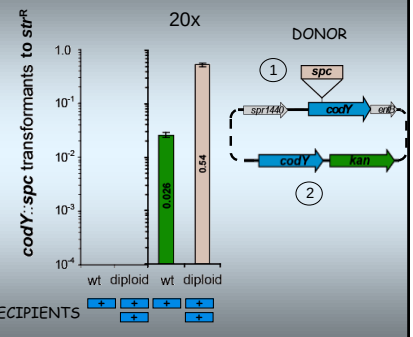

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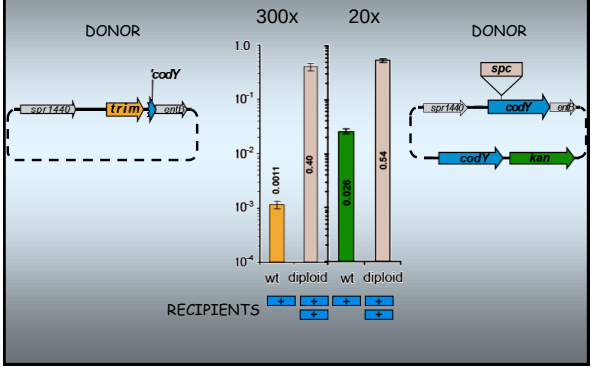

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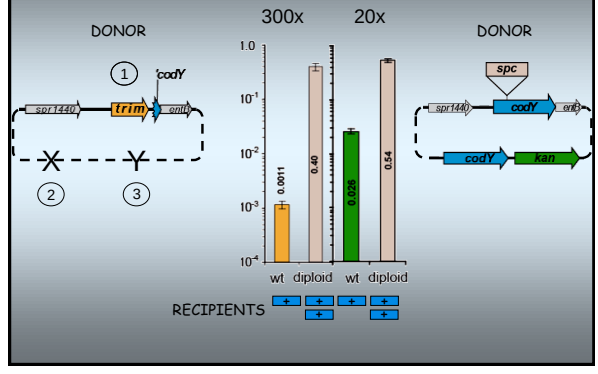

Transfer of *codY*::*trim* by transformation requires the co-transfer of two unlinked suppressor mutations

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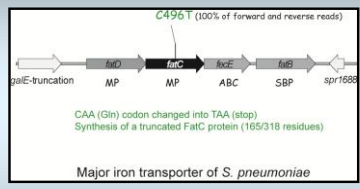
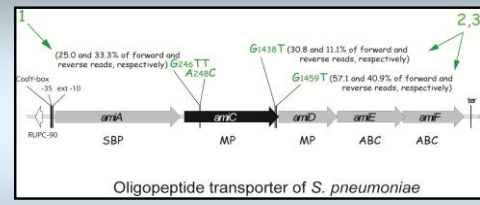
Genome sequence of *codY::trim* mutant to identify the suppressors

Genome sequence of *codY::trim* mutant



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

Genome sequence of *codY::trim* mutant

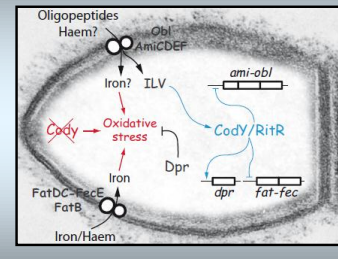


Mutation Y: Inactivation of *amiC* by three different mutations (each present in about 30% of sequences)

Genome sequence of *codY::trim* mutant

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

Genome sequence of *codY::trim* mutant



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

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Repression of glutamine synthesis and uptake (*glnA* and *glnPQ*),
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Expression of *gdhA* is also repressed by the pleiotropic regulator CodY

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

Kloosterman *et al., JBC*, 2006

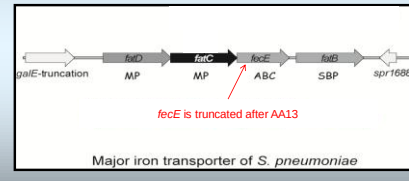
Transfer analysis indicated that

- a *glnR* mutant does not tolerate *codY* inactivation
- the *glnR-codY* double mutant was *fatC*⁺ and *amiC*⁺

Further transfer analysis suggested the presence of <u>two suppressors</u>…

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

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



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
  - Merodiploïds (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…
  - Genome sequencing, a possible way to identify suppressor mutations but…
  - … not necessarily successful if 'suppressor' is not a point mutation
  - Merodiploïds (partial chromosome duplications) can also occur (not easy to detect)
- 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