Juan Farizano

Dr. Juan Farizano has a degree in Biotechnology. Currently, he is a doctoral fellow of CONICET (Consejo Nacional de Investigaciones científicas y técnicas). He has been developing his scientific career in the "Instituto de Química Biológica" (Universidad Nacional de Tucumán-CONICET). His laboratory focuses on the study of the RcsCDB system in the virulence of Salmonella Typhimurium, in order to develop an oral vaccine against Salmonella infections.

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Development and optimization of attenuated bacterial vaccines for the control of Salmonella *infection: effect of the RcsCDB system in the expression of fimbrial genes*

Juan Farizano[1]

[1] *CONICET (Consejo Nacional de Investigaciones científicas y técnicas)*

Background

The genus *Salmonella* includes Gram-negative bacteria that cause foodborne diseases in human and animals (1). *Salmonella* can survive and respond to adverse environmental changes through the regulation of gene expression, and two-component regulatory systems play a major role in such responses. For instance, the RcsCDB system controls virulence, colanic acid biosynthesis (2), regulation of flagellum synthesis (3), cell division (4), O-antigen chain length determination (5), motility (6) and Vi antigen synthesis (7), among other cellular functions.

We are interested in the role of the RcsCDB system in the virulence of *S. Typhimurium*, a serovar that produces gastroenteritis in humans and typhoid fever in mice (8). The *rcsC11* allele activates constitutively the RcsCDB system. As we previously demonstrated that the *S. Typhimurium rcsC11* mutant shows reduced attachment to eukaryotic cells, we postulate that the activation of the RcsCDB system represses expression of fimbrial encoding genes. The goal of this project was the study of the role of this system in the control gene expression of fimbrial genes, in order to explain the inability of *rcsC11* mutant to attach the eukaryotic cells. An additional goal was the possibility of expanding the number of genes of the RcsB regulon involved in the virulence of *Salmonella*.

Main Activities Conducted

In order to study whether fimbrial expression depended on the RcsCDB system in *S. Typhimurium*, we chose the *std* operon. For this purpose, we used a transcriptional fusion *stdA::lacZ*. We measured the β-galactosidase activity in the *rcsB* and *rcsC11* (constitutive activation) backgrounds, and observed that in both genetic backgrounds as well as in the wild type strain the *stdA::lacZ* fusion was not expressed. Previously it had been reported that this operon is repressed when its promoter is methylated by Dam (9). According to this, we decided to measure the β-galactosidase activity in a *dam* mutant and in *dam rcsB* and *dam rcsC11* double mutants. We observed that in the absence of *rcsB* the expression levels of the *stdA::lacZ* fusion increased 2 fold, while in the *rcsC11* mutant these levels decreased 10 fold in comparison with those determined in the *dam* mutant. These results suggest that the RcsCDB system negatively regulates the expression of the *std* operon. To support these results we performed a qPCR of *stdA* in the backgrounds mentioned above, and we observed the same patterns of expression. As the *std* operon contains six genes, we decided to determine if this regulation is maintained in the downstream genes of the operon. For this purpose we used a translational fusion *stdF::lacZ*. When we measured the expression of the fusion in the *dam, dam rcsB* and *dam rcsC11* backgrounds, we observed the same effect described above for the *stdA::lacZ* fusion. Finally we conducted a western blot assay using the *stdF*::3xFLAG fusion in order to determine whether RcsCDB-mediated regulation was likewise observed at the protein level. In this assay we observed that in the *dam rcsC11* double mutant the StdF protein was not detected.

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The next step in our study was to find out if the RcsCDB regulation is direct or indirect. In this sense, HdfR is the only known regulator of the *std* operon. Therefore, we aimed to study whether RcsB might control the expression of *hdfR*. For this purpose we measured the β-galactosidase activity of an *hdfR::lacZ* fusion in the *rcsB* and *rcsC11* genetic contexts. We observed a slight decrease of expression in the *rcsC11* mutant, but such expression was not modified in the *rcsB* mutant. Then we performed a qPCR assay and we found that RcsB does not regulate *hdfR*. Simultaneously, we performed a Western blot assay using the wild type, *rcsB* and *rcsC11* strains carrying an HdfR::3xFLAG construct. We concluded that there were not differences in the level of HdfR between the strains under study. For this reason we discard HdfR as an intermediary in the regulation of the *std* operon by the RcsCDB system. As an alternative possibility, the RcsB effect might be exerted directly on the promoter. To test this possibility we performed an electrophoretic mobility shift assay (EMSA) and a footprinting assay. The EMSA results confirmed that RcsB is able to bind to the promoter region of the *std* operon. The footprinting assay and the bioinformatic analysis of the promoter region are being conducted at this time in collaboration between the two working groups.

Conclusion

In conclusion, we demonstrated that the *std* operon is regulated by RcsB only when the *std* promoter is not methylated by Dam. It was confirmed that this regulation is not mediated by HdfR. Finally, EMSA analysis allowed us to conclude that the regulation of *std* by RcsB is direct, because this regulator binds to the *std* promoter region. Importantly, despite having completed my short training in the laboratory of Dr. Casadesús, we continue working in collaboration in order to perform the footprinting assay as well as bioinformatic analysis. On the other hand, the results obtained suggest that the reduced adhesion of the *rcsC11* mutant may be caused by repression of fimbrial genes like *std*. To demonstrate this, cell culture assays are under way. It is important to highlight that this is the first report indicating that the RcsCDB system regulates the expression of a fimbrial operon, which opens the way to investigate the regulation of other fimbrial genes. Altogether, our results contribute to increase our understanding of *rcsC11* mutant attenuation, and provide relevant information for the development of attenuated or vector vaccines.

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ISID Report of Juan Farizano

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