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Site-specific DNA Recombinases and Genetic Stabilization of Vaccines

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The infectious diseases are the leading cause of death of human beings in the world. Different measures can be undertaken to avoid dissemination of such diseases; by far the most effective one has been proven to be the use of vaccines. Despite the advances in developing other strategies, the first generation of bascterines and virins based on the culture of attenuated microorganism or physicochemically inactivated strains is still utilized for the production of several vaccines. In fact, the most effective vaccines used in eradication campaigns against human and animal diseases belong to this category. The vaccinal properties of bacterial and viral strains depend largely on the stability of their phenotype. For that reason, the establishment of a well-characterized frozen or freeze dried master seed bank is mandatory for large scale production in order to avoid phenotypic changes.

Efficacy and safety, two key vaccines properties, rely on the stability of the strain, which in turn depends on that of its genome. Changes observed in bacterial genomes, also called genome plasticity, are prompted by the interaction with the environment or, in the case of pathogens, with the corresponding host. In bacterial pathogens, genome plasticity is manifested in part by the activity of particular enzymes known as DNA site-specific integrases/recombinases (Int). These enzymes catalyze the interchange of DNA strands at particular spots containing short DNA sequences or direct repeats, which results in integrations/deletions or even inversions of genetic material. Often, Int are linked to the horizontal transfer (HT) of their cognate encoding DNA along with other genes, which means they are responsible for transmission of pathogenic traits among bacterial population [1]. The acquisition of gene clusters or genomic islands (GI) is recognized as the major driving force for prokaryotic evolution. These clusters are part of the flexible or not essential gene pool maintained by the environmental (host) pressure. Interestingly, the activity of Int is tightly regulated by accessory small proteins termed recombination directionality factors or RDFs that determine the balance between integration/deletion of GI within the genome. Although the role of RDFs is architectural rather than catalytic, they form a complex with Int that allows to accomplish excisive recombination, therefore they are also called excisionases.

Frequently, it is overlooked that in bacteria grown under laboratory conditions, the pressure imposed by the environment upon HT acquisitions has been removed. Since bacteria can rapidly evolve in order to adapt to a changing environment, spontaneous gene or even complete GI deletions may occur by the concerted action of Int and RDFs. The result of such deletions is a loss of virulence for pathogenic strains and over attenuation in the case of vaccine strains. This phenomenon is well described in the case of GI-2 of *Brucella*, a group of gram-negative pathogens that cause brucellosis. This GIencodes genes for lipopolysaccharide (LPS) biosynthesis, a major virulent factor for *Brucella* pathogenesis [2]. The presence of a full LPS molecule in the outer membrane is mainly responsible for the smooth (S) colony phenotype shown by virulent, classic *Brucella* species. The lack of the outer part of the LPS, the O-polysaccharide (O-PS), determines the appearance of rough (R), less virulent species and also R mutants. Oxygen limitation and other culture conditions causing energy shortage facilitate the establishment of *Brucella* R variants through the so-called S-R dissociation. We have speculated that such variants not synthesizing the costly O-PS would be more competitive when energy shortage and no selective pressure to maintain the O-PS concur. Regarding to genetic mechanisms of S-R dissociation, we have demonstrated that the Int of GI-2 mediates the excision from the *Brucella* genome of the entire island; the GI is then lost in subsequent rounds of cellular divisions because it cannot replicate [3]. Therefore, the resulting mutant carrying the scar left by the excision changes from S to R phenotype. Strikingly, this finding is not restricted to virulent strains. *B. melitensis* Rev 1, so far the most widely used vaccine against brucellosis for small ruminants, is particularly unstable and for that reason a manufacturing seed-lot system has been adopted to reduce genetic drifts including the appearance of non-immunogenic R types. Despite the precautions taken during its manufacturing, Rev 1 experiences GI-2 excision and R mutants containing the genomic scar can be isolated. Based on this finding and the conserved LPS loci organization among *Brucella* species, we hypothesized that the abrogation of GI-2 excision by means of the deletion of *int* may help us to develop a more stable strain. As expected, the mutation of GI-2Int reduced the level of S-R dissociation, thus improving the S-LPS stability [4].

Similarities between *Brucella* LPS loci organization and other gram-negative bacteria suggest that not only the O-PS was acquired by HT, but also the same dissociation mechanism can occur. Indeed, the S-R dissociation is far from being exclusively an in vitro behavior manifested by the *brucellae*. Several reports and reviews have accounted for this phenomenon in major *Enterobacteriaceae* species [5-8] and even in phylogenetically distant microorganisms. The unraveling of *int* genes and *cis* elements involved in LPS loci deletion pathways open the way to control undesirable excisive recombination events as it has been demonstrated for *Brucella*. Nowadays, in the post genomic era, we are able to design and to carry out specific mutations into the bacterial genomes in order to obtain customized attenuated strains. Since recent insights point out the relevance of site-specific recombinases for the stability of vaccines, we think that this knowledge could be applied to improve the phenotype stability of new vaccine candidates.

A barely explored field of study is the substrate specificity of GI-associated Int. As mentioned before, Int are also implicated in the spreading of GI among bacteria sharing a particular ecological niche. Noteworthy, genome sequencing of bacterial strains responsible for outbreaks has revealed that these novel strains have evolved after incorporating foreign DNA in their genomes by Int-mediated GI transfer [9]. The study of Int target sequences and the environmental

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signals that regulate their activity will provide us some clues for the prediction of HT events that could be involved in the emergence of new pathovars or even pathogens in a near future.

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