ENZYMES OF THE SHIKIMIC ACID PATHWAY ENCODED IN THE GENOME OF A BASAL METAZOAN, THE SEA ANEMONE NEMATOSTELLA VECTENSIS, HAVE MICROBIAL ORIGINS

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Background

• The shikimic acid pathway is responsible for the biosynthesis of many aromatic compounds by a broad range of organisms, including bacteria, fungi, plants and some protozoans.

• Animals are considered to lack this pathway as evidenced by their dietary requirement for shikimate-derived aromatic amino acids.

• We challenge the universality of this traditional view in this novel report of genes encoding enzymes for the shikimate pathway in an animal, the starlet sea anemone Nematostella vectensis.

Methods

The DNA sequence of the N. vectensis genome was downloaded from Stellabase version 1.0 (http://evodevo.bu.edu/stellabase/) and translated into all six reading frames using Transeq (http://www.ncbi.nlm.nih.gov/blast/) and release 20 of the Pfam database (http://www.sanger.ac.uk/Software/Pfam/) were used. Similarity searches used the BLAST service at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

Results 1

Phylogenetic tree showing the relationship of the predicted protein sequence of the N. vectensis aroA-like gene to the predicted murA protein sequences of the seven best hits in a BLAST analysis, as well as those in E. coli and Tenacibaculum. Distances were calculated from a CLUSTAL W alignment using the Jones-Taylor-Thornton matrix and the tree was constructed using the neighbor-joining algorithm in programs of the PHYLIP package (version 3.63). The distance is proportion of amino acid substitutions.

Results 2

Phylogenetic tree showing the relationship of the deduced protein sequence of the arocB-O-methylase protein of N. vectensis to homologous dinoflagellate proteins. Sequences were aligned with CLUSTALW and the tree constructed using the neighbor-joining algorithm with distances derived from the Jones-Taylor-Thornton model (using PHYLIP version 3.63). The tree was rooted using Arabidopsis thaliana as an out group. The distances are the proportion of amino acid substitutions, and the bootstrap values based on 100 samples are shown.

Discussion

• Molecular evidence establishes, for the first time, horizontal transfer of ancestral genes of the shikimic acid pathway into the N. vectensis genome from both bacterial and eukaryotic (dinoflagellate) donors.

• Bioinformatic analysis also reveals four genes that are closely related to those of Tenacibaculum sp. MED152, raising speculation for the existence of a previously unsuspected bacterial symbiont. Indeed, the genome of the holobiont (i.e., the entity consisting of the host and its symbionts) comprises a high content of Tenacibaculum-like gene orthologs, including a 16S rRNA sequence that establishes the phylogenetic position of this associate to be within the family Flavobacteriaceae.

Significance

• These results provide a complementary view for the biogenesis of shikimate-related metabolites in marine Cnidaria as a “shared metabolic adaptation” between the partners.

• The results could also have wider implications for future development of the shikimic acid pathway as a “druggable” target for the discovery of new antibiotics and for the treatment of parasitic infections, including malaria.

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