

The radial-symmetric hydra and the evolution of the bilateral body plan: an old body became a young brain¹

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The radial symmetric cnidarians are regarded as being close to the common metazoan ancestor before bilaterality were invented. It is proposed that a large fraction of this gastrula-like organism gave rise to the head of more evolved organisms. The trunk were added later in evolution from an unfolding of a narrow zone between the tentacles and the blastoporus. This implies that, counter intuitively, the foot of the hydra corresponds to the most anterior part (forebrain and heart) while the opening of the gastric column gave rise to the anus. Two fundamentally different modes of midline formation evolved. In vertebrates, the organizer attracts cells from the both sides of the marginal zone. These leave the organizer as a unified band. The midline is formed sequentially from anterior to posterior. In insects, the midline forms opposite of a dorsal repelling center, i.e., on the ventral side. This occurs more or less simultaneously.

A central issue of evolutionary biology is how the two major body axes of higher organisms, anteroposterior and dorsoventral, evolved from the single oral-aboral axis of simpler radial-symmetric organisms several hundred millions years ago. In recent years, evidence accumulated that the basic regulatory pathways are highly conserved. In the patterning of the freshwater polyp hydra, molecules have been found that participate in higher organisms in the generation of both axes. For this reason, hydra is most likely closely related to the putative common radial-symmetric ancestor of coelenterates and higher animals. Comparative analysis with respect of axis formation and the molecules involved are expected to shed light on the evolution

of the spatial organization of vertebrates and arthropods. Using the term 'hydra-like' for the common ancestor is not to overlook the long evolutionary history of the current hydra with its highly elaborated features such as the nematocyst cell. The term refers only to the elementary body plan. It is assumed that the expression patterns of gene families employed for the basal organization of body axes have been preserved. In contrast, the morphological features these genes control in contemporary animals may have changed completely due to the large phylogenetic and temporal distance.

There is an increasing evidence that the oral opening of a hydra corresponds to the blastopore of a vertebrate gastrula. The *brachyury* homologue of hydra, *Hy-bra1* is expressed around the oral opening¹ (Fig. 1), corresponding to the *brachyury* expression in the in the marginal zone in *Xenopus*² next to the endoderm. A basic question is how the dimension extending from the oral opening to the opposite region of the radial symmetric ancestor, conventionally called the head-foot axis of hydra, is related to the body axes of the bilateral animals.

Two conflicting hypothesis have been put forward (see ref. 3-5 for review). According to the protostomia - deuterostomia concept, a widening of the blastoporus took place in one dimension, resulting in a slit-like geometry. By this 'Amphistomy'⁵, the blastoporus obtained a long extension that forms the future midline. One end of this slit becomes anterior, the other posterior. Either the mouth (protostomia) or the anus (deuterostomia) remained as an opening of the original gastric cavity. The paired arrangement of the nerve cord on both sides of the midline is assumed to be derived from the ring of high nerve cell density around the blastoporus⁴⁻⁶. According to this

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concept, the mouth opening of the hydra-like ancestor became after elongation the future midline and thus the neural side of higher organisms (i.e., dorsal in vertebrates and ventral in arthropods and insects). To accomplish this ring-slit transition, a signal for “most anterior” and “most posterior” must be generated on the ring at opposing positions. This hypothesis implies that the neural – anti-neural (mediolateral) axis was first in evolution and the anteroposterior axis was the new invention.

An alternative view is that the oral – aboral axis of a radial-symmetric hydra-like ancestors gave rise to the anteroposterior axis of higher animals. The elongated column of the hydra, the food intake at the oral ‘head’ and fixation to the ground with the aboral peduncle (‘foot’) makes this assignment reasonable and the use of the terms ‘head’ and ‘foot’ are suggestive. Many genes expressed in the head of higher organisms are expressed in the hydra head. Examples are *Otx*⁷, *Aristaless*⁸ and *gooseoid*⁹. This suggest that the expression patterns of genes characteristic for head development became established before the invention of bilaterality (for review ref. 10). In this view, the AP axis of higher animals would be primary and mediolateral the newly invented one.

Such an assignment does not necessary imply that the *orientation* of the AP axis of higher animals corresponds to the head-to-foot axis of hydra. In many organisms the blastopore becomes the anus^{11,12}. It has been pointed out by Wu and Lengyel¹³, that genes such as *caudal*, *wnt* and *brachyury* are expressed at the posterior pole of *Drosophila* and, likewise, around the blastopore of vertebrates. In hydra, *wnt* and *brachyury* are also expressed around the opening of the gastric column, suggesting that this part corresponds to blastoporus and thus to the most posterior structure of higher organisms. How can this be reconciled with the evidence that head-specific genes of bilateral animals are expressed in the head of hydra? The explanation proposed is that the most of the body of the hydra-like ancestor gave rise fore- and midbrain in vertebrates. This region ranges in hydra from the foot very far into the body and includes the tentacle area (which is usually considered as part of the head region). Only the narrow zone between tentacles and the gastric opening gave rise to the trunk and anus. Therefore, gene systems that were used in the hydra-like ancestor to organize most of the whole body can be found in contemporary animals to organize the brain. The proposition is supported by the following comparison of genes expressed along the AP axes in higher animals and in hydra respectively.

The ancient gastrula-like organism develop into the head; the trunk is added in the course of time

In vertebrates, *gooseoid* and *brachyury* are initially expressed in adjacent regions. While *gooseoid* is involved in head formation, the pan-mesodermal gene *brachyury* is crucial for trunk and tail formation. Thus, the expression

regions of these two genes become separated from each other during the sequential anterior to posterior trunk formation. Their initial border can be regarded as the head/trunk border. In hydra, these two genes are also expressed adjacent to each other in a narrow zone. It is located between the tentacles and the hypostome; the *gooseoid* homologue *Cngsc* closer to the tentacles, *HyBra1* closer to the opening^{8,1}. This suggests that most of the AP body axis of contemporary higher organisms, the trunk, is derived from a narrow ring of the putative hydra-like ancestor located between the tentacle zone and the opening of the gastric column (Fig. 1). In contrast, the major fraction of the original gastrula-like organism became converted into the head and especially into the brain of higher animals. With this orientation of the axes, the foot of the hydra corresponds to the most anterior part of the brain while a region between tentacle zone and the cone-shaped hypostome would correspond to the posterior end of the brain. This scheme accounts for the fact that the AP axis of a higher organism consist of two parts with very different properties. One part, the head, would be derived from most of the original gastrea-like organisms, whereas the second part, the trunk and the posterior end, resulted from an unfolding of a very small portion of the original radial-symmetric organism. Only during the evolution of the latter process, the notochord and the Hox gene code was invented. It is, therefore, not surprising that in hydra the typical pattern of Hox gene expression has not been found although HOX genes exists^{14–17}: the trunk was not yet there. The sequential elongation of the trunk from a narrow ring during evolution and the elaboration of the sequential co-linear Hox gene pattern arose presumably in a coupled way. In many higher organisms such a sequential elongation of the trunk is recapitulated during the ontogenetic development.

Assuming that the body column of hydra corresponds to the fore- and midbrain of vertebrates, it is expected that corresponding gene expressions can be found there. This is indeed the case. The Hox gene *Cnox-2* is expressed in the body column of the hydra, but not in the hypostome nor in the tentacle region¹⁷. Homologues genes are expressed in the brain of the mouse¹⁸ and in the cerebral vesicle of amphioxus¹⁹, a structure homologous to the vertebrate fore- and midbrain.

The gene *Otx* is expressed in the hydra body column including the tentacle zone⁸. Its homologue in vertebrates, expressed in the front part of the brain, is crucial for the formation of midbrain/hindbrain organizing region (ref. 20-23 for review). In vertebrates, the region posterior to *Otx* expression forms the hindbrain and has already a notochord with *brachyury* expression. Thus, the expression border of *Otx* at the oral side, abutting the region of *HyG-sc* expression, marks the posterior end the of old part of the body that evolved into the fore- and midbrain.

In *Hydractinia*, a colonial marine relative of hydra, an *ems*- related gene has been found. It is expressed between the tentacle zone and the oral opening²⁴. According

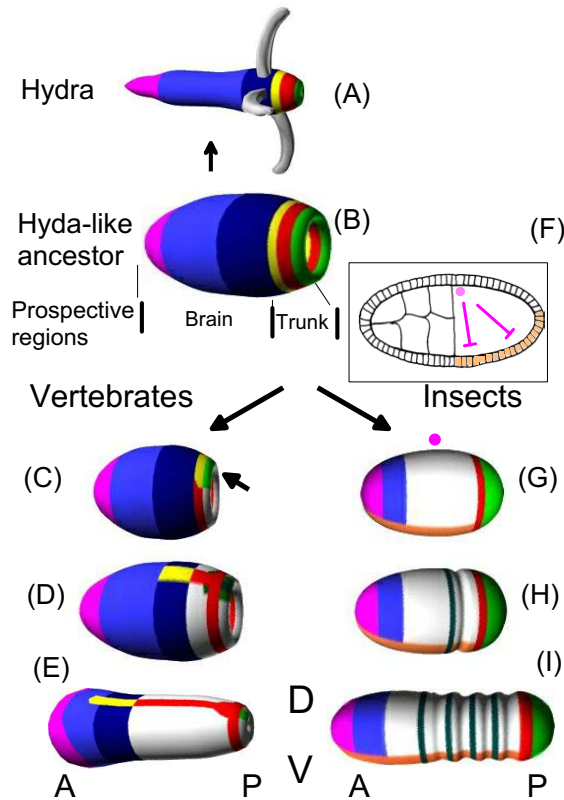


Fig. 1: Proposed equivalence patterns along the AP axis of a hydra-like ancestor and contemporary bilaterians: the foot corresponds to the most anterior structures including the forebrain and heart (Nk2-related genes, violet); the central part of the gastric column (*Otx*, blue) to central part of the brain; the border of tentacle region (*Aristaless*, deep blue) and the hypostome (*Gsc*, yellow) region to the mid/hind-brain region. Only the hypostome with *brachyury* and *Wnt* (red, green) appears as "ancestor area" of the trunk, the opening became the anus. In vertebrates (C-E), the primary organizing region (β -*catenin*, *Tcf*, green) and *Gsc* became contracted to a patch-like extension (arrow) near the blastoporus forming the (Spemann-like) organizer with an anterior (yellow) and posterior part (green). *Gsc* expressing cells (yellow) move anterior to form the prechordal plate, specifying dorsal in the brain region. Cells of the marginal zone including *brachyury*-expressing cells (red) move from both sides towards the organizer and leave them as a unified band. Midline and trunk are formed in a sequential way from anterior (D) to posterior (E). Thus, *brachyury* expressing cells form first a ring around the blastoporus (C) and participate later in notochord formation. In insects (F-I), initial midline formation (orange) is governed by a repelling influence from the dorsal side (violet, see insert for *Drosophila*,). This can proceed simultaneously along the whole ventral extension. *Brachyury/brachyenteron* remains restricted to the posterior pole. During further posterior outgrowth, e.g., in short germ insects established DV organization is maintained and the midline becomes elongated. With increasing distance of the cells to the zone of *wingless* (green) expression at posterior pole, new stripes of *wingless* expression can be initiated. The resulting repetitive pattern participates in segment formation (H - I).

to the proposed assignment of the A-P axis, this is posterior to *Otx*. This fits with the AP-arrangement of both genes in *Drosophila* where *ems* is also expressed posterior to *Otx/Otd*²⁵.

Several genes are known that play a role in foot formation of hydra^{26,27}. An *NK2* related gene is expressed in a graded way towards the foot²⁶. In zebrafish, an *Nk2* gene demarcates a zone of neuronal differentiation in the embryonic forebrain²⁸. Corresponding expressions at very anterior (-ventral) positions has been observed in *Amphioxus*²⁹, *Xenopus*^{30,31}, mouse³² and chick³³. In *Drosophila*, an *NK2* related gene is initially expressed only in future brain region of the embryo³⁴. In *C.elegans*, an *Nk2* gene is involved in pharynx formation³⁵. These observations are compatible with the view that the region corresponding to the foot of hydra gave rise to the anterior-most portion of the body. *Nkx2.5* related genes are involved in heart formation, also a very anterior (-ventral) structure^{36,37}.

Recently it has been shown for hydra that β -*catenin*, *Tcf* and *Wnt* are expressed in the hypostome, with *Wnt* restricted to the tip³⁸. These molecules are known since long to be involved in the vertebrate organizer³⁹. A *WNT* gene, *WNT-3a*, is required for posterior development in the mouse⁴⁰. Without *WNT-3a*, no node and no

streak is formed. Likewise in *Amphioxus*, *Wnt* is expressed at the blastoporus and this expression remains there as this regions develops into the most posterior part of the organism². In *Drosophila* as well as in *Tribolium*, *WNT* is expressed around the posterior pole⁴¹. Wu and Lengyel¹³ emphasized the similarities between posterior *wnt/brachyury* expression in *Drosophila* and around the blastoporus of vertebrates. The corresponding expression patterns in hydra are in full agreement with the stipulation that the mouth opening became the most posterior part, the anus.

In Cnidarians that go through a larval stage, the posterior part of the larva gives rise to the 'head' of the final polyp⁴². This has been interpreted as a polarity reversal. According to the proposed orientation of AP axis no such polarity reversal occurs: the posterior part of a larva gives rise to the posterior part of the polyp, the gastric opening. Thus, not only the adult animal but also the larva can provide information about the ancestral gastrula.

Bilateral symmetry and the formation of a midline

The transition from a radial-symmetric cup-shaped gastrula to a bilateral-symmetric organism requires a symmetry breaking as indicated by the formation of a Spemann-type organizing region close the blastoporal margin. However, a local high concentration on a ring is on itself insufficient to generate two axes that have an orthogonal orientation relative to each other: from a single high point the concentration decreases in all directions; therefore, the early amphibian organizer marks both a most dorsal and a most anterior point of the trunk. At this stage, these axes are not yet separated. The crucial step towards a bilateral-symmetric coordinate system for animals with an extended trunk is the generation of a midline such that positional information for the mediolateral extension can be generated. This requires the formation of a stripe-like organizing region as given by the notochord of vertebrates or by the ventral midline of insects.

A single straight stripe cannot be generated by a single pattern forming mechanism. The reason is easy to understand. A single spot-like local high concentration can be generated by local self-enhancement and a strong long-range inhibition⁴³. Stripe formation occurs if the self-enhancement has an upper bound otherwise the stripe will decay into separate patches^{44,45}. When initiated by uniform initial conditions, the resulting patterns consist of somewhat meandering and branching stripes such as seen in the pigmentation patterns on some tropical fishes or on the skin of zebras. Due to the restricted inhibition, the width of the stripes is of the same order as the width of the interstripe regions. A single stripe would require a strong lateral inhibition, but such an inhibition would cause a decay of the stripe into individual patches. However, by an organizing influence of a single spot-like organizer it can be achieved that only a single stripe is formed and that this stripe is straight. It is proposed that this patterning problem has been solved in two different ways: in vertebrates the organizer causes a local initiation and elongation of the midline. In contrast, insects a repulsion of a dorsal organizer restrict the midline to the ventral side of the embryo. Thus, an understanding of this process may provide a key for this fundamental branching during evolution.

Midline formation in vertebrates: the spot-like organizer elongates the midline

In vertebrates, midline formation can be explained by the assumption that the organizer, located on the germ ring (in zebra fish) or on the marginal zone (*Xenopus*) attracts cells of these rings. Cells move toward the organizer and, after being close once, they change their behaviour (similar as some nerve cells change their orientation af-

ter crossing the midline⁴⁶); they are no longer attracted. Therefore, cells from both sides of the ring move towards the organizer and leave this region as a unified band – the future midline (Fig. 1). It has a 90° orientation in relation to the original ring (for modelling, see ref 47). This is a special mode of the movement generally known as conversion-extension movement⁴⁸. The passage of cells through the organizer has been most clearly demonstrated for the chick⁴⁹. Cells from the organizer may be added to the band and contribute to structures such as the notochord and floor plate. In the course of time this leads to a conversion of the ring- into a rod-shaped arrangement of the mesoderm, most clearly seen in the *brachyury* expression during gastrulation^{2,50}. Thus, the organizer (or node) can be regarded as a spot-like device that elongates the midline by first attracting and then reprogramming (and perhaps eventually repelling) the migrating cells. The organizer is therefore always located at the tip of the midline, causing its sequential elongation from anterior to posterior. The time at which cells leave the organizer fixes presumably their anteroposterior identity by stopping a sequential activation of more posterior HOX-genes.

This midline formation starts with notochord formation at the contemporary hindbrain level posterior to the *Otx* expression. Therefore, a separate mechanism is required that allows a mediolateral organization of the fore- and midbrain. This is proposed to be achieved by the anterior movement cells to form the prechordal plate. *Gooseoid*-expressing cells, originally localized at the organizer region move anteriorly and are able to accomplish an anterior/dorsal re-specification⁵¹. Thus, *gooseoid* may be a part of the expected machinery required for the anterior elongation of the midline into the fore- and midbrain region.

During trunk formation, the distance of the posterior organizer to anterior regions increases to and becomes thus less and less precise in supplying positional information for the organization of the brain. This is presumably the reason why the fading posterior organizer becomes replaced by a new one that remains in place - the istmic organizer at the midbrain/hindbrain border^{20–23}.

Midline formation in insects: a repelling influence from dorsal leads to ventral midline formation

In *Drosophila* a spot-like organizing region is first formed dorsally, i.e., *opposite* to the future ventral midline (Fig. 1F). It is based on a signalling between the oocyte nucleus and the follicle cells by members of the EGF pathway – a signalling system that leads also to the dorsal appendages^{52–54}. This inhibition restricts *pipe* expression to the ventral follicle cells^{55,56}. The asymmetry is sufficient to generate a single ventral midline. In *Drosophila* this mode of midline formation does not occur in a sequential fashion as in vertebrates but can happen more or less simultaneously in the early embryo over the whole AP ex-

tension. A similar patterning mechanism is also involved in the short germ insect *Tribolium*⁵⁷, although there an elongation of the midline has to take place at later stages. So far, these two holometabolous (that is, highly derived) insects are the only systems for which detailed information for DV patterning are available. It is assumed, however, that this mode of DV patterning – localization of the midline to the opposite side by a repulsion emanated from a spot-like region – is representative for patterning primitive arthropods (see also below). This scheme provides possibly a new inroad to the apparent inversion of the DV axis in insects and vertebrates^{58–60} since in insects midline formation occurs opposite to the spot-like organizing region while in vertebrates, it is formed on the same site as the organizer. Not yet clear is of how these different modes of midline formation led to the employment of the same genes for the actual dorsoventral pattern, *dpp/short gastrulation* in insects and *chordin/BMP* in vertebrates.

This model of midline formation explains why in insects an anterior movement of cells like that for prechordal plate formation in vertebrates does not occur. In insects, the midline starts at the most anterior level, and not, as in vertebrates, only posterior to *Otx*. Therefore, an anterior elongation of the midline is not required for the DV organization of the insect brain.

This model accounts for the different expression patterns of *Brachyury/brachyenteron* in vertebrates and insects (Fig. 1): In vertebrates it is expressed in the notochord, i.e., at the most dorsal level and along the whole AP axis of the trunk. In contrast, in insects, it is expressed at a very posterior position in a ring, i.e., localized at a specific AP level but occupies but all DV levels^{61,13}. For this reason the function of *brachyury* as tail gene has been questioned⁶². According to the model proposed, in vertebrates, *brachyury* participates in the ring – rod conversion and becomes, therefore, expressed along the whole AP axis. In contrast, in insects the expression of the homologous *brachyenteron* remained as it was in the putative hydra-like ancestor: a ring of activity close to the most posterior pole of the animal. While in vertebrates, *brachyury* is essential for mesoderm formation, it is never expressed in the mesoderm of insects, underlining the stipulation that the different modes of midline formation were a fundamental branch during evolution.

A-P Elongation of the DV axis in annelids and arthropods

In many segmented animals, the zone around posterior pole becomes a growth zone (Fig. 1). However, this elongation is different from that in vertebrates. This is most easily understandable by regarding segment formation in leech⁶³ and some lower crustaceans⁶⁴. The trunk pattern is generated from bundles of blast cells that bud off small cells which give rise to the germ band. The blast cell bundles have a fixed mediolateral pattern as do the small cells they give rise to. Thus, during the posterior outgrowth

the early established mediolateral pattern is posteriorly elongated. This is in contrast to vertebrates, where cells obtain the dorsal reference character only as they pass the node or organizer.

The generation of the primary AP axis during hydra embryogenesis

In hydra, after some initial cell divisions, first a hollow sphere is formed that consists of a single-layered cell sheet. Cells delaminate from this sheet, move to the inside and form eventually the endoderm⁶⁵. Although the molecular basis of this process is not yet known, it is expected to be based on a conserved pathway in which known genes responsible for endoderm formation play a role, for instance, *VegT*^{66,67}. On theoretical grounds it is expected that autoregulatory loops are involved in this activation (e.g., *VegT* and *derriere*⁶⁸ or other *nodal*-related factors). Minor changes in the parameters can either lead to a salt-and-pepper – like pattern as in hydra or to large coherent patches as required for determination of hemispheres as in amphibians⁴⁷.

After the formation of the concentric diploblastic ectodermal/endodermal spheres a genuine de-novo patterning process is required to generate an AP axis. The capability for that is demonstrated by experiments in which hydra body columns are disaggregated into individual cells. After their re-aggregation first a sorting out of ecto- and endodermal cells occurs, recapitulating early stages of embryonic development. Eventually viable polyps are formed⁶⁹. In the spherical aggregates, high levels of *β -catenin* and *TCF* expression are turned on³⁵. Initially this expression is uniform as expected on theoretical consideration^{70,43}. Later discrete maxima are formed. This suggests, that *β -catenin/TCF* is part of a pattern forming reaction that defines the posterior pole. At the highest points of this pattern, more localized spots of *Wnt* expression emerges, causing presumably the actual gastric openings of this bi-layered hollow sphere.

In hydra, in addition to the primary signal for the most posterior pole and blastoporus, a further pattern forming system becomes active at the antipodal site causing foot formation. Analogously in vertebrates, in addition to the organizer, a separate pattern forming system seems to be required to form the anterior part of the head (for review see ref. 71-73). Once these two counter-oriented gradients exist, a reliable subdivision into discrete zones is possible. A corresponding model for hydra⁷⁰ found recently support from observations at the molecular level^{8,26}. On the basis of the proposed homology of foot formation in hydra and of the anterior structures in head development common pathways are expected.

The model allows several predictions. *Caudal*¹³ is expressed both in vertebrates and insects. It was therefore also likely present in the common precursor, suggesting that it can be also found in Hydra. It is expected at the most posterior position, the hypostome. In vertebrates,

several genes have been isolated that are required to induce anterior head structures. The genes *Hex*, *Cerberus* and *Dickkopf* belong to this group (see ref. 71-73). It is expected that homologous genes will be found in Hydra that are required for foot formation. In part, these genes are *Wnt*-antagonists. From a model of hydra patterning⁷⁰, hypostome and foot formation are expected to be antagonistic processes. Since *Wnt/β-catenin* is involved in hypostome formation, the involvement of *Wnt* antagonists for foot formation is in accordance with this expectation.

Conclusion

The freshwater polyp hydra can be regarded as a living fossil that allows insights into axis formation of a putative metazoan ancestor before bilaterality and trunk formation were invented. In relation to contemporary animals, originally a very small anterior part were present that that evolved towards the head. In contrast, only a small fraction of these hydra-like ancestors gave rise to the trunk that now forms such a large part of the body of higher animals. Cnidarians are among the lowest phyla in which specialized nerve cells exist. In hydra discrete neuronal centers such as ganglia are not yet present. The existing regionalization of the ancestral body column, however, was able to regionalize the brain that evolved from most of this column

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