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Review Article

Is Gastrulation the Most Important Time in Your Life?

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Gastrulation is considered a fundamental process of metazoan embryogenesis, as the layers that give place 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 (Haeckel 1872).

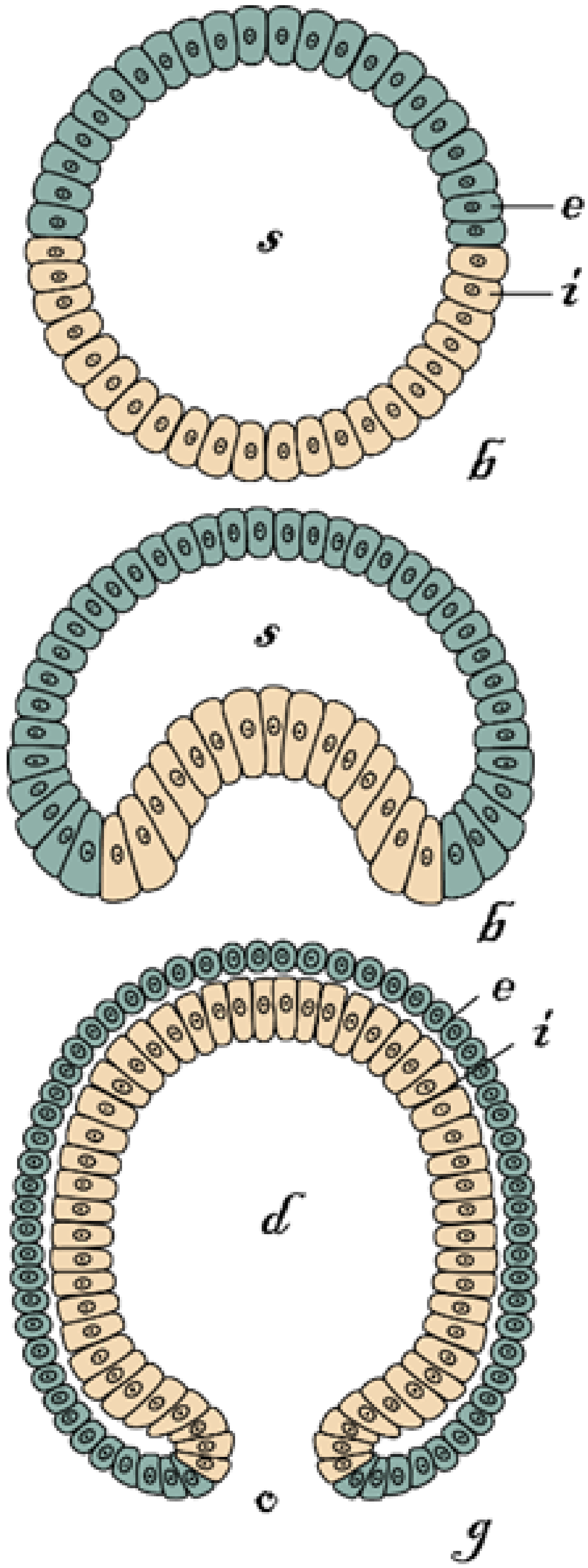

Figure 1. Gastrulation by invagination according to Haeckel. [*s*: germ cavity (*Furchungshöhle*); *e*: ectoderm (*Ektoderm*); *i*: endoderm (*Entoderm*); *d*: primary intestine (*Urdarmhöhle*); *o*: primary mouth (*Vrmund*); *b*: blastula; *g*: gastrula] [Redrawn from Haeckel (1874) Table II].

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 (Levit et al. 2022). 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 (Levit et al. 2022), 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 (Steventon et al. 2021; Uesaka et al. 2022).

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 the 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 (McClay et al. 2021). 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 (Stern 1992), 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 (Nakanishi et al. 2014) (Fig. 2). Based on this

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observation, some authors have stressed that the common feature of all metazoans is not gastrulation, but its morphogenetic component only (Ereskovsky and Dondua 2006).

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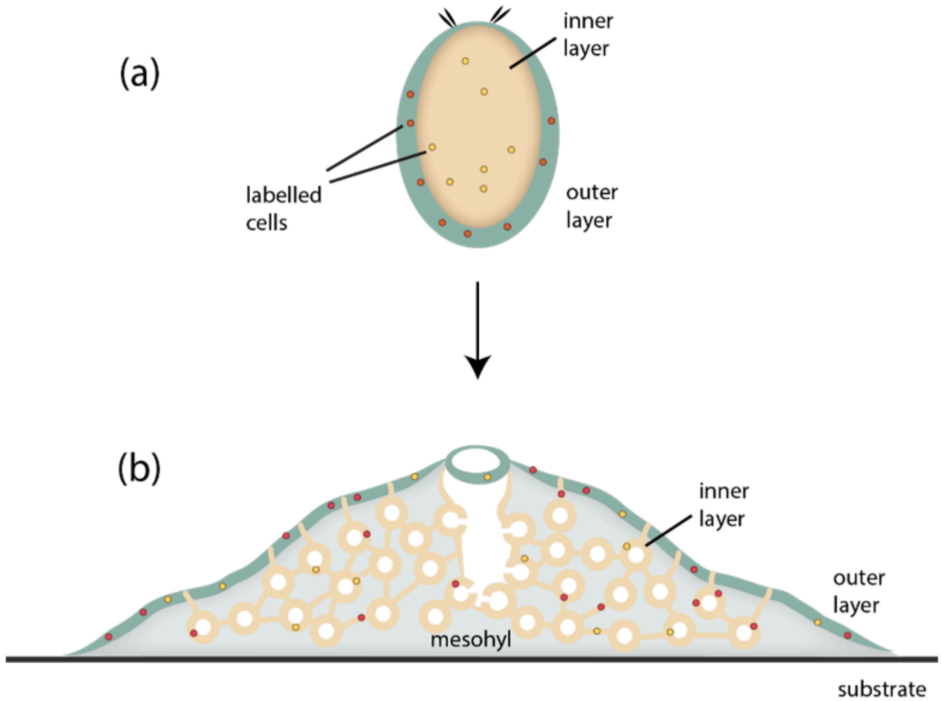

Figure 2. Lack of germ layer stability in sponges. A longitudinal section of a larva (a) and a juvenile (b) showing the inner and the outer layer. Cell tracking has revealed a lack of correspondence of the germ layers between these stages: cells of the larva's outer layer not only contribute to the formation 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. (2014)].

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" (Hopwood 2022). In *Xenopus*, the whole body axis is established during gastrulation, except 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 (Handrigan 2003). This incompleteness is more remarkable in amniotes, as only the head and the most anterior structures have been specified at the gastrula stage (Stern et al. 2006). 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 (Stern et al. 2006). 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 (Solovieva et al. 2022a; Solovieva et al. 2022b). When most of the trunk structures have been established, the node forms part of the tailbud, which continues elongating the body (Guillot et al. 2021; Solovieva et al. 2022b).

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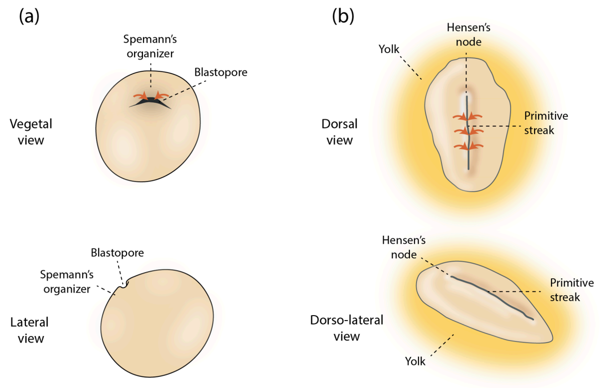

Figure 3. Initiation of gastrulation in the frog and the chicken embryo. (a) In frogs, cells of the blastula's outer layer internalize (brown arrows) through the blastopore. This movements are regulated by the organizer (Spemann's organizer). (b) In chickens and humans, the blastula forms a disc-like 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 Martínez-Arias and Steventon 2018).

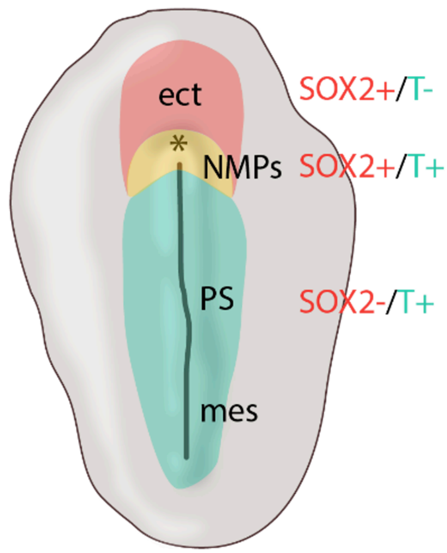

Figure 4. Neuromesodermal progenitors are not part of a germ layer. The figure shows the dorsal view of a chicken embryo (HH5) immunostained for SOX2 (red) and BRACHYURY/T (green). The epiblast has differentiated into ectoderm (red), mesoderm (green) and endoderm (not shown, as it has been already internalized). 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. (2021)].

Recent studies have shown the existence of pluripotent cells capable of differentiating into neural tube and paraxial mesoderm located at the node in chickens (Guillot et al. 2021; Solovieva et al. 2022a), and the node-streak border in mice (Solovieva et al. 2022a). A new study has shown these cells can also give place to the notochord in zebrafish (Lange et al. 2023). 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 (Handrigan 2003). Located at the interface between the ectoderm and the mesoderm (Wood et al. 2019), 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 (Sato et al. 2023).

NMPs reside at a region of divergent tissue flows (Lange et al. 2023). This means that they can be displaced to different tissue territories during the elongation of the body axis. If already committed to 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 (Theise and Wilmut 2003).

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

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[Beck 2015]), assuming a similar contribution of NMPs between the chick and the human embryo (Guillot et al. 2021; Solovieva et al. 2022a). 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 (Steventon et al. 2021), gastrulation would be the most important time to 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 them would hide key embryological differences that could help to better understand the evolution of their body plans.

References

- Beck CW (2015) Development of the vertebrate tailbud. Wiley Interdiscip Rev Dev Biol 4:33-44.
- Ereskovsky AV, Dondua AK (2006) The problem of germ layers in sponges (Porifera) and some issues concerning early metazoan evolution. Zool Anz 245:65-76.
- Guillot C, Djeffal Y, Michaut A, Rabe B, Pourquié O (2021) Dynamics of primitive streak regression controls the fate of neuromesodermal progenitors in the chicken embryo. Elife 10.
- Haeckel E (1872) Die Kalkschwämme: Eine Monographie. Verlag von Georg Reimer, Berlin.
- Haeckel E (1874) Anthropogenie oder Entwickelungsgeschichte des Menschen. Gemeinverständliche wissenschaftliche Vorträge über die Grundzüge der menschlichen Keimes- und Stammesgeschichte. Wilhelm Engelmann, Leipzig.
- Handrigan GR (2003) Concordia discors: duality in the origin of the vertebrate tail. J Anat 202:255-267.
- Hopwood N (2022) 'Not birth, marriage or death, but gastrulation': the life of a quotation in biology. Br J Hist Sci 55:1-26.
- Lange M, Granados A, VijayKumar S, Bragantini J, Ancheta S, Santhosh S, Borja M, Kobayashi H, McGeever E, Solak AC, Yang B, Zhao X, Liu Y, Detweiler A, Paul S, Mekonen H, Lao T, Banks R, Jacobo A, Balla K, Awayan K, D'Souza S, Haase R, Dizeux A, Pourquié O, Gómez-Sjöberg R, Huber G, Serra M, Neff N, Pisco AO, Royer LA (2023) Zebrahub – Multimodal zebrafish developmental atlas reveals the state transition dynamics of late vertebrate pluripotent axial progenitors. bioRxiv March 07.
- Levit GS, Hoßfeld U, Naumann B, Lukas P, Olsson L (2022) The biogenetic law and the Gastraea theory: From Ernst Haeckel's discoveries to contemporary views. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 338:13-27.
- Martínez-Arias A, Steventon B (2018) On the nature and function of organizers. Development 145.
- McClay DR, Croce JC, Warner JF (2021) Conditional specification of endomesoderm. Cells Dev 167:203716.
- Nakanishi N, Sogabe S, Degnan BM (2014) Evolutionary origin of gastrulation: insights from sponge development. BMC Biol 12:26.
- Sato Y, Narasaki I, Kunimoto T, Moriyama Y, Hashimoto C (2023) The complete dorsal structure is formed from only the blastocoel roof of Xenopus blastula: insight into the gastrulation movement evolutionarily conserved among chordates. Development Genes and Evolution.
- Solovieva T, Lu HC, Moverley A, Plachta N, Stern CD (2022a) The embryonic node behaves as an instructive stem cell niche for axial elongation. PNAS 119.
- Solovieva T, Wilson V, Stern CD (2022b) A niche for axial stem cells - A cellular perspective in amniotes. Dev Biol 490:13-21.
- Stern CD (1992) Vertebrate gastrulation. Curr Opin Genet Dev 2:556-561.
- Stern CD, Charité J, Deschamps J, Duboule D, Durston AJ, Kmita M, Nicolas JF, Palmeirim I, Smith JC, Wolpert L (2006) Head-tail patterning of the vertebrate embryo: one, two or many unresolved problems? Int J Dev Biol 50:3-15.
- Steventon B, Busby L, Martínez-Arias A (2021) Establishment of the vertebrate body plan: Rethinking gastrulation through stem cell models of early embryogenesis. Dev Cell 56:2405-2418.
- Theise ND, Wilmut I (2003) Cell plasticity: flexible arrangement. Nature 425:21.
- Uesaka M, Kuratani S, Irie N (2022) The developmental hourglass model and recapitulation: An attempt to integrate the two models. J Exp Zool Part B 338:76-86.
- Wood T, Kyrsting A, Stegmaier J, Kucinski I, Kaminski CF, Mikut R, Voiculescu O (2019) Neuromesodermal progenitors separate the axial stem cell zones while producing few single- and dual-fated descendants. bioRxiv May 13.

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Declarations

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