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Regular paper

Chlamydial genomic MinD protein does not regulate plasmid-dependent genes like Pgp5

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Chlamydia **has a unique intracellular developmental cycle, which has hindered the single protein function study of *Chlamydia*. Recently developed transformation system of *Chlamydia* has greatly advanced the chlamydial protein's function research and was used to find that a chlamydial plasmid-encoded Pgp5 protein can down-regulate plasmid-dependent genes. It is assumed, that chlamydial genomic MinD protein has a similar function to Pgp5. However, it is unknown whether MinD protein regulates the same plasmid-dependent genes. We replaced pgp5 gene in the shuttle vector pGFP::CM with minD gene of *C. trachomatis* (CT0582) or *C. muridarum* (TC0871). The recombinant plasmid was transformed into plasmid-free organisms-CMUT3 and qRT-PCR was used to detect the transcription level of plasmid-encoded and -dependent genes in these pgp5 deficient organisms. As a readout, GlgA, one of the plasmid-regulated gene products was detected by immunofluorescence assay. After recombination, transformation and plaque purification, the stable transformants CT0582R and TC0871R were generated. In these transformants, the plasmid-dependent genes were up-regulated, alike in the pgp5 premature stop mutant and pgp5 replacement with mCherry mutant. GlgA protein level was also increased in all pgp5 mutants, including CT0582R and TC0871R. Thus, our study showed that genomic MinD protein had different function than Pgp5, which was useful for further understanding the chlamydiae.**

Key words: *Chlamydia*, MinD, Pgp5, Transformation system

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Abbreviations: EB, Elementary body; RB, Reticular body; PCR, polymerase chain reaction; C. *trachomatis*, *Chlamydia trachomatis;* C. *muridarum*, *Chlamydia muridarum;* qRT-PCR, quantitative real-time polymerase chain reaction; GFP, green fluorescent protein; CPAF, chlamydial protease-like activity factor; WT, wild type; PBS, phosphate buffer saline; CT0582R, CMUT3-pGFP::CM CT0582Rpgp5; TC0871R, CMUT3-pGFP::CM TC0871Rpgp5; pgp5S, the pgp5 premature stop mutant organism; mCherryR, the pgp5 replacement with mCherry mutant organism

Supplementary Table 1: Primers for generation of the recombinant plasmids

Plasmids	PCR product	Forward primer	Reverse primer
CT0582 Replace pgp5	F1	cttgtcgtcatcgtccttgtagtccatcgttagagtaaaacatctattctgccttag	ttagagaatcgttctctttgag
	F2	cacaaacaagacttttcccagaggacact	ctcaaagagaacgattctctaa
	F3(CT0582)	cggggcggtggtgggtcgggtggcggcggatctaaaacaatcgctgttaatagtttcaaag	tctgggaaaagtcttgtttgtgttagatgttccttaacaaaaatagcagttc
	F4(CT0582)	gactacaaggacgatgacgacaagtctggtggcggtggctcgggcggtggtgggtcggg	tctgggaaaagtcttgtttgtgttagatgttccttaacaaaaatagcagttc
TC0871 Replace pgp5	F1	cttgtcgtcatcgtccttgtagtccatcgttagagtaaaacatctattctgccttag	ttagagaatcgttctctttgag
	F2	cacaaacaagacttttcccagaggacact	ctcaaagagaacgattctctaa
	F3(TC0871)	tctgggaaaagtcttgtttgtgttagatgtccttaacaaaaatagcagttc	tctgggaaaagtcttgtttgtgttagatgtcccttaacaaaaatagcagttc
	F4(TC0871)	gactacaaggacgatgacgacaagTcctggtggcggtggctcgggcggtggtgggtcgggt	tctgggaaaagtcttgtttgtgttagatgtcccttaacaaaaatagcagttc

Supplementary Table 2: Primers for PCR screen to detect the insertion fragment in the recombinant plasmids

	Forward primer	Reverse primer
Primers for detecting the insertion fragment CT0582	aaaacaatcgctgttaata gtttcaaag	Gagaaacggattcctgattt tatttaaaaaagacgctagct
Primers for detecting the insertion fragment TC0871	aaaacaatcgctgttaaca gttttaaagg	Gagaaacggattcctgattt tatttaaaaaagacgctagct