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Current Opinion in Genetics & Development

Remaining questions related to the hourglass model in vertebrate evolution

Naoki Irie[1,2]

Despite the phenotypic divergence of early embryogenesis among vertebrates (*e.g.*, the wide variety of cleavage and gastrulation patterns), all species converge into phenotypically similar mid-embryonic stages (particularly pharyngula embryos, which show the typical anatomical features of vertebrates, such as the pharyngeal arch), and evolutionary divergence occurs again thereafter. This observation coincides well with the recently supported developmental hourglass model; however, little is known about the nature of this conserved pharyngula period in vertebrates, and it is unclear why this mid-embryonic period has been conserved. By highlighting recent molecular-based studies, this review focuses on known information and what should be known on this topic, with a focus on vertebrate pharyngula embryos.

Addresses
[1] Department of Biological Sciences, The University of Tokyo, Tokyo, Japan
[2] Universal Biology Institute, The University of Tokyo, Tokyo, Japan

Corresponding author: Irie, Naoki (irie@bs.s.u-tokyo.ac.jp)

Current Opinion in Genetics & Development 2017, **45**:103–107

This review comes from a themed issue on **Developmental mechanisms, patterning and evolution**

Edited by **Christian Hardtke** and **Yoshiko Takahashi**

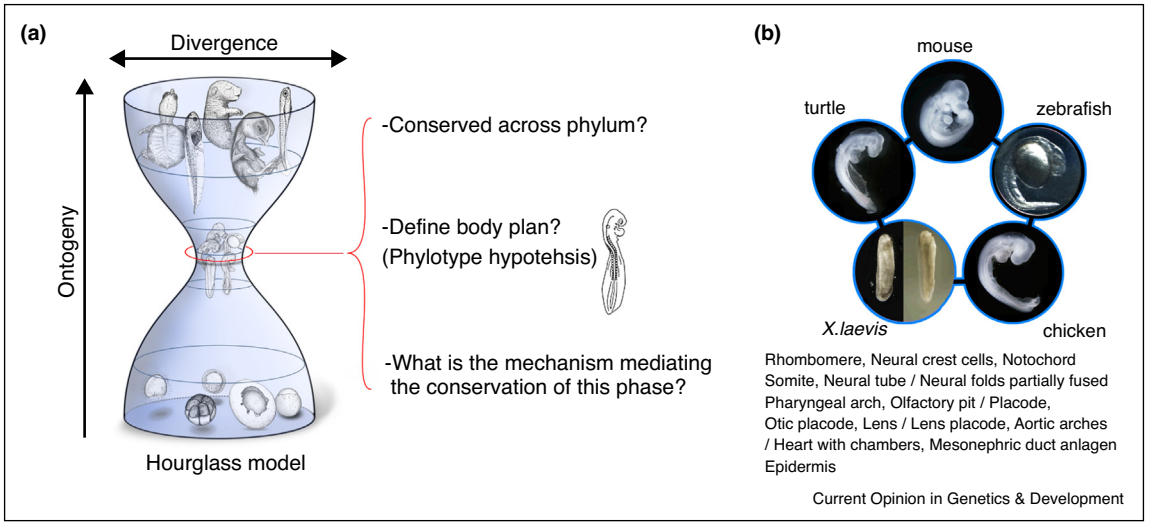
http://dx.doi.org/10.1016/j.gde.2017.04.004

Introduction

Early developmental processes in vertebrate embryogenesis (*e.g.*, cleavage, germ layer formation, body axis patterning, and gastrulation) provide a basis for subsequent developmental processes (*e.g.*, neural induction from ectoderm, somite segmentation along the anteroposterior axis, and lung bud formation from foregut). Accordingly, it is reasonable to assume that changes in early developmental processes can have a dramatic impact on subsequent developmental processes, and this importance (or responsibilities toward subsequent developmental processes) of the early processes could lead to the strong evolutionary conservation of such early processes. Although it has not been thoroughly tested, this potential mechanism, that is, that the importance of early processes could lead to stronger evolutionary conservation of early stages, was first proposed by Garstang as the stepping-stone model [1] and later by Riedl [2] and others [3], on the basis of the assumption that the earliest stages are the most conserved stages during development. The simple morphological appearance of early embryos among different species (*e.g.*, fertilized eggs of vertebrates are all 'single celled') were considered to support this early conservation model (originally proposed by Karl von Baer [4] and Ernst Haeckel [5] in the 19th century); however, no quantitative or empirical evidence has been obtained to support this hypothesis, whereas several counterproposals have been casted [6–13]. For example, the idea that the mid-embryonic stages are the most conserved and the early and late stages are rather divergent was first proposed by Sander [6], and later formulated by Duboule [7] and others [8,14]; this concept is now known as the developmental hourglass model (Figure 1). The conserved expression of *Hox* cluster genes along the anteroposterior axis of various bilaterians (*e.g.*, mouse, *Drosophila*, and *Xenopus* embryos) is one of the most frequently cited examples supporting the evolutionary conservation of mid-embryonic stages [7,8,15]. In addition, based on the similar appearances of conserved mid-embryonic stages, the developmental hourglass model further predicted [7,8] that this period matches the previously proposed concept of the phylotypic period [6]. The phylotypic period is a hypothetical concept in which the morphological features in the conserved mid-embryonic period define the body plan (*i.e.*, the basic anatomical features for each animal phylum). In vertebrates, the pharyngula stages (mid-embryonic stages with a variety of primordial organs such as the pharyngeal arch, notochord, and dorsal hollow nerve cord) are considered attractive candidates for the phylotypic period.

If a clear description of phenomena (evolutionary conserved embryos, in this case) should precede investigation of potential mechanisms mediating the evolutionary conservation of animal embryos, the central question should be: which developmental stages, if any, have been conserved the most during animal evolution? While such arguments originated in the field of comparative embryology and have lasted for more than a century, quantitative morphological comparisons still have not reached a consensus [5,9,11,12,16]. However, many molecular-based studies are now supporting mid-embryonic conservation. Using an expressed sequence tag

Figure 1



(a) The developmental hourglass model that explains how divergence can be observed during development. The model was originally formulated by Duboule [7]. Figure adapted and modified from Wang *et al.* [24]. Three major unsolved problems are listed to the right. **(b)** Potential phylotypic period for five vertebrate species (embryonic day 9.0 for *Mus musculus*, 24 hour post fertilization for *D. rerio*, Hamburger Hamilton stage 16 [HH16] for *Gallus gallus*, stage 28–31 for *X. laevis*, and Tokita and Kuratani stage 11 [TK11] for *Pelodiscus sinensis*) identified by similarities of cross-species, whole embryonic gene expression patterns [22]. Anatomical structures shared among these embryos are listed underneath the images.

(EST) dataset, researchers evaluated sequence-oldness or ancestrality of genes expressed at each developmental stage in mice [17,18] and *Drosophila* [19,20] and demonstrated the conserved nature of mid-embryonic stages. Taking advantage of comprehensive expression profiles, such as microarray and RNAseq data, recent studies further supported the conservation of mid-embryonic stages by showing cross-species similarities of whole embryonic orthologous gene expression [21–26] and sequence-oldness of expressed genes [27] in various species (*Drosophila*, nematodes, *Anopheles gambiae*, mice, chickens, turtles, *Xenopus*, and *Danio rerio*) [28]. Because the same genetic machinery used in different species (orthologous gene expression) is a strong indicator of evolutionary conservation, similar expression profiles obtained from cross-species studies seem to reliably support the conserved nature of mid-embryos. To conclude, it would be reasonable to assume that early stages do not always retain more information of ancestral organisms than subsequent stages do, as once proposed by Ernst Haeckel [5,29], but rather mid-embryonic stages may retain such information [30]; however, questions remain. Obviously, similarities in whole embryonic expression profiles do not directly indicate morphological similarities, but at best reflect similarities in homologous cell compositions between species. Then, what is known about the nature of conserved mid-embryonic stages? How deep in evolution (or wide in phylogeny) can we find the conservation of the mid-embryonic phase? Is it the period that defines the body plan for each animal phylum, as predicted by the phylotype hypothesis of the hourglass model? Accordingly, in this review, I discuss the nature of conserved mid-embryonic stages with a special focus on chordate embryos.

Features of conserved mid-embryonic stages in chordates

One of the most important questions regarding the conserved mid-embryonic period is whether this period represents the phylotypic period, as proposed in the hourglass model [7,8]. Notably, the hourglass model was originally proposed to explain embryonic divergence among vertebrates [7], and Raff further expanded the idea to explain it in phylum-wide species [8]. Thus, given that the conserved mid-embryonic stages represent phylotypic period hypothesis, mid-embryonic conservation should be observed among species across the phylum Chordata, and the conserved stages should show shared anatomical features of chordates, that is, body plan elements of segmental muscles, a dorsal nerve cord, pharyngeal gill slits, and a notochord [31]. However, most studies are one step short of answering this question.

Regarding the context I discuss in this review, chordates are the most thoroughly investigated phylum, and studies to date have demonstrated that hourglass-like conservation can be observed in organisms as diverse as gnathostomes [23–25,28,31]. The anatomical features shared among conserved stages (pharyngula embryos) of gnathostomes contain the chordates' body plan elements (Figure 1b). Based on a report showing that hourglass-like conservation does not cross different phyla [32•],