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Unraveling the Evolution and Function of the Retinoic Acid Signaling in the Mollusc *Mytilus galloprovincialis*

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ABSTRACT

Vitamin A (retinol) and its derivatives play crucial roles in metazoan, primarily through the active metabolite all-*trans* Retinoic Acid (atRA or RA), which, in vertebrates, modulates transcription via the Retinoic Acid Receptor (RAR). While the evolution of RA signalling remains largely unknown, mollusc Rars seem to have lost the ability to bind RA due to mutations in key amino acids of the ligand-binding domain (LBD). Although molluscs can produce RA, its biological role remains elusive, and it is also unclear whether Rar contributes to RA-dependent signal transduction. In this study on the bivalve mollusc *Mytilus galloprovincialis* (*Mg*), we screened its pan-genome and identified orthologues of RA signalling-related genes. Subsequent sequence and computational analyses of the *Mg*Rar-LBD revealed amino acid signatures suggestive of a reduced responsiveness to RA, which was confirmed by *in vitro* assays demonstrating an absence of its activity. However, exogenous RA treatment during embryogenesis resulted in specific phenotypes, and a differential transcriptome analysis revealed a significant upregulation of a *MgCyp26* gene, encoding an RA-metabolising enzyme, supporting a negative feedback mechanism not previously reported in lophotrochozoans. A conserved physiological role of Cyp26 was demonstrated by treating embryos with both RA and a Cyp26 inhibitor, which induced phenotypic alterations resembling those caused by high RA doses. Ongoing analyses will determine if *MgCyp26* is directly regulated by MgRar or if other nuclear receptors, such as MgRxr that we showed is able to respond to RA, could mediate RA signalling. *M. galloprovincialis* thus represents a valuable model for elucidating both the function and the evolution of RA signalling in metazoans.