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GENOMIC POSITION OF ATP SYNTHASE GENES IMPACTS *Vibrio cholerae*'s PHYSIOLOGY

Oglini, Nicolás¹ - Larotonda, Leticia^{1,2} - Andrey Giovanni Gomes De Oliveira^{1,3} - Comerci, Diego¹ and Soler Bistué, Alfonso^{1,2}

1) Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde" UNSAM/CONICET, San Martín, Buenos Aires, Argentina.

2) Institut Pasteur, Université de Paris Cité, Unité Plasticité du Génome Bactérien, Paris, Francia.

3 Australian Research Council Centre of Excellence in Synthetic Biology, School of Natural Sciences, Macquarie University, Sydney, Australia.

Contacto: nicolasoglini@gmail.com

Growth rate is a key parameter of bacterial physiology that varies widely among microorganisms. However, its genetic basis remains unclear. Gene order along the chromosomes could play a role: in fast-growing bacteria, several genes important for cell metabolism and viability are located near the origin of replication (*oriC*). One case is that of the genes encoding ATP synthase (ATPsyn), a protein complex responsible for synthesizing ATP, one of the main sources of energy for the cell. In particular, a recent bioinformatic study shows that the ATPsyn locus is in close proximity to *oriC* independently of their growth capacity. To assess the effect of perturbing the conserved location of ATPsyn genes we employed recombineering techniques to manipulate the genomic location of the *atpIBEFHAGDC* locus in *Vibrio cholerae* (Vc). After relocating it to increasing distances from *oriC*, we measured the growth rate of this strain set at optimal growth conditions. Relocation of the locus close to its original location shows no phenotype indicating that neither the transposition process nor the exact genetic context impacts *atpIBEFHAGDC* function. In fast growing conditions, *atpIBEFHAGDC* relocation to increasing distances from the *oriC*, such as the middle of the chromosome, *ter1* region, *ter2* region, etc., led to an increased generational time (GT). These results suggest that the relocation of *atpIBEFHAGDC* far from *oriC* affects the GT. Since the decrease in growth rate is incremental, we link the genome location of ATPsyn genes to gene dosage during exponential growth. In optimal growth conditions, fast-growing bacteria overlap replication rounds, a process called multi-fork replication (MFR). Hence, genes close to the *oriC* benefit from a higher dosage during exponential growth with respect to those close to the terminal region (*ter*) increasing their global expression. In work to come we look forward to constructing merodiploid strains with two copies of the locus: one strain with both copies near the *oriC*, one copy in the original site and other next to it; and another strain with one copy next to the original site and with other copy in the terminal region of the chromosome 1. This would determine if the effects seen are due to the dosage or the genomic position of the locus. Overall, this study is a new example of key loci whose

genome location has been selected along evolution to maximize growth when nutrients are abundant and efficiently occupy the niche. The positional bias of ATPsyn genes maximize their expression.

Palabras clave: ATP synthase -- *Vibrio cholerae* -- relocation -- merodiploid