

The sea anemone *Nematostella vectensis* as a model system for the study of the evolutionary origin of triploblasty and bilaterality¹

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Cnidarians represent an early branching animal lineage that may have split about 600 Mio years ago. They are diploblastic, i. e. consist of endoderm and ectoderm only, hence lack the third germ layer, the mesoderm. Further, traditionally they are regarded as radially symmetric. Since Cnidaria are the sister group to the Bilateria they may provide crucial insights into the evolution of the third germ layer and bilaterality. In recent years the sea anemone *Nematostella vectensis* has developed into an emerging model organism among cnidarians (DARLING et al. 2005; GENIKHOVICH & TECHNAU 2009), due to its slow rate of evolution, its accessible embryogenesis, amenable for functional studies. Despite the apparent morphological simplicity, the gene repertoire is astonishingly complex: an EST survey of *Nematostella* and the coral *Acropora* revealed that not only are most anthozoan transcripts significantly more similar to vertebrates than to ecdysozoan model systems, in fact, anthozoans share a large fraction of genes exclusively with chordates, which are absent in the ecdysozoans (TECHNAU et al. 2005). These genes that previously were thought to be chordate innovations must have been lost from the ecdysozoans. In turn, this means that the common ancestor of Cnidaria and Bilateria was genetically surprisingly complex and much of that complexity was maintained in the anthozoan and vertebrate lineage (TECHNAU et al. 2005). A major breakthrough was the publication of the draft genome of *Nematostella*, in fact the first genome of a non-bilaterian animal that was sequenced (PUTNAM et al. 2007). It corroborated the view of a high genetic complexity of the common eumetazoan ancestor. The detailed analysis showed that in the *Nematostella* genes the degree of sequence conservation extends to the exon-intron structure: 80% of all ancestral intron positions are conserved in *Nematostella* as well as in vertebrates, whereas only about 20% are conserved in *Drosophila* and *Caenorhabditis elegans* (PUTNAM et al. 2007). Even more surprisingly, when allowing for local rearrangements there is a significant synteny conservation between *Nematostella* and humans but has recently been sequenced and has revealed a surprising degree of com-

plexity in terms of gene repertoire and genomic organisation, much of which is conserved in vertebrates (PUTNAM et al. 2007). Thus, these comparative genomic analyses support the view that the common ancestor of Cnidaria and Bilateria was genetically complex. This contrasts with the apparent morphological simplicity and the question arises how the animal actually uses this complex gene repertoire.

Interestingly, despite lacking a mesoderm, the sea anemone genome encodes most of the transcription factors crucial for mesoderm and muscle differentiation in Bilateria. Similarly, all genes used to establish the dorso-ventral axis in Bilateria in a conserved way are present in the “radially symmetric” *Nematostella*. To address the question of the origin of bilaterality and the mesoderm, we characterized these genes by in situ hybridization and Morpholino-mediated gene knockdowns. First we analysed the expression and function of *dpp*, *chordin* and other DV-axis-related genes. Expression studies showed that both *dpp* and *chordin* start being expressed around the blastopore at early gastrula stage (RENTZSCH et al. 2006). Shortly later expression of both genes shifts towards one side of the blastopore, hence becomes asymmetric with respect to the oral-aboral axis. Strikingly, *chordin* and *dpp* are expressed on the same side (RENTZSCH et al. 2006; MATUS et al. 2006a, b; FINNERTY et al. 2004). Morpholino-mediated knockdown of *dpp* leads to a radialisation of *chordin* and *dpp* itself. This suggests a negative feedback on its own expression. In line with this result, treatment of embryos with recombinant human BMP2 leads to an abolishment of *dpp* and *chordin* (SAINA et al. 2009). Analysis of marker gene expression suggests that most effects of *dpp* or *chordin* knockdown are restricted to the endoderm both marking the oral-aboral axis as well as the directive axis (SAINA et al. 2009). Together these results suggest that a molecular system to create asymmetries in body axis using the BMP-Chordin system predated the split of Cnidaria and Bilateria. However, while it is tempting to homologise the directive axis with the dorso-ventral axis, it is far from clear which side in *Nematostella* would correspond

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to dorsal or ventral side, respectively, since a central nervous system is missing. It is at least equally feasible that the BMP/ Chordin system is simply suited to create asymmetries which may have been employed several times in various animal lineages.

Concerning the evolution of the mesoderm, the first thing to note is that *Nematostella* has almost all the transcription factors that are involved in mesoderm formation or differentiation in Bilateria (TECHNAU et al. 2005). In situ hybridisations showed that all these “mesodermal” determinants are expressed at the blastopore (e. g. *brachyury*, *foxA/forkhead*), in the whole endoderm (e. g. *snailA*) or part of the endoderm (e. g. *twist*) (SCHOLZ & TECHNAU 2003; MARTINDALE et al. 2004; FRITZENWANKER et al. 2004). This suggests that the ancestral function of all analysed “mesodermal” genes were involved in gastrulation and/or endomesoderm differentiation.

One of the major derivatives of the mesoderm in Bilateria is the musculature. While Cnidaria do not have a mesoderm they have differentiated myo-epithelial cells. In Anthozoans like the sea anemone, retractor and parietal muscles form at distinct positions in the endodermal mesenteries, which are endodermal folds that reach into the gastric cavity and are connected to the pharynx in the upper region. The retractor muscle expresses specifically a *myosin heavy chain type II* gene (RENFER et al. 2010).

Is the differentiation of the retractor muscle cells in *Nematostella* under similar control of “mesodermal” determinants as in Bilateria? To address this question, we established transgenic *Nematostella* polyps (RENFER et al. 2010). We isolated the upstream region of the *MyHC* gene to drive *mCherry* reporter expression in a transgenic vector that integrates the insert into the genome. This transgenic line expresses *mCherry* in retractor muscles faithfully mimicking the endogenous expression (RENFER et al. 2010). This shows that all cis-regulatory elements necessary to drive correct gene expression are present on the isolated promoter region. Future studies will aim to identify the precise binding sites for the transcription factors crucially involved in the gene regulation.

Striated musculature is a rather complex structure. In vertebrates over 30 proteins interact to form a sarcomere and to regulate the contraction. Striated muscles are considered a homologous structure in Bilateria. Interestingly, hydrozoan medusae contain striated muscles in the sub-umbrella. In order to determine which protein components are shared between Bilateria and Cnidaria we carried out a comparative genomics approach. We found that Cnidaria as well as even the placozoan *Trichoplax* share a core set of proteins shared with Bilateria, suggesting a very ancient origin of these core components of the musculature.

We conclude that ancestral gene networks used for gastrulation and endoderm differentiation have been recruited for mesoderm differentiation in Bilateria. Further, a

signalling system suited to create body plan asymmetries has been adopted and re-employed independently in cnidarian and bilaterian lineages.

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