„Role of specialized controlling protein in gene expression regulation of Csp231I restricton-modification system from Citrobacter sp."
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Restriction-modification (R-M) systems are highly abundant among bacteria and archea, and appear to play crucial roles in modulating horizontal gene transfer and protection against invasive DNAs, such as bacteriophages. There is much to explore about these diverse enzyme systems, especially concerning regulation of their expression. Type II R-M systems consist of genes coding for separate proteins with independent enzymatic activities: a restriction endonuclease (REase) and a protective DNA methyltransferase (MTase). Lack of balance between the REase and MTase activity could be potentially l*ethal* for the host. Thus, R-M systems must have mechanisms that tightly regulate their gene expression. Some R-M systems have an additional gene coding for a transcription factor called the C-protein (C – controlling). C proteins play a vital role in the temporal regulation of R-M gene expression, as well as indirectly influence the R-M system's mobility and stable maintenance in the bacterial population.

The goal of this work was to determine the molecular mechanisms involved in gene expression regulation of the Type II Csp231I R-M system from *Citrobacter sp*. RFL231 bacteria, in particular to understand the role of the C protein of a new, not widely studied family of the C.EcoO109I-like regulators. In the investigated Csp231I R-M system, a bicistronic common transcript of the C and REase genes is produced, similarly to other C-linked R-M systems. The C regulator functions mainly as the auto-repressor of its own transcription *via* a negative feedback loop due to its binding to the so-called C-box DNA sequence located in the C gene promoter region. Thus, the effect of activation or repression of transcription depends on the concentration of the C regulator in the cell, however inhibition of transcription occurs over a wide range. In contrast to other most known C-linked R-M systems, the REase transcript is mainly driven from its own tandem promoters. Further, the C protein only partially controls the REase expression through the bicistronic transcript, but its presence significantly reduces the level of relative restriction of the R-M system, as well as the efficiency of cell transformation for plasmids carrying the R-M system. Further studies revealed that the level of C transcripts is negatively regulated by an antisense RNA driven from a reversed promoter P_{aC} located in the noncoding strand within the C gene. Inactivation of this P_{aC} promoter leads to a drastic increase in the C gene expression and a significant

reduction in the REase mRNA levels, resulting in a low relative restriction. Disturbance in the R-M system's gene expression through absence of antisense RNA or C gene deletion results unexpectedly in loss of R-M system stability regardless of its relative restriction activity. These observations could provide new insights into the mechanisms that control gene expression of selfish genetic elements. In addition, we present a putative, novel regulatory role of the C protein, which not only acts as a transcription factor, but its mRNA also plays a regulatory role at the post-transcriptional level, directly affecting the REase expression.

The presence of a possible multi-layered complexity is not surprising as the R-M systems, similarly to other toxin-antitoxin modules, must be controlled to keep the counter-balancing amounts and timing properties to avoid lethality.