The investigation of RpoN in swarming of uropathogenic Proteus mirabilis

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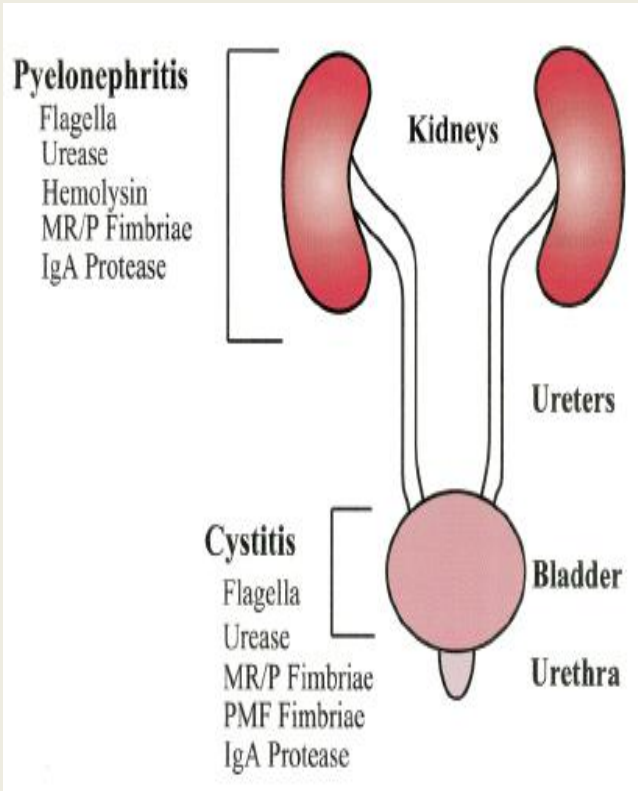
Introduction

Proteus mirabilis

By Bobby Henderson

- Gram-negative, facultative anaerobic Enterobacteriaceae
- Urinary tract infection (UTI)
  - catheter-associated urinary tract infections
  - urolithiasis
  - pyelonephritis

Wound infection, septicaemia, pneumonia



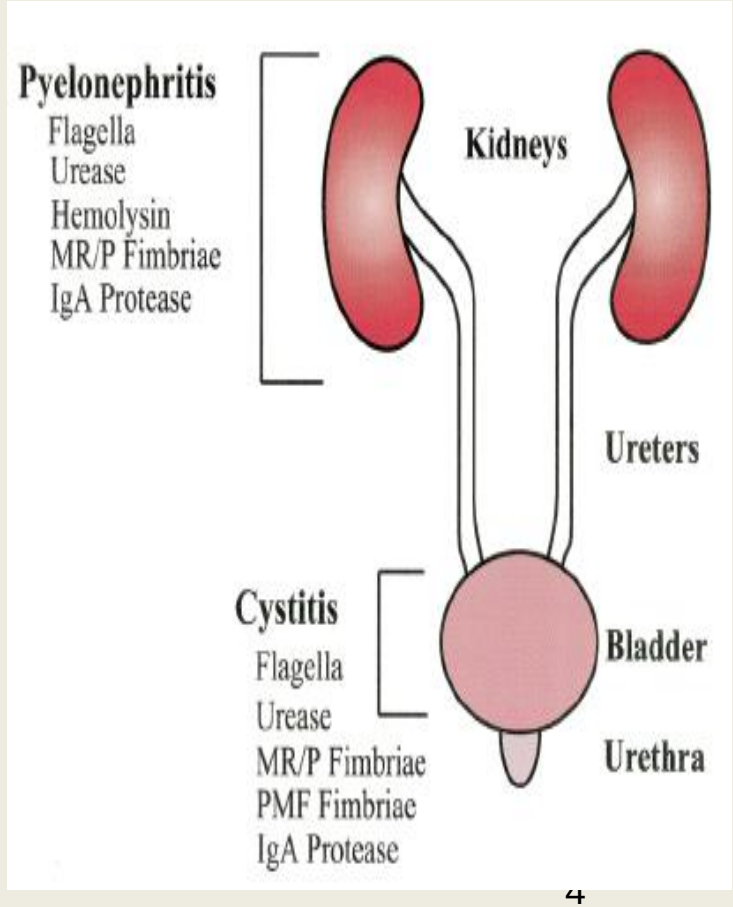
Coker *et al*. Microbes Infect. 2000. 2: 1497–1505.

Proteus mirabilis

- Virulence factors

  Fimbriae: Mrp and P-fimbriae
  Haemolysin
  Urease
  IgA protease
  Flagella

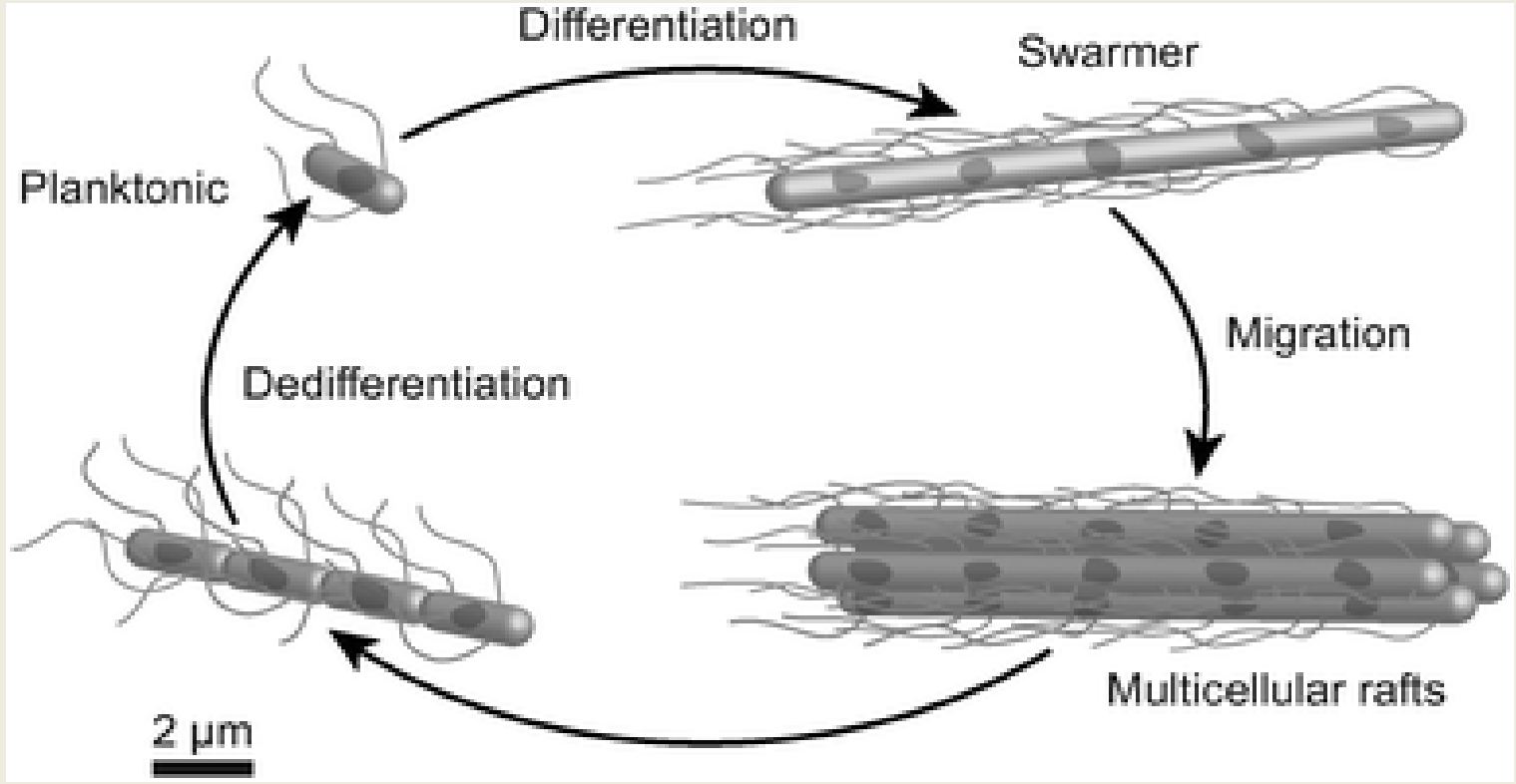
- Swarming
  short rod swimmer cell/
  elongated multicellular
  swarmer cell

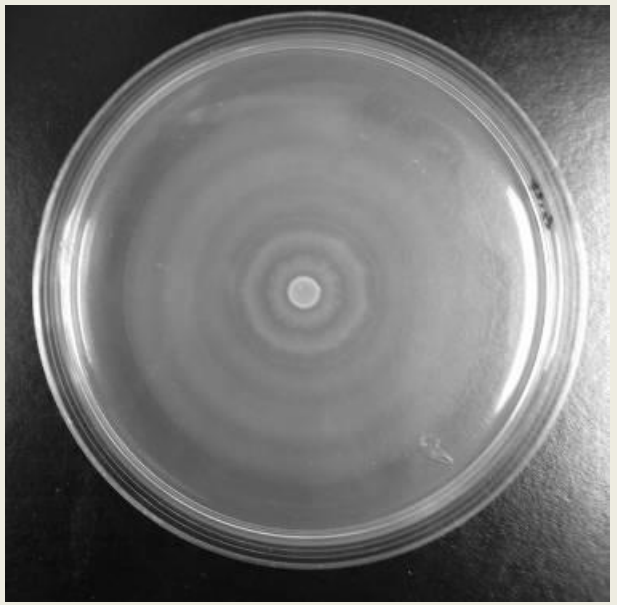


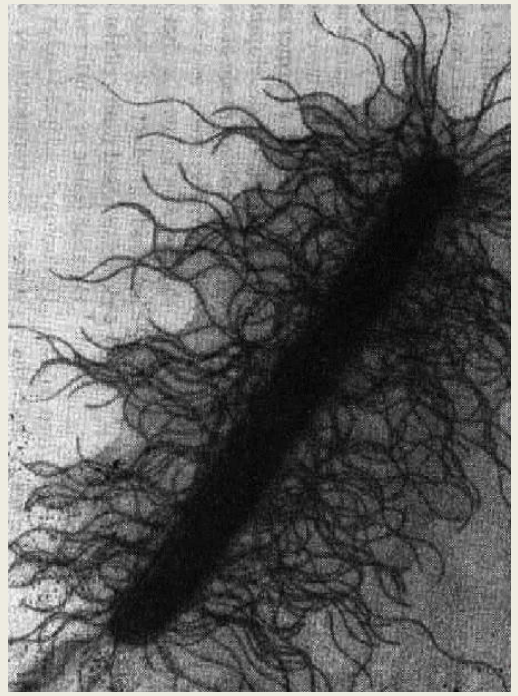
Four separate phases in swarming of P. mirabilis

- Induction of swarmer cell differentiation
- Delay prior to onset of swarming
- Active swarming migration
- Consolidation

Swarming motility



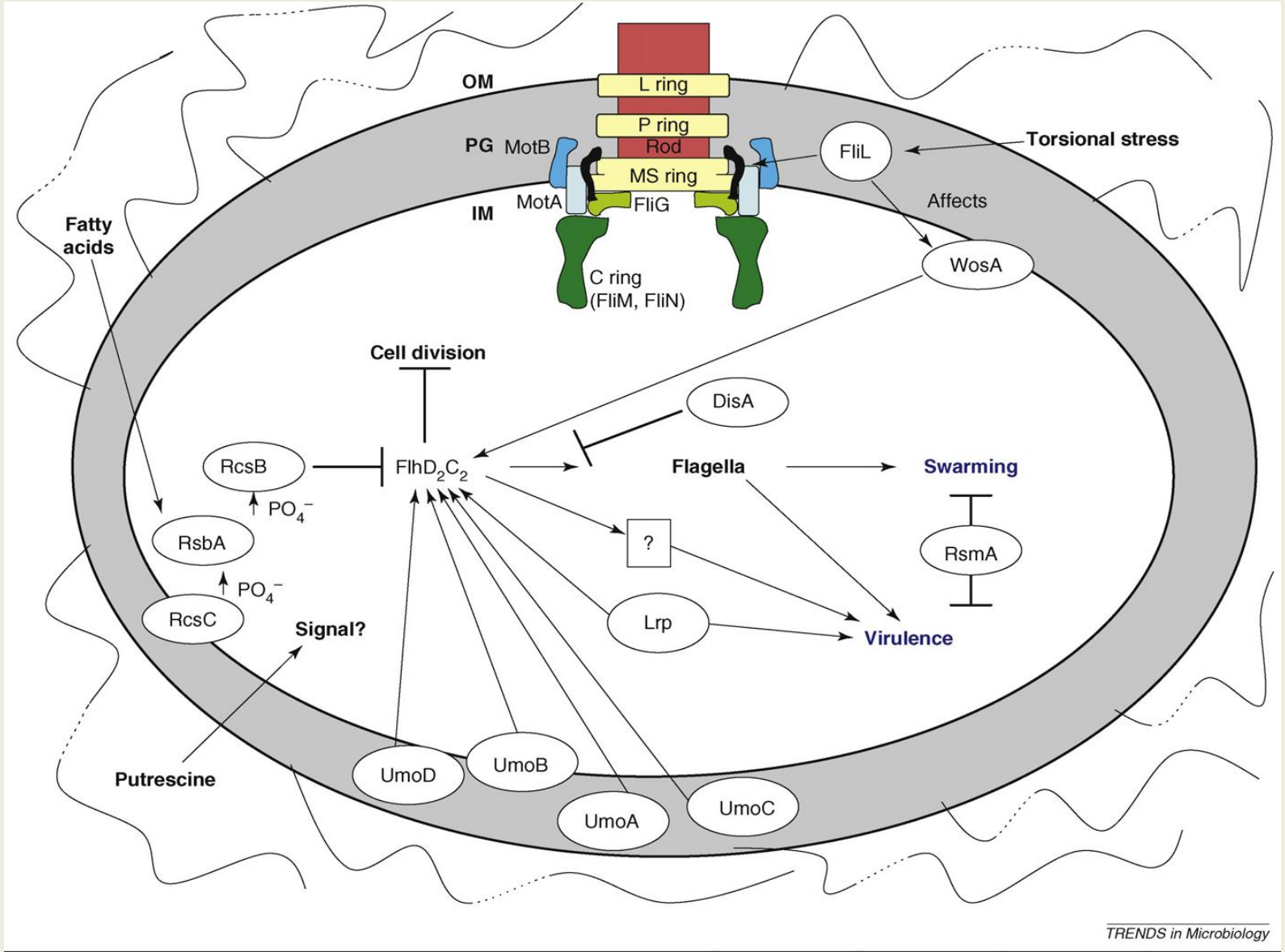




Soft Matter, 2009, 5, 1174-1187

Coordination of virulence factor expression and swarming migration of *P. mirabilis*

P. mirabilis regulatory networks of swarming and virulence



Flagella

Lipopolysaccharide

Chemotaxis

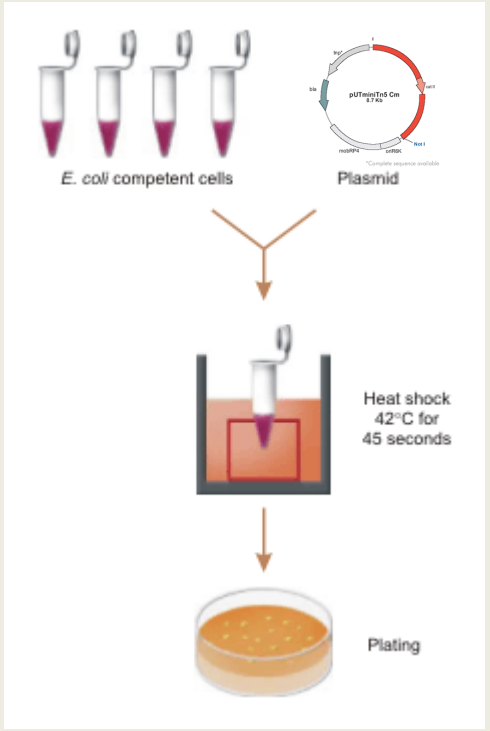
Two-component

Trends in Microbiol 16:496 2008

Results

Tn5 - mutagenesis

S17-1



Km 50µg/ml

Amp 50µg/ml

37°C/overnight

***E. coli* S17-1**

Km^r
Tet^s

***P. mirabilis* N2**

Tet^r
Km^s

10 mM $MgSO_4$

conjugation
(on LSW$^-$ agar)

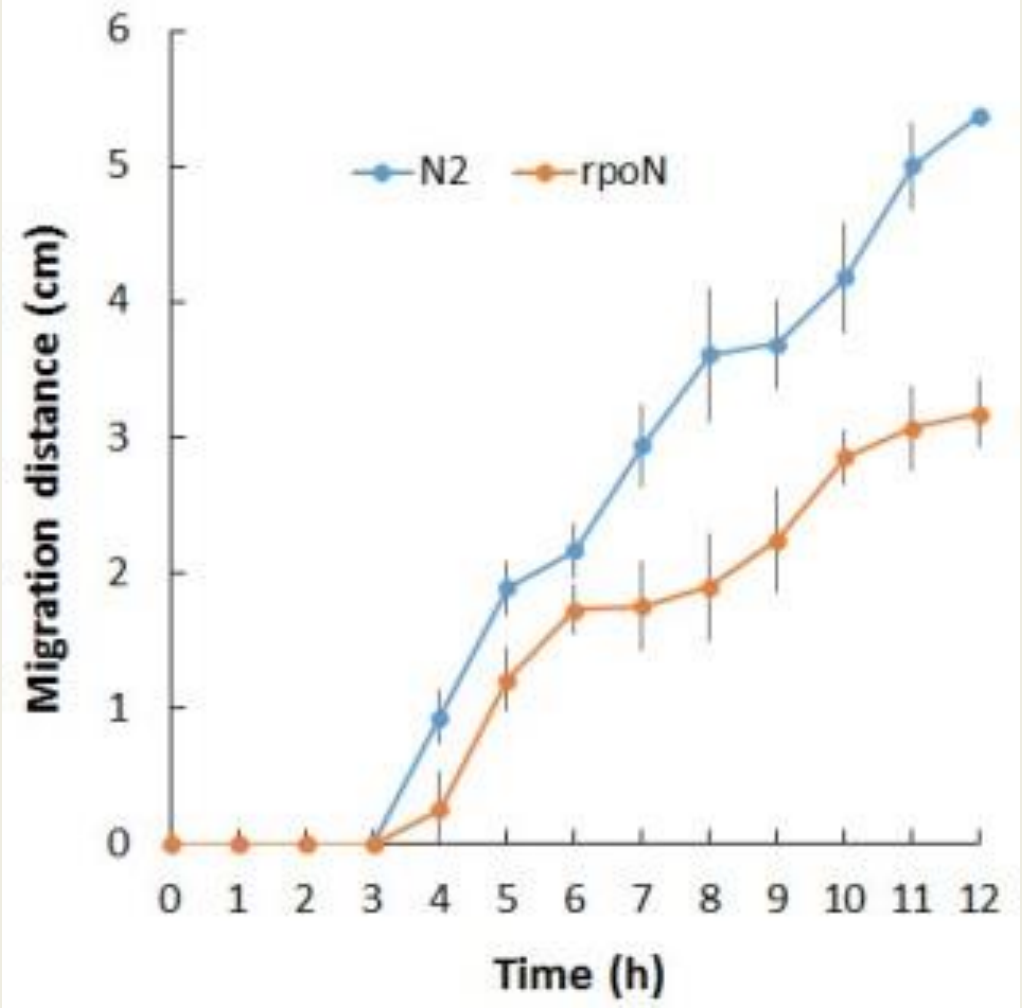
10 mM $MgSO_4$

Plating on selection agar

37°C/overnight

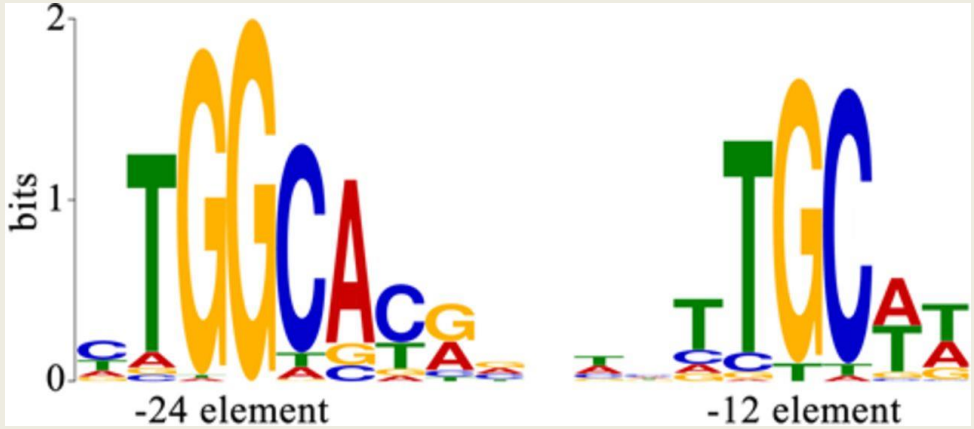
Km 50 µg/ml
Tet 12.5µg/ml

RpoN regulates swarming



RpoN

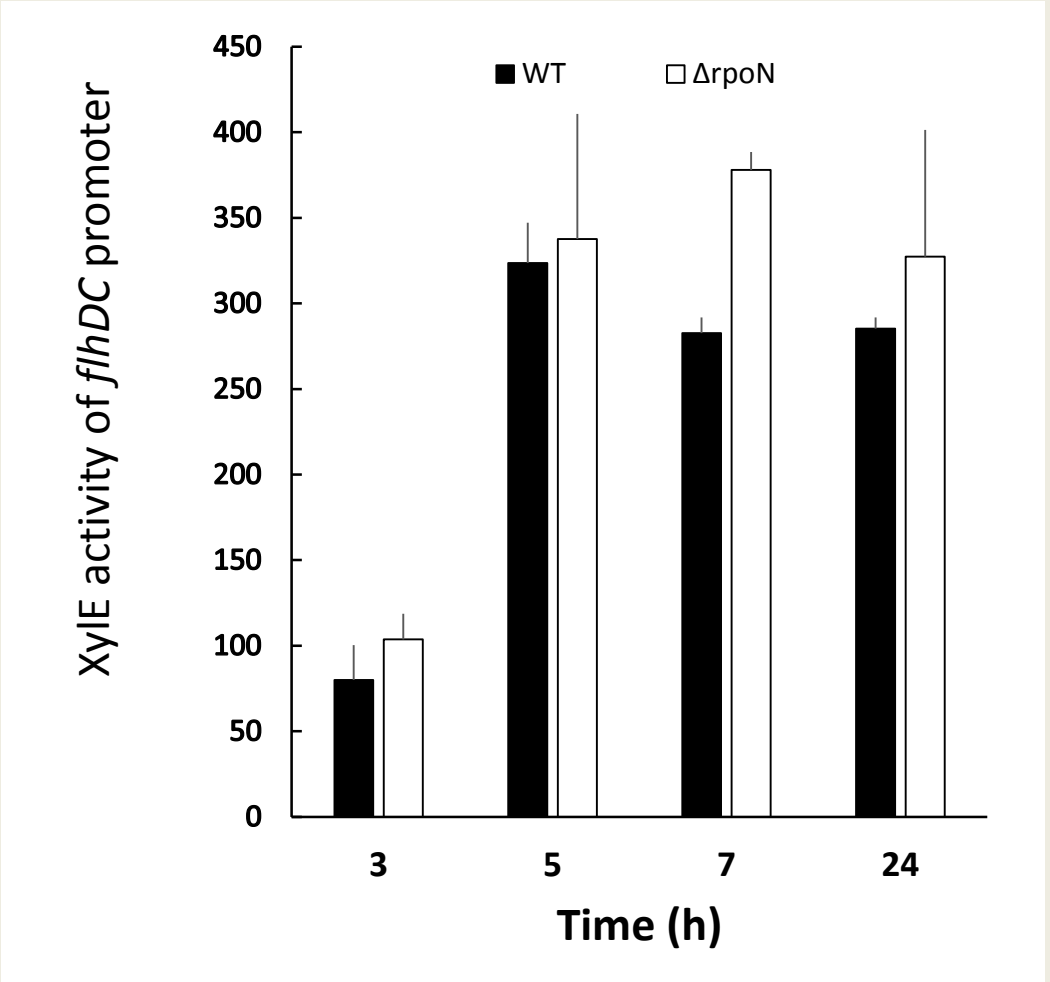
- A bacterial RNA polymerase alternative sigma factor
- RpoN has been shown to play a key role in stress responses (N limitation).
- -24, -12 element NOT -35, -10 element (RpoD)



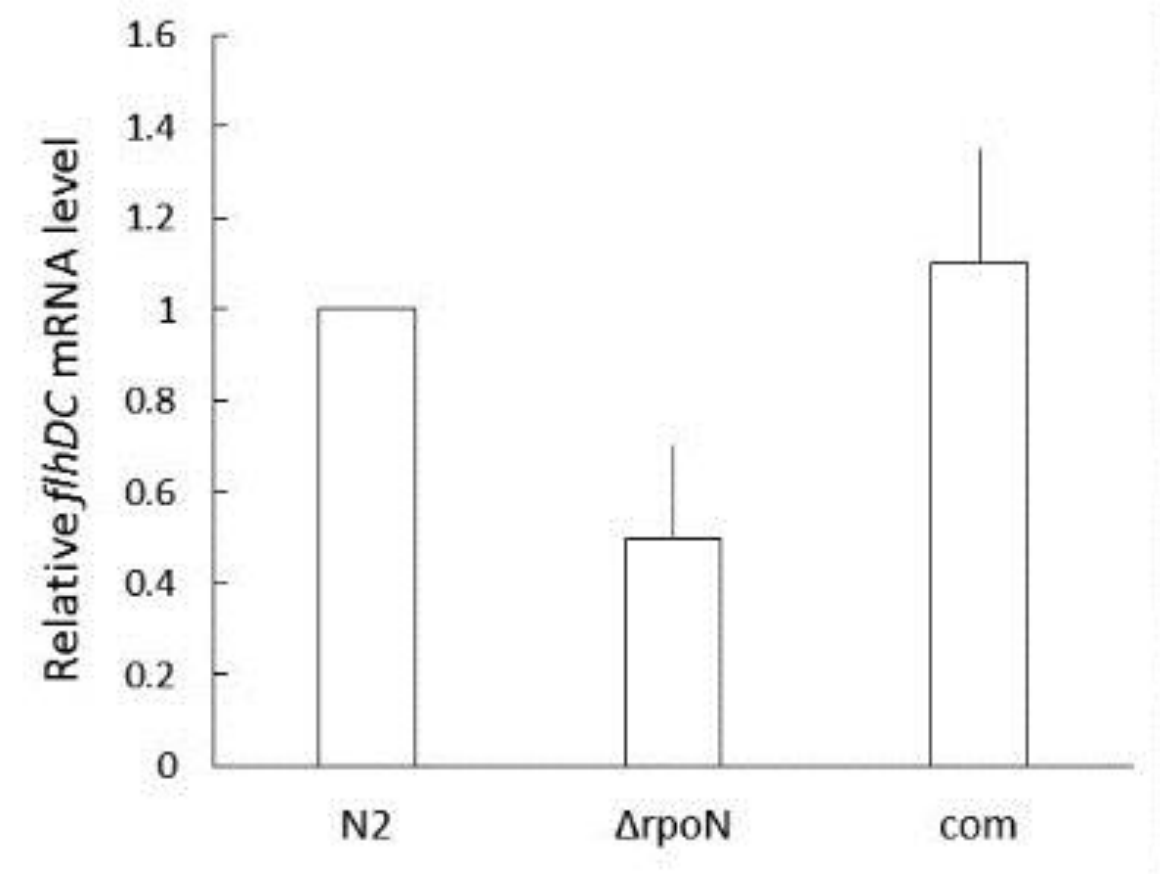
Nucleic Acids Res. 2014 Dec 1;42(21):12961-72.

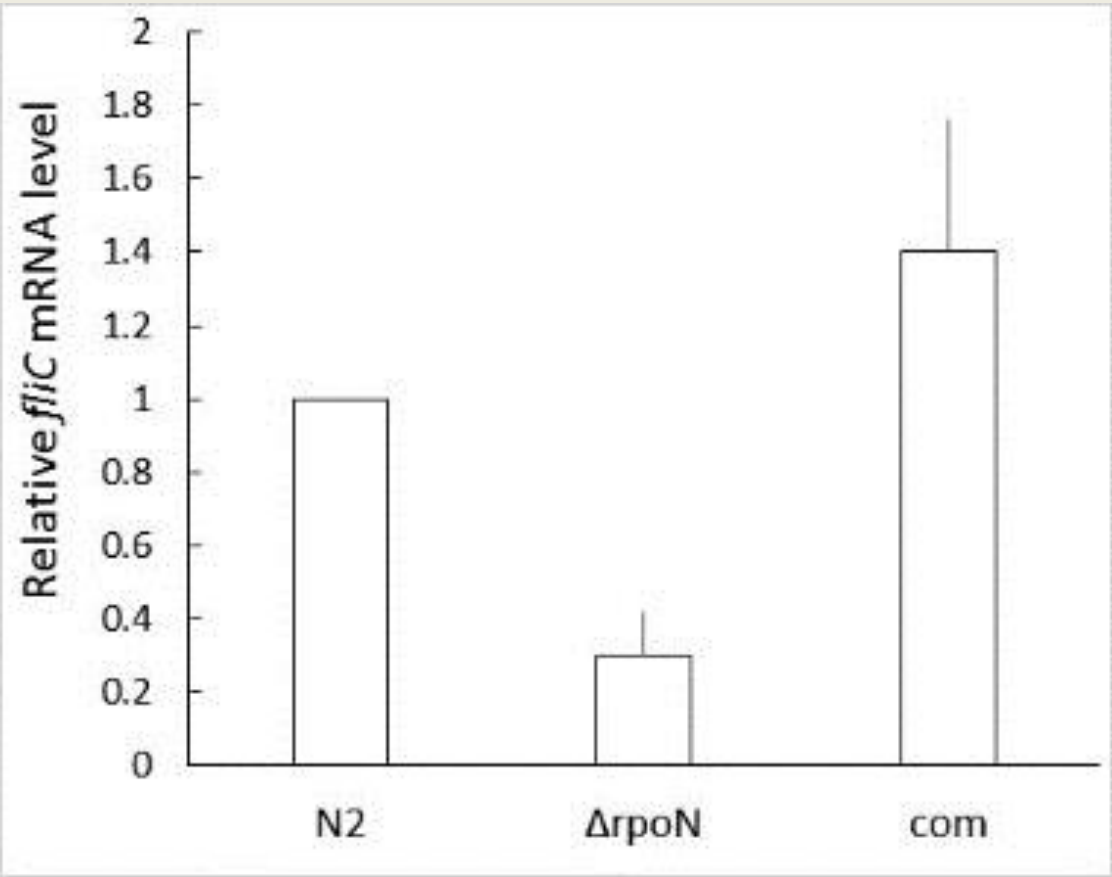
No effect of RpoN on the promoter activity of flhDC

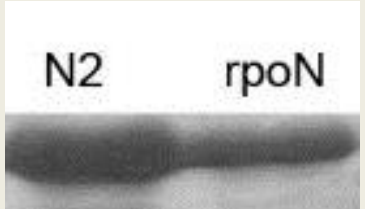
- A master regulator of flagellin synthesis



RpoN affects the mRNA levels of flhDC and fliC

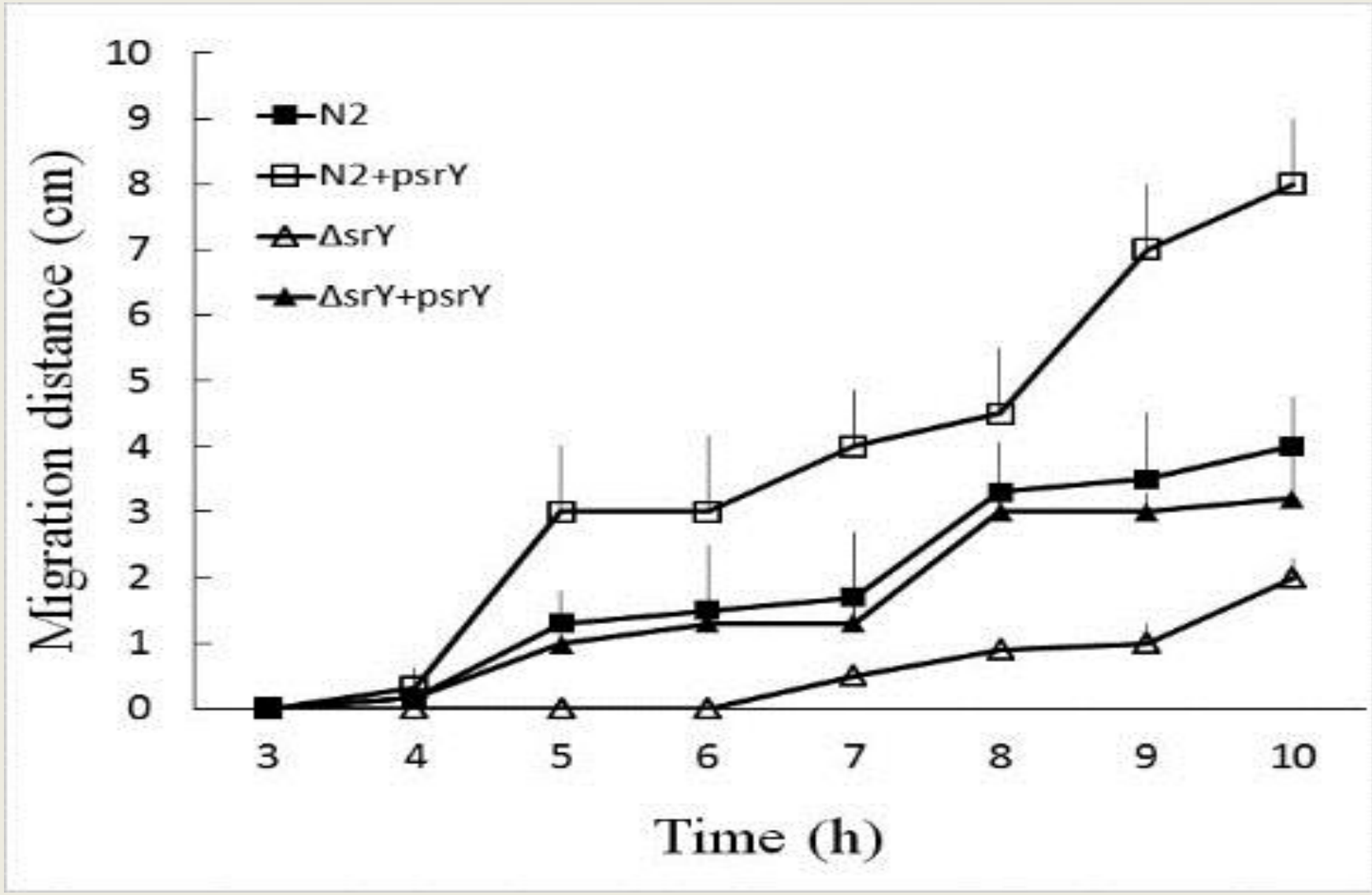






Why can RpoN affect mRNA levels of *flhDC* and *fliC* ?

SrY regulates swarming motility

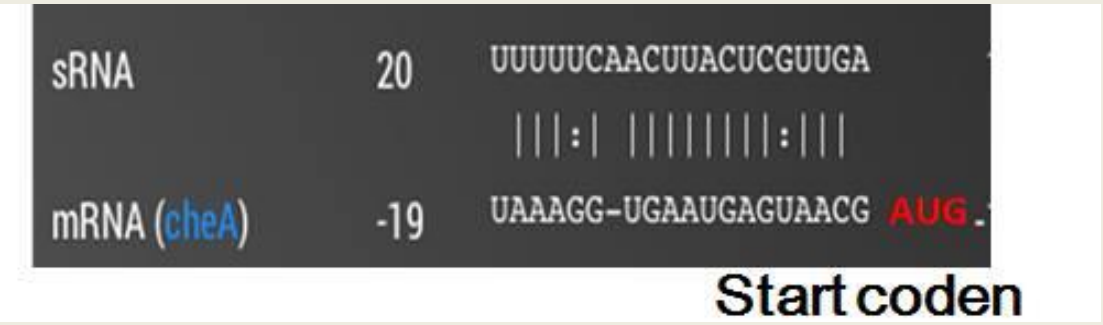


Bacterial small RNAs (sRNAs)

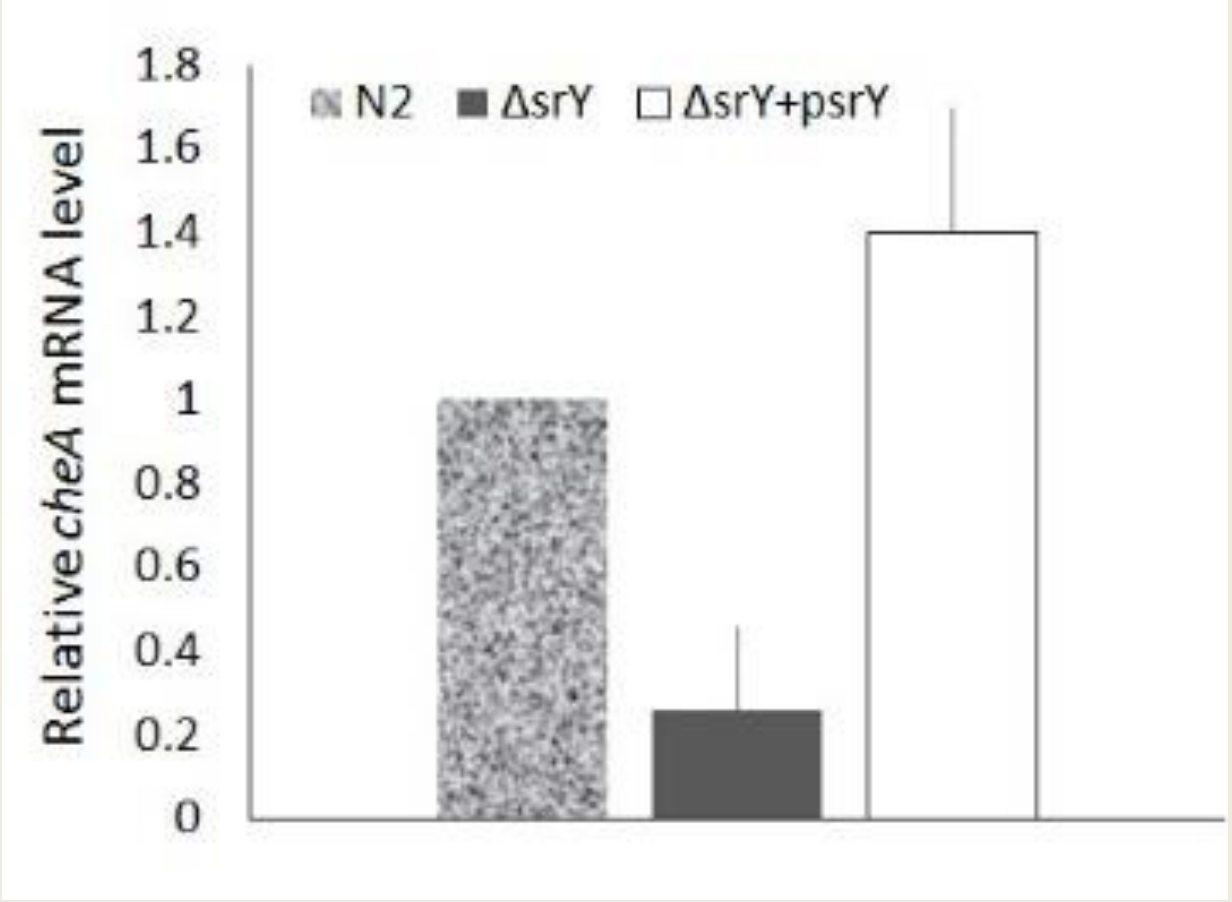
- small non-coding RNA molecules
- sRNAs usually regulate gene expression posttranscriptionally by binding to mRNA targets.

Viegas, Arraiano. *RNA Biol* **5** (4): 230–43 (2008).

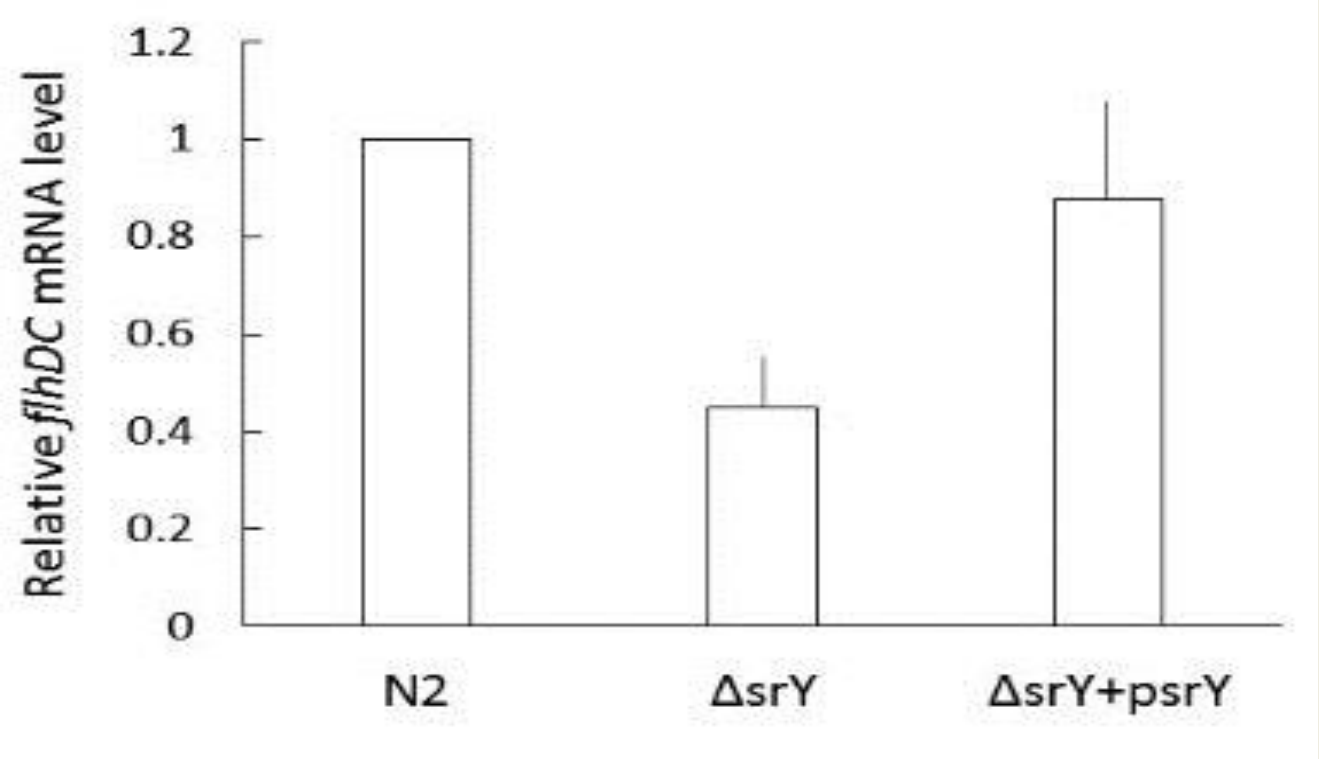
SrY regulates expression of cheA



CheA: a chemotaxis kinase involved in flagellar assembly. The chemotaxis system plays an essential role in swarm cell differentiation and motility

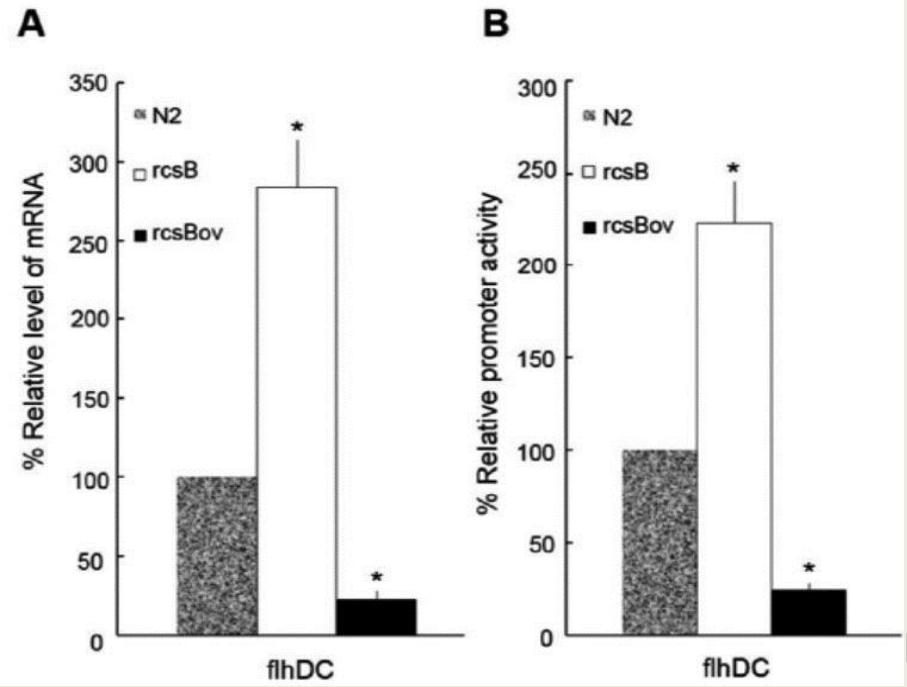


SrY regulates expression of flhDC

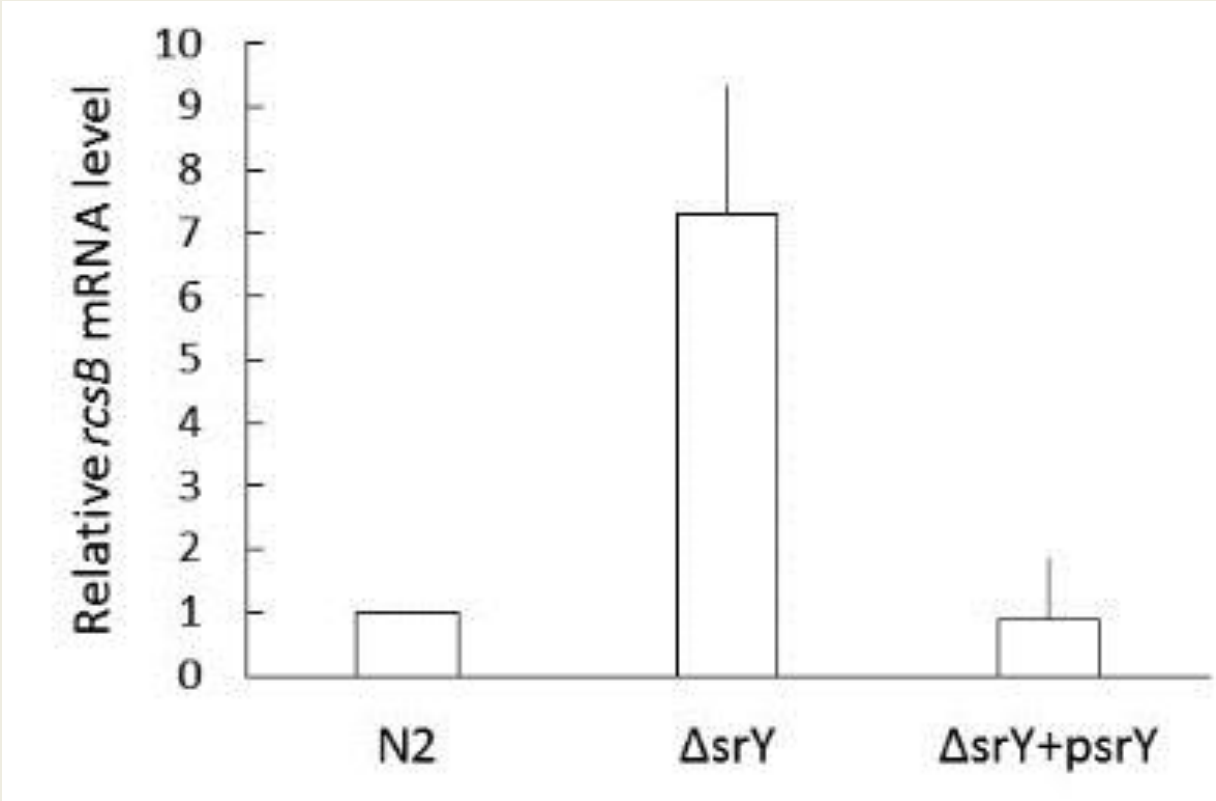


SrY regulates expression of rcsB

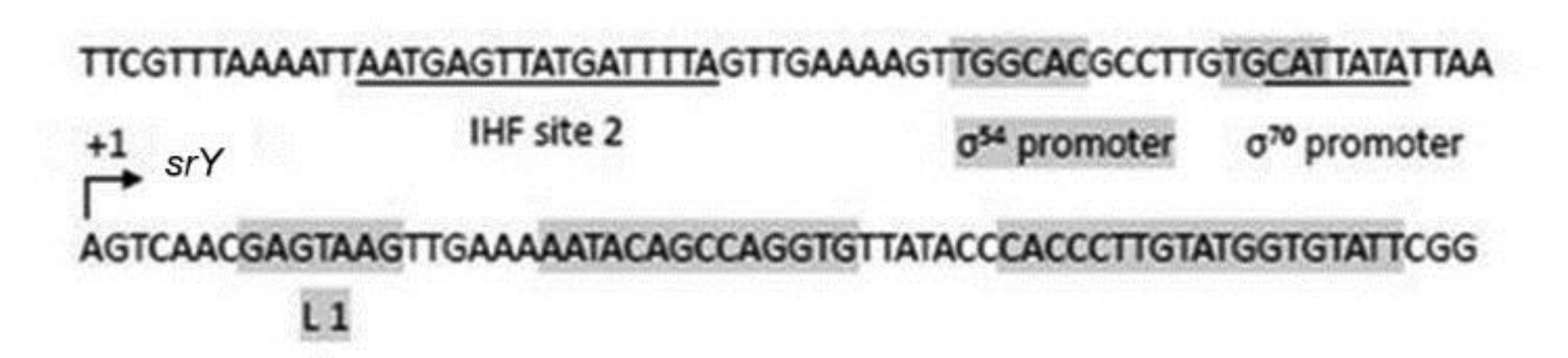
RcsB:
a bacterial two-component response regulator negatively regulating *P. mirabilis* swarming by downregulation of *flhDC*



Liaw et.al.

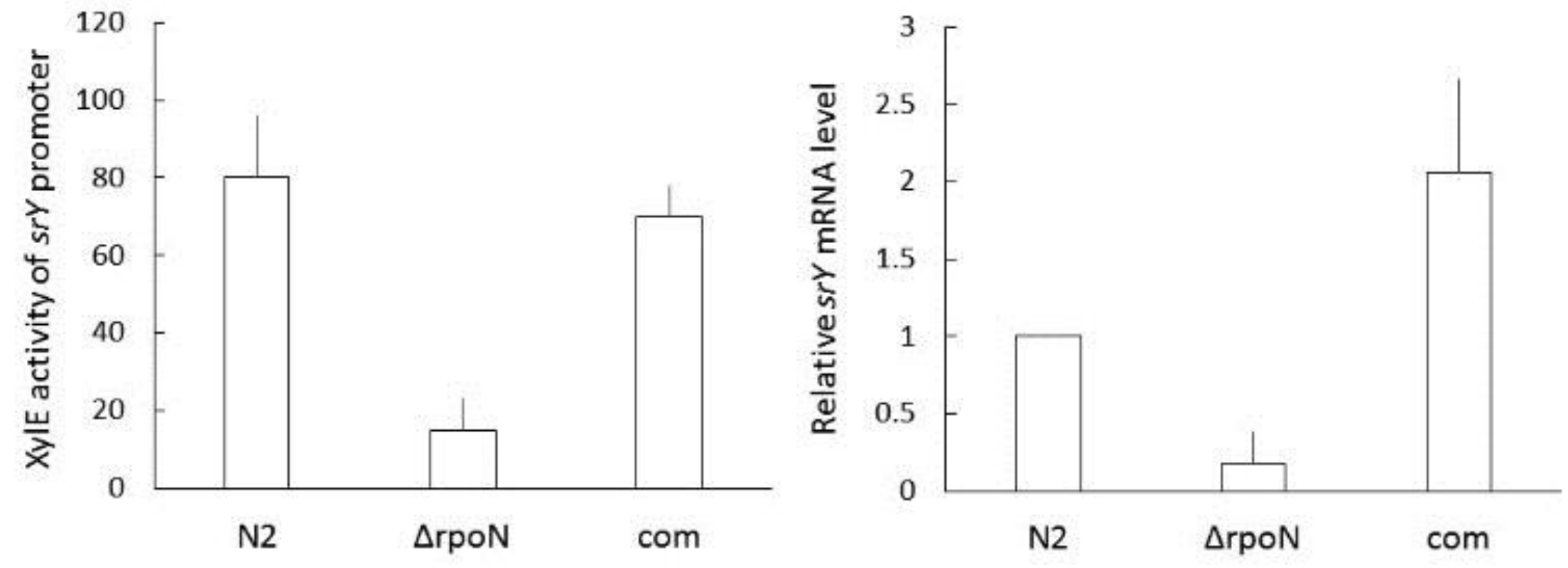


SrY small RNA



May RpoN affect swarming through SrY ?

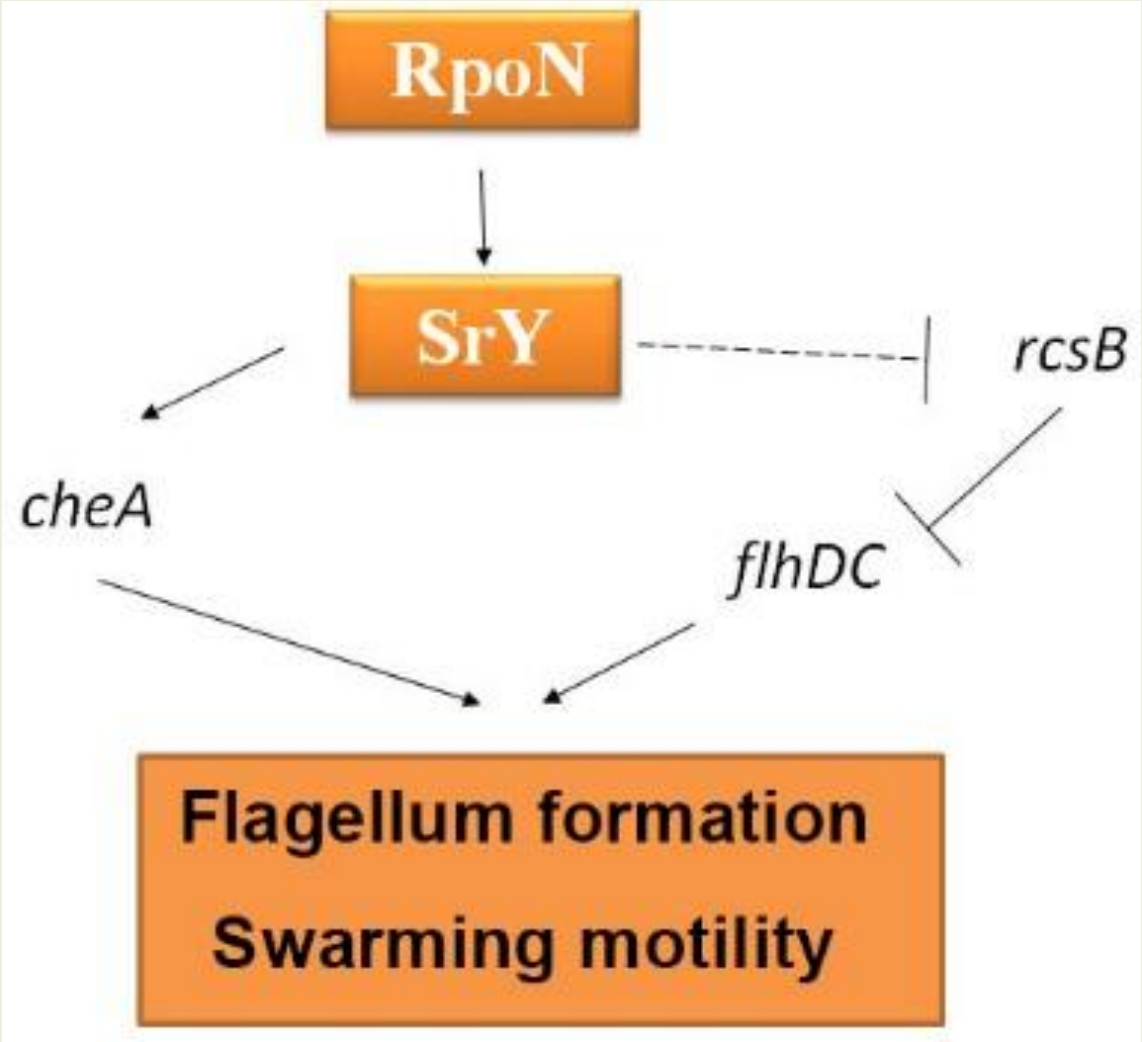
RpoN regulates expression of srY



Conclusions

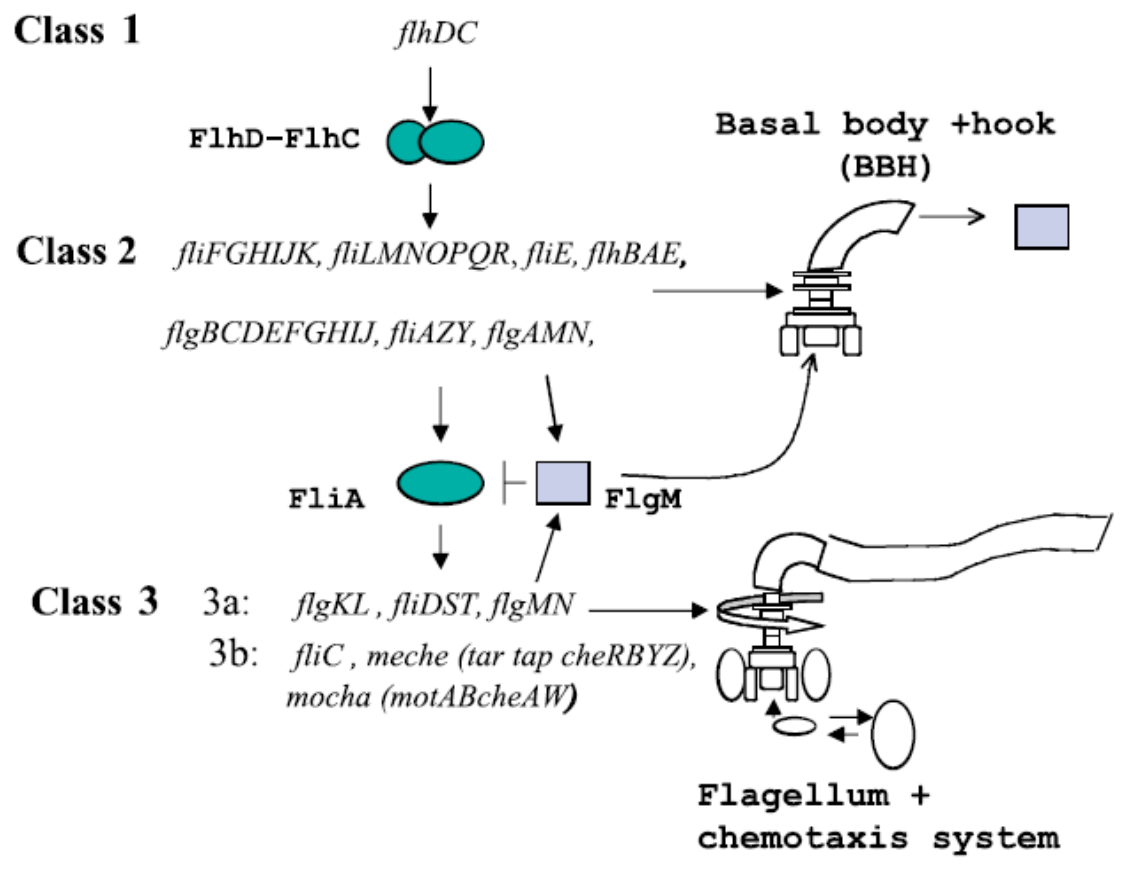
Conclusions

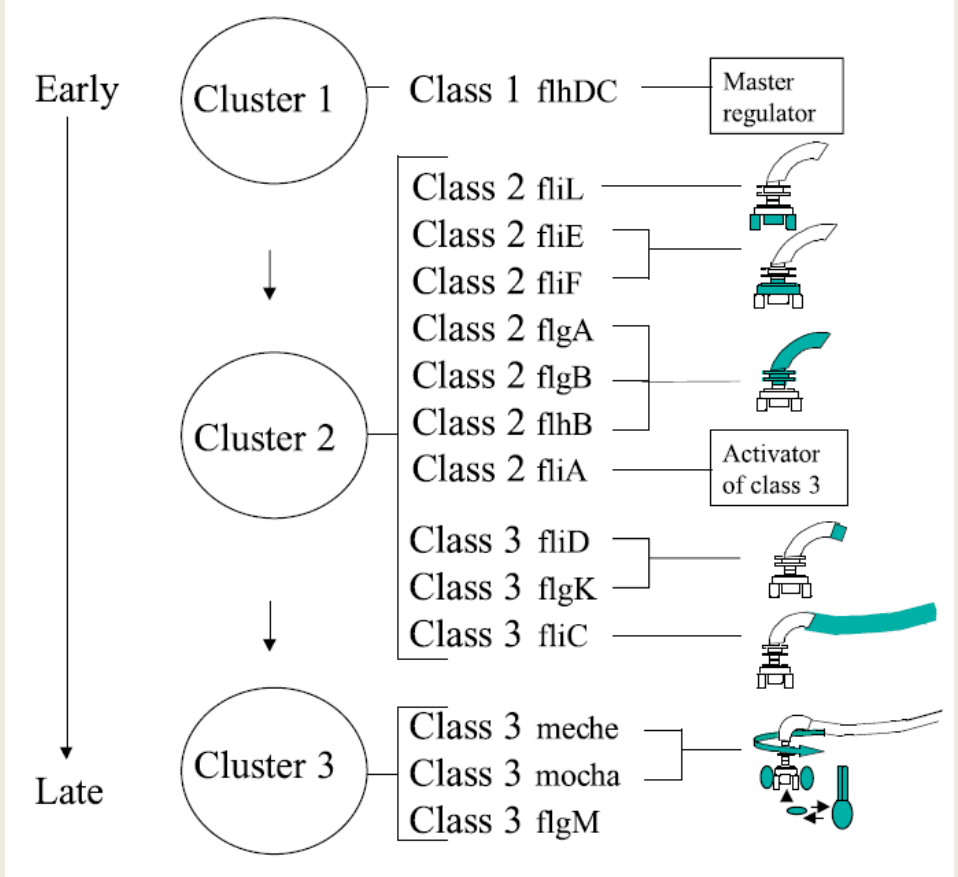
- RpoN positively regulates SrY.
- SrY negatively regulates *rcsB* mRNA level and hence *flhDC*.
- SrY positively regulates *cheA*



Thanks for your attention ~

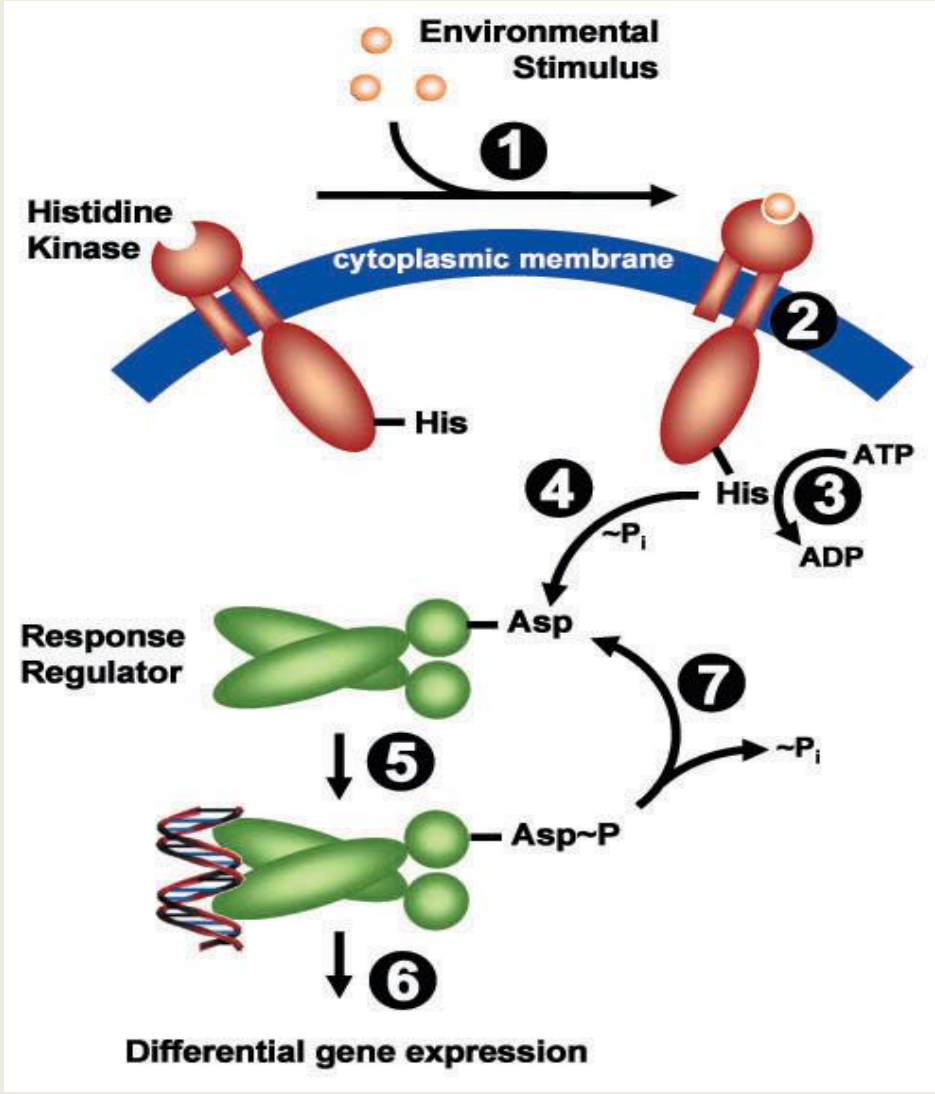
Salmonella flagella

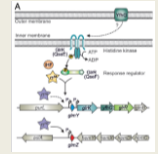




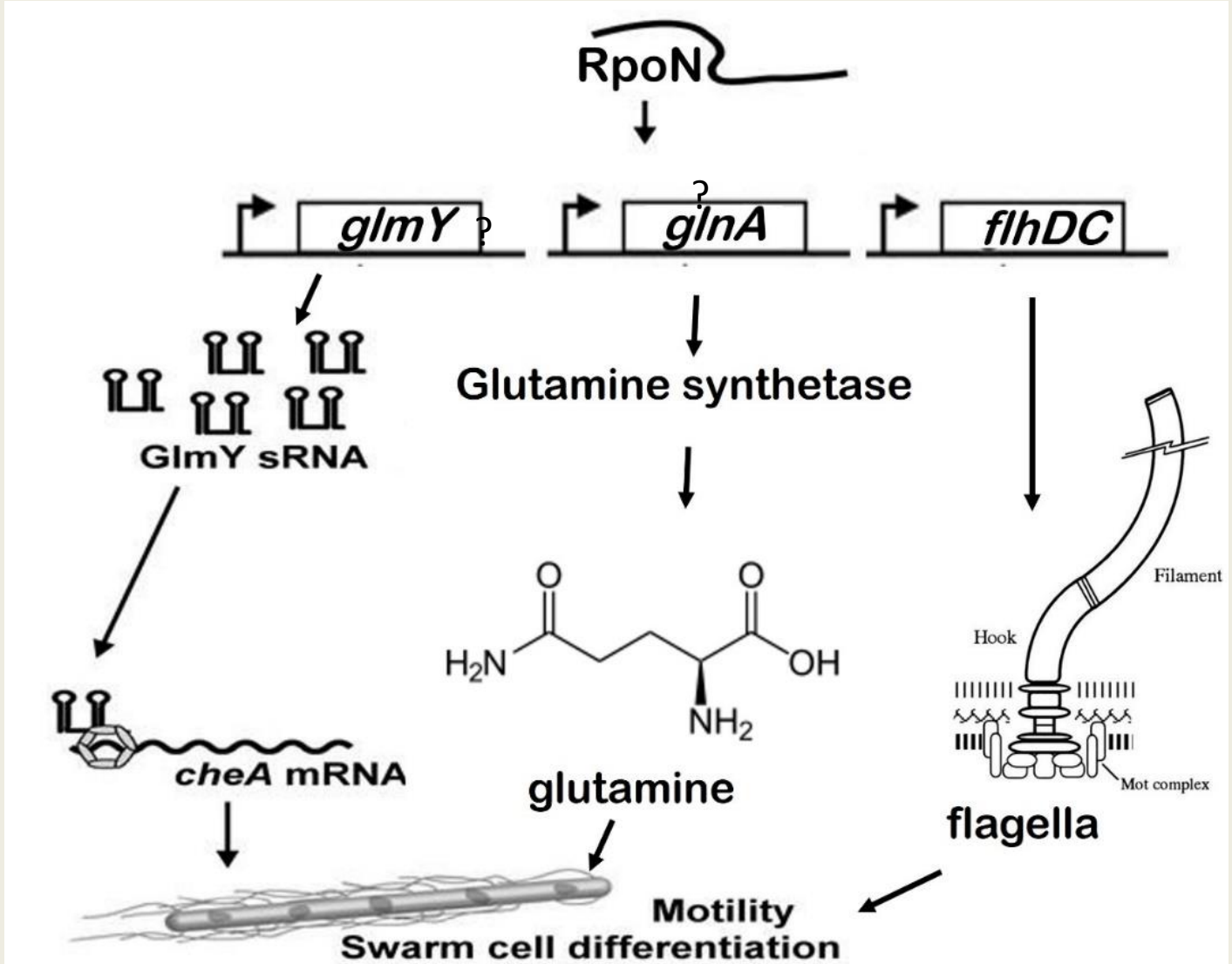
S. Kalir *et al.*
Science **292**, 2080 (2001)

Bacterial two component system



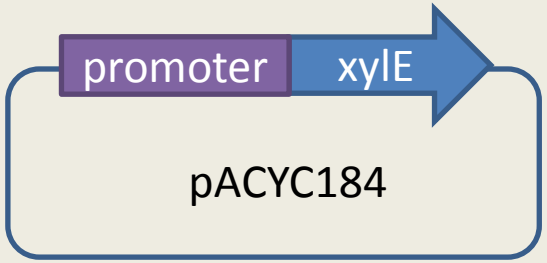


Putative RpoN-swarming regulatory network in *Proteus mirabilis* N2



RpoN modulates *glnA*, *glmY or flhDC* expression

Transcriptional fusion

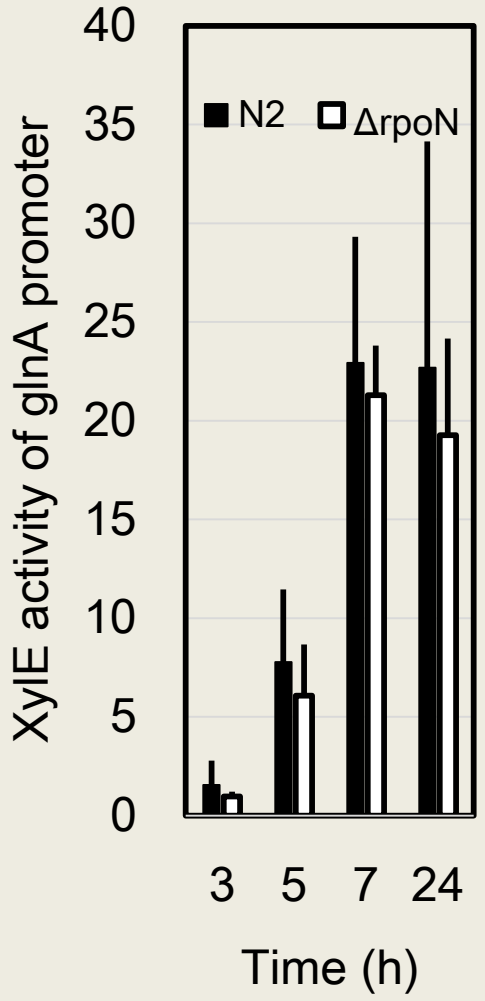


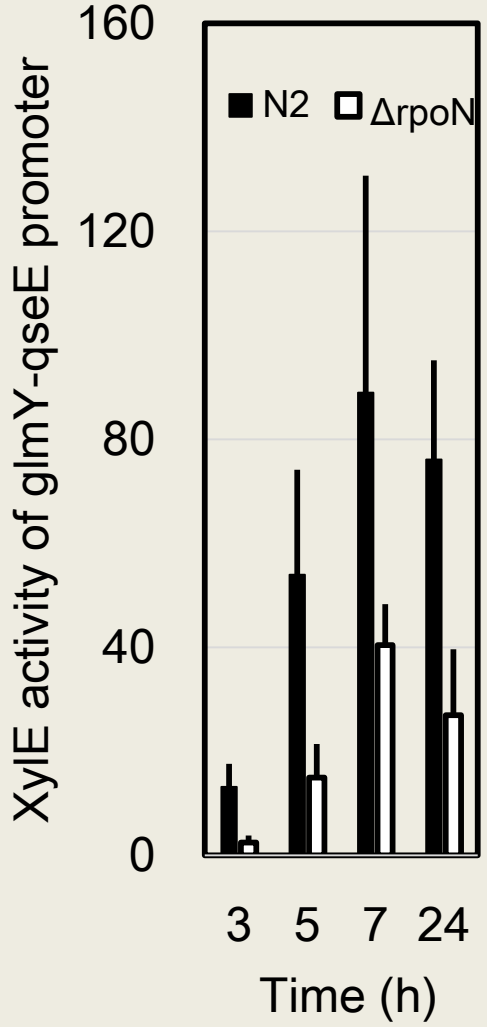
pyrocatechol

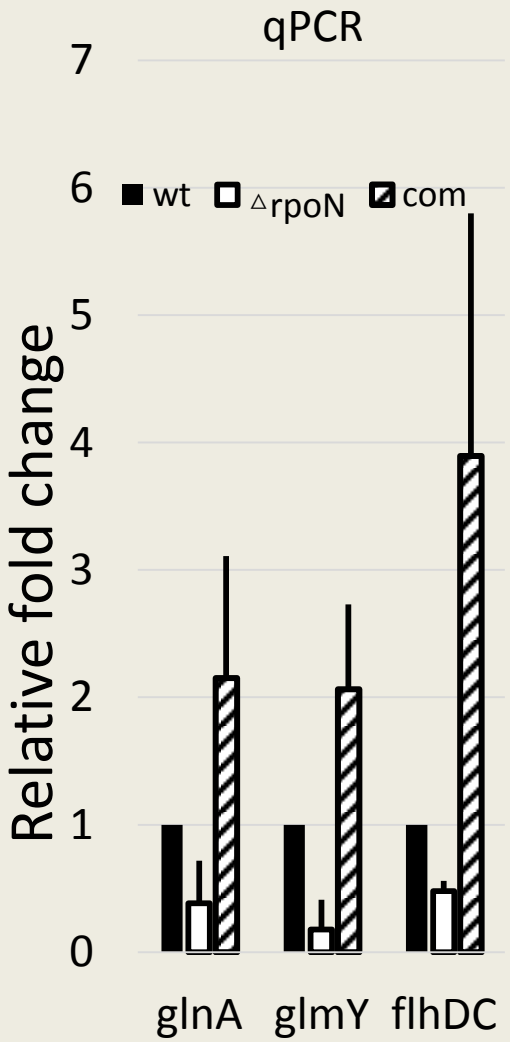
OD375

Wt *ΔrpoN*

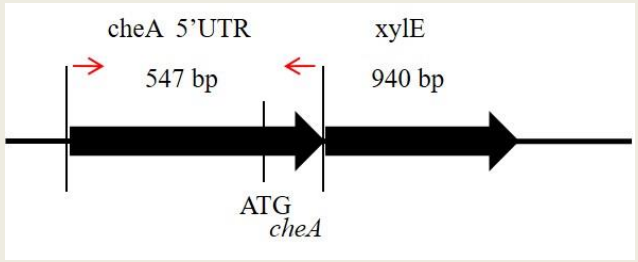
flagellin

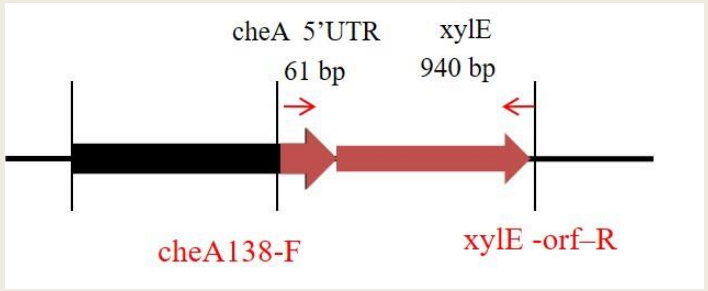






sRNA GlmY regulates *cheA* mRNA





Translational fusion reporter