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Nitric Oxide regulates mouth development in amphioxus

Giovanni Annona[1], Filomena Caccavale[1], Juan Pascual-Anaya[2], Shigeru Kuratani[2], Pasquale De Luca[3], Anna Palumbo[1] & Salvatore D'Aniello[1]

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The development of the mouth in animals has fascinated researchers for decades, and a recent study proposed the modern view of recurrent evolution of protostomy and deuterostomy. Here we expanded our knowledge about conserved traits of mouth formation in chordates, testing the hypothesis that nitric oxide (NO) is a potential regulator of this process. In the present work we show for the first time that NO is an essential cell signaling molecule for cephalochordate mouth formation, as previously shown for vertebrates, indicating its conserved ancestral role in chordates. The experimental decrease of NO during early amphioxus *Branchiostoma lanceolatum* development impaired the formation of the mouth and gill slits, demonstrating that it is a prerequisite in pharyngeal morphogenesis. Our results represent the first step in the understanding of NO physiology in non-vertebrate chordates, opening new evolutionary perspectives into the ancestral importance of NO homeostasis and acquisition of novel biological roles during evolution.

Nitric oxide (NO) is a small and highly diffusible signal molecule that is known to be involved in a wide range of important biological processes. Since its initial discovery as a modulator of vascular activities in mammals, NO has been found to participate in numerous physiological and developmental functions in a wide spectrum of organisms[1]. Our understanding of NO signaling has profoundly changed over recent decades. It was originally considered solely as a toxic substance, but nowadays, although harmful at high concentration, NO is believed to be an essential signaling molecule for living organisms. The function of this ambivalent gas depends on the precise balance between its production and consumption. When produced at high levels, for example during inflammation, NO may interact with cellular components, such as DNA, RNA, lipids, and proteins, leading to mutations and altered cell physiology that may lead to carcinogenesis[2–4]. On the other hand, NO deficiency can cause disorders of endocrine[5], cardiovascular[6], musculoskeletal[7] and immune systems[8].

The biosynthesis of NO is catalysed by the nitric oxide synthase enzymes (NOS), through two successive mono-oxygenation reactions, from L-Arginine to L-Citrulline with Nω-hydroxy-L-arginine (NOHLA) as an intermediate[9]. Mammalian genomes have three paralogous *Nos* genes with distinct expression patterns and specific functions[10,11]: *NosI* or *neuronal Nos* (*nNos*); *NosII* or *macrophage inducible Nos* (*iNos*), and *NosIII* or *endothelial Nos* (*eNos*). All *Nos* genes share a very similar gene structure, with highly conserved intron number, position and phases. At the protein level, they only differ in the presence of the protein-interaction domain (PDZ) in NOSI, which is absent in both NOSII and NOSIII, and in the absence of the inhibitory loop in the region of FMN-binding domain exclusively in NOSII[12,13]. Two of these genes, *NosI* and *NosIII*, are typically constitutively expressed, while *NosII* expression levels increase upon microbial infection, generating high and sustained amounts of NO[14]. Despite their given names indicating a tissue-specificity, all three *Nos* genes are, in fact, expressed in most tissues and organs. Therefore, we prefer to use the *NosI-II-III* nomenclature. In the central nervous system (CNS) the NO produced by NOSI is implicated in neurogenesis, synaptic plasticity, learning and memory[15], while in the peripheral nervous system it is involved in the control of blood pressure, gut peristalsis and vasodilatation[14,16]. NO derived from NOSII, primarily from macrophages, is essential for the control of inflammatory processes induced by intracellular bacteria or parasites[14]. Lastly, NO produced by NOSIII, which is the best characterized of the NOS proteins, is a homeostatic regulator of numerous essential cardiovascular functions, such as vasodilatation, inhibition of platelet aggregation and adhesion to the vascular wall, as well as inhibition of vascular inflammation[14].

[1]Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn di Napoli, Villa Comunale 1, 80121, Napoli, Italy. [2]Evolutionary Morphology Laboratory, RIKEN, Minatojima-minami 2-2-3, 650-0047, Kobe, Hyogo, Japan. [3]RIMAR, Stazione Zoologica Anton Dohrn di Napoli, Villa Comunale 1, 80121, Napoli, Italy. Correspondence and requests for materials should be addressed to S.D. (email: salvatore.daniello@szn.it)