Molecular basis for the crosstalk between two unrelated bacterial transcription factors – the regulatory C protein of a restriction-modification system Csp231I and RacR repressor of a defective prophage in *Escherichia coli* cells

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Fundamental processes in all living systems are mostly regulated at the level of gene expression, which provides adaptation to the rapidly changing environments to increase the chances of survival. This control is multi-layered, but largely operates at the level of transcription. Bacterial gene expression regulation usually impacts large groups of genes (regulons/operons), which together form global transcriptional networks. Their efficient functioning, interconnectivity and orchestration rely mainly on the action of individual DNA binding proteins called transcription factors (TFs). TFs interact not only with their specific target sites (primary sites) determined by DNA sequence motif, but also with secondary (off-target) sites, and vary in their promiscuity. It is not clear yet what mechanisms govern the interactions with secondary sites, and how such rewiring affects the overall regulatory network. This is especially crucial during transfer of a genetic module carrying a TF gene into a new host, e.g. *via* horizontal gene transfer. In the course of our research, we noticed that introduction of genes coding for Type II restriction-modification (R-M) system Csp231I led to manifestation of the abnormal cell filamentation.

The main objective of this work was to determine the molecular mechanism responsible for the formation of elongated bacterial cells (filaments) in the *Escherichia coli* MG1655 strain carrying genes of the Type II Csp231I restriction-modification system, derived from a related bacterium *Citrobacter sp*. RFL231. Our results indicated that the primary source of the cell defect was the interference of the introduced TF (C protein), as a part of a R-M system, with the host genetic network operated by the RacR repressor, an essential regulator (TF) of the cryptic Rac prophage. The C protein exerts a transcriptional cross-talk with another TF of the host, the RacR repressor. We showed that the C protein binds to its unrelated, off-target site within coding sequence of the *racR* gene, in close proximity to the *racR* promoter. In turn, this results in significant reduction in *racR* expression, which unblocks *ydaS* and *ydaT* expression. These genes' function is unknown, but we predict indirectly YdaT activity leads to cell toxicity and loss of their fitness and viability. Under physiological conditions, *ydaT* is completely silent and its transcript is undetectable. The genetic elements taking part in transcriptional cross-talking were identified and the expression of *racR – ydaST* operon was studied extensively by *in vivo* and *in vitro* approaches.

Our results demonstrate an apparent example of horizontal gene transfer leading to adventitious TF cross-talk with negative effects on the recipient's viability or even its death. More broadly, this study represents an experimentally-accessible model of a regulatory constraint on horizontal gene transfer.