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The Role of Acetyl-Phosphate in the Pathogenesis of *Neisseria gonorrhoeae*

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Introduction

Neisseria gonorrhoeae is the etiologic agent of the second most common sexually transmitted disease, gonorrhoea. The gonococcus possesses several virulence factors, however, the mechanisms that regulate them is not fully understood.

Acetyl-phosphate (AcP) is an intermediate metabolite from the phosphotransacety-lase-acetate kinase (PTA-AK) pathway. AcP is involved in the non-enzymatic lysine acetylation and phosphorylation of proteins, acting as a dual donor of either the acyl or phosphate group, respectively. This post-translational modifications (PTM) of pro-teins modulate the activity of enzymes and transcription regulators. Previous studies have shown the relation of this PTM with the pathogenesis and synthesis of virulence factors in bacteria.

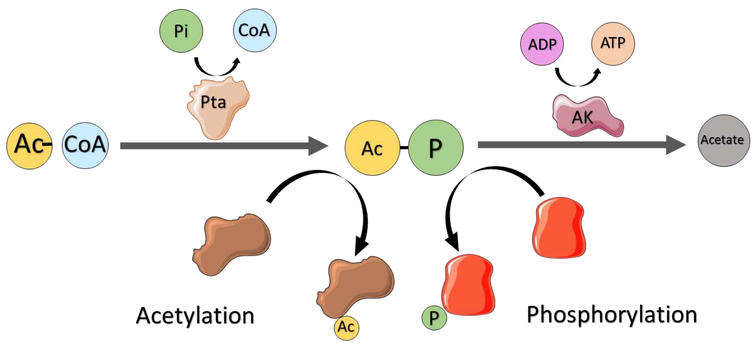

Figure 1. **Dual role of acetyl-phosphate.**

Aims

- To determine the role of AcP in the metabolism of carbon sources and the acetyla-tion rate
- To observe the role of AcP in the virulence of *Neisseria gonorrhoeae* MS11.
- To determine the role of AcP in the gene expression.

Results

A) Construction of isogenic mutant strains with different AcP concentration

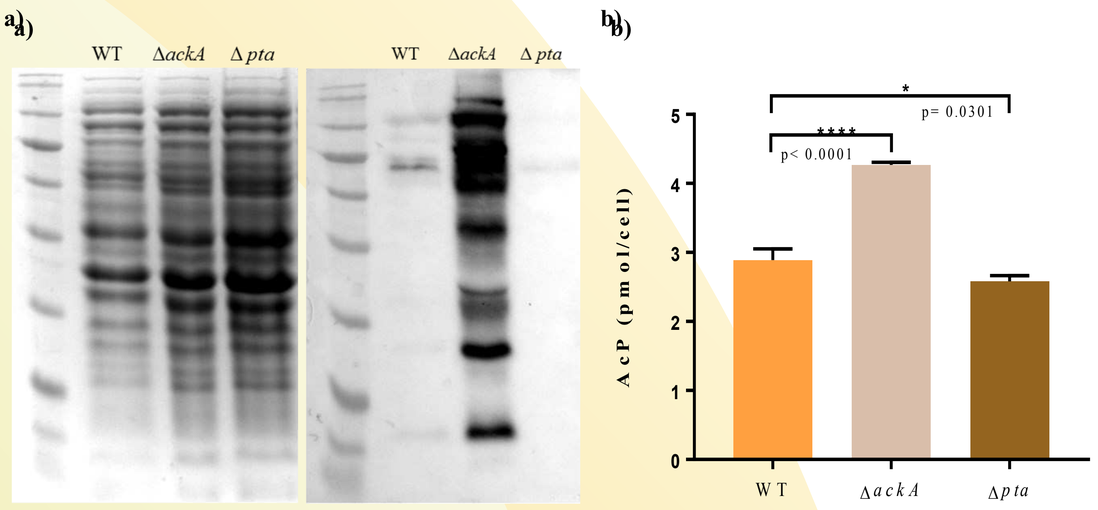

Figure 2. **Characterisation of isogenic mutant strains of *N. gonorrhoeae* MS11.** a) Screening of acetylated proteins. The construction of the two isogenic mutants shows a different rate of acetylation. The Δ*ackA* presents a higher concentration while the Δ*pta* is lower compared to the WT. b) Intracellular concentration of acetyl-phosphate in the three strains of the gonococcus (n=3).

B) The alteration of AcP concentration modifies the metabolism of carbon sources and inhibits the growth in aerobic conditions

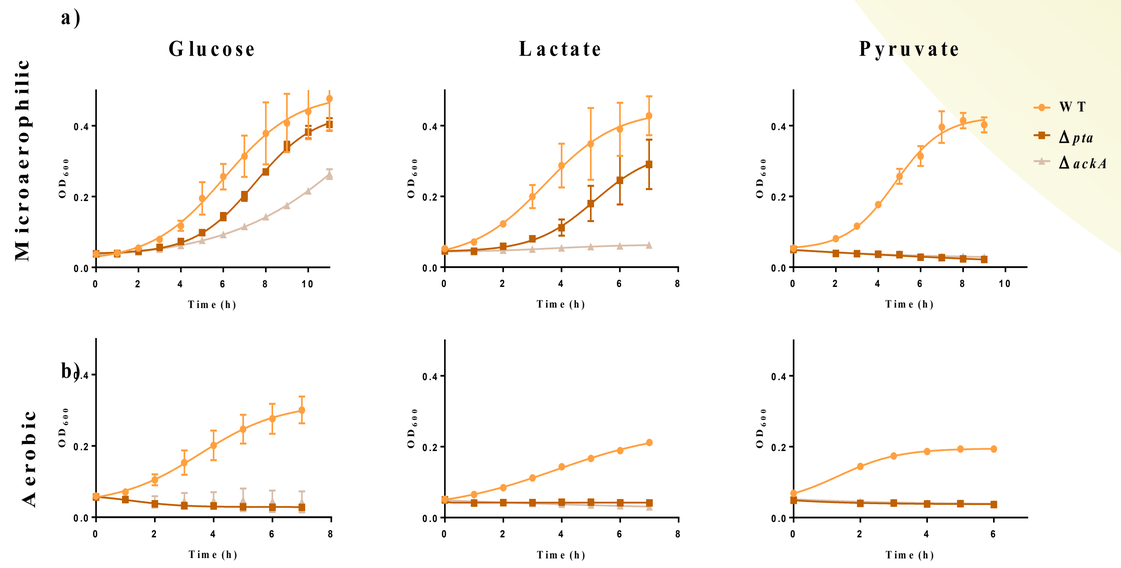

Figure 3. **Growth curves of *N. gonorrhoeae* MS11.** Three gonococcus strains, wild type (WT), isogenic mutant strains Δ*ackA* and Δ*pta* were grown in a chemically defined medium for gonococci . Samples (n=9) were taken every hour to read the optical density at 600 nm until the stationary phase was observed. The strains were grown in both conditions microaerophilic (a) and aerobic (b), and the concentration used for the carbon sources were 10, 20 and 20 mM for glucose, lactate and pyruvate, respectively.

C) Lysine acetylation increases virulence of *N. gonorrhoeae* MS11

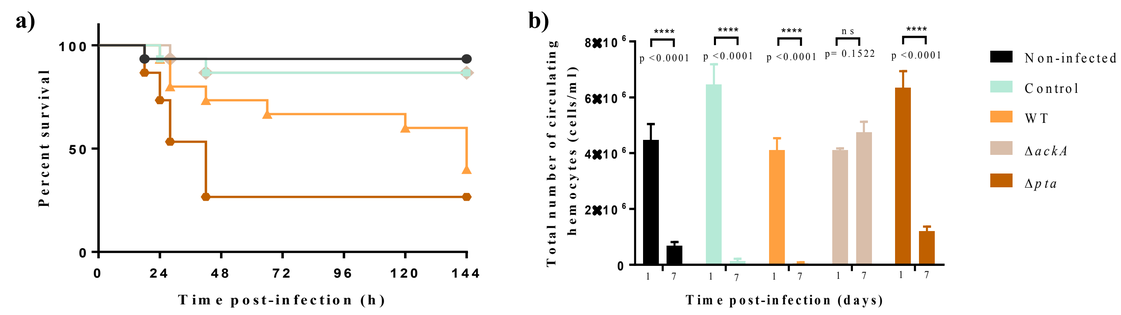

Figure 4. **Lysine acetylation enhances virulence and maintain levels of inflammatory cells in *Galleria mellonella*.** a) Larvae (n=15) were infected with 10µL of a 10^8 bacterial suspension each. A group with no injection and a group injected with PBS were used as controls. b) Number of hemocytes counted from the hemolymph of larvae after infection (n=4).

D) AcP modulates the activity of the two component system (TCS) MisR-MisS

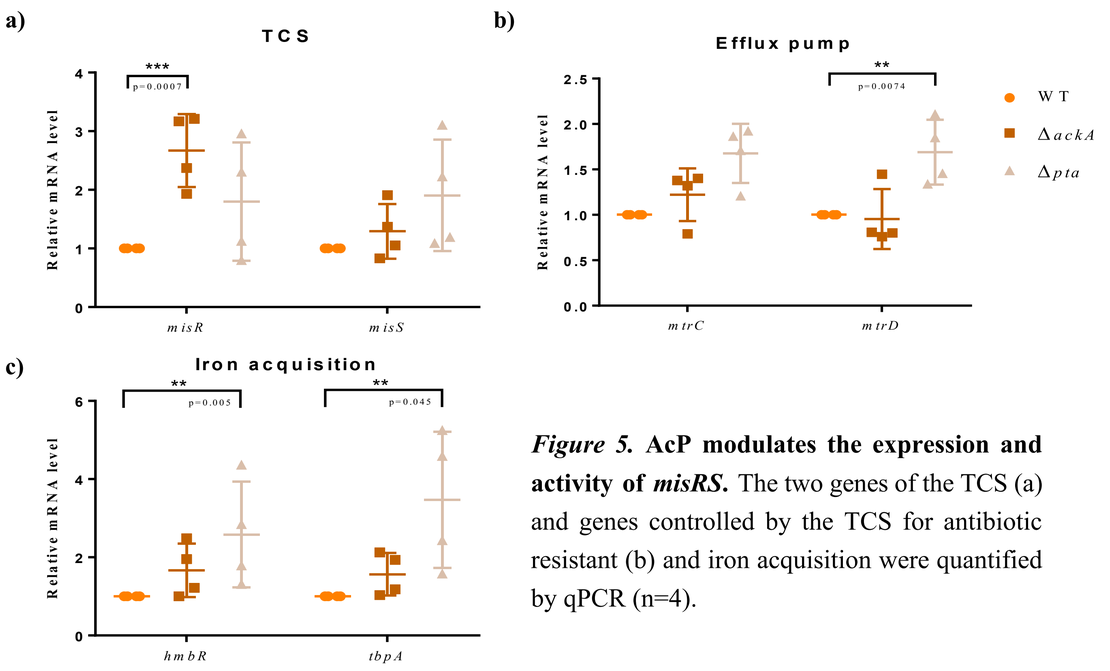

Figure 5. **AcP modulates the expression and activity of *misRS*.** The two genes of the TCS (a) and genes controlled by the TCS for antibiotic resistant (b) and iron acquisition were quantified by qPCR (n=4).

Conclusions

- AcP is the metabolite responsible for the lysine acetylation.
- AcP modulates the metabolism of *N. gonorrhoeae* MS11.
- AcP is involved in the virulence of the gonococcus.
- The activity of the TCS is probable to be modulated by AcP.
- Further analysis are required to better understand the role of AcP.

Acknowledgements

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