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## Review Article

# Is Gastrulation the Most Important Time in Your Life?

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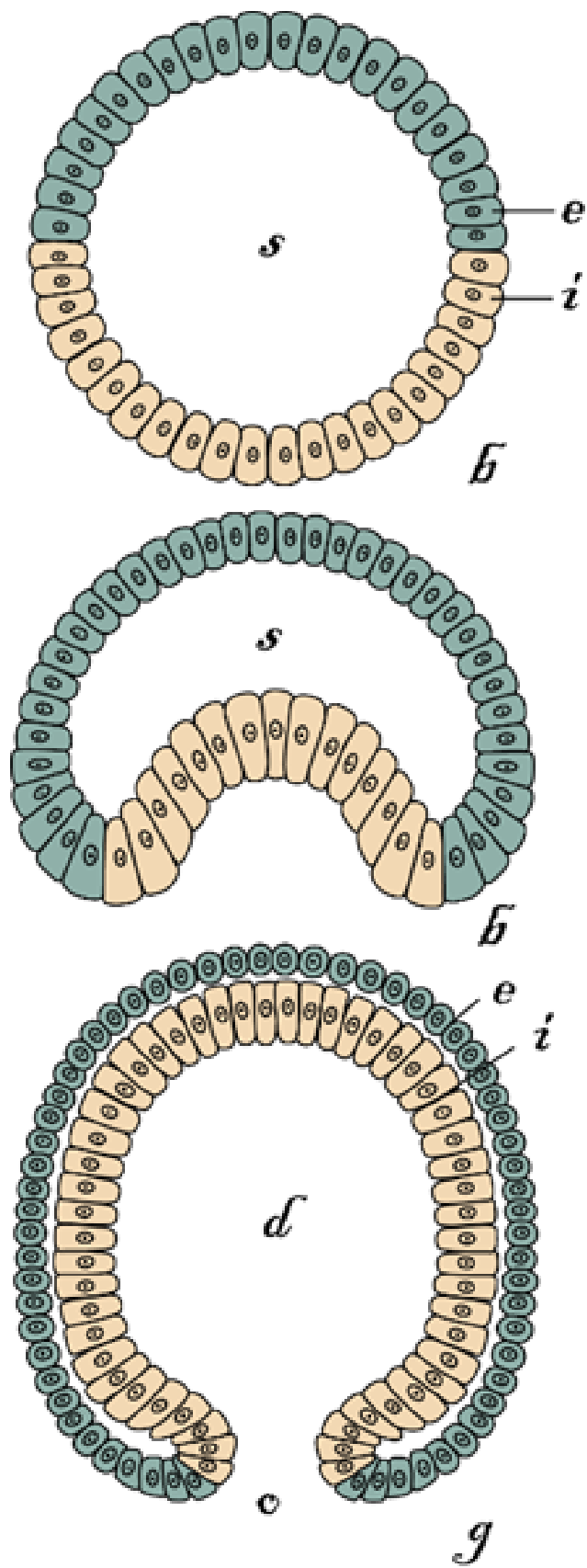
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**Gastrulation is considered a fundamental process of metazoan embryogenesis, as the layers that give rise to all body parts are laid down by extensive cell movements and cell differentiation. It is commonly thought that gastrulation establishes the entire body axis. The discovery of neuromesodermal progenitors is challenging this view. These cells keep their pluripotency after gastrulation, and they are able to differentiate into ectoderm (neural tube) and mesoderm derivatives (paraxial mesoderm, notochord) directly, without passing through a germ layer intermediate. They contribute to the elongation of the body axis and could play a key role in the evolution of the chordate body plan.**

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The term gastrulation was coined by Haeckel to refer to the transformation of an early embryo from a homogeneous, single-layered, hollow sphere (i.e., the blastula) to a heterogeneous, two-layered structure (i.e., the gastrula) (Fig. 1). He proposed that the gastrula was originated by the invagination of the blastula's layer – as when one squeezes a deflated ball with one finger –, and the differentiation of the resulting two layers (outer layer: ectoderm; inner layer: endoderm). This invagination gave place to a cavity lined by the endoderm, the primary intestine (Fig. 1). The term “gastrula” derives from the Greek root “γαστήρ” = *gaster*, which means “gut”, plus the Latin diminutive suffix –“ula”. Translated as “little gut”, Haeckel coined it to name the developmental stage at which this structure is formed<sup>[1]</sup>.



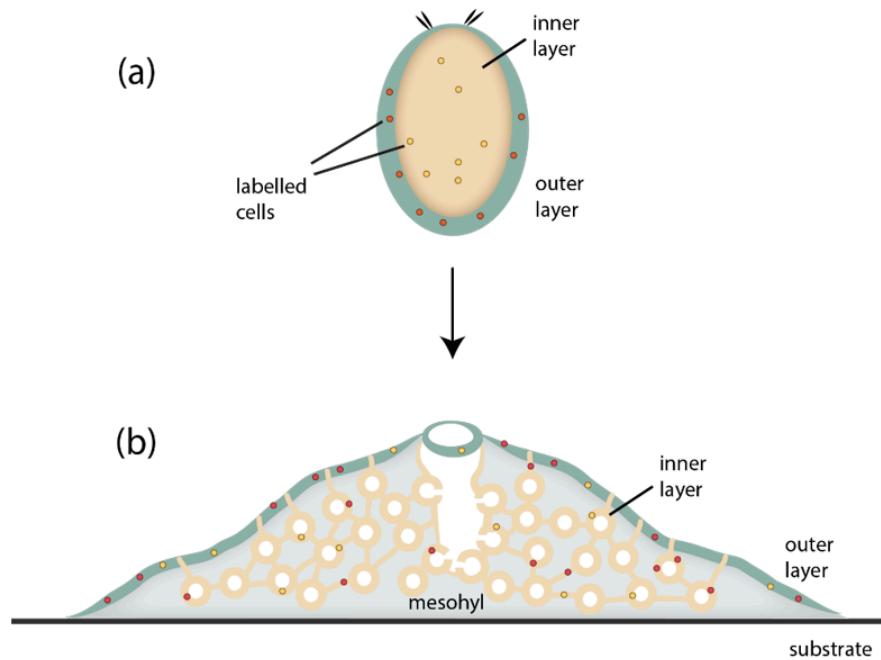
**Figure 1.** Gastrulation by invagination according to Haeckel. [s: germ cavity (*Furchungshöhle*); e: ectoderm (*Ektoderm*); i: endoderm (*Entoderm*); d: primary intestine

(Urdarmhöhle); o: primary mouth (Vrmond); b: blastula; g: gastrula] [Redrawn from Haeckel<sup>[2]</sup> Table II].

He proposed that embryonic development recapitulates the evolution of a taxon, that is, the *adult* forms of their ancestors (called Haeckel's biogenetic law). By identifying a gastrula stage in all metazoans, Haeckel wanted to provide evidence in support of Darwin's theory of common descent. The gastrula would correspond to their common ancestor, a hypothetical animal he called *Gastraea*<sup>[3]</sup>. Contrary to Haeckel's expectations, however, gastrulation is highly variable among metazoans, tissue invagination being one of several mechanisms capable of generating tissue layers. The author also considered cell ingression as a mechanism of gastrulation<sup>[3]</sup>, but the important point in support of his theory was not a specific mechanism, but its conservation across the animal kingdom. Furthermore, mechanisms of gastrulation are not mutually exclusive: they can conjointly contribute to the formation of a gastrula. This has led to re-thinking how embryonic development could have evolved<sup>[4][5]</sup>.

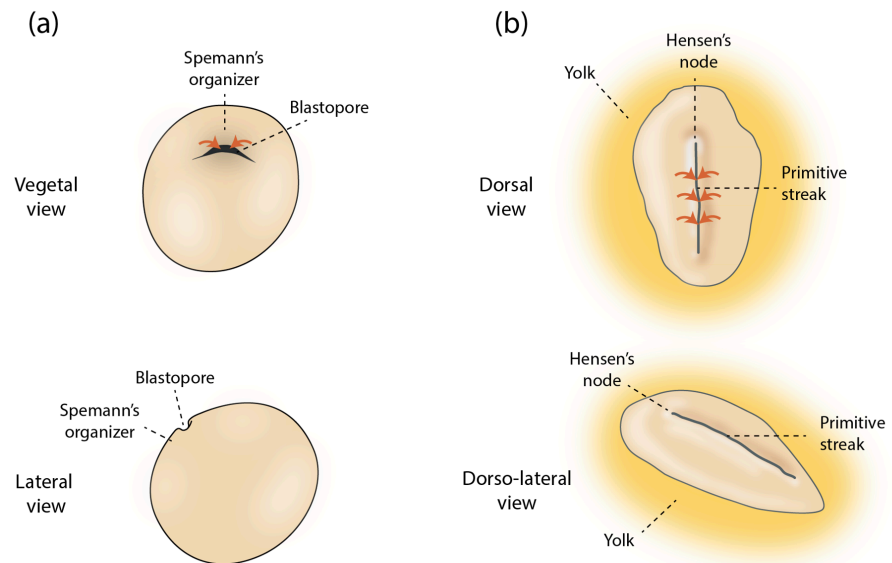
According to Haeckel's *Gastraea* theory, all metazoans would pass through a two-layered gastrula stage, which means that the third layer of triploblastic metazoans (i.e., the mesoderm) would develop last. However, in many metazoans, the mesoderm derives from the endomesoderm, or mesendoderm, a cell/tissue capable of differentiating into endoderm and mesoderm, i.e., the two layers differentiate simultaneously<sup>[6]</sup>. Finally, the primary intestine, if vestigially present, does not give place to the gut in some animals (e.g., chicken), i.e., their gastrula does not present the structure that gave it its name.

Although the gastrula does not develop as expected by Haeckel, the term has prevailed to define the stage at which the germ layers develop. This includes both their morphogenesis and their differentiation<sup>[7]</sup>, and it is independent of: 1) the temporal sequence of these two events, 2) the specific morphogenetic mechanism, 3) the number of germ layers, and 4) its relationship with gut morphogenesis. According to it, gastrulation is a common characteristic of all metazoans, except sponges. The latter develop two tissue layers by the morphogenetic mechanisms characteristic of gastrulation, but they lack the fate determination and stability that define them<sup>[8]</sup> (Fig. 2). Based on this observation, some authors have stressed that the common feature of all metazoans is not gastrulation, but its morphogenetic component only<sup>[9]</sup>.



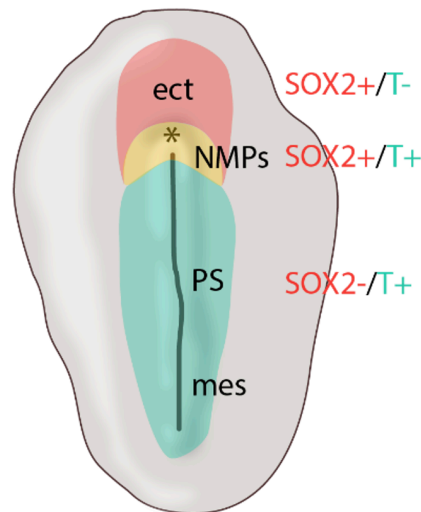
**Figure 2.** Lack of germ layer stability in sponges. A longitudinal section of a larva (a) and a juvenile (b) showing the inner and the outer layer. Cell tracking has revealed a lack of correspondence of the germ layers between these stages: cells of the larva's outer layer not only contribute to the formation of the external pinacoderm, but also to the choanocyte chambers of the inner layer in the juvenile, and *vice versa* [Based on Nakanishi et al.<sup>[8]</sup>].

It is commonly thought that the formation of the germ layers is complete after gastrulation, i.e., that the whole body axis is laid down during this process. This led Wolpert to state “it is not birth, marriage, or death, but gastrulation which is truly the most important time in your life”<sup>[10]</sup>. In *Xenopus*, the whole body axis is established during gastrulation, except for the tail. This has led to a long-standing controversy as to whether the tail forms by the growth of established structures or anew from undifferentiated tissue, independently from gastrulation<sup>[11]</sup>. This incompleteness is more remarkable in amniotes, as only the head and the most anterior structures have been specified at the gastrula stage<sup>[12]</sup>. This is because the process of gastrulation, i.e., the internalization of cells to form different germ layers, continues after the gastrula stage in this group<sup>[12]</sup>. However, it is not the only process that contributes to the elongation of their body axis. In addition, an organizer appears at the anterior region of the primitive streak, called the node (Fig. 3). After the gastrula stage, the node – conjointly with the primitive streak – regresses in a head-to-tail progression, laying down part of the body axis, specifically: the notochord, the floor plate, and the medial somites<sup>[13][14]</sup>. When most of the trunk structures have been established, the node forms part of the tailbud, which continues elongating the body<sup>[15][14]</sup>.



**Figure 3.** Initiation of gastrulation in the frog and the chicken embryo. (a) In frogs, cells of the blastula's outer layer internalize (brown arrows) through the blastopore. These movements are regulated by the organizer (Spemann's organizer). (b) In chickens and humans, the blastula forms a disc-like shape rather than a sphere [compare the lateral views in (a) and (b)]. The equivalent to the blastopore is an elongated groove, the primitive streak, through which cells internalize (brown arrows). The node (Hensen's node in the chick) is located anterior to the primitive streak, and it is commonly considered homologous to the Spemann's organizer (but see [\[16\]](#)).

Recent studies have shown the existence of pluripotent cells capable of differentiating into the neural tube and paraxial mesoderm located at the node in chickens<sup>[15][13]</sup>, and the node-streak border in mice<sup>[13]</sup>. A new study has shown these cells can also give rise to the notochord in zebrafish<sup>[17]</sup>. Therefore, tissues that generally derive from different germ layers (ectoderm and mesoderm) can also be derived from these pluripotent cells, called neuromesodermal progenitors (NMPs), without passing through a germ layer intermediate, i.e., independently from gastrulation (Fig. 4). These results would solve the controversy started by Holmdahl, Vogt, and Pasteels at the beginning of the twentieth century<sup>[11]</sup>. Located at the interface between the ectoderm and the mesoderm<sup>[18]</sup>, the NMPs may help to zip the body axis, assuring its functionality. The zipping of the ectoderm and the mesoderm could play a key role in the evolution of the chordate body plan<sup>[19]</sup>.



**Figure 4.** Neuromesodermal progenitors are not part of a germ layer. The figure shows the dorsal view of a chicken embryo (HH5) immunostained for SOX2 (red) and BRACHYURY/T (green). The epiblast has differentiated into ectoderm (red), mesoderm (green), and endoderm (not shown, as it has been already internalized). Neuromesodermal progenitors are located at the node (asterisk) (yellow), between the ectodermal and the mesodermal region, and they are characterized by co-expressing the neural marker *Sox2* and the mesodermal marker *Brachyury* (*T*) (PS: Primitive streak; *ect*: ectoderm; *mes*: mesoderm, NMPs: neuromesodermal progenitors) [Redrawn from Guillot et al.<sup>[15]</sup>].

NMPs reside in a region of divergent tissue flows<sup>[17]</sup>. This means that they can be displaced to different tissue territories during the elongation of the body axis. If already committed to a neural or mesodermal fate after gastrulation, these cells could end up in the wrong place. By delaying their differentiation, they flow as pluripotent cells until they reach their final destination, thus linking cell differentiation with morphogenesis<sup>[20]</sup>.

The discovery of the NMPs challenges the common view that gastrulation establishes the whole body axis. Coming back to the title question: is gastrulation the most important time in your life? Yes, for most of your body, except part of the spinal cord, vertebral column, ribs, and the coccyx (what remains from the human tail<sup>[21]</sup>), assuming a similar contribution of NMPs between the chick and the human embryo<sup>[15][13]</sup>. This answer is based on the most common definition of gastrulation (i.e., morphogenesis and differentiation of the germ layers), but if redefined as “a process whereby the embryo acquires a system of coordinates to organize and position the primordia for the different tissues and organs” as recently suggested<sup>[4]</sup>, gastrulation would be the most important time for your entire body.

Like in the case of sponges, a definition of gastrulation that leaves out some animal taxa may be seen as “undesired” by some researchers; however, stretching the term to include

all of them would hide key embryological differences that could help to better understand the evolution of their body plans.

## Statements and Declarations

### Author Contributions

ML conceived the topic, researched the literature, wrote the manuscript, and approved the final submitted version.

### Data Availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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## Declarations

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