# Study of the Evolutionary Role of Nitric Oxide (NO) in the Cephalochordate Amphioxus, *ñBranchiostoma lanceolatumò*

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by

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*"Find a job you enjoy doing,*

*and you will never have*

*to work a day in your life."*

*Mark Twain*

# ABSTRACT

Nitric Oxide (NO) is a gaseous molecule that acts in a wide range of biological processes. NO can be produced by enzymatic and non-enzymatic pathways and, in this scenario, the enzyme Nitric Oxide Synthase (NOS) has a great importance for its exclusive role in *de novo* synthesis of NO. In the present study, the role of the NO in the embryonic development was investigated in the cephalochordate *Branchiostoma lanceolatum* (amphioxus) with the purpose of acquiring further knowledge on the ancestral role of animal *Nos* and the acquisition of new NO functions during evolution. Amphioxus has three different *Nos* genes (*NosA*, *NosB* and *NosC*) that are not orthologues of the three *Nos* of mammals (*NosI*, *NosII* and *NosIII*) deriving from an independent duplication occurred in the common ancestor of cephalochordates. The three amphioxus *Nos* genes showed a different temporal and spatial expression during development and a different susceptibility to be induced after immune stimulation. The study of the promoter regions of these genes can be very useful to identify possible diversities in regulation that can lead those peculiar expression features. In amphioxus larva, NO was mainly detected in the developing nervous system and in the pharyngeal area, before and after the mouth opening. The inhibition of NOS activity, and as consequence the enzymatic NO production, during amphioxus neurulation, resulted in the alteration of pharyngeal structures formation in the larvae, in particular opening of the mouth resulted compromised. Moreover, an alteration in larva locomotion was observed. Further studies will be necessary to reveal the exactly molecular mechanisms and the pathways in which

NO acts for establishment of the pharyngeal structures and the neuromuscular junctions early in development. For this purpose, a differential transcriptomic analysis of NOS-inhibited embryos was performed but the results are still very preliminary.