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Did the first chordates organize without the organizer?

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Models of vertebrate development frequently portray the organizer as acting on a largely unpatterned embryo to induce major components of the body plan, such as the neural plate and somites. Recent experiments examining the molecular and genetic basis of major inductive events of vertebrate embryogenesis force a re-examination of this view. These newer observations, along with a proposed revised fate map for the frog *Xenopus laevis*, suggest a possible reconciliation between the seemingly disparate mechanisms present in the ontogeny of the common chordate body plan of vertebrate and invertebrate chordates. Here, we review data from vertebrates and from an ascidian urochordate and propose that the organizer was not present at the base of the chordate lineage, but could have been a later innovation in the lineage leading to vertebrates, where its role was more permissive than instructive.

Introduction

The chordate body plan, represented in extant organisms by urochordates (see Glossary), cephalochordates and vertebrates, is more than half a billion years old. Phylotypic features of chordates include a notochord flanked by muscle, a dorsal nervous system, pharyngeal gill slits and a postanal tail. The key events that led to the evolution of the chordate body plan are unknown, but new observations from both vertebrates and ascidian urochordates could shed new light and overturn long-held assumptions about the role of the organizer in the establishment of the chordate body plan. Although Spemann's organizer of the frog, or its equivalent (Hensen's node in chick, the node of mouse and the shield of zebrafish), has figured large in models of the vertebrate body plan, ascidians appear to have no tissue orthologous to the organizer based on *in situ* hybridization surveys and data on neural induction [1,2]. Nevertheless, ascidians arrive at the same fundamental body plan. This would seem to require one of two scenarios: either the urochordate lineage lost the organizer and has evolved independent mechanisms to generate a chordate body plan, or the lineage leading to vertebrates has added the organizer. We review recent developmental and molecular data that call in question the assumption of the primacy of the chordate organizer, and offer a revised scenario for the history of patterning of the chordate body.

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A revised fate map for *Xenopus*

Spemann's organizer in *Xenopus*, centered on the early involuting blastopore lip, has long been thought to be central in specifying and directing body axis formation. The prevailing model for organizer function in *Xenopus* holds that it is a signaling center that influences embryonic tissues in their dorsoventral fates depending on their proximity to the organizer itself [3,4]. The late blastula fate map – the projection forward in development of blastula territories to their future location in the larval or adult body plan – intimately depends on organizer function under this model: the organizer defines the future dorsal side of the organism, and regions increasingly distant from the influence of the organizer are specified as ventral. Accordingly, manipulations reducing or expanding organizer territory have a profound effect on both patterning and morphogenesis and are interpreted as ventralizing or dorsalizing in their action [5]. The traditional model places the future dorsoventral axis from the organizer to the opposite side of the gastrula (the 'ventral marginal zone'), and this axis, in turn, lies orthogonal to the animal/vegetal axis, which runs from the pigmented portion of the embryo to the yolky portion.

Recently, Lane and others have proposed a radical realignment of the major embryonic axes in *Xenopus* based on new lineage tracing studies and a re-evaluation of older ones [6–8]. The revised model largely redraws the

Glossary

Cephalochordata: also called lancelets, or amphioxus, these are the closest living non-craniate relatives of vertebrates, and contain all chordate features, yet lack the cranial and sensory complexity of the vertebrate clade. Their position in chordate phylogeny makes these organisms ideal for comparisons with vertebrates.

Chordata: the deuterostome phylum that includes urochordates, cephalochordates and vertebrates. Chordates have a notochord flanked by muscle, a dorsal nervous system, pharyngeal gill slits, and a postanal tail. These characters have been modified or lost in some lineages, but are usually present at some point in development.

Urochordata: also called tunicata, because of an outer covering, or 'tunic', in adults, urochordates are the most basal chordates. Adults filter-feed and bear few visible traces of chordate affinity aside from a pharyngeal basket perforated with gill slits. Other chordate features, notably a dorsal nervous system and notochord, are present in the larvae. Ascidian urochordates, such as *Ciona*, *Halocynthia*, *Molgula* and *Styela*, have been used as developmental models for more than a century.

Vertebrata: characterized by a vertebral column, this group includes the jawless lamprey and all jawed vertebrates including cartilaginous fishes (e.g. sharks), ray-finned fishes (e.g. teleost fish) and lobe-finned fishes (e.g. coelacanth, tetrapods). Vertebrates, together with the hagfish, comprise the craniata, a group characterized by a bony or cartilaginous skull as well as numerous sensory innovations. Neural crest and ectodermal placodes are thought to be craniate novelties, but this view has come under scrutiny recently.

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fate map: briefly, the revised map reassigns dorsal-ventral to the animal-vegetal axis, a 90° shift, and anterior-posterior is proposed for the organizer-contraorganizer axis (the 'old' dorsal-ventral axis). The organizer itself, which goes on to form the chordamesoderm, is an exception to these new axes. Its derivatives come to lie dorsally along the length of the anterior-posterior, but only after tissue separation at early gastrula stages between forming chordamesoderm and paraxial mesoderm enables these tissues to slide past one another. Under the revised model, somites, dorsal mesodermal derivatives, arise from mesoderm near the organizer, but also from opposite the organizer (previously the 'ventral marginal zone'), the 'ventral'-most territory on the fate map under the traditional model. Presumptive somites nearer to the organizer are demonstrated to be increasingly anterior, following an anterior-posterior gradient along the organizer-contraorganizer axis. Blood islands, ventral mesoderm, are found to arise from territories opposite the organizer, as on the traditional fate map, but also nearer to the organizer, that is, 'dorsal' under the previous schema. Like somites, blood islands that map closer to the organizer are more anterior on the fate map than those opposite. Dorsal-ventral is reassigned as animal-vegetal, occupying an embryonic axis along which no future body axis previously had been mapped. Somites (dorsal) map to the marginal zone closer to the animal pole, whereas the blood islands (ventral) map to the marginal zone closer to the vegetal pole. The two sets of axes are orthogonal to one another and appear to be independent; for example, they do not appear to run either anterodorsal or ventroposterior. So, blood island territories are always more vegetal, and therefore more ventral, than prospective somites, but can still be closer to or farther from the organizer, more anterior or posterior on the fate map, depending on their ultimate anterior/posterior assignment. The revised map has profound consequences for the interpretation of organizer function, particularly for manipulations traditionally viewed as 'dorsalizing' or 'ventralizing' in their effect.

Convergence of fate maps for vertebrate and invertebrate chordates

One striking consequence of the revised fate map is that the general distribution of major tissue territories within the embryo, including epidermis, endoderm, neural tissue and muscle, bear a much closer correspondence with the distribution for the same territories in the ascidian urochordate one-cell zygote. Simply put, the fate maps are more alignable under the new model (Figure 1). Muscle flanking notochord, or somites in vertebrates, arises from a much broader swathe, not just a restricted region close to the presumptive notochord or the site of gastrulation. This is important, because the new fate map shows clearly that muscle is derived from areas previously thought to be too distant from the vertebrate organizer to be influenced by its 'dorsalizing' signal. The similarity of the *Xenopus* and ascidian fate maps suggests that the chordate body plan might have a corresponding generalized fate map. Despite the similarity of topography in presumptive tissue territories, the mechanisms of early development are thought to be largely different between urochordates and vertebrates. Ascidian development is thought to be largely determinative, and vertebrate development inductive, with the organizer figuring largely in the induction. New findings challenge both of these views. Many inductive events have been described in the ascidian, and indications of organizer-independent prepatterning of those tissues thought to be induced by the organizer are being uncovered in *Xenopus*. All of this leads one to ask whether the organizer was fundamental to the evolution of the chordate body plan, or even present in the primordial chordate.

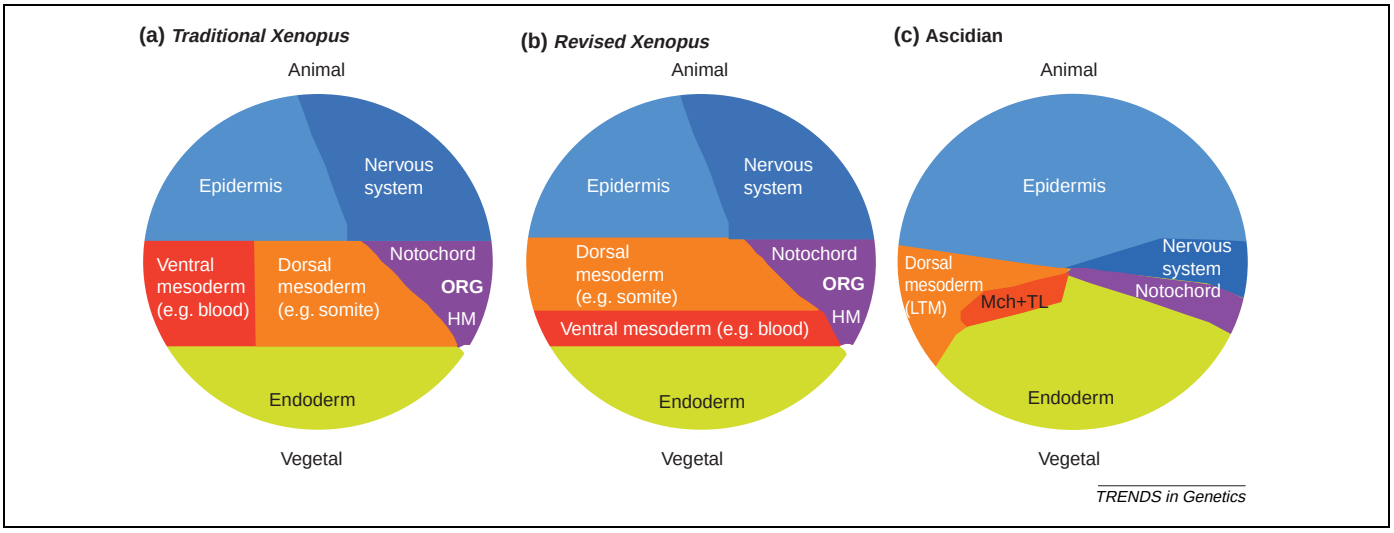

Figure 1. Schematic of traditional and revised fate maps for the late blastula and early gastrula frog, *Xenopus laevis*, and the ascidian urochordate one-cell zygote. The traditional map (left) depicts a dorsal-ventral polarity in mesodermal derivatives along the axis from the organizer (ORG) to the opposite side. In the revised model (middle), mesodermal derivatives closer to the organizer are anterior, whereas those further from the organizer are increasingly posterior; consequently, for example, anterior somites fate to positions adjacent to the organizer whereas posterior somites fate to the opposite side. The newly assigned axes for mesodermal fates do not apply to chordamesoderm (see main text). The ascidian fate map (right) is depicted without an organizer, but nevertheless has a similar topology of tissue fates as the revised *Xenopus* map, albeit with differently proportioned domains. Like the revised *Xenopus* map, the ascidian map includes an anterior-posterior polarity for mesodermal fates along the horizontal axis (right to left in the figure). Abbreviations: HM, head mesoderm; LTM, lateral tail muscle; Mch, mesenchyme; TL, trunk lateral cells.

Organizer-independent prepatterning suggests a different role for the organizer

If the organizer were a more recent addition within the chordate lineage, it might be expected that some aspects of patterning the body could occur independently of organizer function. Observations support such a claim, showing that a great deal of patterning in frog is evident pregastrula, even before the formation of the organizer. *myoD* (which encodes the myoblast determination 1 protein) marks the prospective somitic regions of the embryo – the broad band of *myoD* prefiguring the somites is present early, at blastula stages, and appears even if organizer formation is prevented by UV treatment or molecular ablation [9,10]. (Early *myoD* expression in 'ventral' territories of the embryo had been described more than a decade ago and its incongruence with the older fate map noted [9,11]; the new fate map resolves this incongruence.) When the organizer is removed, *myoD* expression is not maintained and somites fail to form, pointing to a permissive/maintenance role for the organizer in the myogenic pathway. The initial *myoD* prepattern is dependent on a maternal factor, eFGF, the *Xenopus* ortholog to fibroblast growth factor 4 (FGF4) [12]. This *myoD* expression does not depend on zygotically transcribed genes, suggesting that, as in ascidians, the muscle prepattern is determined maternally [13,14]. Likewise in blood formation, marginal zone explants isolated at the blastula stage already contain a prepattern specifying the vegetal-most region as competent to form ventral blood islands, independently of organizer function [15].

Much as *myoD* marks presumptive somitic regions in an organizer-independent manner, neural-specific transcripts mark prospective neural territories even before organizer formation. Sox3, SoxD and Geminin, which causes epidermis to adopt a neural fate when ectopically expressed, are expressed in the late blastula, before gastrulation and organizer formation, and Sox3 is restricted to presumptive neural ectoderm before gastrulation [16–18]. Likewise, bone morphogenetic protein (BMP) signaling is restricted to presumptive epidermal territories of the ectoderm before organizer formation [19]. Finally, in frog, zebrafish, chick and mouse, neural differentiation and neural plate formation will take place even when the gastrula organizer is removed, although these experiments should be interpreted cautiously, because complete removal of all traces of the organizer by surgical means is difficult to confirm [20–23]. This prepatterning of territories within the fate map, in an apparent organizer-independent fashion, is consistent with a scenario in which the organizer has a permissive role for further differentiation of cells or a maintenance role later in development, stabilizing gene expression patterns.

Initiation of neural specification by FGF is conserved in chordates

The 'default model' for the neural specification of ectoderm holds that the organizer preserves a default neural state through the secretion of BMP antagonists [24]. Where BMP signaling is unopposed, further from the organizer in the animal hemisphere, the ectoderm adopts an epidermal fate. An expanded or reduced organizer will result in expanded or reduced neural tissue, or the opposite outcome for epidermis, showing the importance for organizer function on these ectodermal derivatives during normal embryogenesis [25,26]. Recent studies from both amniote and anamniote vertebrates, however, show that the default model provides an incomplete description of neural specification and that, specifically, BMP inhibition alone is not sufficient to induce neural fates [27–29]. In *Xenopus*, ectodermal explants express *Bmp4* and develop as epidermis. When explants are cultured with BMP antagonists, mimicking conditions near the organizer, neural induction occurs [30]. If the cells from these explants are dissociated, abolishing cell–cell contact, cells will again develop neural fates [31]. If BMP4 is added to dissociated explants, the fate of the ectoderm will revert to epidermis [32]. These results have been taken to indicate the sufficiency of BMP inhibition for neural specification. The explant procedure, however, induces autocrine *Fgf* expression [33,34], which subsequent investigations have shown is also required for neural specification, in addition to BMP inhibition. Disruption of FGF receptor function demonstrates that even in the presence of organizer signals, cells require FGF signaling to acquire a neural fate [29]. The details in relative timing and in the degree of interdependence of BMP antagonists and FGF signaling need to be resolved, but the requirement for FGF appears to be conserved in *Xenopus*, chick and zebrafish as well [29,35–37]. More strikingly, neural induction in ascidians also employs FGF signaling as a first step, although orthologs to vertebrate organizer-derived BMP antagonists appear to have no role in ascidian neural induction [2,38]. This apparent broad conservation has led to the proposition by Delaune and others that FGF signaling is a 'conserved initiator of neural specification' in chordates [29].

Urochordates and the chordate body plan

Urochordates, or tunicates, exhibit all the important features of the chordate body plan at some time during their life cycle. Historically, urochordate development was seen as mosaic; a stereotyped cell lineage and manipulations separating early blastomeres led investigators to believe that induction and regulative development were less important in urochordate ascidians [39]. This view was not borne out experimentally; important inductive interactions occur during ascidian embryogenesis – endoderm specification by dorsal/ventral determinants, induction of the notochord by the endoderm, and induction of neural tissue by the spinal cord [40–42]. Other interactions, for example induction of the spinal cord by the notochord, remain to be demonstrated, and yet others, such as the specification of muscle cells, are known to be determined by intrinsic factors [43]. For many who investigate the mechanisms of chordate evolution, the small number of cells (relative to vertebrates) of the urochordate embryo, a stereotyped cell lineage, and a compact, polymorphic genome point to an organism that is simplified to such a degree that it might not reliably provide clues of its chordate ancestry. However, the nature of the chordate common ancestor is unknown, and specific

features of its development, including size, cell number, and whether it displayed a stereotyped cell lineage, remain points of speculation. All organisms, whatever their taxonomic grouping, are a collection of basal and derived features; and the ascidian urochordate, like other organisms, requires care when used to make broad phylogenetic conclusions. Data from ascidians will ultimately be a part of our picture of the chordate common ancestor, alongside developmental data from other organisms, sequence comparisons across phyla and the fossil record.

One possible scenario is that the chordate ancestor did have an organizer, but that tunicates have lost most or all aspects of organizer function in development. According to this view, the relatively small number of cells comprising the urochordate embryo and the stereotyped cell lineage did not require more than simple inductive interactions – the simplified urochordate tadpole retains chordate features, but discards the organizer or greatly reduces its role because the rules of development for such a simplified chordate have changed and the chordate body plan can be established by other means. The vertebrate organizer gives rise to notochord and head mesoderm; some authors contend that the urochordate notochord precursor cells might still retain organizer function [44], albeit reduced, although this has been difficult to test directly because of the small size of ascidian gastrulas and the difficulty of tissue transplant. If the urochordate lineage has lost the organizer, we might expect gene expression patterns that characterize the organizer to persist in the urochordate notochord precursor either because some reduced organizer function is retained, or because gene expression that originally characterized the organizer has not been entirely eliminated, even if this expression is nonfunctional insofar as it contributes to organizer function.

Ascidian orthologs of vertebrate organizer genes (http://ghost.zool.kyoto-u.ac.jp/tfst.html) [1] do not appear to be expressed similarly to vertebrates. This could indicate that a loss of organizer function is complete and no traces of an organizer-dependent past remain. An alternative is that these genes are not active near the site of gastrulation in ascidians because they never were; these 'organizer' genes were only later deployed for that function, in the lineage leading to cephalochordates and vertebrates, after its divergence from the urochordate lineage. The mere presence of ascidian orthologs to vertebrate organizer genes should not be taken as evidence of an organizer in the common chordate ancestor; orthologs to these genes are also present in basal deuterostomes and in protostomes, where they can have entirely different roles. Nevertheless, the formal possibility remains that in spite of extensive efforts to characterize urochordate and vertebrate genomes and to describe gene expression patterns in these models, common genes for the organizer might exist, but simply have not yet been characterized. We favor the view that the absence of organizer-type gene expression patterns in ascidians, rather than pointing to the deficiencies of the ascidian as a study organism for chordate relations, could in fact be informative.

Concluding remarks

Ultimately, the early evolution of chordates might be difficult to reconstruct. Our own hypothesis could hinge on whether urochordates represent a derived or ancestral state with respect to organizer function, and this can only be addressed through further comparative studies. Gene expression and functional data from hemichordates, which, with echinoderms, make up the nearest outgroup to chordates, could be used to determine whether any 'pre-organizer' activity of orthologs to vertebrate organizer genes is evident, directly testing whether the lack of such expression in ascidians is representative of the chordate ancestor or merely an indication of idiosyncrasies within the urochordate clade. Data from another non-vertebrate chordate, the cephalochordate amphioxus, show that at least some orthologs to vertebrate organizer genes are expressed in a similar fashion [45,46]. Additionally, transplants in amphioxus appear to provide support for the presence of an organizer [47]. Most currently accepted phylogenies place urochordates as the outgroup to a cephalochordate/vertebrate clade; the amphioxus data would place a latest date on the appearance of the organizer to the cephalochordate/vertebrate common ancestor. Amphioxus could be particularly valuable in determining to what extent the organizer functions in a permissive or maintenance role, and not in the initiation of patterning. A broader survey within the vertebrates might tell us not only whether *Xenopus* is typical, but surely will also indicate cases in which the relation between the organizer, the establishment of the fate map, and the stabilization of pattern leading to differentiated tissue types has undergone significant modification with changing developmental modes, as could have occurred within the amniotes (within which, e.g. *myoD* is not a good marker for presumptive somitic regions because its expression is first detected long after gastrulation [48,49]).

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