The developmental hourglass model: a predictor of the basic body plan?

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ABSTRACT

The hourglass model of embryonic evolution predicts an hourglass-like divergence during animal embryogenesis — with embryos being more divergent at the earliest and latest stages but conserved during a mid-embryonic (phylotypic) period that serves as a source of the basic body plan for animals within a phylum. Morphological observations have suggested hourglass-like divergence in various vertebrate and invertebrate groups, and recent molecular data support this model. However, further investigation is required to determine whether the phylotypic period represents a basic body plan for each animal phylum, and whether this principle might apply at higher taxonomic levels. Here, we discuss the relationship between the basic body plan and the phylotypic stage, and address the possible mechanisms that underlie hourglass-like divergence.

KEY WORDS: Body plan, Evo-devo, Evolution, Hourglass model, Phylotype, Transcriptome

Introduction

Animals that belong to the same phylum or to the same (usually large) phylogenetic group are considered to share a basic body plan (Jane et al., 2013; Wallace, 2000), which is key to grouping different animal species together (see Box 1). For example, despite the various niches inhabited (aquatic, terrestrial and aerial environments) and the over 1000-fold difference in body size (the smallest vertebrate is a frog less than 1 cm long (Rittmeyer et al., 2012) and the largest is the 30 m blue whale), all vertebrates share a set of anatomical features, such as a dorsally located central nervous system, segmented trunk muscles, vertebrae along the anteroposterior axis, a complex head with sensory organs (eyes, inner ear and nose), and organs such as the brain, heart and liver (Benton, 2004). Similarly, various other animal groups are also said to have a conserved basic body plan within their phyla. But what underlies this conservation across evolution? Why should basic anatomical features but not, for example, body mass or colour, be conserved across evolution? Is this anatomical conservation inevitable?

Why is the body plan conserved?

Considering hundreds of millions of years of animal evolution, with highly variable interactions between organisms and their environment, it is not easy to explain why the basic body plan is conserved within each phylum. The concept that the basic body plan arises from a set of conserved morphological elements found at a specific stage of embryogenesis (Fig. 1A,B) was suggested by classical morphological studies, and has recently been lent support by molecular analyses. Various concepts have been advanced to explain why specific embryonic patterns should be conserved (Fig. 1C). For example, the idea of ‘developmental constraint’ argues that the nature of the developmental process imposes certain limitations on phenotypic variability, leading to evolutionarily conserved phenotypes (Maynard Smith et al., 1985). Similarly, the concept of ‘developmental burden’ (Riedl, 1978) suggests that, because developmental processes are hierarchically organized and interdependent (e.g. somite segmentation requires the prior establishment of the anteroposterior axis), strong conservation is inevitable (discussed further below). As Garstang proposed in his ‘stepping-stone model’ (Garstang, 1922; Holland, 2011), we often see that specific embryonic structures at one developmental stage require structures or developmental processes that arise or occur at an earlier stage. This type of dependency could have been a force that ensured conservation of embryonic structure.

Although these concepts have been around for several decades, no consensus has been reached as to whether these mechanisms actually take place to conserve basic body plans. Meanwhile, recent analyses at the genomic and transcriptomic level are providing new insights into the problem, identifying the conserved mid-embryonic period from a molecular perspective and allowing scientists to extract shared morphological patterns that are conserved in this molecularly identified period, which may perhaps serve as a source of the basic body plan. In this Review, we first introduce the current models in an attempt to lay out a generalized relationship between animal development and evolution – focussing particularly the ‘developmental hourglass model’ (Fig. 2; discussed further below) that potentially explains the conservation of animal body plans. We then evaluate the predictions of and questions raised by the hourglass model, especially in terms of how a basic animal body plan can be explained on the basis of this model.

Conservation of embryonic development

Despite their widely divergent final appearance, all vertebrates go through a broadly similar set of developmental stages, starting from a single-celled fertilized egg, proceeding through broadly

Box 1. Basic body plan and the phylotypic period

Basic body plan has been defined as ‘an assemblage of morphological features shared among many members of a phylum-level group’ (Valentine, 2004). Chordate basic body plan, for example, includes anatomical features such as notochord, pharyngeal gill slits, brain, dorsal hollow nerve cord and post-anal tail. For unknown reasons, body size, coat colour or body weight are not well conserved in phylum-level animal groups, and are much less commonly characterized as morphological features of basic body plans. The developmental hourglass model hypothesizes that basic body plans are established at the most conserved embryonic period, or phylotypic period – which occurs during mid-embryogenesis (see also Fig. 2).
Fig 1. The logic underlying the hourglass model-predicted body plan hypothesis. (A) The basic body plan is conserved within each animal phylum but no consensus has been reached as to how or why this is the case. (B) Of the various possible reasons that may explain the shared basic body plan, the contribution of the conserved embryonic pattern is now gaining interest due to studies that support the developmental hourglass model. In brief, a set of conserved, anatomical features found at the mid-embryonic period contribute to the basic body plan of a given animal phylum (or other taxonomic group). However, it is largely unknown why the mid-embryonic period should be conserved. (C) Many hypotheses have been proposed, which may not be mutually exclusive, but no consensual empirical evidence has been obtained.

The hourglass model
In the 1990s, based on the observation that early embryonic processes (e.g. cleavage and gastrulation) of different vertebrates are rather divergent, as well as the discovery of conserved Hox cluster gene expression along the anteroposterior axis of animal embryos, Duboule (1994) proposed the developmental hourglass model (Fig. 2). This model argued for evolutionarily diverged early and late stages, with an intermediate period of embryogenesis being most conserved. A similar concept was also proposed by Raff (1996). The hourglass model predicts that the most conserved embryonic stage of animal phyla (Duboule, 1994; Raff, 1996) is not the earliest, fertilized egg stage, but a mid-embryonic period called the ‘phylotypic period’, during which the common anatomical features of the basic body plan are defined (see Box 1). In addition, these seminal papers also proposed possible evolutionary mechanisms that could explain the hourglass-like pattern of conservation during embryogenesis. Duboule (1994) proposed that the spatial and temporal co-linearity of Hox cluster gene expression would make fundamental changes to the organization along the embryonic anteroposterior axis unlikely, further leading to body plan conservation. Meanwhile, Raff (1996) proposed that the highly inter-dependent molecular signalling among developmental modules in the mid-embryonic stages makes this period developmentally constrained, thus leading to evolutionary conservation (Fig. 2B). In brief, these two hypothetical mechanisms (which currently lack empirical verification) attribute mid-embryonic conservation to the fundamentally important nature of the developmental system itself; changes in the molecular network during this mid-embryonic period could have fatal consequences, thus leading to evolutionary conservation.

In response to the proposal of the hourglass model, the quest to uncover the relationship between development and evolution was renewed. Some researchers proposed different models for different animal groups (Salazar-Ciudad, 2010), whereas others tested possible models by evaluating evolutionary divergence during embryogenesis based on quantitative measurements of morphologically homologous traits of various vertebrate embryos (Bininda-Emonds et al., 2003; Poe, 2006; Poe and Wake, 2004; Richardson et al., 1997, 1998; Richardson and Keuck, 2002; Galis and Metz, 2001; Hall, 1997). However, these analyses have proved inconclusive, and it has remained controversial whether the hourglass model holds.

A number of alternatives have been proposed. For example, based on comparative morphological analyses, Richardson et al. suggested an alternative model, the ‘adaptive penetrance model’, that questions the existence of a conserved mid-embryonic stage (Richardson et al., 1997). The model attributes the highly divergent mid-embryonic, organogenesis stages to a higher tendency for beneficial mutations, because this is the period when the basic body plan is established and when there is the potential to generate adult innovation. Others have proposed an ‘ontogenetic adjacency model’ that does not assume a temporal difference of evolutionary conservation during development (Poe and Wake, 2004). Based on the observed lack of trends in heterochronic changes during embryogenesis, the authors suggest that ‘evolutionary change is easier between ontogenetically adjacent events’.
The confusion relating to the timing of evolutionary divergence is primarily associated with the difficulty in using morphological characteristics to evaluate quantitatively the degree of conservation at each developmental stage given qualitatively different morphological features. For example, although one can quantitate differences in, for example, blastomere number at an early stage there is no method for quantitatively comparing this variation against the variations of somite number at a later stage. Meanwhile, some researchers have tested the models by investigating developmental features that may potentially result in certain evolutionary conservation (e.g. Raff proposed that the phylotypic period of the hourglass model arises because the organogenesis period is constrained, with changes during this period tending to result in a lethal or less adaptive phenotype). In concordance with this viewpoint, some researchers have tested the models by reviewing the studies that applied teratogens to rodent embryos (reviewed by Galis and Metz, 2001), to identify the stages that are particularly sensitive to these treatments. They identified enhanced sensitivity (in terms of a higher frequency of abnormality of lethality) to teratogens during the organogenesis stages. However, it is not surprising that teratogens cause more malformations during the period of organogenesis because these agents are, by definition, chemicals that cause abnormal organogenesis. Therefore, it remains to be clarified whether the organogenesis stages are actually most susceptible to failure or lethality due to genetic mutations, and whether this can cause an hourglass-like divergence in evolutionary timescale. Similarly, Roux and Robinson-Rechavi pointed out that genes expressed in early stages are often indispensable (Roux and Robinson-Rechavi, 2008); however, the data were not comprehensive and, as argued by Kalinka and Tomancak (2012), loss-of-function analysis does not address the issue of evolvability of different periods of development.

**Testing models with molecular approaches**

Since the models discussed above were introduced to explain the divergence of embryos over hundreds of millions of years (Myr) of evolutionary time (~550 Myr ago for vertebrates), clarifying the possible evolutionary mechanism underlying embryonic body plan conservation is far more challenging than evaluating the degree of conservation. Traditionally, studies assessing divergence/conservation have used morphological approaches, but the rise of sequence-based analysis for evolutionary studies, based on genomic or transcriptomic data, has provided a new tool for researchers in this field. Thus far, and not surprisingly given the challenges mentioned above, most molecular studies have focused on evaluating the divergent or conserved nature of embryos by quantitatively measuring expression profiles of genes during animal embryogenesis.

Pioneering studies took advantage of expression profiles from single species (Hazkani-Covo et al., 2005; Irie and Sehara-Fujisawa, 2007), and more recently from multiple species to make cross-species comparisons of expression profiles (Kalinka et al., 2010; Irie and Kuratani, 2011; Levin et al., 2012; Yanai et al., 2011; Wang et al., 2013; Schep and Adryan, 2013). Measuring orthologous gene expression profiles from whole embryonic RNA samples discards all the morphological information, but the measured expression similarity between samples can be regarded as an index that reflects the degree of similarity in cellular composition between embryos. Therefore, it is potentially a useful alternative approach to identify conserved embryonic stages. Moreover, such molecular studies seem to have some advantages over morphological approaches because they more directly assess the inherited entities (the DNA sequences and transcribed information). This is similar to what has happened in the field of phylogenetics, where molecular phylogeny now dominates morphology-based data.

The pioneering molecular studies (Hazkani-Covo et al., 2005; Irie and Sehara-Fujisawa, 2007; Artieri et al., 2009; Cruickshank and Wade, 2008) had two major limitations. First, they were based on a limited number of genes [e.g. they used EST (expressed sequence tag) data]. Second, their evaluation was largely based on the sequence conservation of expressed genes in single species, such as mice (Hazkani-Covo et al., 2005; Irie and Sehara-Fujisawa, 2007) or Drosophila (Artieri et al., 2009; Cruickshank and Wade, 2008), rather than incorporating data from multiple species. It is important to bear in mind that these experimental animals were...
selected as models because they share useful features, such as a short generation period and quick developmental time, and that this may represent a highly derived mechanism of development compared with other animals – thus potentially having limited use for evolutionary studies (Hall, 1997). Rapid advancements in technology, such as microarray and massively parallel sequencing, have addressed the first limitation mentioned above. However, the second limitation still applies to many studies (Domazet-Loso and Tautz, 2010; Piasecka et al., 2013; Quint et al., 2012; Roux and Robinson-Rechavi, 2008). Although analysing the expression profiles of single species [e.g. using the ancestor index (Irie and Sehara-Fujisawa, 2007) and the transcriptome age index or TAI (Domazet-Loso and Tautz, 2010)] does not directly identify whether the evolutionarily equivalent genetic program is working in other species, these studies have stimulated debate on this long-standing problem. Many of these single-species-based expression analyses show that the expression profiles at mid-embryogenesis stages have the highest ratio of sequence-conserved genes, thus supporting the hourglass model (Cruickshank and Wade, 2008; Domazet-Loso and Tautz, 2010; Hazkani-Covo et al., 2005; Irie and Sehara-Fujisawa, 2007; Quint et al., 2012). However, some studies favour the funnel model (Artieri et al., 2009; Roux and Robinson-Rechavi, 2008), and the authors of these studies have proposed a co-existence model using zebrafish that incorporates both the hourglass model for morphological variations and the funnel model for molecular processes (Comte et al., 2010; Piasecka et al., 2013). It should be noted, however, that one of the most detailed morphological studies on vertebrate embryos is not consistent with the hourglass model (Bininda-Emonds et al., 2003).

Considering that the estimation of evolutionary conservation using extant animal taxa largely relies on the observation of shared features between different species, it is essential to include cross-species evaluation of shared gene expression in these types of molecular studies. Ancestor Index (Irie and Sehara-Fujisawa, 2007) and TAI (Domazet-Loso and Tautz, 2010) analyses, on the other hand, can evaluate only the abundance of sequence-conserved genes in embryos of each species, and these do not necessarily tell us whether the evolutionarily equivalent genetic program is commonly taking place among different species. More recently, a number of studies have included cross-species comparisons of orthologous gene expression (Table 1). In general, these have supported hourglass-like divergence, with the most conserved expression profile at mid-embryonic stages. Importantly, these cross-species studies clearly indicated that hourglass-like divergence can be found irrespective of platform (microarray or massively parallel sequencers), normalizations and signal calculations, and similarity calculation methods. However, the diversity of measurements used also means that consensus has yet to be reached. Given that these robust studies included cross-species comparisons of shared expression of orthologous genes, it seems reasonable to accept that hourglass-like divergence, which is a prerequisite for the developmental hourglass model, can be observed in a variety of animal species.

**Table 1. Summary of cross-species transcriptome comparisons that test the hourglass model**

<table>
<thead>
<tr>
<th>Compared species (groups)</th>
<th>Divergence time (Myr ago)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five nematode species</td>
<td>30*</td>
<td>Levin et al., 2012</td>
</tr>
<tr>
<td>Six Drosophila species</td>
<td>40†</td>
<td>Kalinka et al., 2010</td>
</tr>
<tr>
<td>X. tropicalis and X. laevis</td>
<td>50-81§</td>
<td>Yanai et al., 2011</td>
</tr>
<tr>
<td>P. sinensis (turtle) and chicken</td>
<td>248-268§</td>
<td>Wang et al., 2013</td>
</tr>
<tr>
<td>A. gambiae and Drosophila</td>
<td>240**</td>
<td>Schep and Adryan, 2013</td>
</tr>
<tr>
<td>X. tropicalis and D. rerio</td>
<td>476‡</td>
<td>Irie and Kurihara, 2011</td>
</tr>
<tr>
<td>Mouse, chicken, X. laevis and D. rerio</td>
<td>476‡‡</td>
<td>Irie and Kurihara, 2011</td>
</tr>
<tr>
<td>Mouse, chicken, Xenopus, D. rerio and A. gambiae</td>
<td>993§§</td>
<td>Levin et al., 2012</td>
</tr>
<tr>
<td>X. tropicalis and C. elegans</td>
<td>1177§§</td>
<td>Levin et al., 2012</td>
</tr>
</tbody>
</table>

*Cutler, 2008; †Kalinka et al., 2010; ‡Evans et al., 2004; ‡‡Wang et al., 2013; *§§You et al., 2013; ††Blair and Hedges, 2005; §§Benton and Ayala, 2003.

vertebrates (Duboule, 1994) and each phylum (Raff, 1996) is expected to be established. Three major issues must be resolved to verify this prediction. First, assuming that the phylotypic period represents the basic body plan for each phylum, what morphological elements can be found during this period, and how can we define the basic body plan from the embryonic point of view? To solve this issue, studies based on sufficient coverage of phylum-wide species must be carried out. Second, we still do not know the range of species or phylogenetic groups, that can be explained by this model: does it apply at the level of the phylum, as was initially proposed, or can it explain conservation within smaller or larger taxonomic groups? Third, what mechanisms might the conservation during embryogenesis predicted by the hourglass model. Below, we discuss each of these challenges in turn.

**The morphological features of the phylotypic period**

Comparing embryonic expression profiles among six *Drosophila* species that split 40 Myr ago, Kalinka et al. (2010) found that the extended germband stage has the most conserved expression profile; this stage is generally considered to be the arthropod phylotypic period (Sander, 1976). Meanwhile, by comparing *D. melanogaster* and *A. gambiae* and using expression analysis limited to transcription factor-coding genes, Schep and Adryan (2013) reported that there are two periods of high conservation peaks between these species, one at the extended germband stages as Kalinka et al. reported, and the other at a later stage (stage 17 in *Drosophila*). *Drosophila* embryos at this later stage have a more complex set of features than at the extended germband stage: they have undergone head involution and various structures – including the atrium, the ventral nerve cord, the proventriculus, the hindgut and the posterior spiracles – have formed (Campos-Ortega and Hartenstein, 2013). Which stage could be regarded as the potential phylotypic period for arthropods? In other words, which of these stages contain the morphological elements that explain the three primary features of the arthropod body plan (Jane et al., 2011): anteroposteriorly segmented structures, the external skeleton and articulated limbs? Given that imaginal discs that later become articulated limbs have formed (Campos-Ortega and Hartenstein, 2013). Which stage could be regarded as the potential phylotypic period for arthropods? In other words, which of these stages contain the morphological elements that explain the three primary features of the arthropod body plan (Jane et al., 2011): anteroposteriorly segmented structures, the external skeleton and articulated limbs? Given that imaginal discs that later become adult limbs already exist at the extended germband stage, and segmented trunk of this stage contributes to adult segments, it can be said that two out of three features of the arthropod body plan can be traced back to an extended germband stage. However, the other key feature of the arthropod body plan, the external skeleton,
cannot be explained by morphological elements found at either of these stages. Cells that later produce external skeleton at these embryonic stages contribute to the exoskeleton of only the pupa, rather than that of the adult. However, both these studies have limitations: the first (Kalinka et al., 2010) was based on a shallower degree of evolutionary depth (only 40 Myr since the Drosophila species diverged, compared with 250 Myr since Drosophila and Anopheles diverged), while the latter (Schep and Adryan, 2013) was based on only a subset of genes – transcription factors. Therefore, a more comprehensive study is warranted to cover the arthropod phylum.

The situation is similar for studies on chordate species. By using the gene expression profiles of four vertebrate species (mouse, chicken, X. laevis and zebrafish) that separated 400 Myr ago, we previously identified the pharyngular embryo as a potential vertebrate phylotypic period (Irie and Kuratani, 2011). This was achieved by making all-to-all comparisons of the developmental stages from each species and identifying the most conserved set of embryonic stages from the four vertebrates in terms of expression profile similarity. More recently, we have added another vertebrate species, the Chinese soft-shell turtle (Pelodiscus sinensis), to the analysis (Wang et al., 2013). Although the turtle has a very different body plan to that of other vertebrates (Nagashima et al., 2009), we found that the most conserved embryonic period between turtle and chicken in terms of expression profiles matched the same vertebrate phylotypic period that had been identified previously (Irie and Kuratani, 2011) (Fig. 2C).

Do these stages share the anatomical features that can explain the basic body plan of the vertebrates, or chordates, as predicted by the hourglass model? Based on the hypothetical form of the vertebrates’ common ancestor deduced from paleontological studies (Benton, 2004), the shared morphological features of vertebrates that constitute the basic body plan can be defined as: the notochord; dorsal nerve cord; head with nostril, eye, ear, muscle blocks, horny teeth and pharynx with slits; trunk with the heart, liver, stomach, gonad and kidney; and tail with anus. Almost all of these features, or at least organ primordia that later give rise to most of these features, can be found in the identified phylotypic period, except for horny teeth. However, as in studies with arthropods, we have to admit that this result may not be conclusive, as these studies did not include early diverged vertebrates such as cyclostomes (lamprey and hagfish). The features found at the identified phylotypic period of the vertebrates are much more complex than the defined key elements of the chordate body plan: the notochord, pharyngeal gill slits, brain, dorsal hollow nerve cord and post-anal tail (Jane et al., 2011). One might therefore imagine that including invertebrate chordate species in the analysis would shift the most conserved stage to an earlier phase of development, thus defining a different phylotypic period for chordates than for vertebrates. Obviously, further studies with more species are needed to investigate this apparent discrepancy.

As described above, it seems too early to conclude whether the anatomical elements found in the most molecularly conserved embryonic stages of arthropods and chordates correspond to the basic body plans of the group concerned, and two issues need to be addressed to test this phylotypic period hypothesis. The first is that the number of species covered by these studies is still not broad enough to cover phylum-wide animals; the second is that the developmental stages investigated in these studies are not comprehensive. For example, none of the studies listed above that focused on identifying the arthropod phylotypic period covered stages later than the embryonic period, such as metamorphosis stages. Thus, there still remains the possibility that even later stages show the most conserved expression profiles. Addressing both these issues is feasible with current technologies, and should shed significant light on the degree to which the developmental hourglass model can explain evolutionary conservation.

**Phylogenetic units and the hourglass model**

Can the hourglass model be applied to groups of animals that are wider, or narrower, than the phylum? Although more than 30 animal phyla are known to exist (Carroll et al., 2001), only three phyla (chordates, arthropods and nematodes) have been the focus of cross-phylum molecular studies during embryogenesis (see also Table 1). Levin et al. performed a cross-phylum comparison by using embryonic expression profiles of C. elegans and X. tropicalis (Levin et al., 2012), and reported that the ventral enclosure stage of C. elegans and the X. tropicalis tailbud stage showed the highest expression similarity, further indicating that the phylotypic period overlaps with the stage when the body plan for the whole animal is established, as has been hypothesized Slack et al. (1993). Our previous study also indicated a similar implication based on the finding that the segmentation stage of A. gambiae showed the highest similarity with the expression profiles of mid-embryonic (around gastrula to organogenesis) stages of four vertebrate species (mouse, chicken, X. laevis and zebrafish; Irie and Kuratani, 2011). These studies, together with attempts to identify conserved molecular modules (Gerstein, 2014), may provide a way to investigate what molecular and morphological features the urbilaterian ancestor possessed (Hejnol and Martindale, 2008), though the number of species studied so far is limited and further investigation with a broader range of animals is needed.

With respect to the experimentally identified conserved period of the hourglass, it is of particular interest that these organogenesis periods (i.e. the pharyngular stage for vertebrates) were found to be maximally conserved irrespective of the phylogenetical distance of the species being compared. For example, the most molecularly conserved period found in X. laevis when compared with three other vertebrates (mouse, chicken and zebrafish) was the pharyngular stage (Irie and Kuratani, 2011); this stage (the tailbud stage of Xenopus – stages 28-31) was also found to be most conserved when compared against the phylogenetically closer species X. tropicalis (Yanai et al. 2011). Why should this be? The observation suggests that the anatomical features of the most conserved embryonic stages do not necessarily reflect shared, adult anatomical features of the species being compared. In other words, anatomical features of the conserved embryos always show features of the body plan of that phylum. In fact, the most highly conserved stage identified in various comparisons between tetrapods is, as discussed above, the pharyngular stage (Irie and Kuratani, 2011; Wang et al., 2013) – before limbs develop. This is despite the fact that the adults forms of all embryos compared have limbs. Importantly, this tendency – so-called ‘persistent conservation’ – was also observed in other phyla, nematodes and arthropoda. The highest conservation among nematode species was at the ventral enclosure stages, and these stages also showed the highest expression similarity to the X. laevis tailbud stages (Levin et al., 2012). Similarly, the highest conservation between fly species was at the extended germband stage, showing much simpler morphological features than those commonly found in Drosophila adults. In the next section, we will further discuss this ‘persistent conservation’ in the context of underlying mechanism for the hourglass model.
Evolutionary mechanisms underlying the hourglass model

Despite the recent advances in the field, the issue of why hourglass-like divergence is observed remains unresolved. Some researchers have proposed that divergence found in early and late embryonic stages is the result of adaptation to particular types of reproductive strategy (Slack et al., 1993), or to diverse ecological niches (Kalinka and Tomanek, 2012); these imply that the phylotypic period is just in between diversifying stages, and is a ‘period of calm’ with much less selective pressure. However, considering rapid neutral evolution, it would be interesting to determine why the phylotypic period has remained both molecularly and morphologically conserved after hundreds of millions of years of evolution. Moreover, it would be interesting to know how animals could have tolerated changes in early developmental stages while conserving the phylotypic period (discussed by Irie and Kuratani, 2011). Similarly, we still do not know why the most conserved period should be the organogenesis phase. In contrast to ideas proposed by Slack et al., and as mentioned above, Duboule and Raff ascribed this conservation to characteristics of embryogenesis; Duboule (1994) attributed the conservation of the phylotypic period to the co-linearity of Hox cluster gene expression, whereas Raff (1996) attributed it to the particularly complex signalling modularity within organ primordia found in the phylotypic period (Raff, 1996).

In accordance with this, the ‘persistent conservation’ of the potential phylotypic period irrespective of the species being compared suggest that evolutionary diversification of this embryonic period has been strictly limited throughout the hundreds of millions of years of evolution, and that this constraint might still apply extant animal embryos. In this context, it would be interesting to see whether the variations among inbred strains, natural populations and genetically identical individuals are also the smallest during the phylotypic period.

If the intrinsic characteristics of embryogenesis are indeed the main reason for this persistent conservation of the phylotypic period, two opposite possibilities can still be considered: fragility or robustness of phylotypic period embryos. For example, hypotheses proposed by Duboule and Raff suppose the fragility, or a limited flexibility, of developmental systems during the mid-embryonic period – such that no species could withstand genetic mutations that lead to drastic changes in the phylotypic period. As mentioned above, studies reviewed by Galis and Metz (2001) also seem to support this idea: organogenesis stages of rodents were prone to die when treated with teratogen, although there are caveats to these studies. The other possibility is that the phylotypic period is ‘robust’ against induced changes, thereby conserving molecular and morphological features, as proposed, for example, for the segment polarity network (von Dassow et al., 2000). Mechanistically, such robustness could potentially be implemented by a capacitor such as heat-shock protein HSP90 (Rohner et al., 2013), which buffers the effects of mutation-induced misfolding of proteins and eventually masks the abnormal phenotype. No empirical evidence has been obtained so far, however, and quantitative measurements of the fragility/robustness of phylotypic stage embryos in various animals would provide insights to this problem.

Conclusions and future perspectives

In conclusion, although recent molecular studies have demonstrated hourglass-like divergence in various animal species, and the identified bottleneck periods – the potential phylotypic periods – show morphological similarities across species, it is still perhaps premature to conclude that the phylotypic period really represents the body plan for a given animal group. The range of species studied, and the developmental stages covered, are limited; further analysis is required to fill these gaps. The observed ‘persistent conservation’ of the phylotypic period, as discussed above, suggests this period could conceivably reflect the basic body plan at a higher taxonomic level than the species being compared in any particular analysis, but whether or not the phylotypic periods represent a phylum-specific body plan is not yet clear. In addition, it is still not clear why embryonic evolution exhibits hourglass-like divergence, and this is an important issue that needs to be addressed. Not only do we not know whether hourglass-like divergence arises from developmental constraints, but we still have no effective tools to measure these developmental forces that may conserve embryonic patterns. Comprehensive measurements of embryonic gene expression profiles have fostered this field in recent years, but intervening experiments such as adding mutations and fluctuations to embryonic stages are required to test the concept, and to investigate the relationship between the robustness or fragility of embryos and evolutionary conservation. Answers to these problems should shed light on the issue of why no new animal phylum has appeared since the Cambrian explosion, and help us to better understand our own body plan and how it relates to our vertebrate relatives.

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Competing interests

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References


