Preprint del artículo publicado en: Journal of Molecular Evolution 82(4-5): 173-175 (2016)

ACCEPTING FOREIGN GENES

LUIS BOTO

Dpto. Biodiversidad y Biología Evolutiva.
Museo Nacional de Ciencias Naturales (CSIC)
C/ José Gutierrez Abascal 2
28006 Madrid
Spain
Phone: (34) 914111328 Ext 1131
Fax: (34) 915645078
e-mail: mcnb119@mncn.csic.es

Acknowledgements.

Author is supported by a grant of Spanish DGI (CGL2013-41375-P). Author thanks to Elena Bulmer for English editing of the manuscript

Key words: Evolution, Horizontal gene transfer, integration of foreign genes, contingency

ABSTRACT:

Three recent papers underline the importance of the host genomic background in allowing the stable maintenance of horizontally acquired genes. These studies suggest that post-transfer changes in both, host genome and acquired genes contribute to the stable integration of foreign genes.

In the last twenty five years horizontal gene transfer has been recognized as a force modulating the evolution of bacterial, archaeal, unicellular eukaryotes, plants, fungi and, to a less extent, metazoan genomes, and consequently as a force that modulates the evolution of the life on earth. A search in the Thompson Web of Science using "*horizontal gene transfe*r" as query yields more than ten new publications coming out per week, which clearly reveals to us that this is an active research field.

Over the past twenty five years many genes have been identified as being horizontally transferred between organisms. Moreover, insights in mechanisms that allow the transfer and stable integration of foreign genes in the new genomic context have been revealed. This includes for example the characterization of barriers that horizontally acquired genes need to overcome to become integrated in the receptor genomes (Thomas and Nielsen 2005; Baltrus 2013; Boto 2015).

However, the role performed by the recipient genetic background and the post-transfer responses in the stable integration of foreign genes has been hardly studied. Three recent papers (Michener et al. 2014; Pascuan et al. 2015; Llorente et al. 2016) that combine the identification of horizontally transferred genes with experimental genetic engineering approaches have shed light in this topic revealing that the assimilation of foreign genes by an organism is contingent to the evolution of both, the host genome and the acquired gene.

Methylobacterium mutations allow the adaptation to the presence of a foreign gene.

Some natural strains of *Methylobacterium extorquens* harbor a dehalogenase *dcmA* gene, which has probably been acquired through horizontal gene transfer

(Vuilleumier et al. 2009). This gene is essential for the catabolism of Dichloromethane and consequently for the growth of the bacteria in the presence of this toxic compound. Using an *in vitro* evolution approach, Michener et al. (2014) have recently provided suggestions of how this gene becomes a part of the *M. extorquens* genome.

Transformation with *dcmA* of different *Methylobacterium* species and *M. extorquens* strains lacking the gene results initially in the minor growth of transformants (compared to the wild strains of *M. extorquens* containing the gene) in presence of Dichloromethane. However, after 150 generations of in vitro evolution in presence of this compound, transformant strains with better fitness that the original strain appear.

Genome sequencing of these evolved strains have enabled authors to identify regulatory mutations in four genes that appear to be associated to the improved fitness of these strains. Some of these mutations affect genes involved in the chloride ions (a byproduct of the metabolism of Dichloromethane) efflux to the extracellular medium, which may explain the observed fitness increase.

Furthermore, the sequencing of the *S. extorquens* natural strains containing the *dcmA* gene have revealed mutations on the *clcA* (one of the genes identified in evolved strains which encodes an antiporter protein associated to chloride efflux) promoter that improve gene expression as compared to strains lacking the *dcmA* gene.

What this study shows is how important is the recipient genetic background to ensure the stable integration of an acquired gene. The evolution of the recipient genome post-gene transfer, or the presence of a receptor possessing a permissive genetic background, may allow the exploitation of the potential advantages that the acquisition of a new (although initially harmful) gene can provides

.

Sequential horizontal transfer in *Pseudomonas* allows the stabilization of horizontally acquired nitrogenase genes.

In the wild some *Pseudomonas* strains have acquired horizontally nitrogenase genes that enable bacteria to fix nitrogen. However in the laboratory, the transformation of *Pseudomonas* strains with nitrogenase genes results in phenotypes that reveal the constitutive expression of the acquired genes (Setten et al. 2013) suggesting that acquisition of these genes may initially be harmful to recipient.

A recent study by Pascuan et al. (2015) suggest the way by which nitrogenase genes may have become a part of *Pseudomonas* genomes. In this study, the authors identify horizontally acquired genes involved in the biosynthesis of Polyhydroxibutyrate (PHB) in strains of *Pseudomonas* that fix nitrogen naturally. Next, authors transform recombinant strains of *Pseudomonas protegens* harboring nitrogenase genes with the PHB biosynthesis genes. Results clearly show that the presence of PHB genes contributes to the regulation of the expression of nitrogenase, suggesting that the presence of PHB genes in the receptor alleviate the problems caused by the presence of nitrogenase genes.

In this case, sequential horizontal gene transfer seems to be an important contributor towards the stable integration of the acquired genes. As in the case of *Methylobacterium*, the evolution of the recipient genome through the acquisition of new genes or the transfer to a recipient that has previously acquired permissive genes leads to the stable acquisition of an initially detrimental gene. In this way, it is possible to exploit the advantages that the new gene may provide.

Post-transfer gene modifications allow the stable integration of foreign genes.

Several studies have shown the importance of amelioration of horizontally acquired genes for their stable and long term maintenance in the recipient organism (Marri and Golding 2008).Over time, changes in codon usage and base composition improve the transcription and translation of the acquired gene in the new host.

In a very recent paper Llorente et al. (2016) go one step ahead, underlining the role of the acquisition of new sequences that enable the correct cellular targeting of the gene product in the host cell. In this paper, the authors identify in plants a genomic gene acquired from bacteria which are different from the cyanobacteria precursors of chloroplasts. This gene encodes a plastid Polyphenol oxidase (PPO) and contains plastid targeting signals that allow the gene product to travel from the nucleus to the chloroplast.

Using a genetic engineering approach, the authors show that the deletion of the targeting signals leads to the cytosolic localization of the gene product and that the cytosolic localization of the enzyme reduces the plant growth. In this way, authors conclude that the acquisition of targeting signals after the horizontal gene transfer is necessary for the stabilization of the acquired gene.

To conclude, these three studies give us important new keys to understand how foreign genes become successfully integrated in a new host, and how this integration provides the recipient organism with novel options to exploit new habitats and resources.

References

Baltrus DA. (2013) Exploring the costs of horizontal gene transfer. Trends Ecol. Evol. 28:489-495

Boto L (2015) Evolutionary change and phylogenetic relationships in light of horizontal gene transfer. J. Biosc. 40:465-472

Llorente B, De Souza FSJ, Soto G, Meyer C, Alonso GD, Flawiá MM, Bravo-Almonacid F, Ayub ND, Rodriguez-Concepción M (2016) Selective pressure against horizontally acquired prokaryotic genes as a driving force of plastid evolution. Sci. Reports 6:19036

Marri PR, Golding GB (2008) Gene amelioration demonstrated: the journey of nascent genes in bacteria. Genome 51:164-168

Michener JK, Camargo Neves AA, Vuilleumier S, Bringel F, Marx CJ (2014) Effective use of a horizontally-transferred pathway for dichloromethane catabolism requires post-transfer refinement. Elife 3:e04279

Pascuan C, Fox AR, Soto G, Ayub ND (2015) Exploring the ancestral mechanisms of regulation of horizontally acquired nitrogenases. J. Mol. Evol. 81:84-89

Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. 3:711-721

Setten L, Soto G, Mozzicafreddo M, Romina Fox A, Lisi C, Cuccioloni M, Angeletti M, Pagano E, Diaz-Paleo A, Ayub, ND (2013) Engineering Pseudomonas protegens Pf-5 for nitrogen fixation and its application to improve plant growth under nitrogen-deficient conditions. Plos One 8:e63666

154 Vuilleumier S, Chistoserdova L, Lee MC, Bringel F, Lajus A, Zhou Y, Gourion B,
155 Barbe V, Chang J, Cruveiller S, Dossat C, Gillett W, Gruffaz C, Haugen E, Hourcade E,
156 Levy R, Mangenot S, Muller E, Nadalig T, Pagni M, Penny C, Peyraud R, Robinson
157 DG, Roche D, Rouy Z, Saenampechek C, Salvignol G, Vallenet D, Wu Z, Marx, CJ,
158 Vorholt JA, Olson MV, Kaul R, Weissenbach J, Medigue C, Lidstrom ME
159 (2009) Methylobacterium genome sequences: A reference blueprint to investigate
160 microbial metabolism of C1 compounds from natural and industrial sources. Plos One
161 4:e5584

162

163