argC Orthologs from Rhizobiales Show Diverse Profiles of Transcriptional Efficiency and Functionality in Sinorhizobium meliloti

Rafael Díaz, Carmen Vargas-Lagunas, Miguel Angel Villalobos, Humberto Peralta, Yolanda Mora, Sergio Encarnación, Lourdes Girard, and Jaime Mora*

Programa de Genómica Funcional de Procariotas, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Av. Universidad, Cuernavaca, Morelos C.P. 62210, Mexico

Received 24 August 2010/Accepted 31 October 2010

Several factors can influence ortholog replacement between closely related species. We evaluated the transcriptional expression and metabolic performance of ortholog substitution complementing a Sinorhizobium meliloti argC mutant with argC from Rhizobiales (Agrobacterium tumefaciens, Rhizobium etli, and Mesorhizobium loti). The argC gene is necessary for the synthesis of arginine, an amino acid that is central to protein and cellular metabolism. Strains were obtained carrying plasmids with argC orthologs expressed under the speB and argC (S. meliloti) and lac (Escherichia coli) promoters. Complementation analysis was assessed by growth, transcriptional activity, enzymatic activity, mRNA levels, specific detection of ArgC proteomic protein, and translational efficiency. The argC orthologs performed differently in each complementation, reflecting the diverse factors influencing gene expression and the ability of the ortholog product to function in a foreign metabolic background. Optimal complementation was directly related to sequence similarity with S. meliloti, and was inversely related to species signature, with M. loti argC showing the poorest performance, followed by R. etli and A. tumefaciens. Different copy numbers of genes and amounts of mRNA and protein were produced, even with genes transcribed from the same promoter, indicating that coding sequences play a role in the transcription and translation processes. These results provide relevant information for further genomic analyses and suggest that orthologous gene substitutions between closely related species are not completely functionally equivalent.

Synteny, gene neighboring, or conservation of chromosomal gene order has been proposed to be a result of the interdependence between a gene product and its genomic context. Conservation of a syntenic block could be favored by selective pressure because the arrangement allows the sequential or coordinated expression of genes that correctly integrate important metabolic functions (41, 64, 69). By a comparative analysis of four Rhizobiales species (namely, Sinorhizobium meliloti, Agrobacterium tumefaciens, Mesorhizobium loti, and Brucella melitensis), we found that syntenic genes exhibit striking differences compared to nonsyntenic genes, including increased operon and network organization, high sequence conservation, diminished evolutionary rates, essential functional role, and specific phylogenetic associations (32; H. Peralta, G. Guerrero, A. Aguilar, and J. Mora, unpublished data).

Orthologs encode similar functions sharing a common ancestor and exhibiting various degrees of conservation, due in large part to functional adaptation and cellular role in different species (45). We have proposed a new parameter termed “species signature” to extract the amount of amino acid residues specific for a given species, based on multiple sequence alignment. We hypothesized that the species signature represents the proportion of a particular sequence that responds to adaptation (32); it is a useful evolutive measure because we found a high direct correlation with the nonsynonymous substitution rate (Peralta et al., unpublished). Although orthologous genes of Rhizobiales encode proteins with high degrees of identity among several species, the proteins differ in many respects, including isolectric point. Small differences in protein sequence may be the result of evolutionary adaptation in response to the particular intracellular environment of a given species. In this scenario, specific amino acid changes would be selected for optimal performance in a particular genetic background. Knight et al. (40) carried out a virtual analysis of proteomes of nearly 100 organisms and showed that there was a correlation between theoretical proteomes and ecological niches; conversely, there was no correlation between phylogeny and differences observed in the theoretical proteomes. Our previous work has shown that mutation of a single gene, anIA (a carbon flux regulator), produced a proteomic alteration of approximately 800 proteins (16, 22), indicating that the absence or modification of a single gene can result in complex changes in global gene expression. In this context, syntenic orthologs are ideal to evaluate the functional importance of species signature in related organisms.

The Rhizobiales order is a versatile group of bacteria that present very interesting features, such as a huge amount of genes, abundance of genes acquired by horizontal transfer events, a symbiotic or pathogenic association with higher organisms such as plants or animals, and the ability for nodulation and nitrogen fixation in some species (9, 13, 29, 31, 50, 54,

---

*Corresponding author. Mailing address: Programa de Genómica Funcional de Procariotas, Centro de Ciencias Genómicas-UNAM, Apdo. postal 565-A, Cuernavaca, Morelos C.P. 62220, México. Phone: 52 777 3114661. Fax: 52 777 3175094. E-mail: jmora@ccg.unam.mx.
† Supplemental material for this article may be found at http://jb.asm.org.
‡ Present address: Centro de Investigación en Biotecnología Aplicada-IPN, Ex Hacienda San Juan Molino, Tlaxcala C.P. 90700, Mexico.
‡‡ Published ahead of print on 12 November 2010.