Critical Focus

The Diverse Early Embryonic Development of Vertebrates and Implications Regarding Their Ancestry

David W. Swift*

Development Office, St John's College, Cambridge CB2 1TP, UK

Abstract

It is well known that the embryonic development of vertebrates from different classes (e.g., fish, reptiles, mammals) pass through a "phylotypic stage" when they look similar, and this apparent homology is widely seen as evidence of their common ancestry. However, despite their morphological similarities, and contrary to evolutionary expectations, the phylotypic stages of different vertebrate classes arise in radically diverse ways. This diversity clearly counters the superficial appearance of homology of the phylotypic stage, and the plain inference is that vertebrates have not evolved from a common vertebrate ancestor. The diversity extends through all stages of early development—including cleavage and formation of the blastula, gastrulation, neurulation, and formation of the gut and extraembryonic membranes. This paper focuses on gastrulation, during which the germ layers originate and the vertebrate body-plan begins to form. Despite its key role in embryonic development, gastrulation occurs in fundamentally different ways in different classes of vertebrates. The inference against common ancestry becomes progressively stronger as more is discovered about the genetic and molecular mechanisms that implement development. It is increasingly evident that these are of such complexity that it is unrealistic to think that undirected variations (random mutations) could produce constructive changes to development, such as those required to account for a diversification of development from that of a common ancestor, especially while retaining a similar phylotypic stage.

Cite as: Swift D (2022) The diverse early embryonic development of vertebrates and implications regarding their ancestry. BIO-Complexity 2022(1):1–10. **doi:**10.5048/BIO-C.2022.1.

Editor: Douglas D. Axe

Received: November 29, 2021; **Accepted:** May 25, 2022; **Published:** July 21, 2022

*davidwswift@cantab.net

INTRODUCTION

Darwin was convinced of the truth of evolution because it explained a range of disparate observations.[1] One of these was embryology: it made sense that embryos of apparently related organisms (those that had been grouped taxonomically) would look similar if they had evolved from a common ancestor. Shortly after the *Origin of Species*, Haeckel produced drawings in which he emphasized the similarities of embryos from different classes of vertebrates. Despite their failings,[2] his drawings have been reproduced widely (e.g., in many textbooks on evolution) and have become well known. Partly because of this, it is now widely believed that the embryos of diverse vertebrates develop in similar ways, and this is seen as evidence that they have evolved from a common vertebrate ancestor. In effect, the similar parts of different vertebrate embryos, if not the embryos

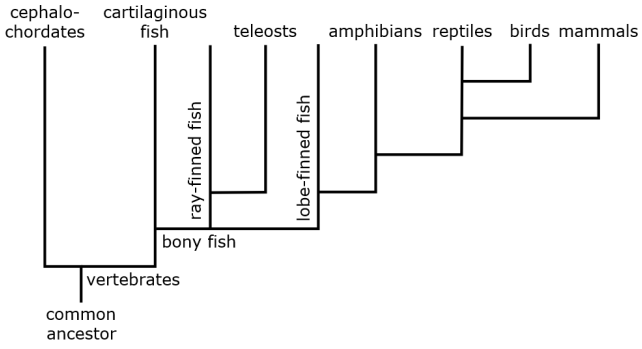

Figure 1: A phylogeny of the vertebrates and cephalochordates. **doi:**10.5048/BIO-C.2022.1.f1

[1] "The present action of natural selection may seem more or less probable; but I believe in the truth of the theory, because it collects, under one point of view, and gives a rational explanation of, many apparently independent classes of facts." (See [1], pp. 13–14.)

[2] Some authors, notably Michael Richardson (see [2]), have pointed out that Haeckel's drawings are inaccurate: they exaggerate the similarities of vertebrate embryos, to try to support his theory that ontology recapitulates phylogeny, which was soon discredited. Here I am not pursuing these inaccuracies, which are insignificant compared with the actual diversity of early vertebrate embryonic development.

as a whole, are regarded as homologous in an evolutionary sense—derived from the same tissues of a common ancestor, even if subsequently modified very differently.

Figure 1 shows the generally-accepted evolutionary relationships of the major groups of vertebrates, along with the cephalochordates, which are thought to resemble the invertebrates from which vertebrates are believed to have evolved.

If common ancestry is the explanation for homologies, not only should homologous organs be derived from equivalent embryonic tissues (the cardinal criterion for homology) but they should also develop by comparable processes. Rudolf Raff, a prominent proponent of evolutionary embryonic development ("evo-devo"), expressed it like this:

> As first pointed out by von Baer in the 1820s, animals within a phylum, such as the vertebrates, share a common body plan, and in their development share a phylotypic stage in which the body plan elements characteristic of the phylum appear. *The process of early development from the egg to the phylotypic stage should be at least as conserved as the pattern of the phylotypic stage.* One might reasonably expect mechanisms of early development to be especially resistant to modification because all subsequent development derives from early processes. [3, emphasis added]

However, despite their morphological similarities and contrary to evolutionary expectations, the striking fact is that the "phylotypic stages" of different groups of vertebrates arise in remarkably diverse ways, even with key tissues such as the germ layers (see below) deriving from completely different early embryonic sources. *These observations clearly refute the presumed evolutionary homology of the vertebrate phylotypic stage, and hence undermine the inference of common ancestry based on that supposed homology*.

Unfortunately, although many aspects of this diverse development have been known for a long time (some since the nineteenth century), the diversity of development is still not widely acknowledged. Contemporary evolutionary texts continue to present the similarities of the phylotypic stage as evidence of common ancestry, with no mention of the diversity preceding this stage. For example, in a recent textbook on evolution from a major academic publisher, we find:

> an examination of vertebrate embryos reveals a remarkable similarity of form indicating that *they have descended from a common ancestor* and so form part of a monophyletic group (common ancestor plus all its descendants). Embryos of chicken, fish, rabbits and humans all look remarkably similar (see Figure 5.1) [which reproduces Haeckel's drawings]. [4, emphasis in original]

In the 1990s Denis Duboule [5] accommodated this early diversity by proposing an hourglass model[3] for vertebrate embryonic development: a broad diversity in the early stages, a narrow "waist" of morphological similarity at the phylotypic stage, followed by increasing divergence in subsequent development as the various adult forms are generated (see Figure 2). This model has been adopted quite widely by those working in this area; and it has been found that the expressions of some developmental genes follow a similar "hourglass" pattern (reviewed by Irie and Kuratani [7]).

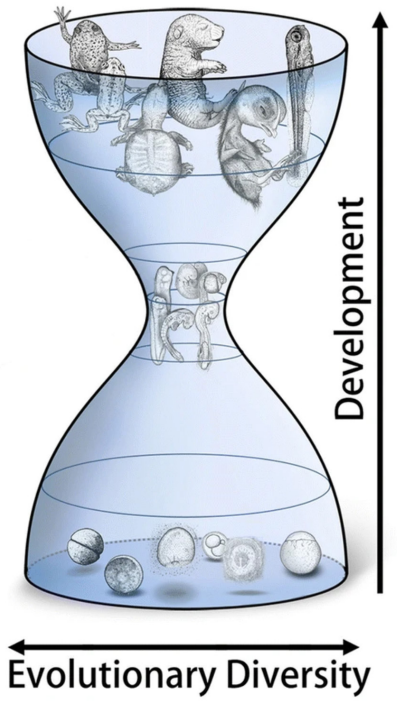

Figure 2: Vertebrate embryonic development depicted as an hourglass. From Figure 5 in [6] in accordance with Creative Commons Attribution License. **doi:**10.5048/BIO-C.2022.1.f2

However, it is important to recognize that even if an hourglass is an informative *description* of vertebrate embryonic development, this does not mean it is a satisfactory *explanation* for the early diversity. Several researchers have explored possible factors that might limit the diversity of the phylotypic stage (also reviewed in [7]); but from an evolutionary perspective, it is also necessary to consider how the diverse early development might have arisen. That is, for evolution to be a coherent explanation for the similarities (as homology) of the phylotypic stage, it is essential that there be credible evolutionary mechanisms by which the early development of a common ancestor could have diversified while maintaining a relatively similar phylotypic stage. I shall return to this below.

First, because there seems to be so little awareness of it, I shall try to convey something of that extensive diversity of the early embryonic development of vertebrates. Especially significant from an evolutionary perspective is that the diversity extends from the earliest stages: the very first cell divisions (cleavage) of the zygote (fertilized egg) occur in significantly different ways. And at later stages, key structures such as the neural tube (the beginning of the nervous system), gut, and even vertebrae

[3] Here Duboule compared embryonic development with an "egg-timer," but "hourglass" became the usual term.

(arguably the defining feature of vertebrates) form in substantially different ways.[4]

Here I focus on the remarkable variety of mechanisms by which the stage known as gastrulation occurs in different classes of vertebrates. In each of the following brief descriptions I start at the preceding blastula stage (which follows cleavage). This shows the variety of structures (which indicates the diversity of the processes by which they are formed) and the substantial differences in the sources of the cells that develop into the actual embryo (rather than extraembryonic tissues). I then outline the main cell movements that take place in the course of gastrulation.[5]

GASTRULATION

Embryonic development is a continuum—every stage is essential and dependent on preceding ones—but if one stage were to be singled out as of central importance probably most would say it is gastrulation. This is because gastrulation leads to the establishment of the germ layers—ectoderm, mesoderm and endoderm—from which all of the body's tissues are derived. And, as Conrad Waddington, a pioneer of genetic embryology, put it: "It is during gastrulation that the fundamental plan of the vertebrate body is brought into existence." [10]

Thus, from an evolutionary perspective we would surely expect gastrulation to be "conserved"—substantially the same throughout the vertebrates which have these three germ layers and similar body-plan. Yet in fact we find that even for this key stage of embryonic development, for almost all of the major classes of vertebrates:

- the mechanism of gastrulation is significantly different from any of the others;
- even the source tissues of the germ layers are different.

Cephalochordates

Although it is not a vertebrate, it is helpful to start with gastrulation in the cephalochordate amphioxus (a somewhat fish-like animal), because it is often presented as the archetypal mode of gastrulation; and, as mentioned above, it is generally thought that vertebrates evolved from creatures similar to these.

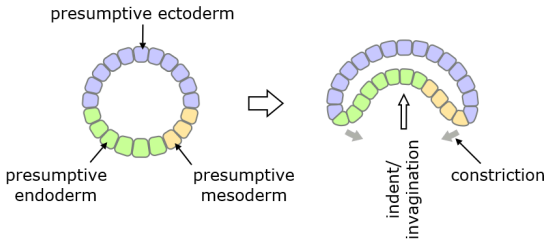

Figure 3: Longitudinal section through an amphioxus blastula, and showing early gastrulation. **doi:**10.5048/BIO-C.2022.1.f3

[4] For overviews of embryonic development in various classes of vertebrates, see Kardong [8]

[5] For descriptions of gastrulation in most vertebrate classes, see Stern [9].

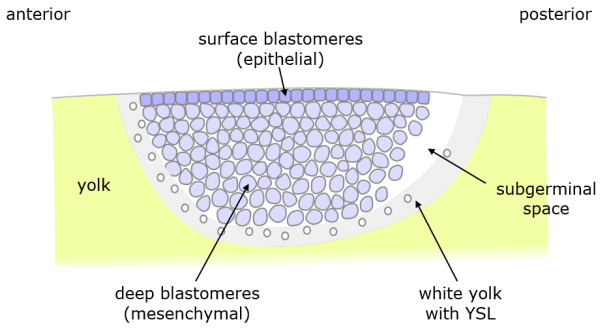

Figure 4: Longitudinal section through a small-spotted catshark blastula. YSL: yolk syncytial layer. **doi:**10.5048/BIO-C.2022.1.f4

Following fertilization, the zygote divides repeatedly (cleavage) and the cells organize into a hollow ball (blastula), see Figure 3. Gastrulation proceeds by one side of the blastula indenting to produce what is at first a shallow cup, and then its edges or "rim" are constricted to enclose a pouch called an archenteron. Cells within the archenteron become endoderm and mesoderm, whilst those remaining on the outside become ectoderm. In due course the archenteron becomes the animal's gut, which is why this stage is called gastrulation (from Greek gaster, "stomach").

Chondrichthyans

Chondrichthyans are cartilaginous fish, notably sharks and rays (but not sturgeons). Following cleavage, the resulting cells (blastomeres) arrange into a blastula (Figure 4) comprising:

- an upper epithelial layer;
- loosely aggregated mesenchymal cells beneath.

In addition, in the surface layer of the yolk where new blastomeres form there is a yolk syncytial layer (YSL) in which the cells' cytoplasm is continuous with the yolk.

The embryo is derived from the upper layer (epiblast), although some of this layer also forms extraembryonic tissues. During the course of gastrulation most of the underlying cells disperse and many become incorporated into the epithelial layer.

Gastrulation proceeds by the upper layer thickening and extending posteriorly such that it overhangs the underlying yolk (Figure 5). The cells in the upper layer proliferate, and some

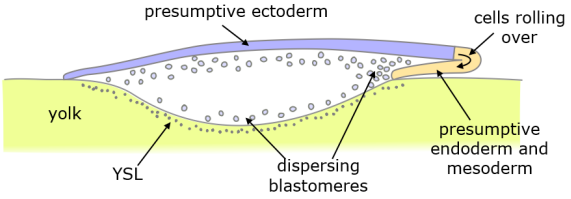

Figure 5: Longitudinal section through a small-spotted catshark embryo during early gastrulation. YSL: yolk syncytial layer. **doi:**10.5048/BIO-C.2022.1.f5

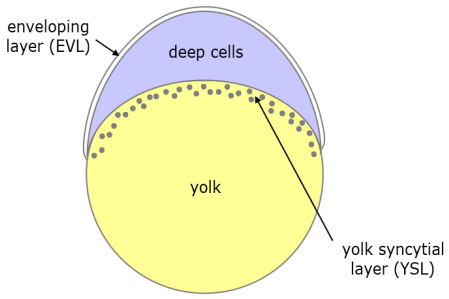

Figure 6: Section through a zebrafish blastula.
doi:10.5048/BIO-C.2022.1.f6

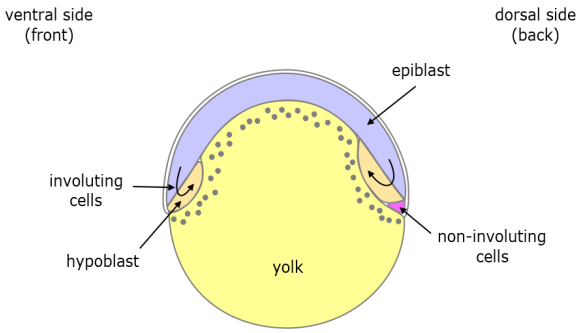

Figure 7: Longitudinal section through a zebrafish embryo at early gastrulation. **doi:**10.5048/BIO-C.2022.1.f7

cells roll over this overhang, which continues to extend. Cells that move to the underside of the overhang become mesoderm and endoderm, whilst those remaining on the upper surface become ectoderm.

Features to note are:

- This process does not include invagination: the space below the overhang is just that; it is not an invagination or archenteron into the embryo.
- Cells roll over the *edge* of the epiblast (mainly at the posterior but also at the sides), i.e., the cells that become mesoderm and endoderm surround those that remain as ectoderm. The significance of this is discussed below.

Teleosts

Teleosts are the major group of bony fish (see Figure 1). They include the vast majority of fish in terms of both number of species and individuals. Their blastula (Figure 6) comprises three layers of cells:

- an outer enveloping layer (EVL) which is one cell thick (having a significant role in implementing epiboly [see below] and gastrulation, but not becoming part of the embryo);
- a population of deep cells which at first is hemispherical or dome-shaped, and from which the embryo forms;
- a yolk syncytial layer (YSL).

There is no blastocoel or subgerminal space.

Gastrulation is preceded by "epiboly," during which the EVL and deep cells start to spread around the yolk. As this occurs, the layer of deep cells becomes of uniform thickness and gradually thins as it spreads, and is now called epiblast.

Gastrulation begins when the epiblast and EVL have spread to around the "equator" of the yolk (Figure 7). As they do so, cells at the margin of the epiblast fold underneath (involute) to form an underlying layer of cells ("hypoblast") which develop into endoderm and mesoderm. This continues as epiboly proceeds to extend over the whole of the yolk. Epiblast cells that remain on the outside become ectoderm.

> Note that, as with chondrichthyans, it is cells on the *edge* of the epiblast that involute, so the cells that become mesoderm and endoderm are derived from those which surrounded the presumptive ectoderm.

Amphibians

The amphibian blastula (Figure 8) is approximately spherical, with a cavity (blastocoel) occupying much of the upper half, and a filled lower half. The dome of the upper hemisphere comprises two layers, although I shall not describe their different fates here.

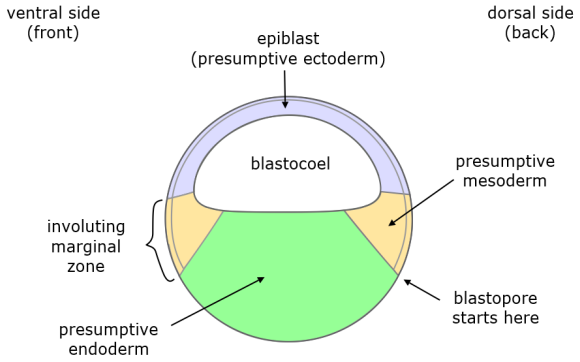

Figure 8: Longitudinal section through a *Xenopus* (frog) blastula.
doi:10.5048/BIO-C.2022.1.f8

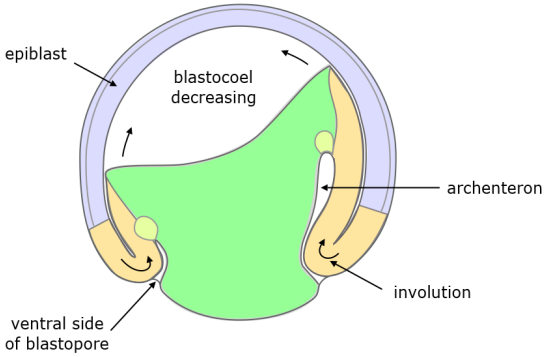

Figure 9: Longitudinal section through a *Xenopus* embryo, early in gastrulation. **doi:**10.5048/BIO-C.2022.1.f9

Amphibians are unusual among vertebrates in that most of the blastula becomes part of the embryo:

- the dome (epiblast) of the upper hemisphere becomes ectoderm;
- most of the lower hemisphere becomes endoderm;
- the cells in between become mesoderm.

The primary process of gastrulation in amphibians is invagination to produce a blastopore, which becomes an archenteron (Figure 9). The blastopore starts as a slit on the dorsal side, which then extends in both directions until its ends meet on the ventral side, such that the blastopore encircles the presumptive endoderm. Also, cells of the epiblast that surround the blastopore move into (involute) the archenteron and become mesoderm. As in amphioxus, the archenteron becomes the animal's gut.

> As in the cases of chondrichthyans and teleosts, the cells that involute into the archenteron are from the *edge* of the epiblast, so cells that become mesoderm surround those remaining as ectoderm.

Reptiles

The main parts of a reptile blastula (Figure 10) are two layers of cells:

- an upper epiblast;
- a lower hypoblast.

There is a blastocoel between the layers. In addition, there are marginal cells which surround these, and a yolk syncytial layer (YSL). There is also a non-cellular vitelline membrane, and there may be a subgerminal space below the hypoblast.

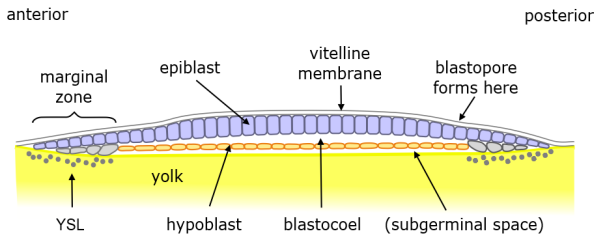

Figure 10: Longitudinal section through a turtle blastula. YSL indicates yolk syncytial layer. **doi:**10.5048/BIO-C.2022.1.f10

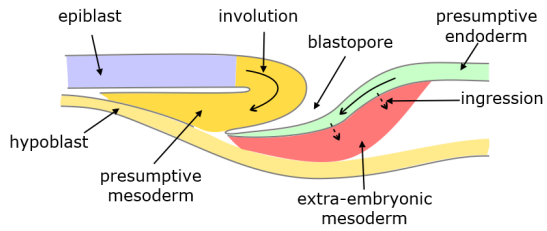

Figure 11: Longitudinal section through part of a turtle embryo at early gastrulation to show cell movements into the blastopore. **doi:**10.5048/BIO-C.2022.1.f11

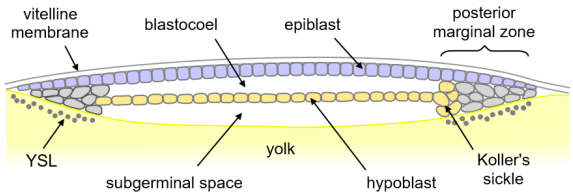

Figure 12: Longitudinal section through a chick blastula. YSL indicates yolk syncytial layer. **doi:**10.5048/BIO-C.2022.1.f12

Only the epiblast develops into the embryo; the other parts form extraembryonic tissues.

Gastrulation (Figure 11) proceeds by means of an invagination (blastopore) which arises towards the posterior side of the upper surface, into which cells move by involution. In general, those on the posterior side of the blastopore become endoderm and those on the anterior side become mesoderm. In addition, some cells on the posterior side of the blastopore ingress (see below) to form extraembryonic mesoderm. Cells of the epiblast that do not enter the blastopore remain as ectoderm.

Other features to note are:

- Unlike in amphibians, the blastopore does not become the animal's gut.
- In contrast to chondrichthyans, teleosts and amphibians, the cells that enter the blastopore are from *within* the epiblast, so the presumptive mesoderm and endoderm are surrounded by presumptive ectoderm.

It is sometimes said that gastrulation in reptiles is via a primitive streak, as in birds (see below). But this is not correct. It has been known since the nineteenth century that gastrulation in reptiles is via a blastopore, and no exception is known (see Bertocchini et al. [11]).[6]

Birds

Following cleavage, the avian blastula arises in two phases. Initially an upper layer of cells (epiblast) forms, along with peripheral marginal zones; then a lower layer (hypoblast) grows from the posterior marginal zone (Figure 12). There is a blastocoel between the layers, a subgerminal space below the hypoblast, and a yolk syncytial layer (YSL) where the marginal zone contacts the yolk.

The embryo forms from the epiblast alone, with the other cells producing extraembryonic tissues.

The key feature of gastrulation in birds is what is called a primitive streak. Initially this is a thickening of the epiblast along its midline, originating close to its posterior end (just forward of Koller's sickle) and then extending anteriorly until it reaches about two-thirds towards the anterior side. In tandem with this thickening of the epiblast, a lower layer of cells (endoblast) spreads from the posterior margin, which displaces the hypoblast anteriorly. When the primitive streak reaches its

[6] In recent years it has become popular to regard birds as being reptiles (not only as having evolved from them), but this major difference in their mechanism of gastrulation clearly demarcates between them (and challenges their presumed evolution from reptiles).

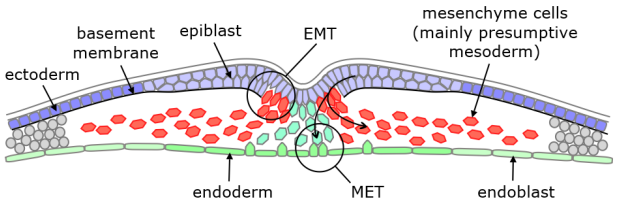

Figure 13: Transverse section (perpendicular to Figure 12) through a chick primitive streak, showing cell movements during gastrulation. EMT indicates endothelial-to-mesenchymal transition; MET indicates mesenchymal-to-endothelial transition. **doi:**10.5048/BIO-C.2022.1.f13

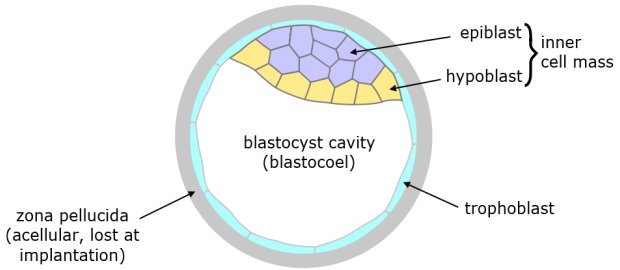

Figure 14: Section through a human blastocyst. **doi:**10.5048/BIO-C.2022.1.f14

maximum length a groove develops on its upper surface, culminating in a funnel-shaped depression at its anterior end, known as the primitive pit or Hensen's node.

The epiblast spreads laterally, and it is through the primitive groove and pit that epiblast cells now ingress between the epiblast and endoblast (see Figure 13). This translocation involves a change from an epithelial nature of the epiblast cells to a mesenchymal nature (epithelial-to-mesenchymal transition, EMT, see below) such that the cells can migrate through tissues.

> Note the major difference between involution, which involves epithelial cells moving as a contiguous layer, and ingression, which involves mesenchymal cells moving individually through tissues.

In the course of gastrulation:

- Some cells entering the primitive groove move across the intervening space and enter the endoblast to become endoderm (progressively displacing the endoblast to the sides of the embryo). Because the endoderm is an epithelial tissue, these migrating cells must revert to an epithelial nature, i.e., undergo a mesenchymal-to-endothelial transition (MET).
- Other cells spread out to form mesoderm between the overlying epiblast (ectoderm) and underlying endoderm.
- Epiblast cells that do not enter the primitive streak remain as ectoderm.

> Note that the primitive streak is located across the *middle* of the epiblast; thus, as with reptiles, the cells that become endoderm and mesoderm are surrounded by presumptive ectoderm.

Mammals (Primates)

In placental mammals, following cleavage the resulting cells arrange into a blastocyst (the mammalian equivalent of a blastula), comprising three distinct populations (see Figure 14):

- an outer trophoblast, which will develop into the placenta;

and an inner cell mass, which consists of:

- epiblast, from which the embryo forms (and some extraembryonic tissues);
- hypoblast, which forms only extraembryonic tissues.

Extraembryonic membranes[7]

In primates there is a substantial additional stage before gastrulation, in which the extraembryonic membranes begin to form (see Figure 15).[8]

- A cavity arises within the epiblast, which will become the amniotic cavity.
- The hypoblast spreads around the inside of the blastocoel which becomes the (primary) yolk sac.

The remaining double layer of epiblast and hypoblast is called the embryonic disc.

A layer of extraembryonic mesoderm then arises between the lining of the primary yolk sac and the cytotrophoblast, and spreads to cover the amniotic cavity as well. As this tissue thickens, cavities form within it, and coalesce to form the chorionic cavity which is lined with extraembryonic mesoderm (Figure 16). In this process some of the primary yolk sac is lost, and what remains is called the secondary yolk sac, and a pocket of this is called the allantois, which is the final extraembryonic membrane.

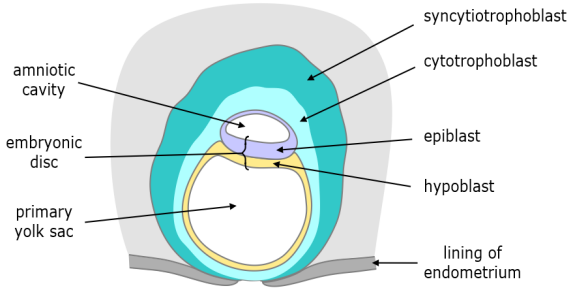

Figure 15: Section through a human embryo just after implantation in the uterus. **doi:**10.5048/BIO-C.2022.1.f15

[7] Reptiles, birds, and mammals have extraembryonic membranes, and are known collectively as amniotes (because one of the membranes encloses an amniotic cavity).

[8] This formation of extraembryonic membranes in primates, by *cavitation*, and *before* gastrulation, is a remarkable difference between these and other amniotes (including other mammals), where the extraembryonic membranes arise by *fusion* (not cavitation), and *after* gastrulation.

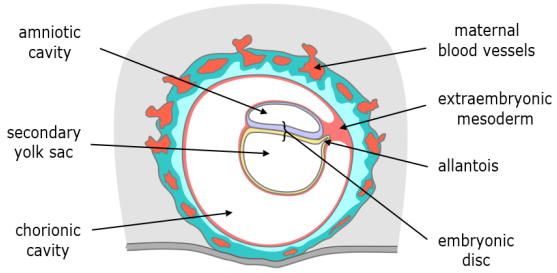

Figure 16: Longitudinal section through a human embryo after formation of the extraembryonic membranes.
doi:10.5048/BIO-C.2022.1.f16

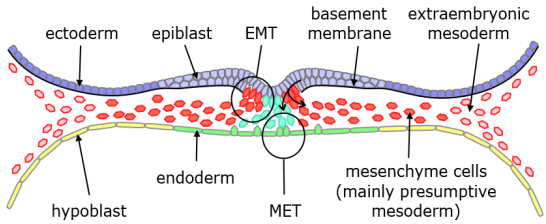

Figure 17: Transverse section (perpendicular to Figure 16) through a human embryonic disc, showing cell movements during gastrulation. EMT indicates endothelial-to-mesenchymal transition; MET indicates mesenchymal-to-endothelial transition.
doi:10.5048/BIO-C.2022.1.f17

Gastrulation

Gastrulation in primates proceeds via a primitive streak which is similar to that in birds. A groove appears near the caudal (posterior) end of the epiblast, it extends about two-thirds of the way along the midline towards the cranial (anterior) end, terminating in a widening with a depression at its centre. This primitive groove and primitive pit are where gastrulation occurs (see Figure 17).

As with birds, in the course of gastrulation cells of the epiblast proliferate and move towards the primitive groove where they transition from epithelial to mesenchymal in character, and ingress below the surface. In the early phase, these ingressing cells enter the hypoblast, reverting to epithelial cells (mesenchymal-to-epithelial transition) to become the definitive endoderm, at the same time displacing the hypoblast cells from the embryonic disc to line the yolk sac. As gastrulation proceeds, further cells ingressing from the epiblast move into the space between the epiblast and endoderm to form a middle layer of mesoderm. (At the edges of the embryonic disc this embryonic mesoderm merges with the previously formed extraembryonic mesoderm.)

> Note that the primitive streak is located across the *middle* of the epiblast; thus, as with reptiles and birds, cells that become endoderm and mesoderm are surrounded by presumptive ectoderm.

A COMPARISON OF GASTRULATION IN VERTEBRATES

Embryonic source tissues

Three important points can be made about embryonic source tissues and homology.

First, the wide variety of structures of the blastulas of different classes of vertebrate challenges the view that the resultant embryonic tissues can be considered equivalent or homologous.

Second, this observation is reinforced in the light of the different types and locations of the parts of the blastulas that become the embryo:

- Chondrichthyans
  It is a one-cell thick epithelial layer, forming the upper surface of the blastula.
- Teleosts
  It is a multiple-cell layer, beneath the overlying enveloping layer.
- Amphibians
  It is the whole of the blastula, comprising the multilayered dome of the upper hemisphere and the mass of cells in the lower hemisphere.
- Reptiles and birds
  It is the upper surface of the blastula, comprising a single-cell thick epithelial layer, overlying the hypoblast.[9]
- Placental mammals
  It is part of the inner cell mass, within the outer trophoblast.

Third, given that gastrulation involves the internalization of some epiblast cells to become endoderm (except for amphibians) and mesoderm, whilst the remaining epiblast cells become ectoderm, it is significant that:

- in amniotes (reptiles, birds, mammals) cells that are internalized arise from a *central area* of the epiblast, i.e., the presumptive endoderm and mesoderm are surrounded by presumptive ectoderm; whereas
- in anamniotes (chondrichthyans, teleosts, amphibians) the cells that internalize are from the *edge* of the epiblast, i.e., the presumptive endoderm and mesoderm surround the presumptive ectoderm.

That is, the relative positions of the presumptive ectoderm and presumptive endoderm/mesoderm are reversed in these two groups.

In the light of these three substantial distinctions—the different overall structure of the blastulas, the different parts of the blastula that become the embryo, and the different relative positions of the presumptive ectoderm and mesoderm/endoderm in amniotes and anamniotes—there is no doubt that the tissues that become the embryo are not equivalent, and hence are far from being homologous across the various vertebrate classes.

[9] For those reading the literature, it may be helpful to note that in anamniotes the cells that *have involuted* in the course of gastrulation are often called "hypoblast," but these are different from the hypoblast that forms *before* gastrulation in amniotes.

Embryonic processes

It is also clear from the above albeit brief descriptions that for all of the major classes of vertebrates the mechanism of gastrulation is substantially different from any of the others:

- Chondrichthyans: by cells rolling over a posterior overhang of the epiblast.
- Teleosts: by involution around the edges of the epiblast as it spreads around the yolk.
- Amphibians: by involution through an annular blastopore.
- Reptiles: by involution through a canal-like blastopore.
- Birds: by cells ingressing through a primitive streak, formation of the primitive streak being accompanied by growth of an underlying endoblast.
- Placental mammals: by cells ingressing through a primitive streak.

Of particular note is the radical difference between involution or rolling over of a sheet of epithelial cells and ingression of individual mesenchymal cells. Before discussing the implications of the non-homologous gastrulation in vertebrates, it is instructive to see something of what is involved in epithelial-to-mesenchymal transition, as an example of what we are now learning about the cellular, genetic, and molecular mechanisms underlying embryonic development.

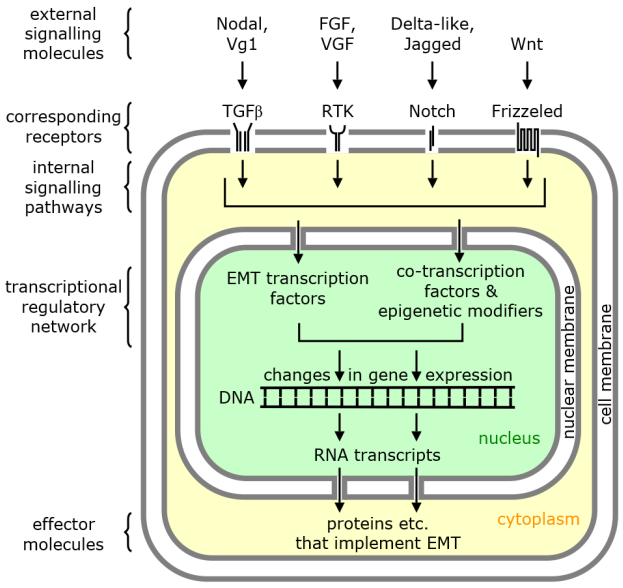

Figure 18: Genetic mechanisms associated with EMT. Based on Figure 3 in Debnath et al. [12]. The graphical representations of the various receptors are to indicate their different molecular structures.
doi:10.5048/BIO-C.2022.1.f18

EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT)

The outer layer of the whole organism, and of many structures within it, is usually an epithelium which has a primary role of maintaining the external integrity of the organism or structure. In general, it comprises a mono- or multilayered sheet of cells which are closely tied to each other. Also, cells of an epithelium are said to have apico-basal polarity, with the basal ("lower") surface of a single layer of cells (or of the lowest where there are several layers) being attached to a fibrous extracellular structure called a basement membrane (shown in Figures 13 and 17). Specialized molecular junctions tie the epithelial cells together and to the basement membrane.

In contrast, mesenchymal cells are generally individual, without cell junctions except transiently when they contact each other, and they are able to migrate within and between tissues. They have front-back polarity, with leading extensions of their cytoplasm (called filopodia), and a trailing pseudopodium.

Due to the substantial differences between them, the transition from epithelial to mesenchymal character involves a coordinated series of cellular changes, including the following, though not necessarily in this order:[10]

- Cell junctions are dismantled and the basement membrane degraded, with a consequent loss of apico-basal polarity.
- Adaptations for locomotion include rearrangement of internal microtubules, construction of an internal network of intermediate filaments, and formation of filopodia, resulting in a front-back polarity.
- So that they can migrate in appropriate directions (and avoid inappropriate areas), the cells produce and locate a battery of surface receptors to detect external cues, and the internal mechanisms whereby activation of these receptors affects the direction of locomotion.
- Because individual cells are generally susceptible to being eliminated by the body's defence mechanisms (apoptosis), mesenchymal cells must also produce the means to protect themselves from these.

Behind the above changes that occur at the cellular level are of course the genetic and other molecular mechanisms that effect these changes. Progress has been made towards elucidating these, and some are depicted in Figure 18.

Key features to note are:

- EMT does not occur spontaneously, but is elicited by external signalling molecules (inducers, which must be produced by other cells).
- Cells undergoing EMT must have the appropriate receptors for these inducers positioned on their cell surface. That is, their preceding differentiation must have included their ability to produce and locate these, which also applies to the following:
- Internal biochemical mechanisms are required, not only to respond to individual inducers, but also to integrate the response from various inducers—which may be to reinforce or attenuate.
- These mechanisms lead to the activation and/or suppression of transcription factors (TFs). As with the external signalling molecules, the various TFs have a variety of

[10] This description focuses on Type 1 EMT, which occurs during embryonic development. Type 2 EMT occurs in wound healing and tissue regeneration, and Type 3 in metastasis and the spreading of cancerous cells.

roles, the integration of which involves other molecules such as non-coding RNAs.
- The TFs affect gene expression—not only to activate but also to suppress—resulting in the production of proteins and other molecules that effect EMT, and suppression of other genes, e.g., for those associated with epithelial character.
- The EMT proteins include the molecular basis for locomotion, which will include producing and locating the receptors to respond to migration cues.

IMPLICATIONS FOR VERTEBRATE ORIGINS

In view of their morphological similarities, it is understandable that the phylotypic stages of different classes of vertebrates were interpreted as homologous and as evidence of common ancestry. However, this apparent homology is refuted by more detailed embryological evidence; despite their similarities, the phylotypic stages are formed embryonically in profoundly different ways. The straightforward conclusion to draw from this radical diversity of their early embryonic development is that it shows the vertebrates have not evolved from a common vertebrate ancestor. This conclusion can be avoided only if there are credible explanations for how such diversity of early development might have arisen from the development prevailing in a common ancestor (whether or not similar to present-day cephalochordates) in an evolutionary way, via changes that (i) had a realistic probability of occurring, (ii) maintained viability, and (iii) offered, in most cases, significant advantage that could be favored by natural selection.

Further, to be taken seriously, such explanations can no longer be based solely on putative morphological changes, but must take account of what we now know about the genetic and molecular mechanisms through which embryonic development is implemented. For example, in a frequently-cited paper from 1999, Arendt and Nübler-Jung proposed how the mode of gastrulation in amphibians might have evolved into that of reptiles and thence to the primitive streak of birds and mammals, via a series of morphological intermediates. [13][11] This approach followed Darwin's nineteenth-century understanding that biological tissues are innately plastic—amenable to more or less unlimited variation in the course of embryonic development, not constrained by developmental genes and processes. However, in the light of what we now know about how development occurs, it is clear that an exclusively morphology-based rationale is totally inadequate. We can no longer regard embryonic development as a "Black Box," but must take account of the genetic and molecular mechanisms that shape morphology.

For all stages of embryonic development, we have begun to elucidate these mechanisms and found them to be remarkably complex, including the orchestrated action of many interdependent genes. The above outline of current knowledge about how EMT is implemented is but one example of how a small part of development is carried out. Comparable genetic and molecular mechanisms are at the heart of every stage of embryonic development. So for any proposed evolutionary explanation for the diversification of embryonic development to be credible, it must take these mechanisms into account: it must propose not so much how the diversity might have arisen through small morphological changes—as if embryonic tissues were plastic—but rather how it could have been achieved through plausible changes (such as undirected mutations) to the underlying genetic systems. In particular, because of the interdependence of the mechanisms that are involved, constructive changes to embryonic development must entail *coordinated* production of and/or changes to several genes, e.g., for transcription factors and the DNA sequences on which they act, which is prohibitively improbable.

It has been found that similar genetic systems are used in various aspects of embryonic development. For instance, some of the same types of signalling molecules and transcription factors involved in EMT (Figure 18) are used to implement other parts of embryonic development as well. This is sometimes referred to as "deep homology" and of course is also seen as evidence of common ancestry: it is assumed that genes initially used for one purpose have been "recruited" for alternative or additional purposes, or developmental networks "rewired" to account for similar genes being used to implement different morphologies. However, as indicated above, even just "rewiring" genetic systems would, at the very least, require constructive and coordinated generation and/or modification of regulatory sequences.

This is not the place for an adequate discussion of the challenges associated with acquiring new genes and/or the regulatory sequences to control their expression. An introduction to early as well as more recent work can be found in Hössjer et al. [14], and Sanford et al. [15] discuss some of the issues that must be considered. However, I shall take the opportunity here to

[11] This paper also illustrates a line of reasoning frequently used by biologists as an explanation for the diversity of early embryonic development in vertebrates, based on the amount of yolk present in the egg. There is very little yolk in the egg of cephalochordates, so it is thought that this would also have been the case in the presumed common ancestor of the vertebrates. Because there is significant yolk content in various vertebrate eggs, it is proposed that in the course of evolution there was a general tendency in these vertebrate lines for the amount of yolk to increase, perhaps because it enables the young to be less dependent on finding an early food supply.

Further, it is presumed that there were various changes to early development to accommodate this increased yolk content. For example, whereas the first cell divisions can pass right through the zygote (holoblastic cleavage) where there is little or no yolk, this becomes more difficult as yolk content increases (giving rise to meroblastic cleavage) until, where there is a definite yolk structure (chondrichthyans, teleosts, reptiles, and birds), cell divisions do not pass through the yolk at all (discoidal cleavage). Then, arising from these presumed changes in cleavage, it is thought there were consequent changes in how subsequent stages of development (including gastrulation) occur.

What is lacking from this rationale is (i) it does not give due weight to the genetic and molecular challenges to changing embryonic development—of increasing yolk in the maturation of female gametes (a late stage of development), or of associated changes to early development (cleavage, etc.), and (ii) it implies that evolution has foresight. In reality, of course, evolution does not have foresight: it cannot know in advance of the advantages of increased yolk content, nor of appropriate changes in early development to accommodate more yolk. Changes to development will only arise opportunistically, and be retained only if an advantage is realized. Neither can increased yolk in some way prompt appropriate changes in early development. So it would be necessary for *coordinated* changes in yolk content and other developmental changes to arise, and to do so *opportunistically*. Given the challenges to obtaining even minor constructive changes to development (see main text), it seems all the more unrealistic to think that coordinated changes on very different aspects of development (egg formation and early development) such as this could arise.

address a common misperception. It is widely thought that the challenge facing an evolutionary origin of new genes, control sequences, etc. is only that of their initial improbability—that even a highly improbable sequence need arise only once. But this prima facie improbability is only part of the issue; it is also necessary to take into account the realities of population genetics. Most new sequence variations (whether arising from a single or multiple mutations)—even selectively favorable ones—are likely to be lost due to the vagaries of inheritance (referred to as genetic drift). What this means is that new sequences need to arise independently many times before it is likely that one will spread throughout the population (become "fixed"). Sanford et al. observed that the longest part of the time required for a new sequence to be adopted by a population is waiting for the right sequence to arise enough times for one to become fixed [15]. Further, even for the "successful" sequence, the process of spreading through a population takes considerable time, at least many hundreds if not thousands of generations. During this time some advantageous sequences can be lost through degradation (back mutation), which not only slows the process of spreading, but can also contribute to a favorable sequence being lost altogether. So it is not surprising that investigators have found that the time required for even modest changes is a formidable challenge to supposed evolutionary scenarios, as it is generally far in excess of the time available.

It is more than 20 years since Raff wrote: "One might reasonably expect mechanisms of early development to be especially resistant to modification because all subsequent development derives from early processes" [3], and the more we find out about how embryonic development is implemented at the genetic and molecular levels, the more it reinforces this commonsense conclusion. Many other authors have also commented on why we would expect early embryonic development to be resistant to change (for examples see Irie and Kuratani [7]). Yet, when it comes to the diverse embryonic development of presumed homologous organs or body-plans, the usual assumption is that their early development must somehow have derived from that of a common ancestor, no matter how improbable the changes required, rather than accept the plain inference that the similar organs etc. are not homologous, at least not in an evolutionary sense.[12] This expectation seems to reflect an ideological commitment to the theory of evolution rather than an objective assessment of the embryological facts.

ACKNOWLEDGEMENTS

I am grateful to Dr Paul Nelson and other reviewers for their helpful comments on an earlier version of the manuscript.

[12] Limitation of space prevents me from discussing further the evolutionary responses to the anomalous development of presumed homologous tissues, including suggestions that have been made as to how the concept of homology might be modified to accommodate diverse development.

1. Darwin C (1875) The Variation of Animals and Plants under Domestication. 2nd Edition. Volume 1. John Murray (London).
2. Richardson MK (1995) Heterochrony and the Phylotypic Period. Dev Biol 172: 412–421. **doi:**10.1006/dbio.1995.8041
3. Raff RA (1999) Larval homologies and radical evolutionary changes in early development. In: Bock GK, Cardew G, eds. Homology: Novartis Foundation Symposium 222. John Wiley & Sons (Chichester) pp 110–120. **doi:**10.1002/9780470515655.ch8
4. Cassidy M (2020) Biological Evolution: An Introduction. Cambridge University Press (Cambridge). Chapter 5. p95
5. Duboule D (1994) Temporal colinearity and the phylotypic progression: A basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. Dev Suppl 1994:135–142.
6. Irie N, Satoh N, Kuratani S (2018) The phylum Vertebrata: A case for zoological recognition. Zoological Lett. 4(32):1–20. **doi:**10.1186/s40851-018-0114-y
7. Irie N, Kuratani S (2014) The developmental hourglass model: A predictor of the basic body plan? Development 141:4649–4655. **doi:**10.1242/dev.107318
8. Kardong VK (2012) Vertebrates: Comparative anatomy, function, evolution. 6th edition. McGraw-Hill (Boston) Chapter 5 Life History.
9. Stern CD ed. (2004) Gastrulation: From cells to embryo. Cold Spring Harbor Laboratory Press (New York).
10. Waddington CH (1952) Modes of gastrulation in vertebrates. Q J Microsc Sci 93(2):221–9. p221
11. Bertocchini F, Alev C, Nakaya Y, Sheng G (2013) A little winning streak: The reptilian-eye view of gastrulation in birds. Develop Growth Differ 55:52–59. **doi:**10.1111/dgd.12014
12. Debnath P, Huirem RS, Dutta P, Palchaudhuri S (2021) Epithelial-mesenchymal transition and its transcription factors. Bioscience Rep 42(1):1–29. **doi:**10.1042/BSR20211754
13. Arendt D, Nübler-Jung K (1999) Rearranging gastrulation in the name of yolk: evolution of gastrulation in yolk-rich amniote eggs. Mech Dev 81(1–2):3–22. **doi:**10.1016/s0925-4773(98)00226-3
14. Hössjer O, Bechly G, Gauger A (2018) Phase-type distribution approximations of the waiting time until coordinated mutations get fixed in a population. In Silvestron S, Malyarenko A, Rančić M, eds. Stochastic Processes and Applications SPAS 2017, Springer Proceedings in Mathematics & Statistics, vol 271. Springer Cham (Cham, Switzerland) pp 245–313. **doi:** 10.1007/978-3-030-02825-1_12
15. Sanford J, Brewer W, Smith F, Baumgardner J (2015) A waiting time problem in a model of hominin population. Theor Biol Med Model 12(18):1–28. **doi:**10.1186/s12976-015-0016-z; pdf: https://tbiomed.biomedcentral.com/track/pdf/10.1186/s12976-015-0016-z.pdf