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Type VI secretion system and expression of flagellar class IV genes in the *Pseudomonas fluorescens* MFE01 strain

Bouteiller Mathilde¹, Gallique Mathias¹, Kosta Artemis², Hardouin Julie³, Konto-Ghiorghi Yoan¹, Barbey Corinne¹, Latour Xavier¹, Chane Andréa¹, Feuilloley Marc¹ and Merieau Annabelle¹

¹Laboratory of Microbiology Signals and Microenvironnement LMSM EA 4312, Normandie Université, Univ. Rouen Normandie, Evreux, France. www.lmsm-lab.fr

² Plateforme de Microscopie de l'Institut de Microbiologie de la Méditerranée IMM FR 3479, Institut

³PISSARO proteomics facility, Université de Rouen, F-76821, Mont-Saint-Aignan, France

INTRODUCTION

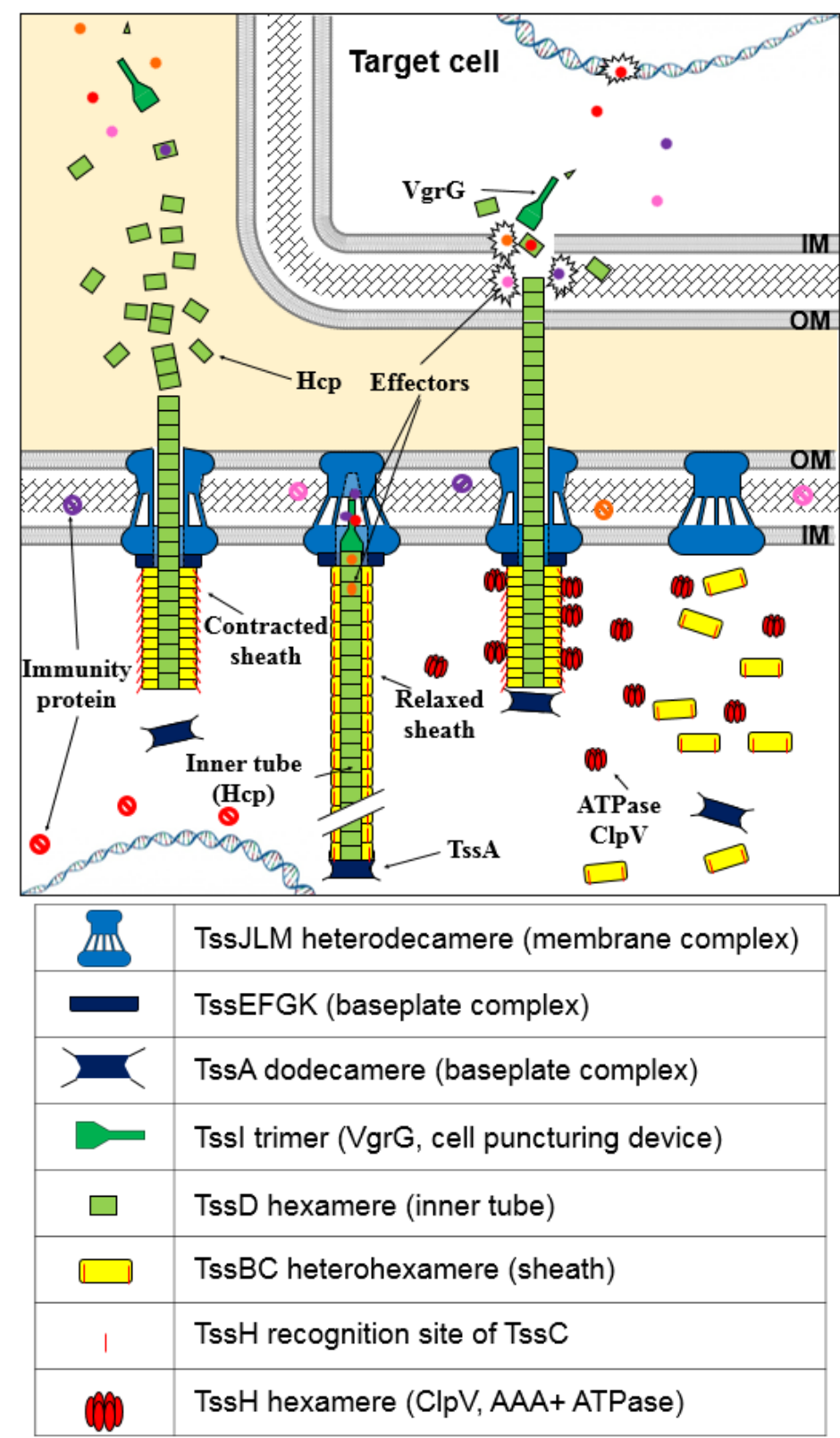


Figure n°1 : Schematic representation of T6SS (adapted from Gallique M. et al., 2017b).

T6SSs are macromolecular machineries that are utilized by bacteria to inject diverse effectors into prokaryotic and/or eukaryotic cells by using a contractile sheath (TssB/C). These systems are constituted by a membrane complex, a baseplate and a tip that is expelled in association with effectors (figure n°1). Nowadays, the most studied effectors exhibit antibacterial activity acting on peptidoglycan, lipid membrane or DNA.

The *Pseudomonas fluorescens* MFE01 strain possesses a functional T6SS (Decoin et al., 2014). All genes encoding T6SS membrane complex, baseplate and the sheath are clustered. The presence of three different orphan genes encoding the tube (*hcp1*, *hcp2*, *hcp3*) allows to provide various declinations of T6SS. The T6SSs containing Hcp2 or Hcp3 exhibit an antibacterial activity (Decoin et al., 2014; Gallique et al., 2017a), contrary to the T6SS formed by Hcp1 that acts on bacterial motility (Decoin et al., 2015). We already showed that the deletion of *hcp1* gene suppresses MFE01Δ*hcp1* motility and by the way no flagellin is secreted in this deletion mutant.

Flagella are extracellular appendages, formed by a basal body and a long filament (polymer of flagellin) connected by a hook (FlgE) (figure n°2) that are assembled in a four-tiered transcriptional regulatory in *Pseudomonas* (Dasgupta N. et al, 2003). Briefly, genes that are clustered in class I to class III permit the expression of the basal body and the hook, whereas class IV genes are involved in the filament and flagellar motor production. In order to obtain a functional flagella, the switch between the expression of class III to class IV genes occurs when the hook achieves a correct length. In that case, the anti-sigma factor (FlgM) is secreted through incomplete flagellar apparatus, releasing the sigma factor FliA, that permits the expression of class IV genes. Here we study different deletion mutants (phenotypes, proteomic and transcriptomic analysis) to identify T6SS and flagellar cross-talk.

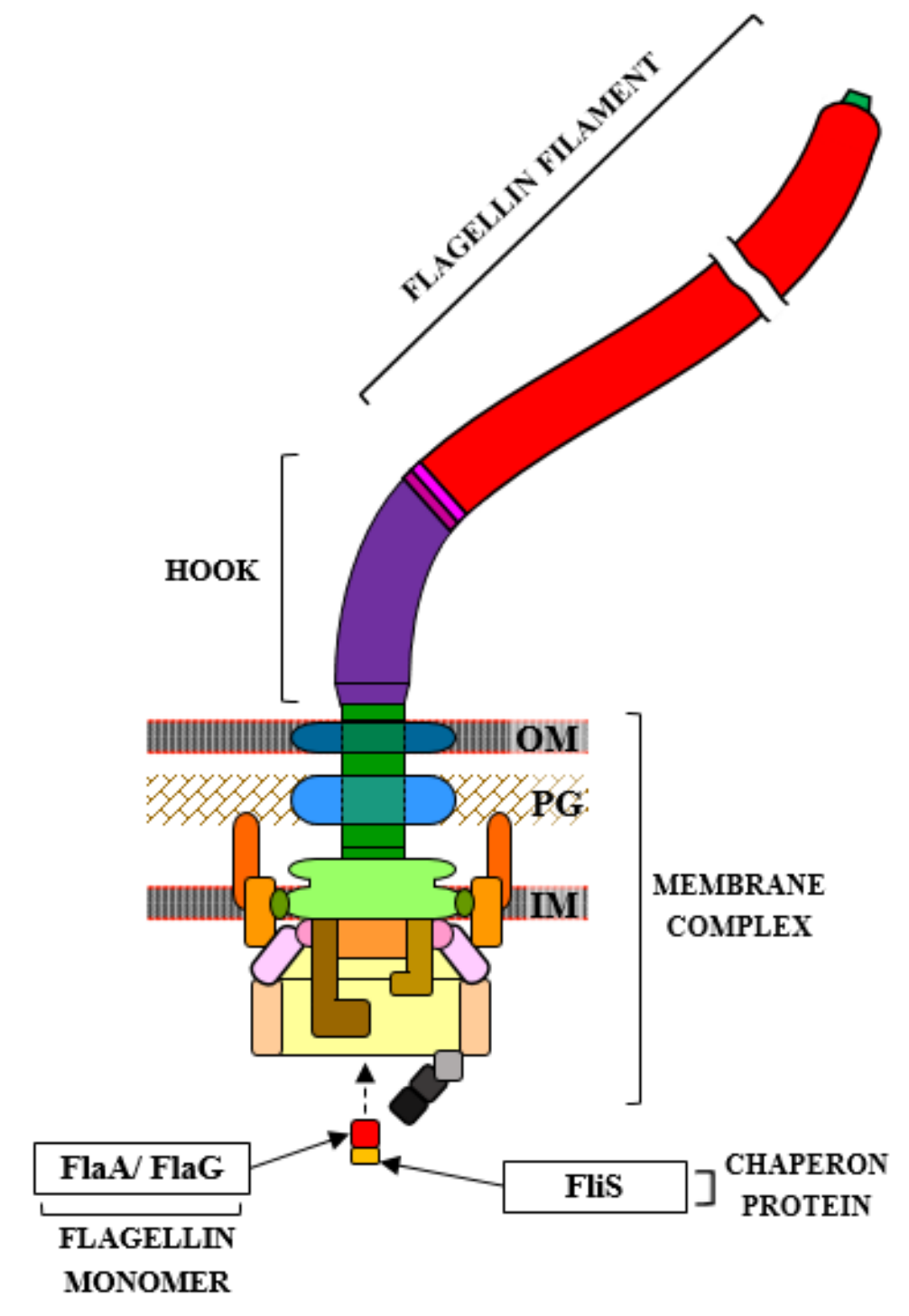


Figure n°2 : Schematic representation of flagellum (unpublished).

1 ELECTRONIC MICROSCOPY

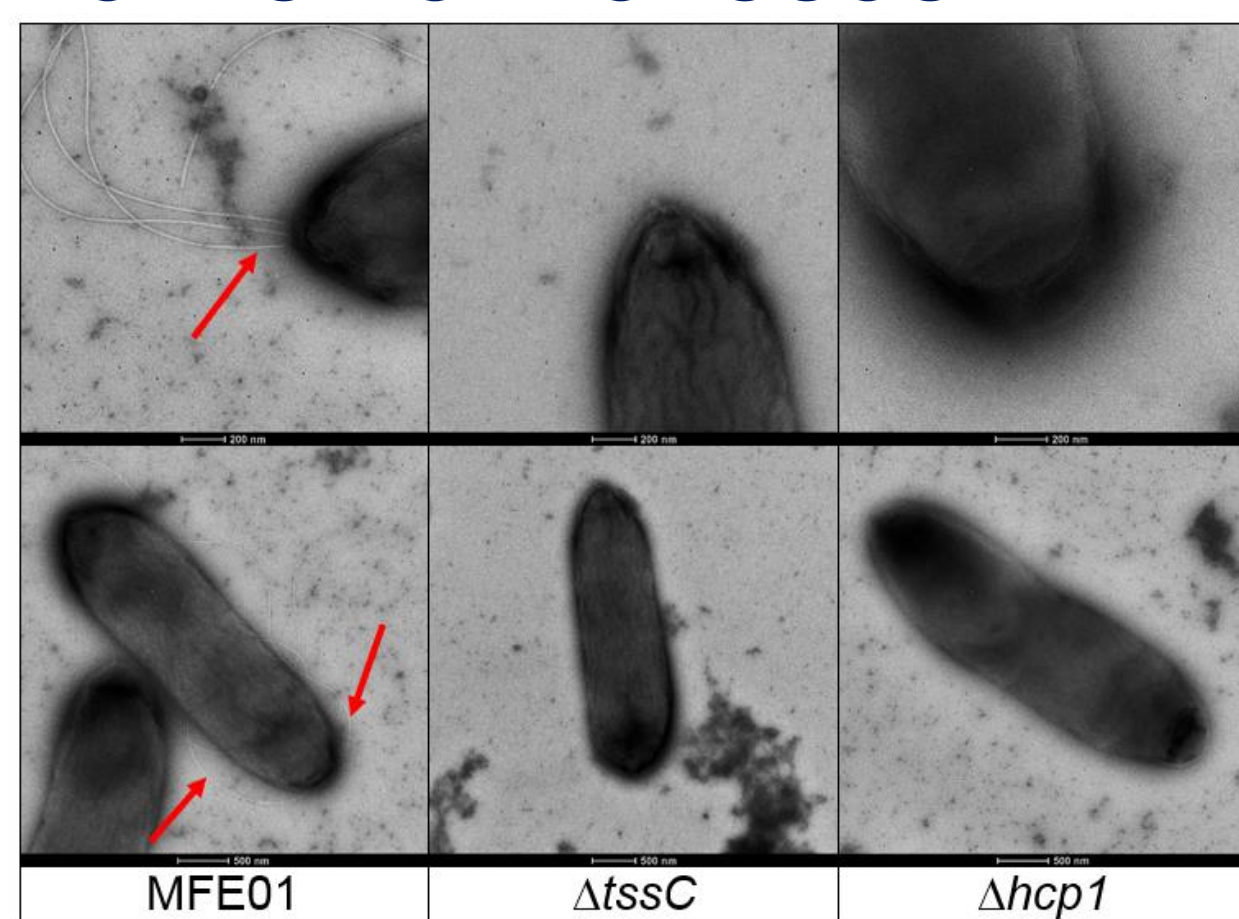


Figure n°3 : Electronic microscopy of MFE01, and different T6SS deletion mutants. Red arrows show flagellar filament (Bouteiller M. et al., in progress).

The lack of T6SS formed with Hcp1, in Δ*hcp1* or Δ*tssC* deletion mutants, disturbs flagellar formation.

2 TRANSCRIPTOMIC ANALYSIS

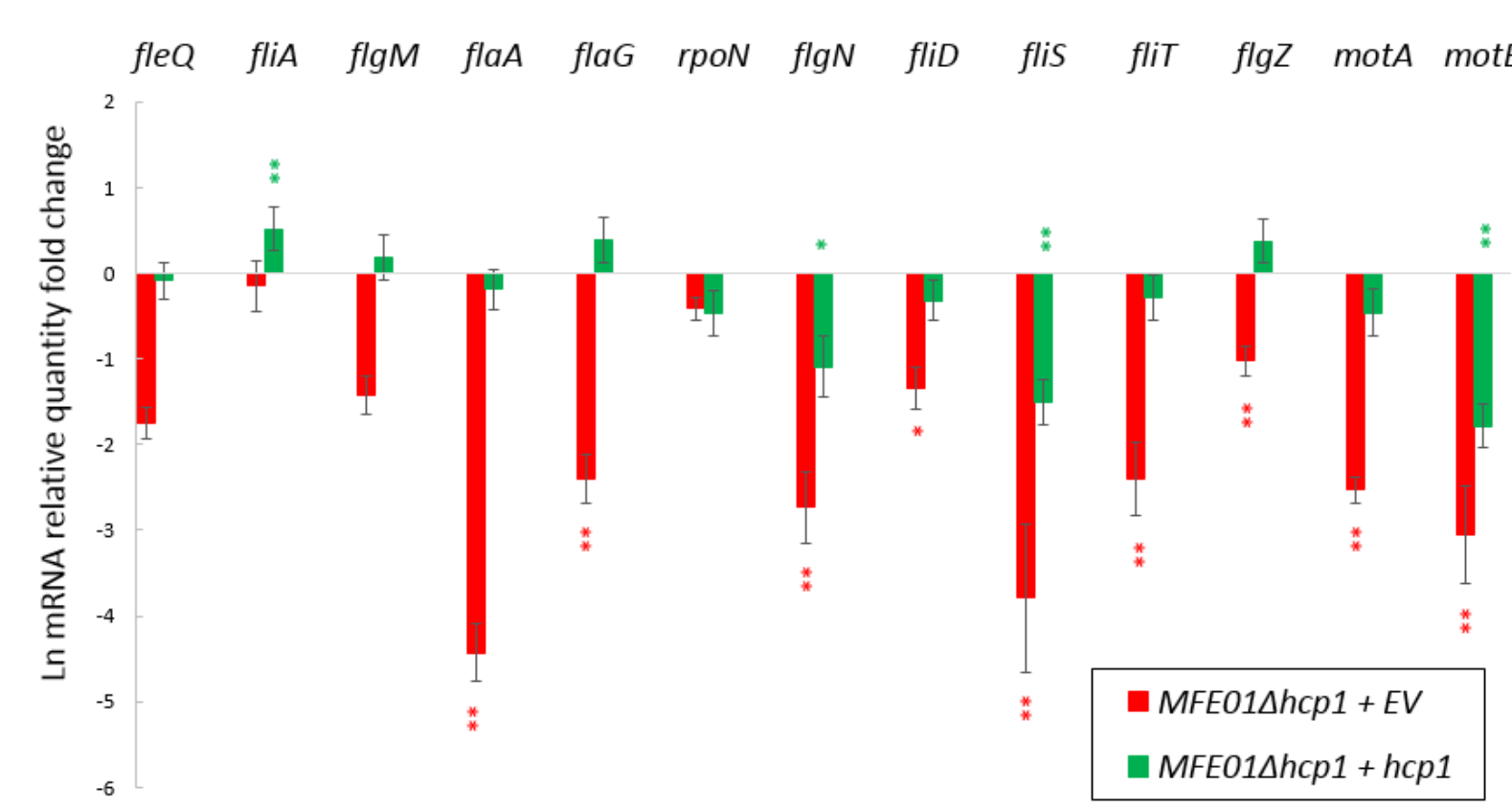


Figure n°4 : Expression of flagellar genes in MFE01 Δ*hcp1* +EV/+*hcp1* in comparison to wild type strain containing empty vector (EV). Data shown represent the mean ± SEM. Significances of differences between mean values were assessed using Mann-Whitney test (n=6) with significance set as *P < 0.05; **P < 0.005 (Bouteiller M. et al., in progress).

The lack of T6SS formed with Hcp1 inhibits the sigma factor FliA activity.

3 OVEREXPRESSION OF FLIA

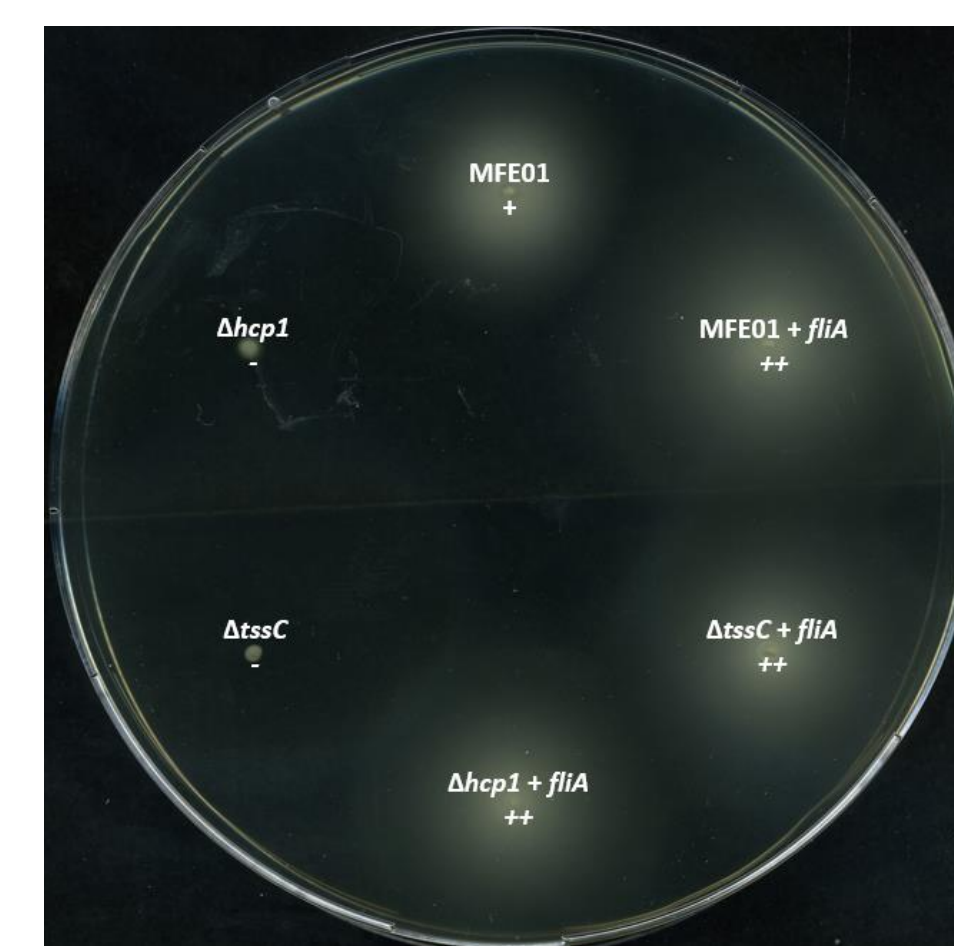


Figure n°5 : Phenotypes analysis of Δ*hcp1* or Δ*tssC* that overexpress *fliA*. Motility on LB 0,3% agar plate (Bouteiller M. et al., in progress).

The overexpression of *fliA* gene restores the production of flagella and by the way the "swimming" motility.

Only FliA activity is disturbed by the lack of T6SS formed with Hcp1.

4 FLGM SECRETION

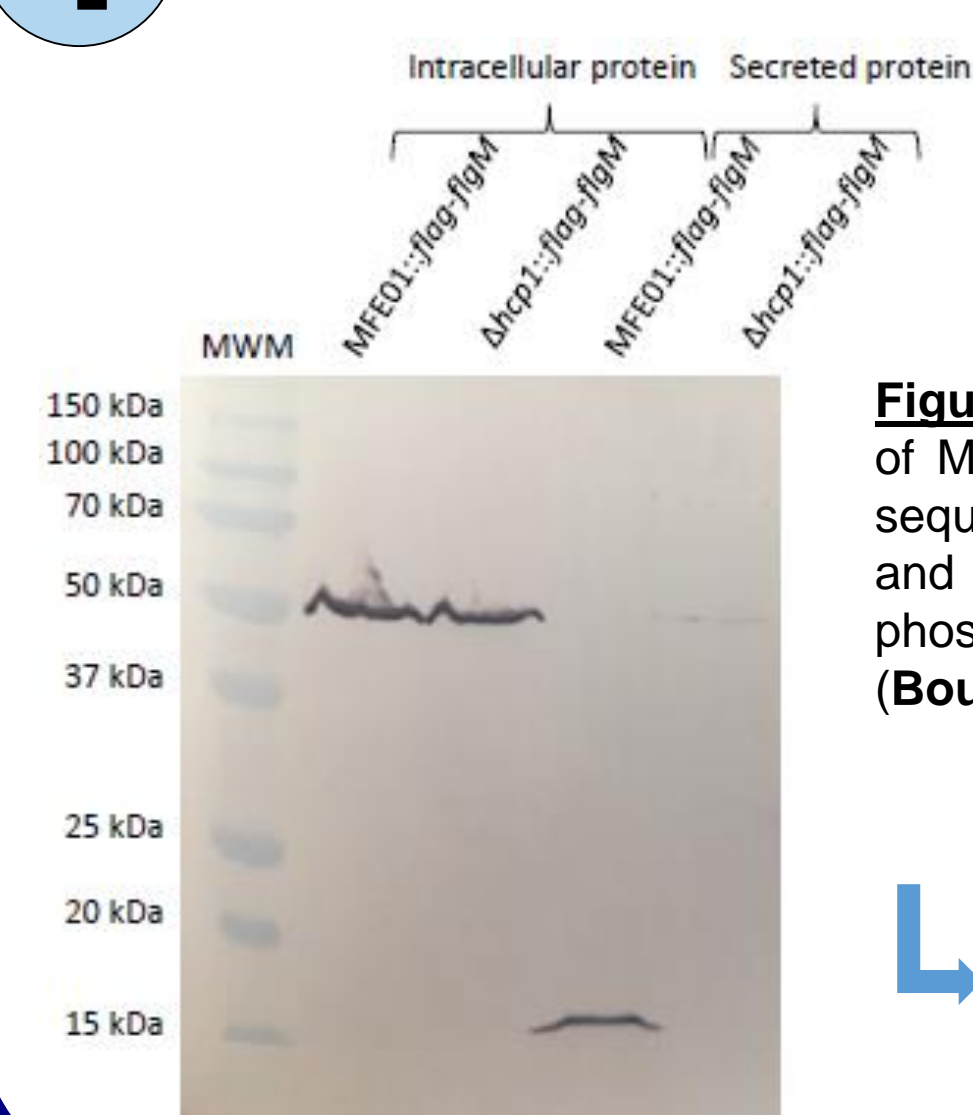


Figure n°6 : Western blot on different protein fractions of MFE01 or Δ*hcp1* after a translational fusion of *flag* sequence with *flgM* gene. An anti-Flag primary antibody, and a secondary antibody coupled with alkaline phosphatase were used to highlight Flag-FlgM protein. (Bouteiller M. et al., in progress).

The wild type strain is able to secrete FlgM anti-sigma factor, in contrary to *hcp1* deletion mutant.

5 FLGM INTERACTIONS

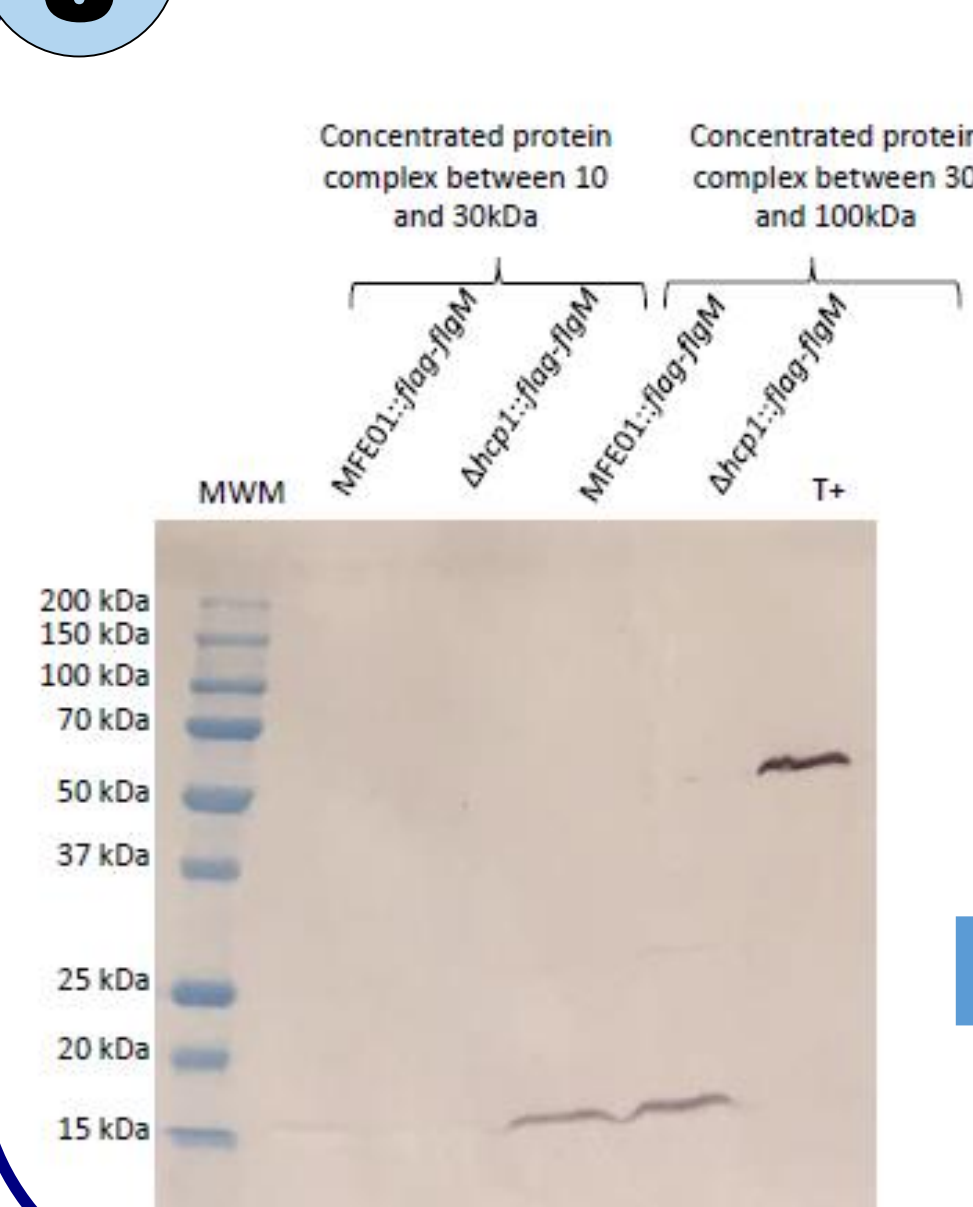


Figure n°7 : Western blot on different concentrated intracellular protein complexes of MFE01 or Δ*hcp1* after a translational fusion of *flag* sequence with *flgM* gene. An anti-Flag primary antibody, and a secondary antibody coupled with alkaline phosphatase were used to highlight Flag-FlgM protein. (Bouteiller M. et al., in progress).

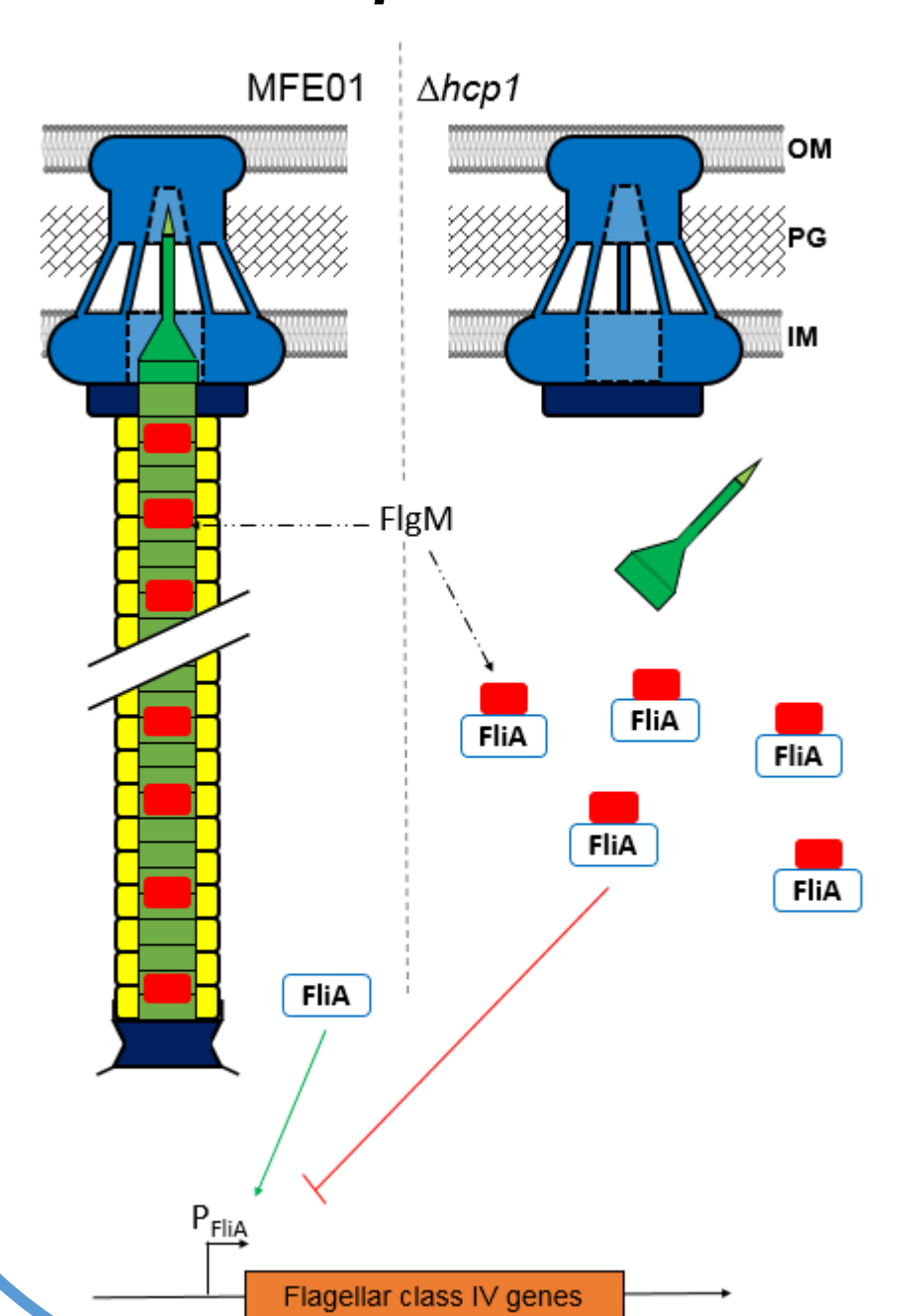
FlgM seems to be strongly associated with other protein(s) during protein extraction.

CONCLUSION

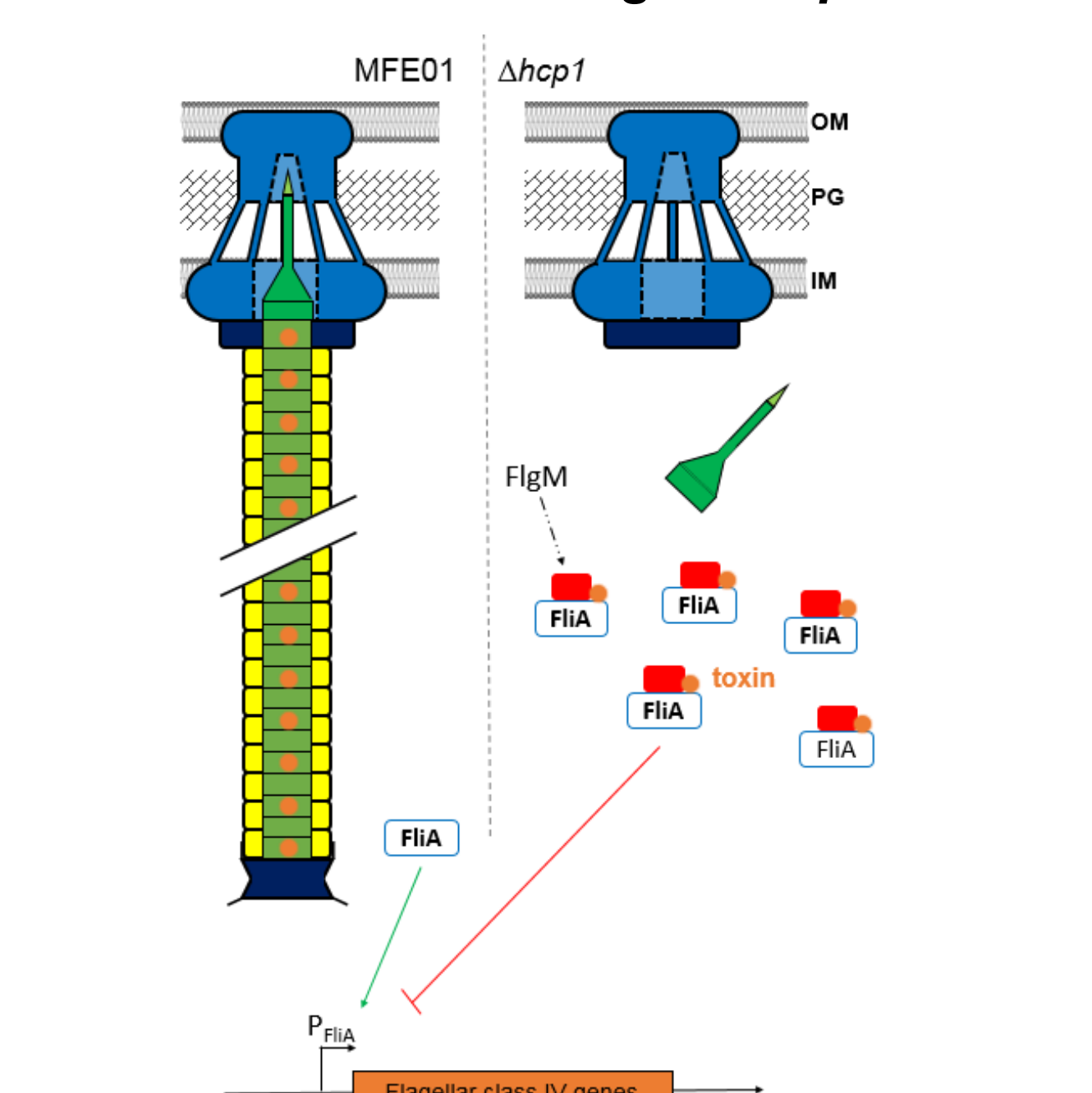
Here we show that the deletions of *hcp1* or *tssC* genes induce the loss of flagella and decrease the expression of flagellar class IV genes, that are regulated by the sigma factor FliA. The overexpression of this sigma factor, in Δ*hcp1* or Δ*tssC* strains, restores the wild type phenotype such as flagellin secretion (data not shown), flagella proper assembly and by the way "swimming" motility. By western blot and LC-MS-MS analysis, we show that the anti-sigma factor FlgM is not secreted in Δ*hcp1* deletion mutant and could bind free FliA in the cytoplasm. This result may explain the less expression of flagellar class IV genes.

Our first hypothesis (1) is that this anti-sigma factor FlgM could be secreted through the T6SS formed with Hcp1 in *Pseudomonas fluorescens* MFE01. The second hypothesis (2) is that a toxin secreted through T6SS formed by Hcp1 could stabilize the FliA/FlgM complex. Our last hypothesis (3) is that this toxin could interact directly with FliA, and inhibits by the way its activity. In order to test these hypothesis, interactomic studies with FlgM and Hcp1 protein by co-immuno-precipitation are in progress.

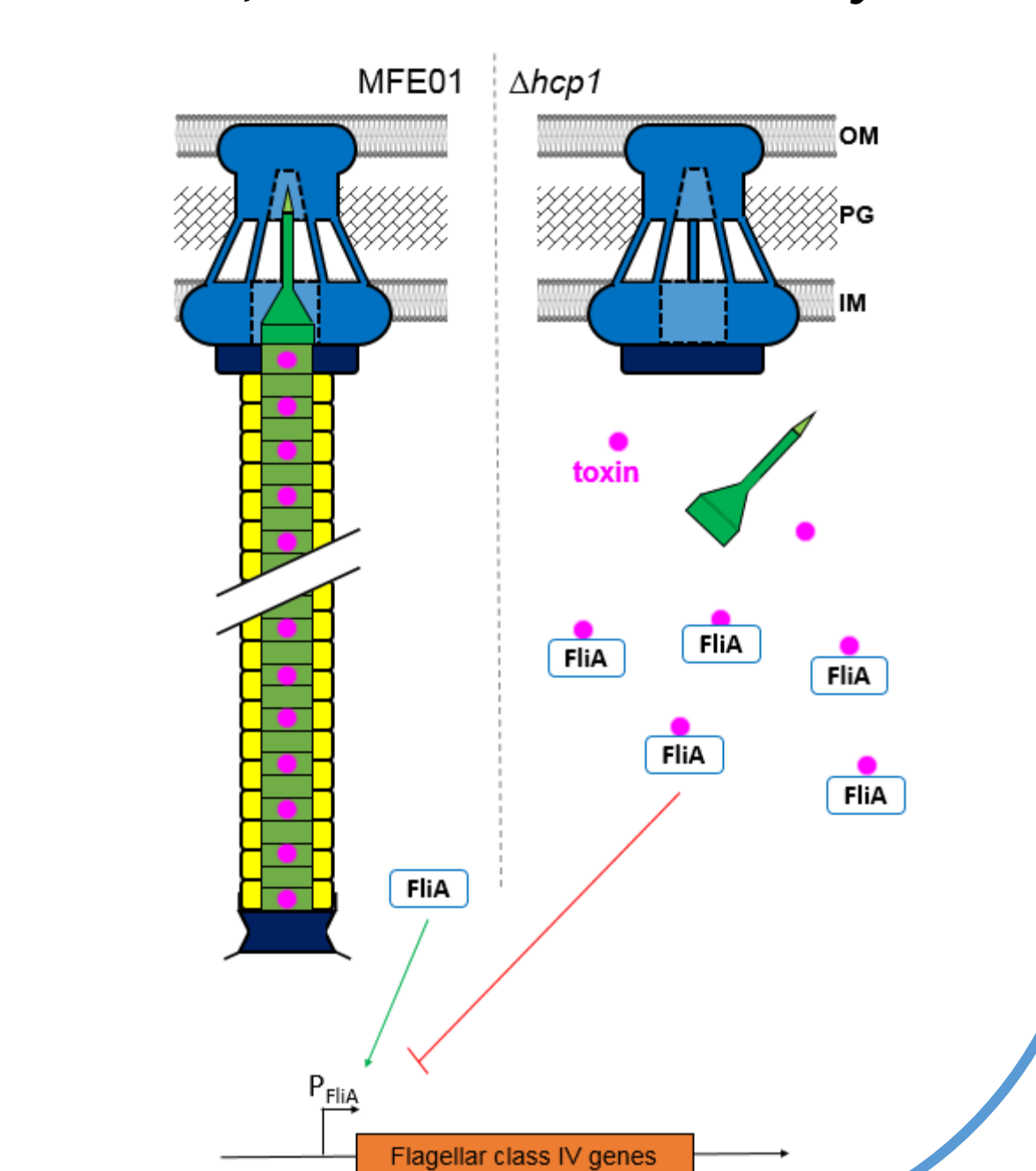
(1) FlgM is secreted through Hcp1-T6SS



(2) The toxin secreted through Hcp1-T6SS stabilizes FliA/FlgM complex



(3) The toxin interacts directly with FliA, and inhibits its activity



Contacts: annabelle.merieau@univ-rouen.fr
mathilde.bouteiller@etu.univ-rouen.fr

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