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The Development of Form: Causes and Consequences of Developmental Reprogramming Associated with Rapid Body Plan Evolution in the Bilaterian Radiation

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Abstract Organismal form arises by the coordinated movement, arrangement, and activity of cells. In metazoans, most morphogenetic programs that establish the recognizable body plan of any given species are initiated during the developmental period, although in many species growth continues throughout life. By comparing the cellular and molecular development of the bilaterians (bilaterally symmetrical animals) to the development of their closest outgroup, the cnidarians, it appears that morphogenesis and the cell fate specification associated with germ layer formation during the process of gastrulation are separately controlled by two distinct downstream pathways (canonical and planar cell polarity) of Wnt signaling. Furthermore, a fundamental change in the early developmental program, the positioning of the site of gastrulation, allowed the spatial separation of gene regulatory networks that facilitated the rapid diversification of bilaterian body plans.

Keywords Axial organization · EvoDevo · Oral–aboral axis · Wnt signaling

There is no escaping the fantastic diversity of organismal form on this planet. From single-celled organisms like radiolarians and diatoms, to meiofaunal animals that live between grains of sand, to large, charismatic megaflora/fauna such as some trees and mammals, these forms arise across many scales of magnitude through complex interactions of environment and cellular design. D'Arcy Thompson's *On Growth and Form* (1917) recognized that organisms were made of cells, and that cells had certain inherent material properties that both dictated, and limited, the outcomes they could generate. Thompson's ideas had a clear influence on the field of evolutionary development, particularly in the "pregenomic era"—from using soap bubbles to model minimum free energy for the packing of embryonic blastomeres, to examining the costs of generating sister cells of unequal cell sizes at mitosis, to exploring modifications of allometric growth of developmental fields as a means to generate variation of form in adult structures. This lesson, that the cell is the fundamental biological unit of all biological form, is all the more relevant now as improvements in optical imaging, computational modeling, and molecular techniques have provided us with new approaches for understanding cellular mechanisms driving morphological diversity in a phylogenetic context.

A more modern understanding of the origin of biological form requires not only an appreciation of material constraints, but of their evolutionary origins and developmental trajectories. More robust sampling and molecular phylogenetic techniques have given us new confidence in establishing ancestral character states and the direction of evolutionary change. This foundation, along with sophisticated methods of studying cellular behavior during development, has provided new insight into the evolution of morphogenesis. This review examines how recent advances help shape our understanding of the molecular regulation of cell behavior during development of a group of animals (cnidarians) that represent

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an evolutionary springboard for the rapid radiation of animal body plans.

Gastrulation Plays a Pivotal Role in Early Body Plan Evolution

One of the most ancient and fundamental mechanisms driving morphogenesis (the change of form) in multicellular animals is epithelial folding. Epithelia are sheets of cells connected laterally by cell adhesion molecules and junctional proteins, and were likely to be the first tissue type that appeared in metazoan evolution (Tyler 2003). It has long been hypothesized that the earliest metazoans were ciliated epithelial organisms (reviewed in Willmer 1990). During morphogenesis, epithelial sheets can be modified in a number of ways, such as invagination, evagination, branching morphogenesis, and epithelial-to-mesenchymal transformations, to affect the size and shape of adult structures during the developmental period. These modifications are not only relevant to the evolution of the three-dimensional form of resulting structures, they are also important emergent properties in the evolution of morphological complexity due to their roles in embryonic induction and patterning events. The differential growth of epithelial tissues affects not only the size and shape of adult structures, but also plays an important role during development by bringing different tissues into close proximity and allowing novel inductive tissue interactions. A classic example of this type of epithelial interaction, which results in the formation of complex integrated structures in the correct location, is the formation of the vertebrate eye where the interaction of the optic cup with the overlying surface ectoderm initiates a sequence of inductive events that give rise to the lens, optic vesicle, and cornea (Jacobson 1966; Grainger 1992).

Triploblastic animals (also called bilaterians) possess derivatives of three definitive germ layers (called ectoderm, mesoderm, and endoderm) that arise through the process of gastrulation, in which a distinct population of endomesodermal precursor cells (that subsequently gives rise to both endoderm and mesoderm) becomes spatially and molecularly distinct from overlying ectodermal epithelia during embryogenesis. Endodermal tissues give rise to the absorptive lining of the gut and its derivatives such as digestive glands and lungs in terrestrial animals, while mesodermal tissues give rise to muscle, blood, connective tissue, coelomic cavities, nephridia/kidneys, and the somatic portions of gonads. The ectoderm gives rise to skin and nervous tissue. Mesodermal and endodermal tissues arise by the activated differential expression of genes in subpopulations of the endomesodermal precursor cells (Leptin 2005).

From ontogenetic and phylogenetic standpoints the role of epithelial morphogenesis during gastrulation is critically significant for understanding the developmental basis of body plan evolution. Both endoderm and mesoderm are clearly important tissues because not only do they allow for more complex topological morphological states, but their origin also allows functional specialization of each tissue, thereby promoting the evolution of new cell types and tissue specialization. During gastrulation cellular behaviors mediate the internalization of cells either as intact epithelia (invagination), or as individual cells (ingression and delamination). Thus, the positioning of cells in appropriate places in the embryo and the activation of specific cellular signaling pathways lead to the normal development of each germ layer. Gastrulation, therefore, provides a powerful system for understanding both the control of cellular behavior responsible for the morphogenetic movements that drive the origins of form, and also the molecular control of cell fate specification and diversification that results in the origins of unique cell types.

Phylogenetic Insight into the Origins of Complex Metazoan Body Plans

Considerable insight into the evolution of epithelial morphogenesis and its role in gastrulation can be revealed by examining early-branching metazoan lineages whose common ancestor had less complex germ layers and cell types relative to later-appearing bilaterian animals. One such lineage is the Cnidaria, the well-accepted sister-group of all bilaterian animals (Hejnol et al. 2009; Fig. 1). Cnidarians, which include sea anemones, corals, and medusoid "jellyfish," are important model organisms as diploblastic "epithelial animals" consisting of an outer epidermis (i.e., ectoderm) and an inner gastrodermis that lines the gastric cavity (i.e., endoderm), but lacking a middle cellular layer (i.e., mesoderm) that triploblastic bilaterian animals possess (Technau and Steele 2011). Thus, the developmental and morphological elaboration of cnidarian body plans occurs largely by epithelial morphogenesis, although epithelial-to-mesenchymal transitions, another important morphogenetic process well studied in bilaterian animals, are also well-documented (Mergner 1971; Byrum and Martindale 2004). The ability to form a functional cnidarian body plan by epithelial folding is exemplified by the process of gastrulation in the anthozoan developmental model system, *Nematostella vectensis,* which generates its gastrodermis (the lining of the gut cavity), and subsequently its pharynx (the thickened epithelial region leading to the oral opening), by coordinated cell shape changes and epithelial buckling (Kraus and Technau 2006; Magie and Martindale 2008) during gastrulation (future oral pole).

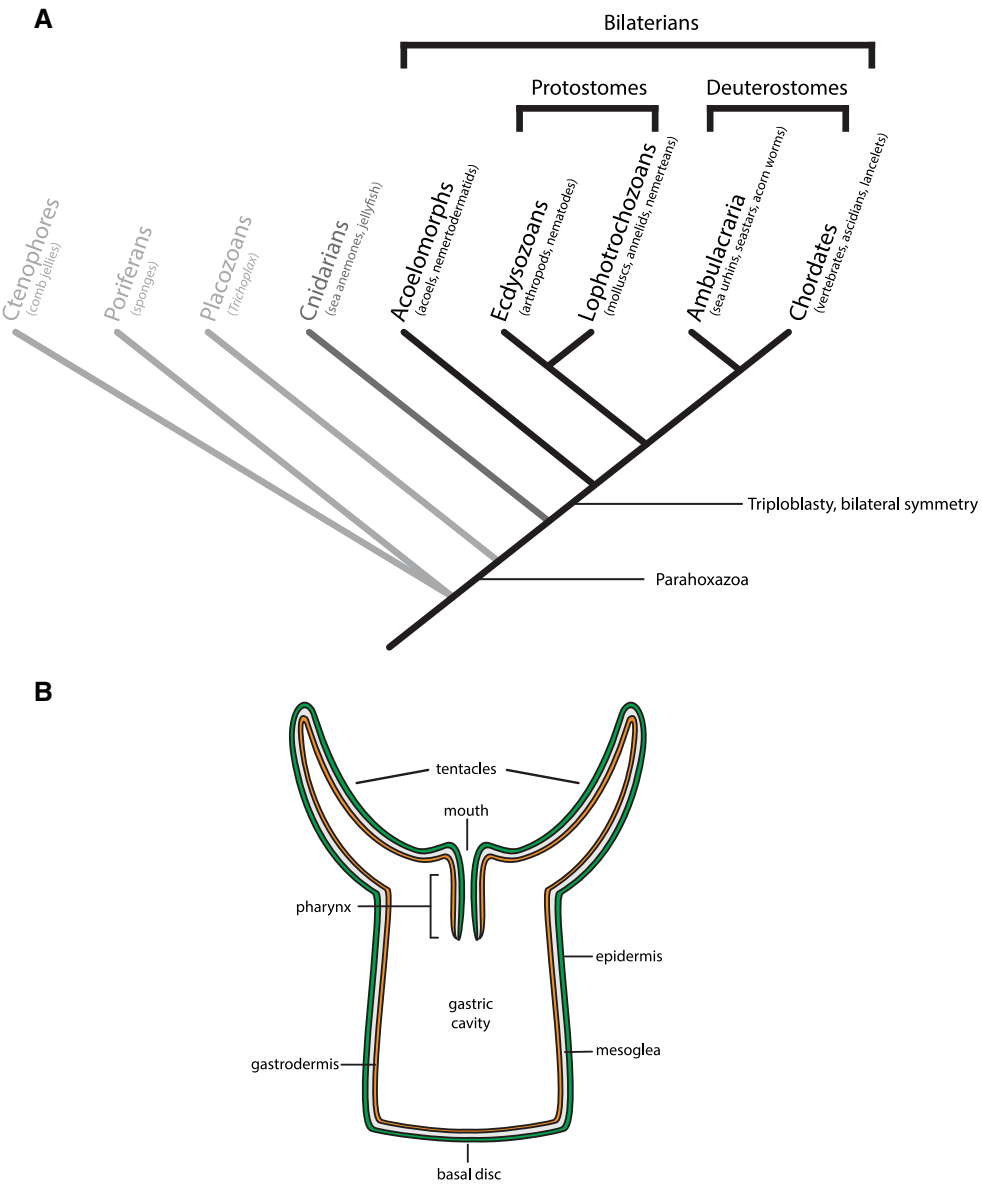

Fig. 1 **a** Phylogenetic tree showing the position of cnidarians as the sister-group to the triploblastic bilaterian clade (Hejnol et al. 2009). Note that it is currently not possible to place the relative branching order of ctenophores and sponges at this time (Ryan et al. 2010). **b** Schematic diagram of the anthozoan cnidarian body plan. A single opening to the gastric cavity (mouth) leads to the pharynx. The entire gastric cavity, including the tentacles, are lined by the gastrodermis, an endomesodermal tissue containing a complex nerve net formed from cells derived from the animal pole of the embryo

Epithelial Animals and Modeling Cell Behavior

Mathematical modeling of the morphogenetic process of gastrulation in *Nematostella* has provided unique insight into the origins of epithelial morphogenesis in metazoan body plan formation. Tamulonis et al. (2010) used a cell-based computational model to identify crucial components of the gastrulation process and tested it against high-resolution confocal micrographs from which accurate morphometric data were obtained. They found that an apical surface stiffness ten times greater than basolateral surfaces was required to maintain blastula wall integrity, and that among the principal parameters driving gastrodermal invagination was a reduction in lateral cell adhesion, which spontaneously formed bulging "bottle" cells and "squatters" (cells that maintain their lateral edge adhesivity and retain parallel lateral edges). The apical constriction of cells causes elongation along the apical–basal axis, and is sufficient to buckle the epithelial wall, which is under surface strain. The model also showed that the increase in the volume of gastrodermal cells is, in part, a function of the phagocytosis of blastocoelic fluid by gastrodermal cells (Magie et al. 2007), and that gastrodermal filopodia play an important role in the "zippering" of gastrodermal cells as they are the motor that drives the continued invagination of gastrodermis toward the aboral pole (Tamulonis et al. 2010). Thus, an integrated understanding of the properties and behavior of cellular phenomena gives insight into the origins of complex morphological form.

Molecular Regulation of Cell Shape Change and Initiation of Gastrulation Movements

The simulations of Tamulonis et al. (2010) demonstrate how visco-elastic forces and simple changes in cell behavior can drive epithelial morphogenesis; however, these models do not predict the underlying molecular regulation or the location of the organized changes in cell shape and adhesion that occur in the developing embryo and that are largely associated with the site of gastrulation. These events do not occur at random sites in the embryo. In cnidarians, the site of gastrulation (also called the blastopore) corresponds to the site of the future oral opening, and thus defines the polarity of the oral–aboral axis (Fig. 1).

Recent work using immunohistochemistry and functional molecular knockdowns (Kumburegama et al. 2011) has shown that in *Nematostella* the cell shape changes responsible for epithelial buckling and the invagination of presumptive gastrodermis are controlled by a highly conserved signaling pathway (Fig. 2), called the Planar Cell Polarity (PCP) pathway. This is a non-canonical Wnt pathway that regulates cell shape changes and cytoskeletal dynamics in a wide variety of systems (Wang and Nathans 2007; Simons and Mlodzik 2008). In *Nematostella*, the membrane-associated PCP pathway protein, Strabismus, is maternally expressed and enriched at the animal pole of the egg. During cleavage and subsequent development, Strabismus becomes progressively more restricted to the animal pole and to the precise location of the site of gastrulation. Functional experiments using Strabismus-specific gene knockdown in *Nematostella* showed that this protein is necessary for the apical constriction that initiates bottle cell formation and epithelial buckling at the site of gastrulation (Kumburegama et al. 2011). The maternal localization of this protein in *Nematostella* is interesting because in bilaterians, a transmembrane Wnt receptor called frizzled is required to activate the PCP pathway through Strabismus, and in *Nematostella*, a *frizzled* gene (*frizzled-10*) co-localizes with Strabismus at the animal pole (Kumburegama et al. 2011). The maternal localization of Strabismus and frizzled to a highly restricted spatial domain is responsible for the activation of the PCP pathway and determination of the site of epithelial folding that initiates the gastrulation morphogenetic process. This is an example of how intercellular signaling pathways like the Wnt pathway, which have multiple roles throughout later stages of embryonic development, are initially used to pattern the embryo even before the first mitotic cleavage (in the sister group to the Bilateria).

Distinct Regulatory Pathways Regulate Morphogenetic Versus Cell Fate Decisions

The demonstration that downstream components of the PCP pathway are positioning the site of gastrulation and regulating epithelial folding by polarizing the *Nematostella* zygote is also important for elucidating what the PCP pathway is *not* doing. Kumburegama et al. (2011) showed that gastrodermal gene expression requires the canonical Wnt signaling pathway (or the Wnt/β-catenin pathway), which operates through the stabilization and subsequent nuclearization of the bifunctional protein, β-catenin (Fig. 2). The stabilization of β-catenin is mediated by the Disheveled (Dsh) protein, which is also enriched in the cell membrane at the site of gastrulation and endomesoderm formation by virtue of its association with the pronuclear envelope at the site of polar body formation (Lee et al. 2007; Kumburegama et al. 2011). Conversely, blocking canonical Wnt signaling using a Dsh dominant-negative does not affect primary invagination in *Nematostella*, demonstrating that the two processes can be uncoupled from one another. Thus, it appears that distinct components of Wnt signaling pathways are used to specify the fate of an epithelium via networks of differential gene expression (Wnt/β-catenin pathway), and to control the morphogenetic movements of an epithelium through cell shape change and differential adhesivity (PCP pathway).

The dissociation of morphogenetic movements of subsets of cells at gastrulation from the ultimate fates of internalized cells by different components of the Wnt signaling pathway begs the question of which one might be more ancient in metazoan patterning. The Wnt pathway is a metazoan invention and components of the pathway have been shown to exist in all three metazoan lineages that branched prior to the cnidarian-bilaterian clade (sponges, ctenophores, and *Trichoplax*) (Adamska et al. 2007, 2010; Srivastava et al. 2008; Pang et al. 2010), although there is no evidence that this pathway was involved in gastrulation prior to the origin of the Cnidaria. Thus, downstream components of a single extracellular signaling pathway were exploited for distinct cellular and developmental trajectories during metazoan evolution. Without additional functional studies on earlier branching taxa, it is currently impossible to determine the relative order in which different components of the Wnt pathway were co-opted in the evolution of gastrulation.

The Site of Gastrulation has Changed in Bilatarians

While it is often difficult to reconstruct the precise details of the construction of elaborate molecular pathways and their role in the evolution of complex morphological traits,

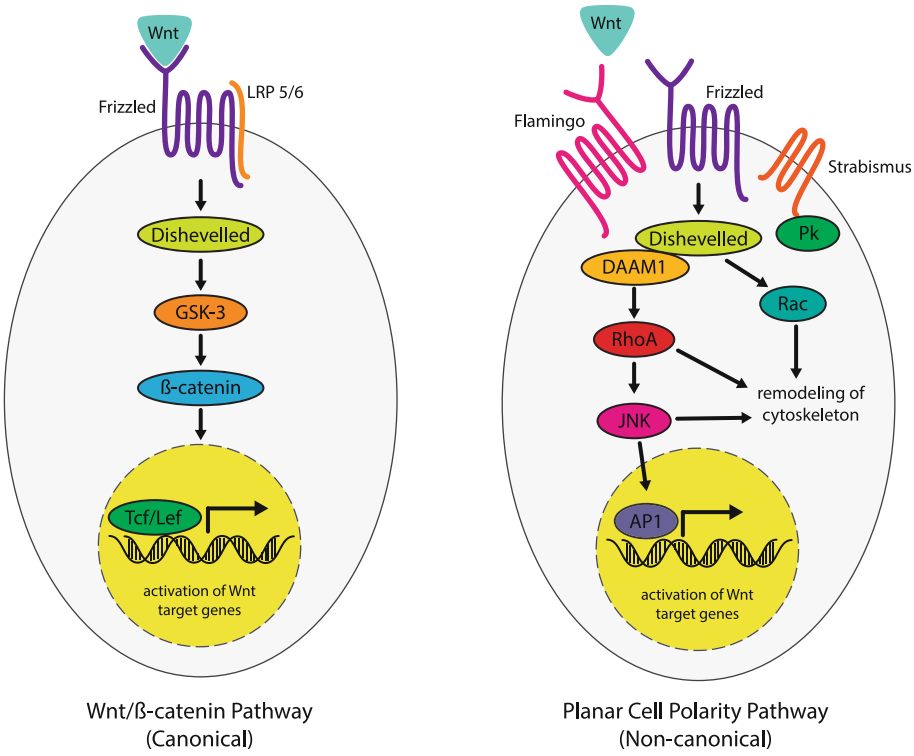

Fig. 2 Diagram indicating two different signaling pathways downstream of the extracellular Wnt ligand. The canonical pathway operates through GSK-3 and the nuclear localization of β-catenin to influence target gene expression. The non-canonical PCP pathway (planar cell polarity) is β-catenin independent and has primary effects on the target cell's cytoskeleton, affecting cell shape and behavior. (Adapted from Lee et al. 2006; Lai et al. 2009)

it is clear that the vast majority of gene families, cell–cell signaling systems, and tissue types existed in the common ancestor of cnidarains and bilaterians (Putnam et al. 2007)—yet 99 % of all animal species are found in bilaterian lineages (Collins and Valentine 2001). What could have happened in the line leading to bilaterian lineages that gave rise to the dramatic increase in body plan morphological complexity and their ecological success? Comparative genomic studies have shown that the genomic complexity is surprisingly similar in cnidarians and bilaterians, eliminating the massive invention of new genetic potential to explain the bilaterian radiation (Putnam et al. 2007). We suggest several fundamental changes in developmental processes that played an important role in facilitating this observed body plan diversification (Martindale 2005; Martindale and Hejnol 2009).

One traditional tool used by developmental biologists to compare the development of different metazoan species is called fate mapping. Fate mapping entails labeling a defined region of a developing embryo and determining the ultimate contributions that these labeled cells/regions make to the functional larval or adult organism. It is the only way to determine the embryological origin of larval and adult structures or cell types and provides additional insight into the homology of morphological structures between related organisms. In some animal taxa, such as those that display spiral cleavage (Wilson 1898; Henry and Martindale 1998; Hejnol et al. 2007; Lambert 2010), embryonic cells can be uniquely identified in every embryo examined, making fate-mapping experiments highly informative. However, in many other metazoan groups such a stereotyped cleavage program is not present, limiting the kinds of comparisons that can be made between distantly related organisms.

One feature of animal development that can be readily compared by fate-mapping experiments is the axial organization of the fertilized egg. Eggs have a polarity called the animal–vegetal axis. The animal pole is defined as the site on the oocyte where the polar bodies, products of the meiotic reduction divisions, are extruded. The vegetal pole is defined as the region of the embryo that is opposite the animal pole. The animal and vegetal poles can be identified and labeled in most animal taxa making these sites amenable for comparative developmental fate mapping, even in very distantly related groups.

Experiments labeling the embryonic poles reveal one of the fundamental changes in the early developmental process during the cnidarian–bilaterian transition that may have led to the rapid diversification of bilaterian animals. In virtually all bilaterian embryos examined, if one labels cells derived from the vegetal pole, they can be found in the developing endodermal gut and its associated derivatives (Fig. 3). If one labels cells derived from the animal pole they give rise to the anterior head (ectoderm) and brain region of the adult. Thus, in bilaterians, the vegetal pole corresponds to the site of gastrulation/endomesoderm formation while the animal pole region corresponds to the "head" region.

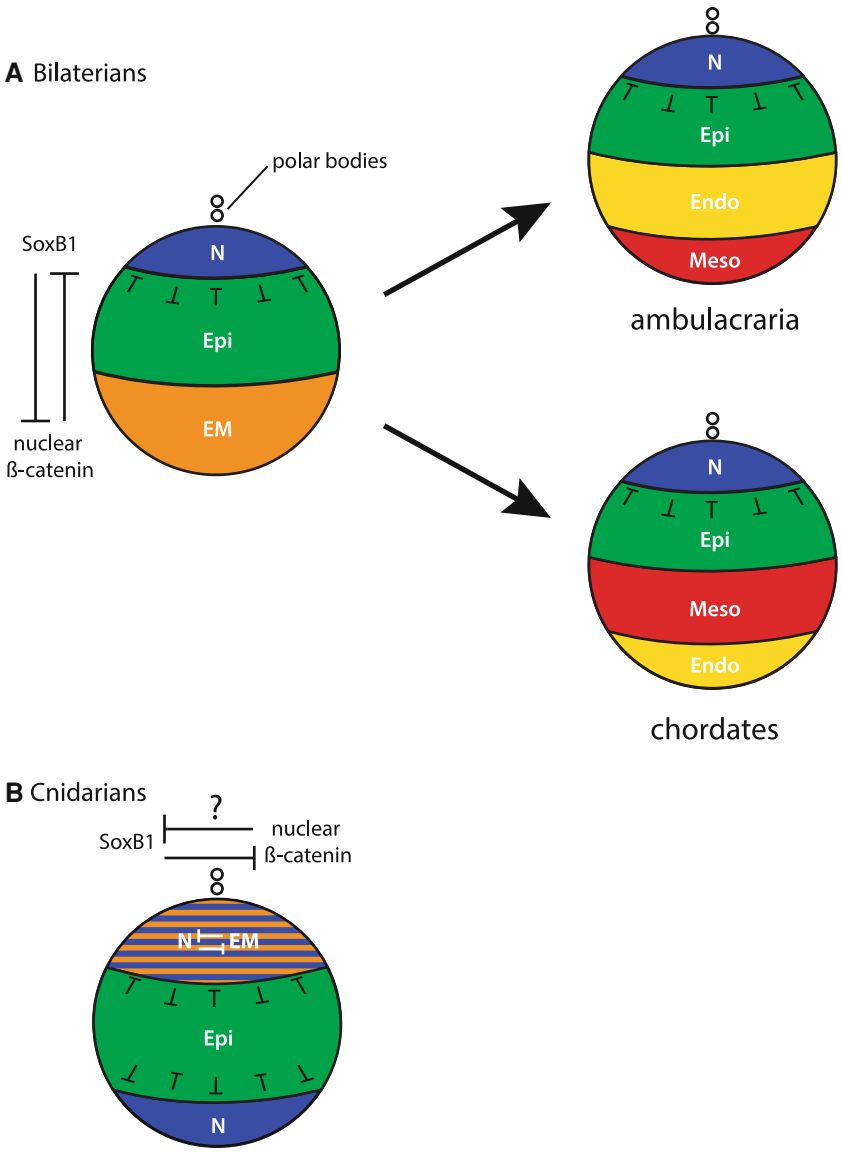

Fig. 3 The site of gastrulation is different in bilaterians and cnidarians (and ctenophores). **a** In bilaterians the origin of endomesoderm (EM), the region that gives rise to endodermal and mesodermal derivatives, occurs at the vegetal pole. Note that mesodermal tissues are derived from the vegetal pole in echinoderms and hemichordates (ambulacraria) but in chordates, endoderm arises from the vegetal pole. Tissues derived from the animal pole region (indicated by the two small polar bodies) give rise to neural (N) and epidermal (Epi) fates. Molecular studies have shown that there is a dynamic interaction between genes such as β-catenin and *Sox* transcription factors that regulate endomesodermal and neural fates along the animal vegetal axis. **b** In cnidarians, the site of gastrulation that gives rise to endomesoderm arises at the animal pole, not the vegetal pole. Endomesodermal tissues in cnidarians are also heavily neuralized, indicating that complex cell fate decision-making is made in these animal pole derivatives. Additional neural tissue, a transient larval apical tuft of sensory cilia, is also generated at the vegetal (aboral) pole of cnidarians. Interestingly, both Sox and β-catenin are expressed in cells from the animal pole. It is of interest how the change in the site of gastrulation influences the interaction of the canonical β-catenin signaling pathway and neural cell type specification in derivatives of the animal pole

If the same fate-mapping experiment is performed in cnidarians or embryos from the phylum Ctenophora (the comb jellies) (Freeman 1977), another group of animals that evolved prior to the cnidarian–bilaterian clade (Fig. 1a), however, a much different result is seen (Freeman 1981a, b; Martindale 2005; Lee et al. 2007; Martindale and Hejnol 2009). If the animal pole region of the embryo is labeled, it marks the site of gastrulation and labels the gastrodermal cells that line the gastric cavity (Fig. 3). If the vegetal pole of cnidarians is labeled, the side opposite the mouth (the aboral pole) is labeled in juvenile polyps. Thus, the ancestral site of gastrulation and the formation of endoderm occurs at the animal pole of these embryos, not the vegetal pole, as is seen in all other animal

taxa, and this scenario likely represents that of the ancestral eumetazoan (all metazoans except for sponges, which have no structure homologous to a mouth or endoderm).

The Gastrodermis of Cnidarians Represents the Evolutionary Precursor of Both Bilaterian Endoderm and Mesoderm

The gastrodermis of cnidarians is a complex tissue. It not only contains cells that absorb nutrients digested in the gut cavity, it also contains myoepithelial cells that have basal actin-myosin based projections capable of generating planar contractile forces against adjacent epithelial cells. Because of these two distinct properties, the bifunctional gastrodermis can be thought of as an endomesodermal tissue. This designation is supported molecularly, as the gastrodermis in *Nematostella* expresses genes that are normally expressed in the endoderm or the mesoderm of bilatarians (Technau and Scholz 2003; Fritzenwanker et al. 2004; Martindale et al. 2004; Technau et al. 2005). These anatomical, functional, and molecular data are consistent with the notion that the cnidarian bifunctional endomesoderm is the evolutionary precursor of the distinct endodermal and mesodermal germ layers found in bilaterian lineages.

Recent cellular and molecular investigations reveal that the cnidarian gastrodermis is even more complex than was previously appreciated (Nakanishi et al. 2008; Marlow et al. 2009; Seipp et al. 2010; Piraino et al. 2011). In addition to digestive/absorptive and contractile properties, the gastrodermis also contains the vast majority of neural complexity found in the organism. While the outer ectoderm possesses a variety of different sensory cells and cnidocytes (the "stinging" cells unique to cnidarians), the gastrodermis contains a complex array of ganglion cells that generate the "endodermal nerve net" (Marlow et al. 2009). These ganglion cells make contact with other neural cells, and likely also regulate global contractile functions of the gastrodermis and feeding tentacles (also lined by gastrodermal cells). Precursors of these neural cells appear to arise locally from gastrodermal tissue that forms during the gastrulation process. Thus, it could be argued that, like the majority of neural tissue in bilaterian forms, the majority of neural progenitors in cnidarians arise from the animal pole.

Is it Possible to Change the Site of Gastrulation in Metazoan Development?

If there were a shift in the site of gastrulation from the animal pole to the vegetal pole during metazoan evolution, how might this have occurred? In most bilaterian embryos the animal–vegetal axis is maternally specified. In bilaterians, endomesoderm formation is restricted to the vegetal pole by the localized stabilization, nuclearization, and activation of the Wnt/β-catenin pathway in endomesodermal precursors at that pole (Weitzel et al. 2004; Petersen and Reddien 2009). There is molecular data from *Nematostella* that may provide insight into how the site of gastrulation became dissociated from the formation of the oral opening in bilaterians. Regulators of downstream components of the Wnt signaling pathway (Disheveled for the canonical pathway and Strabismus for the PCP pathway), are found to be associated with the membrane of the female pronucleus in *Nematostella* and some corals (Lee et al. 2007; Kumburegama et al. 2011). In cnidarians, the female pronucleus is normally located at the animal pole and becomes localized to the site of first cleavage (Lee et al. 2007). However, this polarity is not strictly and irreversibly determined in cnidarians, and the site of first cleavage and subsequent gastrulation/endomesoderm formation can be altered by spatially moving the position of the female pronucleus prior to first cleavage (Tessier 1931; Freeman 1981a, b). Thus, the position of gastrulation and endomesoderm formation in bilaterian embryos may have become fixed at the vegetal pole by the maternal localization of components activating the Wnt/β-catenin signaling pathway. This provides a concrete and plausible cellular and molecular mechanism by which the position and formation of the oral opening can become dissociated from the site where the tissue that will ultimately give rise to endomesoderm occurred in the bilaterian lineage of animals.

Significance of the Change in the Site of Gastrulation for Bilaterian Body Plan Evolution

These simple observations suggest the following evolutionary scenario. In cnidarians (the sister group to the Bilateria), the oral opening forms at the animal pole. The cnidarian animal pole also gives rise to a complex gastrodermal tissue that possesses both endomesodermal and neural cell types. Prior to the radiation of the Bilaterian lineage, the site of gastrulation (origin of endomesoderm) changed from the animal pole (seen in cnidarians and ctenophores) to the vegetal pole, while the mouth and the primary region of adult neurogenic potential remained at the animal pole (Fig. 3) (Martindale and Hejnol 2009). This spatial dissociation of tissue forming regions would have had profound evolutionary consequences for both regions. Formation of multiple cell types in the gastrodermis of cnidarians appears to be the result of complex molecular pathways of cell–cell interactions regulating

gene regulatory networks (GRNs) that give rise to a variety of neural and endomesodermal cell types. Presumably the change in location of gastrulation in the bilaterian ancestor relaxed the antagonism between these feedback networks and might have allowed the expansion of new regulatory pathways leading to the evolution of novel cell types.

The consequences of the change in the site of gastrulation on cell-type specification can be tested by examining the deployment of molecular regulators of tissue-specific gene regulatory networks between cnidarians and bilaterians. For example, an existing interaction between the nuclearization of Sox transcription factors and activation of canonical Wnt/β-catenin pathway signaling supports the proposed consequences of the change in the site of gastrulation. In a wide variety of animals, many *Sox* gene family members, including the *Sox B* group (*Sox B1*, *Sox B2*, and *Sox B3* in vertebrates), are involved in neural specification (Pevny et al. 1998; Sasai 2001; Overton et al. 2002). A large body of work in bilaterians, particularly in developing sea urchin embryos (deuterostomes), has shown that there are direct and indirect antagonisms between *Sox B* group genes (e.g., *Sox B1*) and nuclear β-catenin signaling (Kenny et al. 2003; Zhang et al. 2003; Angerer et al. 2005). In bilaterian animals β-catenin becomes nuclearized in presumptive endomesderm at the vegetal pole, while *Sox* gene expression becomes stabilized in ectodermal derivatives of the animal pole where it appears to function in maintaining cell pluripotency in preparation for neural specification (reviewed in Pevny and Placzek 2005). In contrast, the two *Sox B* genes (Magie et al. 2005) are expressed at the animal pole in the same cells in which β-catenin becomes localized to the nucleus in *Nematostella* (Wikramanayake et al. 2003).

Exactly how both endomesoderm and ganglion cell specification are regulated in derivatives of the same spatial domain in *Nematostella* remains unclear. In bilaterians, the change in location of the site of gastrulation and endomesoderm formation from the animal pole to the vegetal pole would presumably alleviate any direct antagonism of the Sox and canonical Wnt regulatory pathways in the same tissue domain. Neurogenic regions derived from the animal pole would then be free to expand and diversify to give rise to increased number and complexity of sensory organs (e.g., eyes, olfactory) associated with feeding, predator avoidance, and settlement/habitat choice as well as more complex integration of sensory motor function, including the elaboration of oral feeding structures and the consolidation of neural structures into primordial anterior ganglia (brain) could evolve. Thus, the movement of the site of gastrulation (endomesoderm formation) to the vegetal pole facilitated the evolution of the molecular pathways for novel neural cell types and neural integration at the anterior region of developing bilaterian ancestors.

The Evolution of a New Germ Layer: Mesoderm

Another likely consequence of the movement of endomesodermal tissue to the vegetal pole in the bilaterian lineage that would have had profound consequences on body plan evolution was the appearance of two distinct germ layers, mesoderm, and endoderm, from the bifunctional endomesoderm (Fig. 4). Endodermal cells became largely responsible for digestion and absorption of food in the gut. The origin of mesodermal tissues, including muscle, connective tissue, the circulatory system, blood cells, gonads, and osmoregulatory organs/nephridia is arguably one of the most crucial innovations in metazoan animals, allowing the rapid diversification of body plans, including muscular locomotion, the appearance of lateral appendages, and an increase in body size that promoted distinct feeding strategies and invasion of unique ecological niches.

Insight into how these new germ layers appeared will be facilitated through a better understanding of the gene regulatory networks that operate in these distinct tissues. Driven by work initiated in echinoids, good molecular evidence from a variety of metazoans supports the idea that in the last common ancestor of bilaterians there was a core set of genes involved in the specification of endomesoderm from ectoderm during development (Loose and Patient 2004; Davidson 2006; Davidson and Erwin 2006). Many of the genes found in bilaterian endomesodermal GRNs are also expressed in the *Nematostella* bifunctional endomesoderm, suggesting that this ancestral GRN was present prior to the segregation of distinct endodermal and mesodermal germ layers and subsequently divided into two distinct germ layer-specific GRNs in bilaterian lineages. However, other genes such as members of the Nodal signaling pathway, important for mesodermal specification and patterning in deuterostome embryos (Agius et al. 2000; Gritsman et al. 2000; Shen 2007; Duboc et al. 2010), are absent in the *Nematostella* (Matus et al. 2006a; Rentzsch et al. 2006; Putnam et al. 2007), sponge (Srivastava et al. 2010), and *Trichoplax* (Srivastava et al. 2008) genomes, consistent with their later recruitment into mesodermal patterning. Comparative functional genomics therefore provides exciting opportunities to understand how new genes were incorporated into existing GRNs. Continued work on elucidating the *Nematostella* endomesodermal GRN will provide unique insight into how developmental potential was segregated asymmetrically into different tissue layers, how GRNs and their complex subroutines evolved, and how their "logic" became stabilized in distinct tissues and evolutionary lineages.

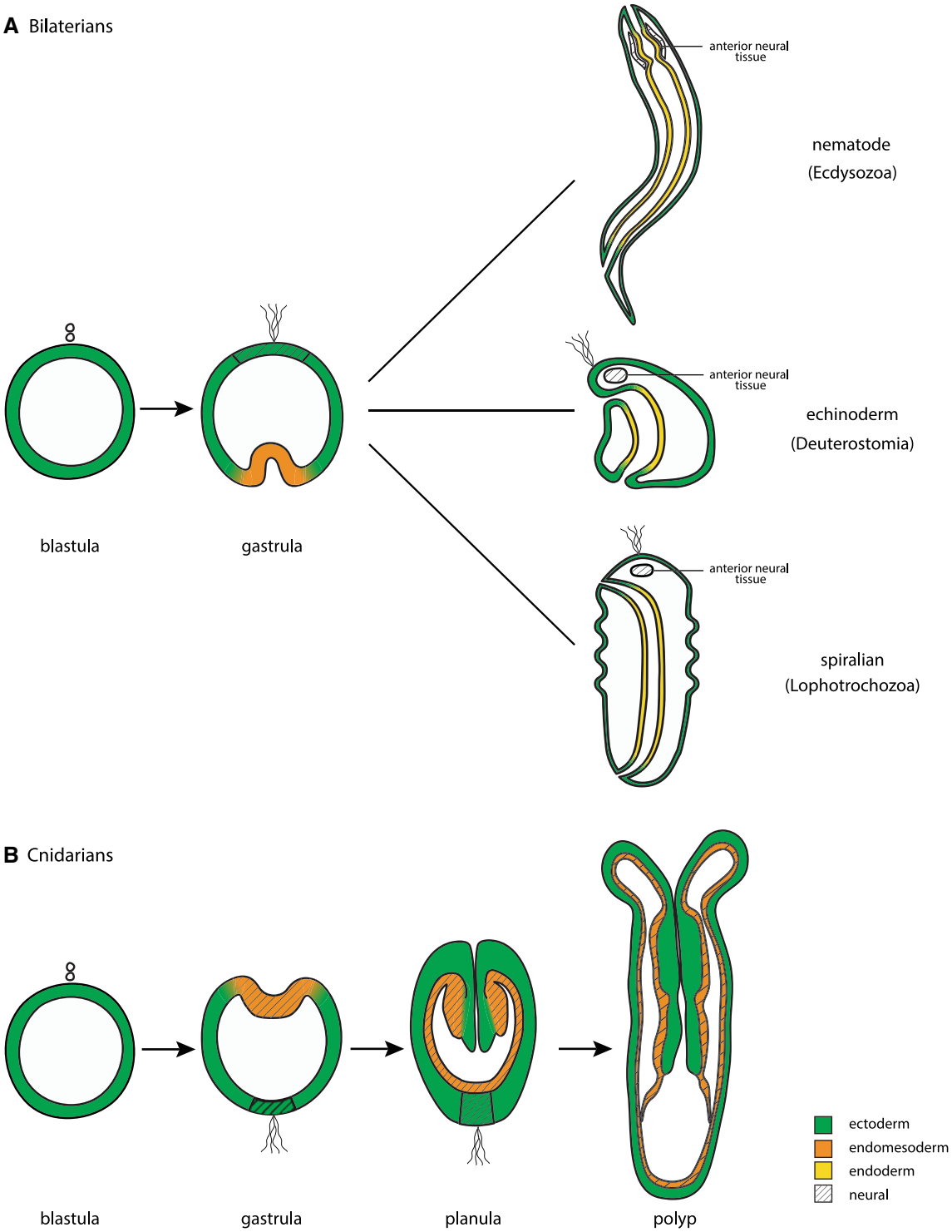

Fig. 4 **a** In most bilaterian embryos, there is a relationship of the animal–vegetal axis to the adult body plan. The anterior region of the animal, including the mouth and brain, is derived from the animal pole, while the gut and its derivatives are generated from the vegetal pole. In some animal groups, growth continues throughout the post-embryonic stages through a terminal growth zone. **b** In cnidarians, the oral opening and majority of the nervous tissue are also generated from the animal pole; however the lining of the gut, the gastrodermis, is also generated from this site. By relocating the site of gastrulation to the vegetal pole, bilaterians were able to more efficiently specialize anterior neural regions and vegetal/endomesodermal regions

Life History Evolution

Another consequence of the change in the site of gastrulation to the vegetal pole was its potential effect on life history evolution. Most bilaterian embryos are known to display an "anterior to posterior progression of development," with anterior regions differentiating before more posterior regions. For example, vertebrate embryos continue to add new somites posteriorly while anterior segments are carrying out their normal pattern of development and differentiation. When the site of gastrulation moved away from the site of anterior regional specification, it allowed the dissociation between anterior structural differentiation (e.g., head, mouth parts, brain) from the generation of new endodermal and mesodermal tissues posteriorly. By prolonging the developmental period, the patterning and "organizing" activity associated with the site of gastrulation (blastopore), mediated by genes such as FGF-, Wnt-, TGF-β-, hedgehog-, and Notch-associated signaling pathways (Kusserow et al. 2005; Matus et al. 2006a, b, 2007, 2008; Rentzsch et al. 2006, 2008), could continue to operate over extended periods to add new tissues posteriorly, leading to an increase in body size by a process known as terminal growth (Jacobs et al. 2005). This type of growth is seen in forms such as crustaceans and annelids in which "larval" forms with functional anterior regions function in pelagic ecological environments while continuing to add posterior regions of their bodies until adulthood (and sometimes even throughout adult life; Seaver et al. 2001; Damen 2007). An increase in body size has obvious selective advantages in terms of avoiding predation, mate choice, and reproductive success and the change in the site of gastrulation posteriorly, using the same embryonic patterning modules in a juvenile terminal growth zone, provides a plausible mechanism to account for this phenomenon without invoking novel adult growth control mechanisms.

Conclusions

Studying outgroups to the most diverse animal radiation of triploblastic bilaterally symmetrical animals is informative. Cnidarians, the accepted sister group to the triploblastic clade, clearly possess the cellular repertoire to generate complex shapes through tissue morphogenesis. Relatively simple changes in cell shape, adhesivity, and mobility that begin early in the developmental period and extend into adult life can account for much, if not all, of the complex body forms seen in this diverse group. These observations argue for a deep origin for the fundamentals of tissue dynamics in animal evolution that provided a springboard for future exploration of morphospace in later branching animal groups.

A careful comparison of the development of outgroup taxa provides a possible explanation for the observation that the vast majority of extant animal forms belong to the Bilateria. A notable difference between cnidarians (and ctenophores) and bilaterian embryos is the spatial change in the site of gastrulation, the position in the embryo in which the future endomesoderm arises. This seemingly simple difference had profound effects on subsequent body plan evolution. It provided the dissociation between the position of the mouth and the site of gastrulation, it allowed neural and endomesodermal tissues to evolve independently in discreet spatial locations, and it promoted the separation of distinct endodermal and mesodermal tissue types from an ancestral bifunctional endomesoderm. This developmental change promoted a spatial separation of anterior head development and differentiation from the region of new germ layer generation, and the prolongation of the developmental period allowed the amplification of the anterior to posterior developmental gradient to increase body size (e.g., terminal addition of body parts posteriorly).

Recent molecular investigations provide compelling explanations for how this change in development occurred and give clear, testable predictions for future studies. Comparative functional analyses of representative diverse taxa will provide important cellular and molecular insight into how the tremendous diversity of organismal form arises.

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