A WNT of things to come: Evolution of Wnt signaling and polarity in cnidarians

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Abstract

The conserved family of Wnt signaling molecules mediates various developmental processes including governing cell fate, proliferation, and polarity. The diverse developmental functions of the Wnt genes in bilaterians have obscured the evolutionary origin of this important signaling pathway. Recent work in the Cnidaria has shown the diversity of Wnt genes, and regulatory components of Wnt signaling, evolved early in metazoan evolution, prior to the divergence of cnidarians and bilaterians. Evidence from Hydra and the sea anemone, Nematostella, demonstrates a role for Wnt signaling in axis formation and patterning, as well as gastrulation and germ-layer specification. In this review, we examine what is currently known about Wnt signaling in cnidarians, and discuss what this group of “simple” animals may reveal about the evolution of Wnt signaling and polarity.

Keywords: Wnt, Cnidaria, Nematostella, Polarity, Review

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Abbreviations: APC, adenomatous polyposis coli; CamKII, calcium/calmodulin-regulated kinase; DAG, diacylglycerol; Dsh, disheveled; Fzd, frizzled; GSK-3β, glycogen synthase kinase 3β; H9252, lithium chloride; LRP, low density lipoprotein receptor-related protein; PCP, planar cell polarity; PKC, protein kinase C; sFRP, secreted Frizzled-related protein; TCF/LEF, T-cell factor/lymphocyte enhancer factor; WIF-1, Wnt inhibitory factor-1

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1. Introduction

The Wnt signal transduction pathway is an evolutionarily conserved signaling pathway that regulates many aspects of metazoan development. The Wnt signaling pathway is also involved in adult tissue homeostasis, and disruptions in normal Wnt signaling are associated with degenerative diseases, as well as cancer (reviewed in refs. [1,2]). A secreted Wnt signal is transduced through one of three signaling pathways, the canonical Wnt/β-catenin pathway, the Wnt/planar cell polarity (PCP) pathway, or the Wnt/Ca\(^{2+}\) pathway (reviewed in refs. [1,3,4]) (Fig. 1).

In the highly conserved Wnt/β-catenin pathway, secreted Wnt ligands signal by binding a Frizzled/LRP (Fzd/LRP) receptor complex on the target cell surface (Fig. 1). The signal is transmitted through the cytoplasmic protein Disheveled (Dsh), which inhibits the activity of the kinase GSK-3\(β\). GSK-3\(β\) forms a complex with co-regulatory proteins, Axin and the tumor suppressor, adenomatous polyposis coli (APC), which controls the cytoplasmic levels of β-catenin by targeting it for degradation. Inactivation of the destruction complex by Dsh results in the stabilization, accumulation, and nuclear translocation of β-catenin. Once in the nucleus, β-catenin forms complexes with Tcf/Lef DNA-binding factors leading to the transcriptional activation of Wnt target genes (reviewed in refs. [1,2]). Wnt/β-catenin signaling regulates the expression of genes controlling many aspects of development (e.g., cell proliferation, polarity, fate specification, and differentiation) (reviewed in ref. [5]), and is involved in axis formation and patterning in vertebrates (reviewed in refs. [6,7]); posterior specification in cephalochordates [8]; endoderm formation in ascidians [9]; endomesodermal patterning along the animal-vegetal axis in sea urchins [10,11]; and endoderm specification in nematodes [12].

Less is known about β-catenin independent, non-canonical Wnt signaling through the Wnt/PCP and Wnt/Ca\(^{2+}\) pathways. The Wnt/PCP pathway (Wnt/JNK pathway in vertebrates) utilizes components common to the Wnt/β-catenin pathway (e.g., Fzd and Dsh), as well as Rho family GTPases, Jun N-terminal kinase (JNK), and novel proteins Flamingo/Starry night, and Strabismus/Van Gogh (Fig. 1). This pathway specifies cell polarity by regulating cytoskeletal organization, and is required for the proper polarity of sensory bristles and ommatidia in Drosophila (reviewed in refs. [4,13]) and for convergent extension movements during vertebrate gastrulation (reviewed in ref. [14]). The Wnt/Ca\(^{2+}\) pathway also signals through a Fed receptor and regulates release of intracellular calcium and activation of calcium/calmodulin-regulated kinase (CamKII) and protein kinase C (PKC) [15] (Fig. 1). The downstream targets of CamKII and PKC are not well known, and the biological function of this pathway is still not clear.

Functional analyses in Xenopus have shown that different classes of secreted Wnt ligands signal through either canonical or non-canonical pathways [3,4]. This functional division in the Wnts is also reflected in the Fzd family of proteins. Some of the targets of Wnt signaling are, themselves, components of the Wnt pathway, indicating that feedback control is one level at which Wnt signaling is regulated. Wnt signaling is also controlled by secreted factors, including Wnt inhibitory factor (WIF-1), Dickkopf (Dkk), and secreted forms of the Fzd receptor (sFRP), all of which inhibit ligand–receptor interactions [16–18].

Despite the ubiquity and importance of Wnt/β-catenin signaling in bilaterian development, its multiple and diverse developmental roles have obscured the evolutionary origin of this...
pathway. What was the ancestral function of Wnt/β-catenin sig-
naling in eumetazoa? How many Wnt genes were present in the
eumetazoan ancestor? Is there a relationship between Wnt
signaling and the evolutionary origin of molecular mechanisms
governing polarity and axial patterning in metazoans? In the
past several years, experimental and molecular data from the
Cnidaria have led to a better understanding of these questions.
In this review, we will focus on recent studies that suggest a role
for Wnt signaling in regulating axis formation and patterning,
gastrulation, and germ-layer specification in these basal meta-
zoans, and what these data may reveal about the evolution of
Wnt signaling and polarity.

2. Cnidarians may hold clues to the ancestry and
evolution of Wnt signaling

The Cnidaria is a highly successful and diverse phylum,
which includes hydroids, sea anemones and corals, box jellies,
and the true jellyfish. Cnidarians have a simple form, possess
only two epithelial germ layers (outer ectoderm and inner endo-
derm), and have traditionally been considered radially symmet-
rical around the main longitudinal body axis, referred to as the
oral–aboral axis. Within the Cnidaria, the Anthozoa (corals and
sea anemones) is considered the sister-group to the more com-
plicated “Medusozoa” (i.e., Hydrozoa, Cubozoa, and Scyphozoa),
a clade defined by a life history with a medusa (jellyfish) stage
[19–21]. The polyp-only life history of the Anthozoa is con-
sidered to be representative of the ancestral cnidarian condition
[19–20].

As a sister-group to the Bilateria, the Cnidaria is an impor-
tant phylum potentially providing key insight to the ancestry
and evolution of developmental pathways. Recently, much effort
in cnidarian research has been directed towards cloning evolution-
arily conserved genes known to play critical developmental
roles in bilaterians [22–25] (reviewed in refs. [26–28]). Data
from several taxa indicate that cnidarians possess most of the
gene families found in bilaterians, and in many cases expan-
sion of the gene families occurred before the cnidarians diverged
[29–32]. Moreover, it appears that cnidarians have retained many
ancestral genes that have been lost in Drosophila and C. elegans
[31,32]. Therefore, cnidarians provide insight into the contents
of the “genetic tool kit” present in the cnidarian-bilaterian ances-
tor. In particular, as cnidarians are one of the first metazoans to
exhibit a defined adult body plan with a nervous system, tissue-
level of organization, and a main body axis, they are potentially
very informative with regards to Wnt signaling and the evolution
of axis formation, polarity, and germ-layer specification.

3. Wnt signaling in Hydrozoans—the freshwater polyp, 
Hydra

The freshwater hydrozoan, Hydra, has long served as a devel-


opmental model system, and the processes of axis formation and
axial patterning are well understood at the tissue level (reviewed
in ref. [28,33]). The adult polyp consists of a radially symmet-
rical, bilayered cylinder with a defined body axis marked by
a head (including hypostome, mouth, and tentacle rings) at the
apical end and a foot (disk of adhesive cells) at the basal end.
Maintenance of the adult polyp requires continuous cell pro-
liferation, migration, and differentiation. Epithelial cells in
the body column continuously divide and are displaced longitudi-
nally, where they differentiate into head- or foot-specific cell
types in the appropriate region, and then are either sloughed
off at the extremities or into new lateral buds. Consequently,
molecular signaling events directing axial patterning must be
constantly active to maintain the adult body form (reviewed in
ref. [28]). Additionally, the molecular mechanisms responsible
for initiating axis formation must also be continuously active as
bud formation during asexual reproduction is an ongoing pro-
cess in adult polyps. Hydra also has an impressive capacity for
regeneration (reviewed in refs. [34,35]). When a head or foot
is removed from a polyp, the appropriate structure is quickly
regenerated. If both distal ends are removed, the body column
maintains its polarity and will regenerate a head at the apical
end and a foot at the basal end. Hydra is even capable of de novo
axis formation and reconstitution of complete animals from an
aggregate of dissociated cells in which the original polarity of
the animal has been lost [36]. Therefore, during maintenance,
budding, and regeneration events in Hydra signaling pathways
are continuously active, enabling cells to sense their position
and then translate positional information to the developmental
pathways regulating cell fate.

The head organizer, located at the apical tip of the hypostome,
is known to be the source of a signal responsible for axis for-
mation and patterning in Hydra [24,36,37]. Experiments have
shown that transplantation of the hypostome to ectopic positions
along the body column of a host polyp can organize a secondary
body axis by recruiting host tissue, characteristic of a true de-
velopmental organizer [37]. The hypostome, which is considered
homologous to the cnidarian blastopore [25], has been compared
to the vertebrate organizer based on both its ability to induce a
secondary axis and conserved patterns of gene expression [38].
Despite what is known at the tissue level, the molecular basis for
the head organizer has not been well understood. Recently how-
ever, several lines of evidence indicate that the Wnt/β-catenin
pathway is active in the head organizer and is involved in axial
patterning in Hydra.

3.1. Components of the Wnt/β-catenin pathway are
expressed in the head organizer in Hydra

The main components of the Wnt/β-catenin pathway have
been cloned from Hydra, including Wnt, Disheveled, GSK-3β,
β-catenin, and Tcf/Lef [39,40]. A single Hydra Frizzled ortholog,
the putative Wnt receptor, has also been identified and char-
acterized [41]. The expression patterns of the Wnt signaling
pathway members suggest a role for the Wnt/β-catenin path-
way in the maintenance of the head organizer in adult Hydra.
Hy/Wnt is expressed in a small cluster of ectodermal and endo-
dermal epithelial cells at the apical tip of the hypostome, at the
location of the head organizer [40]. HyTcf is also expressed in
the hypostome but in a slightly broader domain than that of
Wnt, and with a graded distribution highest at the apex. HyDsh
and HyGSK-3β are uniformly expressed throughout the polyp
at low levels, although HyGSK-3β transcripts are absent in the foot region [40,42]. Hyβ-cat transcripts are also expressed uniformly throughout the polyp at low levels [40], consistent with an additional function for this protein in cell adhesion. However, the intracellular localization of Hyβ-cat protein is not uniform across all cells of the polyp. Cells in the hypostome have higher levels of nuclear β-catenin than cells in the body column [24], indicating that Wnt signaling is active in the hypostome. The receptor gene, HyFrizzled, is uniformly expressed at high levels in endoderm along the entire body column, excluding the tentacles [41]. It is only very weakly expressed in the ectoderm, suggesting that the main target of Wnt signaling is endoderm, and that HyFrizzled may function to maintain the endoderm as a distinct layer from the ectoderm [41].

3.2. Expression levels of Wnt/β-catenin signaling pathway components are upregulated during budding and regeneration in Hydra

In addition to a role in maintaining the state of the head organizer, Wnt/β-catenin signaling is also active in axis formation during asexual budding and regeneration in Hydra [36,40]. During bud formation, HyTcf and Hyβ-cat expression are upregulated prior to HyWnt expression in a band of cells encircling the body column in the region where the bud will emerge. HyTcf expression later becomes restricted to the hypostome of the new polyp, similar to its expression domain in adult heads. Hyβ-cat expression remains high in the apical end of the bud until the new polyp detaches [40]. The onset of HyWnt expression coincides with the beginning of tissue evagination and is restricted to a few ectodermal cells at the tip of the prospective bud [40]. The HyWnt expression domain remains localized to the apex of the polyp, but expands as bud formation progresses until it reaches the size seen in the hypostome of adult heads.

During head regeneration, HyTcf and Hyβ-cat are also upregulated prior to HyWnt, and both are expressed in broad domains at the apical end of the regenerating tip within 1 h of decapitation. HyWnt expression is restricted to a small region at the apex of the tip [40]. HyGSK-3β expression remains uniform along the body column, and there is no transcriptional alteration during regeneration [42].

Perhaps the most compelling indirect evidence that Wnt/β-catenin signaling functions in axis formation is the expression patterns of Wnt signaling components in aggregates of Hydra cells. Adult Hydra can be dissociated into suspensions of single cells and then centrifuged to form an aggregate in which the original polarity of the animal has been completely disorganized [36]. Within 24 h ectoderm and endoderm form, and after 2–3 days head organizers form de novo, organizing the surrounding tissue into new body axes that separate into individual polyps [35,36]. HyTcf and Hyβ-cat are expressed prior to HyWnt throughout the aggregate by 24 h, and are later downregulated except in regions where new head organizers are forming. HyWnt is first expressed 24 h following aggregate formation in small clusters of 10–20 endodermal cells, corresponding to the minimal number of cells that can act as an organizer [36,40]. The spots of HyWnt expression later expand, and eventually become localized to the tip of the hypostome of new polyps [36,40].

3.3. Pharmacological inhibitor experiments demonstrate Wnt/β-catenin signaling is involved in establishing, maintaining, and patterning the body axis in Hydra

Recently, the role of Wnt signaling in head organization and axial patterning in Hydra has been directly demonstrated through loss-of-function experiments using alsterpaullone, a kinase inhibitor which specifically blocks GSK-3β function in Hydra [24]. First, treating adult Hydra with alsterpaullone results in the formation of ectopic tentacles, and in some cases ectopic heads, along the body column. Similar phenotypes were observed in earlier experiments using diacylglycerol (DAG) [43] and lithium chloride (LiCl) [44], both of which are known to be capable of inhibiting GSK-3β activity [45,46]. Second, alsterpaullone treatment increases levels of β-catenin in all cells of the adult polyp. In untreated polyps, the concentration of β-catenin in cells of the body column is maintained at low levels in the absence of Wnt signaling, and nuclear β-catenin is restricted to cells in the hypostome. Blocking GSK-3β activity with alsterpaullone increases both intracellular β-catenin levels, as evidenced by accumulation of β-catenin in cell membranes, and the nuclear localization of β-catenin in cells of the body column, which do not normally receive Wnt signal [24]. Third, alsterpaullone treatment confers hypostome characteristics on cells of the body column, as indicated by expansion of the normal expression domains of HyWnt, HyTcf, and HyBrα1, the Hydra brachyury ortholog. HyWnt is normally only expressed in the head organizer, and HyTcf and HyBrα1 expression is normally restricted to the hypostome [40,47]. Inhibiting GSK-3β activity results in expansion of HyWnt expression to the entire hypostome, and eventually to regularly spaced ectopic spots of expression in the upper regions of the body column, which presumably correspond to the ectopic tentacles and heads observed in alsterpaullone treated animals. Both the HyTcf and HyBrα1 expression domains were expanded to cells throughout the body column [24]. Finally, transplantation experiments using alsterpaullone-treated animals as donors demonstrate that blocking GSK-3β activity promotes the formation of the head organizer in cells of the body column, as defined by its ability to induce a second axis, produce a head formation inhibitor, and transmit a signal to establish the head activation gradient [24].

The results of these experiments provide direct evidence that the Wnt/β-catenin pathway plays a role in the formation and maintenance of the head organizer in Hydra.

3.4. Wnt/β-catenin signaling and axis specification in other hydrozoans

Wnt/β-catenin signaling appears to function in axis initiation and patterning in another hydrozoan, the colonial species Hydractinia echinata [48]. Hydractinia colonies consist of two functionally diverse types of polyps, feeding gastrozoids and sexually reproducing gonozooids, which are physiologically integrated through a network of stolons. Inhibiting GSK-3β
activity with alsterpaullone results in the formation of ectopic tentacles, and eventually ectopic hypostomes, on gastrozooids in the colony; increases the frequency and density of asexual bud formation; and affects the relative timing of tentacle formation in new polyps [48]. In addition, a Hydra (hydranth) ortholog of the Fos/CDB receptor has recently been characterized [49].

3.5. Is Wnt/β-catenin signaling active in axis formation during embryogenesis in hydrozoans?

Wnt signaling has a demonstrated role in axis formation and patterning in adult Hydra, however its function during embryogenesis is not clear. Embryonic development in Hydra involves holoblastic cleavage divisions, which give rise to a coeloblastula. Gastrulation by multipolar ingestion produces a gastrula with an outer ectodermal epithelial layer surrounding a mass of unorganized cells, which will eventually organize into the endodermal epithelium. Gastrulation is followed by the secretion of a thick cuticle in which the embryo persists in diapause from 2–24 weeks [50]. Juvenile Hydra eventually hatch from the cuticle stage, bypassing the typical planula larva stage common to most cnidarians.

The temporal and spatial expression patterns of several Wnt signaling components during Hydra embryogenesis have been reported [51]. RT-PCR analyses indicate that Hydra orthologs of Fzd, β-cat, and Tcf are maternally supplied and persist throughout embryogenesis. HyWnt transcripts, however, were not detected until the cuticle stage, after gastrulation is complete [51]. In situ hybridization experiments indicate that HyTcf and Hyβ-cat are expressed uniformly throughout the embryo up to the gastrula stage, but spatial expression patterns for HyFzd and HyWnt were not reported [51].

At present, the embryonic gene expression data available are insufficient to establish a role for Wnt/β-catenin signaling in axis formation/patterning during Hydra embryogenesis. It will be of interest to determine precisely the spatial expression patterns of HyWnt and Hyβ-catenin during embryogenesis, and to examine HyTcf and Hyβ-catenin expression at later stages of development to see if they are region or germ-layer specific. Further experiments might also address the dynamics of β-catenin protein regulation during embryogenesis to establish whether or not nuclear localization of β-catenin is associated with axis formation and endoderm formation in Hydra. Experimental data are available from several other hydrozoan species (e.g., Podocoryna, Clytia (=Phialidium), and Hydra) with regards to how axial properties are established and the role of polarity during embryogenesis [52–54]. Examining Wnt signaling in these embryonic systems will be crucial for understanding the role of this pathway in developmental patterning.

4. Wnt signaling in Anthozoans—the sea anemone Nematostella vectensis

The use of several hydrozoan model systems has provided important insights into how embryonic polarity is established and how body axes are generated and patterned. It must be kept in mind however, that hydrozoans, and Hydra in particular, may not display ancestral cnidian developmental patterns [19,20]. The anthozoan Nematostella vectensis is proving to be a valuable addition to the traditional cnidian model systems for studying the origin and evolution of developmental signaling pathways [55]. The starlet sea anemone, N. vectensis, is a small, solitary anemone with a simple life cycle. Nematostella is easily cultured in the lab, can be induced to spawn year round, and produces hundreds of embryos in a single spawning event [56,57]. Sexes are separate and fertilization is external. As with other cnidarians, cleavage is unipolar and the zygote undergoes a variable cleavage program giving rise to a hollow coeloblastula. Gastrulation is by invagination and produces a ciliated, bilayered planula. Four to five days after fertilization, the planula transforms into a juvenile four-tentacle polyp constructed of an outer ectodermal layer and an inner endodermal layer, which lines the gastric cavity. Nematostella is also capable of asexual reproduction via transverse fission and regeneration [56].

Unlike Hydra, Nematostella does not enjoy the benefit of a long history as an experimental animal; to the contrary, it is a relatively new player in the field [55]. Despite the short tenure of Nematostella as a model cnidian system, quite an impressive body of work has been amassed over the past couple of years by several labs. Orthologs of genes known to play critical developmental functions in bilaterians, (e.g., Hox, Fox, Brachury, and various ‘mesodermal’ genes) have been cloned and their embryonic expression patterns determined [22,23,25,29,30,58–61]. Furthermore, many molecular and genomic tools have been developed including BAC, EST, and cDNA libraries, in situ hybridization and immunohistochemistry protocols, and a genome project that is near completion (DOE Joint Genome Institute (http://www.jgi.doe.gov); Daniel Rokhsar, JGI Eukaryote Program Lead).

4.1. A diversified family of Wnt genes was already present in basal cnidarians

The Wnt genes comprise a large, multi-gene family in bilaterians. Completed genome projects have revealed the presence of 19 Wnt genes in human and mouse, seven in Drosophila and five in C. elegans. Phylogenetic analyses sort these Wnt genes into 12 subfamilies, of which only six are represented in the ecdysozoans. Additional Wnt sequences from representative lophotrochozoans provide an estimate of nine subfamilies present in the common ancestor of all bilaterians, with subsequent gene loss in Drosophila and C. elegans [62]. Only a single Wnt ortholog (HyWnt3) was recovered from Hydra [40], suggesting that expansion of the Wnt gene family occurred after the divergence of cnidarians and bilaterians. However, a recent paper by Kussnerow et al. [30] has shown that nearly all of the Wnt gene diversity exhibited in chordates was already present at the split between cnidarians and bilaterians.

Twelve Wnt genes, representing 11 of the 12 subfamilies, have been isolated from Nematostella vectensis [30]. The Nematostella genome contains single orthologs of Wnt1, Wnt2, Wnt4, Wnt5, Wnt6, Wnt10, Wnt11, and Wnt4 and two representatives each from the Wnt7 and Wnt8 subfamilies, but lacks a Wnt9 ortholog. The two NvWnt7 and two NvWnt8 sequences...
Wnt/APC regama, personal communication), identified in canonical and non-canonical Wnt signaling cascades have been complete assembly of the sea anemone genome. However establishing if this is the case will need to await com-

lers such as human WntX of the mom2 orthologs representing 11 Wnt cluster existed in the cnidarian–bilaterian NvWnt1–NvWnt6-NvWnt10 clusters and NvDkk1/2/4 (orthology: Fzd1/2/7, Fzd4/9/10, Fzd5/8, and Fzd3/6 [66]. Searches of the NCBI Trace Archives (www.ncbi.nlm.nih.gov) indicate the presence of two representatives from the Fzd1/2/7 class, two from Fzd4/9/10, and two from Fzd5/8 in the Nematostella genome, however no orthologs from the Fzd3/6 class have been found as yet. Four Fzd genes are present in Drosophila, and three in C. elegans. A single Fzd gene, most closely related to human Fzd7, has been identified in Hydra [41]. The pres-

ence of multiple Wnt and Fzd genes in Nematostella, a “simple” metazoan, indicates that expansion of these gene families (and others) occurred very early in evolution, and also highlights the fact that genomic complexity is not necessarily tightly coupled with morphological complexity [30–32].

4.2. Wnt expression patterns in Nematostella suggest a role for Wnt signaling in axial patterning and gastrulation

Expression patterns for all of the Nematostella Wnt genes have been determined by in situ hybridization, and the results are intriguing [30]. Most are expressed along the oral–aboral axis, and are associated with the blastopore of the gastrula and/or the oral region of the planula [30]. Four genes, NvWntA, NvWnt1, NvWnt4, and NvWnt2 are expressed only in endoderm in the region of the blastopore beginning at gastrulation. The expression domains are serially arranged along the oral–aboral axis, with NvWntA at the oral end and NvWnt2 towards the aboral end. Three genes, NvWnt5, NvWnt6, and NvWnt8, are expressed only in the endoderm after gastrulation, at the planula stage. The expression domains in the endoderm are similarly stag-

ered along the oral–aboral axis, with the NvWnt5 domain most oral, and the NvWnt8 domain most aboral. NvWnt1 is first expressed at the oral end of the planula in ectodermal cells surrounding the pharynx, and later in the endoderm of the tentacles and in a few ectodermal cells (Fig. 2). The expression domains for NvWnt11 differ from the pattern previously reported [30].
NvWnt3 and NvWnt16 expression patterns have also been examined in Nematostella embryos by in situ hybridization (Fig. 2). NvWnt3 expression begins at gastrulation in cells around the lip of the blastopore, similar to NvWnt1, NvWnt2, and NvWnt9 [30] (Fig. 2). It continues to be expressed in a ring of pharyngeal ectoderm through the polyp stage at the oral end of the animal. NvWnt16 expression also begins at the planula stage in the developing pharynx, and the expression domain is restricted to the oral-most pharyngeal endoderm (Fig. 2).

The overlapping, but regionally distinct and germ-layer specific, expression patterns of the Wnts suggest that the ancestral role of these genes was in specifying position along the main body axis [30]. It is also suggested that the blastoporal signaling center, defined by the expression of Wnt, Brachyury, Forkhead, Notch, and Caudal, represents an ancestral system responsible for axial patterning and regulating cell movements and differentiation around the blastopore [8,30]. The Wnt expression results in Nematostella invoke many interesting questions. Which of the Nematostella Wnt genes signal through the canonical Wnt pathway? Are the Wnt/PCP and Wnt/Ca2+ pathways present and active during Nematostella embryogenesis? Which Wnts/Fzds are involved in non-canonical Wnt signaling? That many of the Wnts are expressed around the blastopore at the time of gastrulation raises the possibility that a subset of NvWnts may signal through the Wnt/PCP pathway to regulate gastrulation movements, as is the case with convergent-extension movements during vertebrate gastrulation (reviewed in ref. [14]). Examining expression patterns of components in non-canonical Wnt pathways, such as Flamingo/Starry night or Strabismus/Van Gogh, may help to decipher potential associations between specific Wnts and different Wnt signaling cascades. Future functional studies, using techniques for ectopically expressing Wnts or morpholinos for inhibiting Wnt signaling, may begin to elucidate the developmental functions of the Wnt signal transduction cascade in Nematostella.

4.3. Evidence for Wnt/β-catenin signaling in gastrulation and endoderm specification in Nematostella

The timing and location of expression of several Wnt genes [30] suggest that Wnt signaling is involved in gastrulation and axial patterning in Nematostella. Recently, Wikramanayake et al. [67] described the dynamic regulation of β-catenin during embryogenesis and examined the effects of disrupting Wnt signaling on gastrulation events in Nematostella. The spatial and temporal dynamics of β-catenin protein were tracked in vivo during Nematostella embryogenesis using a Nematostella β-catenin-GFP fusion protein [67]. Nvβ-catenin-GFP protein is initially expressed in all blastomeres, but at mid-cleavage stages the protein is degraded in half of the embryo. The asymmetrical distribution of Nvβ-catenin-GFP persists through the cleavage and early blastula stages, at which time the protein becomes localized to the nuclei of cells in one hemisphere [67] (Fig. 3). Gastrulation is initiated in the half of the embryo with nuclear β-catenin, and cells exhibiting GFP fluorescence enter the blastocoele during gastrulation. In vivo results were supported by immunohistochemical localization of endogenous Nvβ-catenin protein with an anti-β-catenin antibody in cleavage, blastula, and gastrula stage embryos. The same results were observed when Xenopus β-catenin-GFP mRNA was introduced to fertilized Nematostella eggs, suggesting that the mechanism responsible for regulating β-catenin levels was already established at the time cnidarians and bilaterians diverged [67].

Interestingly, the expression of Nematostella Tcf (NvTcf) transcripts follows similar spatial and temporal dynamics as Nematostella β-catenin protein. Maternal NvTcf transcripts are initially expressed uniformly and are later down regulated in the same half of the embryo as Nvβ-catenin during early cleavage stages. NvTcf expression later becomes restricted to the region around the blastopore during gastrulation (Fig. 3). This pattern of a broad Tcf expression domain later refined to potential Wnt signaling centers (the blastopore or the head organizer) is rem-

![Fig. 3](image-url)
In early polyp stages, it is expressed endodermally in the mesenteries, but remains absent from the oral-most regions (modified from [30,34,67]). Tcf [24]. In Hydra, Tcf is initially expressed in all blastomeres, but is later down-regulated in the vegetal half of the embryo. Nuclear β-catenin is localized to the animal half of the embryo in the region where gastrulation occurs [67]. In embryos treated with LiCl, an inhibitor of GSK-3β, -catenin was expanded beyond its normal range—consistent with an autocatalytic feedback mechanism (Fig. 4).

In embryos treated with LiCl, an inhibitor of GSK-3β activity, the domain of nuclear β-catenin was expanded beyond its normal range—β-catenin was localized to the nuclei of all cells of blastula and gastrula stage embryos. These LiCl-treated embryos developed into abnormal planulae with an excess of endoderm, but lacked pharynx and tentacles. Similarly, ectopic expression of an "activated" form of Xenopus β-catenin, which cannot be regulated by phosphorylation and degradation, resulted in β-catenin expression throughout both halves of the embryo and translocation into the nuclei of all blastomeres [67]. In a second set of loss-of-function experiments, either the nuclear localization of β-catenin or the transcriptional activity of β-catenin was inhibited by over expressing the cytoplasmic domain of cadherin or a β-catenin-engrailed repressor chimaeric protein, respectively. In both experiments, treated Nematostella zygotes failed to initiate gastrulation even after 30 h of development when control embryos had completed gastrulation [67]. Based on their findings in Nematostella, the authors suggest a conserved function for nuclear β-catenin in establishing the embryonic axis and specifying germ layers. It remains unclear what might be the signal that activates Wnt/β-catenin signaling in Nematostella embryos. As none of the Nematostella Wnt genes are expressed in overlapping domains in both ectoderm and endoderm along the oral–aboral axis, but are absent from the aboral pole, NvDkk1/2/4 is ectodermally expressed at the aboral pole from gastrula to early polyp stages. In early polyp stages, it is expressed endodermally in the mesenteries, but remains absent from the oral-most regions (modified from [30,34,67]).

5. Potential antagonists of Wnt signaling in cnidarians

Tight regulation of Wnt signaling during embryogenesis is essential for normal patterning and development, and is known to operate at several levels. Cytoplasmic components of the path
way such as GSK-3β, Axin, and Dsh, affect the stability and localization of β-catenin (reviewed in ref. [3]). The Wnts themselves have the potential to modulate signaling as non-canonical Wnts can inhibit the canonical pathway (reviewed in ref. [4]) and Wnt signaling can activate expression of Wnt components in a feedback loop. Finally, secreted antagonists of Wnts also mediate Wnt signaling, such as the secreted Frizzled-related protein (sFRP) family, Wnt inhibitory factor (WIF), Cerberus, and the Dickkopf (Dkk) family, which inhibit Wnt signaling by either binding directly to Wnts or by binding to the Wnt receptor complex [17].

Wnt antagonists are absent from the Drosophila and C. elegans genomes, suggesting that these are deuterostome-specific genes. Members of the sFRP and Dkk families of Wnt antagonists, however, have been identified in Nematostella. The eight vertebrate sFRPs are divided on the basis of sequence similarity into three groups, sFRP1/2/5, sFRP3/4, and sizzled/zissled2/crescent [16,17]. A single sFRP sequence present in Nematostella most closely aligns with the sFRP 1/2/5 group in BLAST analyses. Four Dickkopf genes (Dkk-1 to Dkk-4) and a Dkk-related gene (Joggy) are known from vertebrates [18]. Three unique sequences with homology to the Dkk gene family have been cloned from Nematostella. Phylogenetic analyses group two of these sequences within the Dkk-3 subfamily, forming a sister group to the single Dkk-5 related gene identified in Hydra [68]. A putative Dkk-3 sequence has also been recovered from an EST screen of the coral Acropora millepora [69]. The Dkk-3 subfamily, however, has not been demonstrated to modulate canonical Wnt signaling, and its biological function is not clear [17]. In Hydra, HvDkk-3 is expressed in nematocytes at late stages of cell differentiation [68]. The third Nematostella Dkk sequence, NvDkk1/2/4 [69], clusters with a second Hydra Dkk sequence, HvDkk1/2/4, proposed to be a putative precursor to the Dkk1/2/4 subfamily found in vertebrates [69]. Additionally, two sequences reported as orthologs of Dkk-1 and Dkk-4 were found in an EST collection from the tentacle of the jellyfish, Cyanea capillata [70]. In adult Hydra, HvDkk1/2/4 expression is absent in the head but is present throughout the endoderm of the body column, in a pattern inverse to that of HvWnt69. Overexpression studies of HvDkk1/2/4 in Xenopus embryos suggest that it is a functional ortholog of vertebrate Dkk1 and Dkk4, and that it is capable of inhibiting Wnt signaling [69]. In Nematostella, NvDkk1/2/4 is first expressed at the gastrula stage in the aboral ectoderm (Fig. 2), at the pole opposite that of the NvWnt expression domain, suggestive of a role in limiting the range of Wnt activity. In late planulae and early polyps, NvDkk1/2/4 is also expressed in the endoderm of the developing mesenteries but is absent from the oral-most regions of the animal (Fig. 2). In addition to the sFRP and Dkk sequences, an anthozoan ortholog of another vertebrate Wnt antagonist, WIF-1, has been identified in EST data sets from Nematostella and Acropora [32].

Whether or not these potential antagonists actually regulate Wnt signaling in cnidarians, and what their specific functions are, will need to be established experimentally. What is clear, however, is that the presence of several classes of Wnt antagonists in cnidarians suggests that the mechanisms for modulating Wnt signaling may be evolutionarily ancient, and that both expanded Wnt and Fzd gene families and the complex molecular interactions regulating Wnt signaling were likely already in place in the common ancestor of cnidarians and bilaterians.

6. Conclusions

Components of all three Wnt signaling pathways are present in cnidarians, indicating that this signaling pathway is an ancient metazoan patterning mechanism. The surprisingly large inventory of Wnt genes in Nematostella indicates that this developmentally important gene family was already diversified in the cnidian-bilaterian ancestor. Evidence from several cnidarian species indicates that Wnt/β-catenin signaling has an ancestral role in axis formation/patterning and gastrulation, in support of the idea that Wnt signaling was important in the evolution of axial differentiation and germ-layer specification in early multicellular animals. In hydrozoans, Wnt/β-catenin signaling in the head organizer is involved in establishing, maintaining, and patterning the body axis. In the anthozoan sea anemone, Nematostella, nested sets of expression of the Wnt genes in both ectodermal and endodermal layers during gastrulation suggest that the Wnt/PCP pathway may be involved in epithelial patterning along the oral-aboral axis. Furthermore, the selective stabilization of β-catenin at the future site of gastrulation occurs in a Wnt-independent manner, and is required for gastrulation movements and the activation of endomesodermal gene expression [22,60,61]. Despite what is already known about Wnt signaling in cnidarians, considerable work remains to be performed in order to understand how the various Wnt pathways are regulated and what their roles are in embryonic and adult patterning in cnidarians.

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