

germ cells [13]. The *oskar* gene is the only gene known to be both necessary and sufficient to produce germ cells from this localized germ plasm [14] and is present in the genomes of all higher insects that use the germ plasm mode of germ cell specification [15]. In contrast, *oskar* is absent from the genomes of several basally branching insects that specify their germ cells through induction, including aphids, louse, and bugs [15]. This phylogenetic association between the presence of *oskar* and the presence of germ plasm across insects is striking and suggests that *oskar* was an innovation of higher insects enabling the evolutionary transition between the inductive and germ plasm mode of germ cell development [15].

Ewen-Campen and colleagues' [12] discovery of an *oskar* orthologue in a basally branching cricket, *Gryllus bimaculatus*, without germ plasm now forces us to change our current perspective on the phylogenetic origin and evolution of *oskar*. By knocking-down the function of *oskar* during embryogenesis they revealed that *oskar* is not required for germ cell development but instead plays a role in the nervous system. This suggests that *oskar* may have originally functioned in the nervous system and was subsequently co-opted during the evolution of higher insects to enable the transition from an inductive to a germ plasm mode of germ cell specification. Up to this point, this story appears to be a straightforward story of co-option of ancestral genes originally evolved for other functions. However, the expression domains of *oskar* during embryogenesis add an important twist to this story. Even though *oskar* does not function in germ cell specification, Ewen-Campen and colleagues show that it is expressed at low levels throughout the abdominal region including the germ cells (Figure 1). This low-level of non-functional expression may be a pleiotropic consequence of *oskar*'s association with genes involved in both nervous system and germ cell development. Indeed, *oskar* is embedded in a regulatory network of genes, like *nanos*, *pumilio*, and *staufer*, that have been shown in fruit flies to function in both nervous system development and germ cell specification [16,17]. The non-functional expression domain of *oskar* may therefore be the non-adaptive by-product of its network

connections to other genes with multiple roles. This brings us full circle to Gould and Vrba's [11] insight on the role of non-adaptive by-products in co-optive evolution. The non-functional expression domain of *oskar* in the germ cell represents an adaptive potential or 'novelty in the waiting' that likely facilitated *oskar*'s co-option to germ cell specification in higher insects.

Co-option of non-functional variation in gene expression domains may be much more common than we might have initially expected, especially in cases when genes are embedded in networks composed of genes that have multiple functions. An important future goal in evolutionary developmental biology should be to document the prevalence of co-option of non-adaptive-by-products. We should therefore clearly specify the substrate of a co-option event as either an ancestral trait that originally evolved for other functions or as a non-adaptive, non-functional by-product of natural selection on other traits. Understanding the nature of substrates for co-option has important implications for evolutionary theory; co-option of ancestral traits that evolved for other uses may bias future paths of evolution, whereas co-option of non-adaptive by-products may open new adaptive possibilities.

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Department of Biology, McGill University, Montreal, Quebec H3A 1B1, Canada.  
E-mail: [ehab.abouheif@mcgill.ca](mailto:ehab.abouheif@mcgill.ca)

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## Evolution of Development: The Details Are in the Entrails

Historically, the position of the site of gastrulation has been used to understand the developmental basis for body plan diversity. A recent molecular study, however, challenges long-held views and shows that molecular patterning mechanisms can be used to understand body plan evolution despite variation in gastrulation movements.

Mark Q. Martindale

Organismal and evolutionary biologists have long tried to use changes in

developmental features to help explain major transitions in animal body form. The study of gastrulation, in particular the site of gastrulation and the fate of

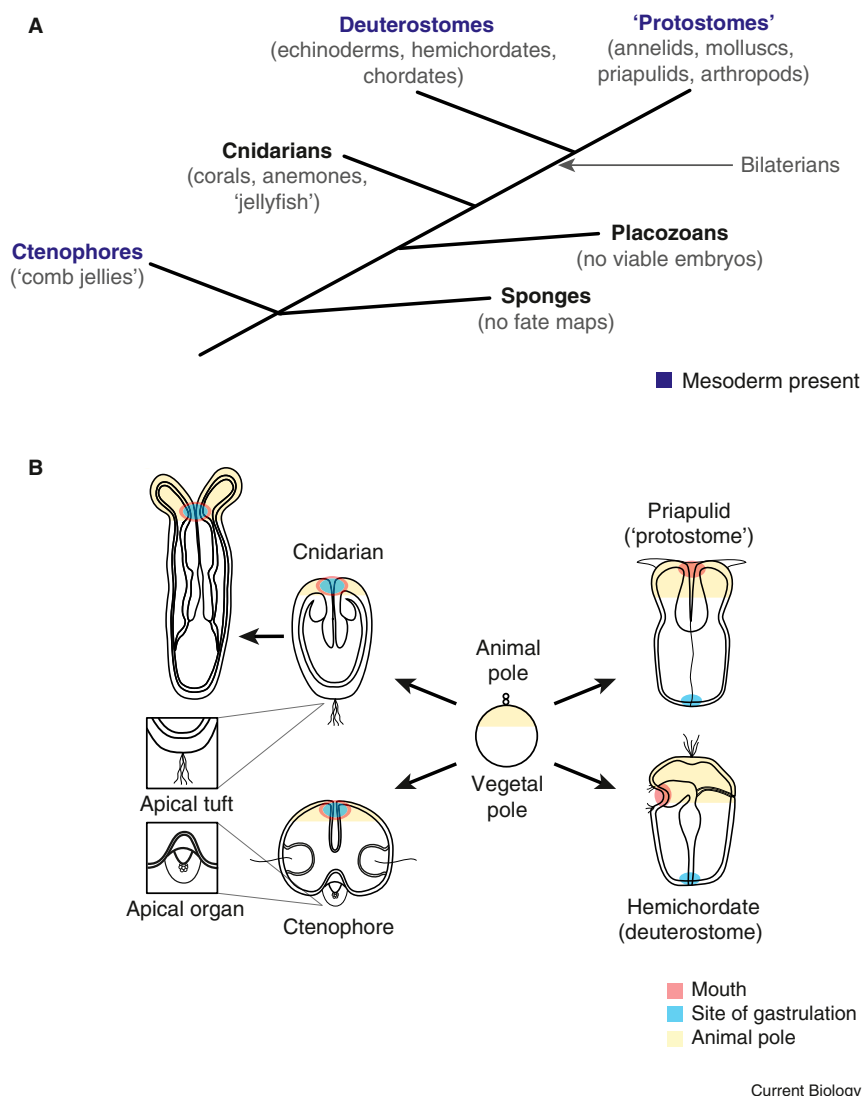


Figure 1. Implications of the site of gastrulation with respect to body plan formation in the Metazoa.

(A) Phylogenetic relationship of major metazoan groups. A recent paper [2] suggests the Protostomes are misnamed because the site of gastrulation does not form the mouth. Note that the relative branching order of sponges and ctenophores remains unresolved. (B) The position of the site of gastrulation and the mouth relative to the animal-vegetal axis in four major metazoan lineages. Note that the two sensory structures derived from the vegetal pole in cnidarians and ctenophores are completely different in structure and function.

the blastopore (the region of the embryo that gives rise to the internal tissues such as the gut), has played a central role in our attempts to understand the evolution of body plan diversity. Indeed, the two major branches of metazoan animals, the Protostomia and Deuterostomia (Figure 1A), were named due to the relationship between the larval/adult mouth and the site of gastrulation [1]. In protostomes, the site of gastrulation is said to give rise to the mouth, while in deuterostomes it forms the anus with the mouth arising from a second

opening distant to the site of gastrulation.

However, a new paper reporting the development of priapulids ('penis worms') published recently in *Current Biology* from Martin-Durán *et al.* [2] shows that this important group of ancient marine animals, firmly established as members of the Protostomia, actually gastrulates exactly like deuterostomes. Furthermore, the expression patterns of molecular markers for the blastopore, mouth, and anus in priapulids follow expression patterns

found in deuterostomes, rather than protostomes. These findings reveal that the terms Protostomia and Deuterostomia as labels for taxonomic purposes are no longer instructive and may actually obscure our understanding of the phylogenetic relationships between metazoan groups [3]. As is now apparent, using gastrulation as a criterion for describing major metazoan radiations turns out to have been an unfortunate choice as many forms of gastrulation (e.g., ingression, epiboly, delamination) do not generate an opening that can be associated with any larval/adult structure. Gastrulation patterns in many 'minor' metazoan taxa have not yet been carefully described and variation in gastrulation patterns even within individual metazoan groups [4,5] have not been thoroughly explored (Figure 1A).

Although previous workers have tried to make broad generalizations from observations of development, fate-mapping experiments are the only way to demonstrate the relationship between the site of gastrulation and the larval and/or adult body plan (Figure 1B). Such experiments have shown that in both protostomes [2,6] and deuterostomes [7] endoderm (i.e., gut) arises from cells derived from the vegetal pole of the egg. Cells from the animal pole give rise to oral and anterior neural structures associated with the feeding apparatus. In all deuterostomes and some protostomes [2], the site of gastrulation persists through development and becomes the anus. However, in protostomes the site of gastrulation (i.e., position of endoderm formation) rarely ever becomes the mouth, although differential growth often displaces vegetal tissue towards the oral opening. These data indicate that endodermal tissue originates from the vegetal pole in both protostomes and deuterostomes and that the mouth bears no conserved relationship to the site of gastrulation. It is tempting to speculate that this may be due to the wide range of modes of gastrulation observed in the protostomes and the conserved persistence of the blastopore in deuterostomes as a constraint of epithelial invagination.

Despite the lack of a relationship between the site of gastrulation and mouth formation, there is a growing body of evidence supporting the

homology of the metazoan mouth [2,3], with the possible exception of the chordate mouth [8]. Fate-mapping experiments in cnidarians (e.g., sea anemones, corals, and ‘jellyfish’) — the sister group to the bilaterian clade (Figure 1A) — and ctenophores (an even earlier branching metazoan taxon) (Figure 1A), show that their mouths form from a region derived from the animal pole, just like bilaterians [9,10] (Figure 1B), and express many of the genes expressed around the mouth in bilaterians [2]. The homology of the metazoan mouth makes sense from a functional perspective as the adaptive significance of an intermediate stage with no, or multiple, mouths is doubtful.

There are two important differences in gastrulation between cnidarians and bilaterians. First, adult cnidarians possess a bifunctional gastrodermal layer lining the gastric cavity and an outer epidermal layer, but do not have a separate mesodermal germ layer (e.g., muscle, parenchyma, nephridia) characteristic for bilaterians. The cnidarian gastrodermis functions in both digestion and contraction and expresses genes typically involved in both bilaterian endoderm and mesoderm development [11–13], suggesting it represents an evolutionary precursor of both bilaterian endodermal and mesodermal tissue layers. Understanding the molecular basis for the development of the gastrodermis in cnidarians might provide tremendous insight into the evolutionary origins of distinct endodermal and mesodermal gene regulatory networks in bilaterians.

The second important difference between gastrulation and the formation of the mouth in cnidarians (and ctenophores) and bilaterians is that gastrulation occurs at the animal pole, not the vegetal pole, making them, by definition, the only true extant ‘protostome’ clades. Unfortunately, we do not know what this relationship is in the two other ‘prebilaterian’ taxa (Figure 1A). No viable embryos have ever been recovered from placozoans (e.g., *Trichoplax*) and a fate-map incorporating the primary egg axis has never been generated in any sponge species (not to mention that there is no clear tissue that can be homologized to bilaterian endoderm). The change in the site of gastrulation from the animal pole in cnidarians and ctenophores to the vegetal pole in bilaterians has

been argued to be the most profound developmental change [9] — not the mode of gastrulation, or the existence of bilateral symmetry — that facilitated the tremendous radiation of bilaterian body plans [14].

A detailed understanding of the components of the cnidarian gastrodermal gene regulatory network is essential to understand the evolution of mesoderm [9] in the Metazoa. For example, ctenophores (Figure 1A) possess several mesodermal cell types (e.g., muscle cell and mesenchymal cells) and branched off from the rest of the metazoan lineage before the cnidarian–bilaterian ancestor. This implies that mesoderm evolved early in the Metazoa and was lost in cnidarians, placozoans, and possibly sponges (depending on the true phylogenetic position of sponges). The loss of mesodermal cell types in these lineages is surprising; however, this scenario assumes that the mesoderm in ctenophores is homologous to the mesoderm found in bilaterian taxa. If mesoderm evolved independently in ctenophores and bilaterians, it provides us with an opportunity to study the molecular basis for the appearance of similar cell types in animal evolution. Functional genomic approaches are being successfully employed to understand the development of mesodermal lineages in different echinoderm clades [15]. An analysis of the gene regulatory network underlying ctenophore mesoderm in comparison to the cnidarian bifunctional gastrodermis will be particularly interesting with respect to the evolutionary origin of mesodermal cell types.

Gastrulation is obviously an emergent property of complex metazoan body plans that allowed not only the formation and specialization of the lining of a digestive surface but, perhaps more importantly, the formation of internal tissues such as endoderm and mesoderm. The appearance of internal tissues allowed the evolution of complex inductive interactions between tissue layers both during and after gastrulation, broadly utilized in all bilaterian development, that would not be possible in a hollow ball of cells. It is of some interest that the lip of the blastopore in the cnidarian *Nematostella* has organizing activity [16] that can induce a new oral–aboral

axis, likely due to the expression of several signaling family molecules such as BMPs, FGFs, and Wnts [17–19]. Thus, when the site of gastrulation changed in bilaterians, not only did the gene regulatory network that activates gastrodermal tissue change its spatial position, but the inductive activity responsible for axial patterning also moved along with it. A better understanding of the molecular basis of gastrulation will keep our bellies full of new insight into the developmental basis for body plan evolution, regardless of where our mouths might form.

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Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI 96813, USA.  
E-mail: [mqmartin@hawaii.edu](mailto:mqmartin@hawaii.edu)

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## Cell Migration: Cooperation between Myosin II Isoforms in Durotaxis

A new study reveals that non-muscle myosin II plays a central role in the durotaxis of mesenchymal stem cells, with the two major isoforms, II-A and II-B, being cooperatively required for this cell movement, and serine phosphorylation of the II-A isoform playing a negative role.

Miguel Vicente Manzanares

Durotaxis is the tendency of most cells to move towards stiffer substrates when they are migrating on a compliance gradient. This type of movement is a cellular behavior based on the mechanical, rather than biochemical, properties of its microenvironment; thus, it can be classified as a process involving mechanotransduction. The physiological relevance of this poorly studied form of migration is beginning to be elucidated, and the ramifications are fascinating. Tissue stiffness favors tumorigenesis [1] as well as cell proliferation [2]. Furthermore, cells spread and migrate more easily on stiff than on compliant substrates [3].

What mediates durotaxis is not well characterized, but major players include integrins and focal adhesion kinase (FAK) [3,4]. Now, based on a new study from Raab and co-workers [5], we can add non-muscle myosin II (NMII) to the list of durotaxis mediators. While studied extensively in the 80s and 90s, NMII has come back into the spotlight more recently due to its pivotal roles in various crucial cellular phenomena, for example, cell migration, division, differentiation and apoptosis (reviewed in [6]). By controlling these processes, NMII is a major integrator of the mechanical properties of the cellular microenvironment, controlling stem cell differentiation and morphology

[7,8], tumorigenesis [9], and cell migration [10].

The relatively simple vision of NMII as a contraction- or force-generating device was complicated by the identification of three major isoforms of the heavy chain and their splice variants, the elucidation of different regulatory sites within the light and heavy chains, and the description of several regulatory kinases and phosphatases that control the contractile, ATPase-based activity of NMII. This picture is even more complex taking into account the fact that, despite their apparent inability to heterodimerize, the different isoforms of NMII cooperate to mediate their biological roles.

In most mammalian cells, there are two major NMII isoforms, which are defined by the nature of the actin-binding, ATPase myosin heavy chain: NMII-A and NMII-B (a third isoform, NMII-C, does exist, but its expression is more restricted, hence its biological significance on a broad context is not yet clear). Both isoforms are implicated in cell migration, but their inhibition produces separable outcomes. NMII-A is implicated in cortical stability [11] and retraction of the cell rear [12], whereas NMII-B is required for cells to polarize and migrate directionally [12,13]. The role of the NMII isoforms in the control of cell shape reflects their subcellular positioning: whereas NMII-A is homogeneously distributed and

localizes everywhere in the cell but the lamellipodium, NMII-B is more confined to the central and rear portions of the cell [14], defining the rear by segregating protrusive signals away from these regions [15,16]. However, an interplay between these isoforms exists because in NMII-A-deficient cells NMII-B is not confined to the central and rear parts of the cell but appears homogeneously distributed and seldom assembles into mini-filaments [15].

Raab et al. [5] now show that NMII-A and NMII-B mediate durotaxis of mesenchymal stem cells. They first demonstrate that NMII-B localizes to the center and rear of primary mesenchymal stem cells on stiff substrates, defining a non-protrusive region. Conversely, on more compliant surfaces, NMII-B is not polarized. On either substrate, NMII-A remains evenly distributed, although its assembly into mini-filaments increases as substrates become stiffer. The authors then probed which of the isoforms played a more prominent role in the control of durotaxis. siRNA-induced inhibition proved that a small reduction in NMII-B was sufficient to impair durotaxis, whereas only a large knockdown of NMII-A produced the same effect. This led the authors to conclude that, although both isoforms are implicated in durotaxis, NMII-B is a more sensitive part of the molecular mechanism that controls it.

To try to explain the differential sensitivity to depletion of each NMII isoform in controlling durotaxis, the authors studied the dependence of the dynamics of both isoforms on the compliance of the substrate. They noticed that NMII-A was more dynamic (which is a proxy for decreased affinity of NMII-A for stable actomyosin filaments) in cells on soft compared to stiff substrates, and this correlated with