# Review of: "Is gastrulation the most important time in your life?"

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Willingly or not, the author illustrates a persistent problem in the evodevo field. Namely, that we are divided into lumpers and splitters as regards the existence of general laws or general developmental processes in the field. In this case the issue is gastrulation. Lumpers think that this being such a fundamental organizing process of zygotic structural development (i.e., definition of longitudinal axis and fundamental germ layers), when facing new evidence we should stretch or otherwise modify the old concepts as needed in order to obtain a definition that satisfies every condition found among animals (preferably including sponges; why leave them out). Splitters contrarily think that every lack of straightforward similarity noted in a taxon (e.g., as in sponges) is proof that the law does not apply, or the process does not occur necessarily as defined, and therefore evolving development is intrinsically more variable and unpredictable than expected. The universe has more freedom this way, true, even if understanding it becomes more difficult.

Though the author of this opinion piece duly cites authors of both groups, reading between lines one perceives (particularly in her response to Green's comment, an apparent lumper) that Linde-Medina is among the splitters. Several of the other commentators may not have decided yet their position in this aspect. Marta apparently sort of likes the idea that NMPs rival the ancient predominance of gastrulation as a general developmental process, and she thus plays around with the consequently diminished value of Wolpert's famous dictum. I tend to be a lumper myself and would desire at least some nominal effort to see whether NMPs can be incorporated into the classic concept of gastrulation, rather than assuming automatically (without discussion) that they represent a different process acting outside of gastrulation upon the embryonic axis and germ layers (though their neural and mesodermal derivatives do integrate into existing germ layers; why do we read that they do not participate in gastrulation? I fully agree here with Green).

The author might give more details about the extent of the NMPs phenomenon (for instance, is it present in invertebrates? Does it distinguish vertebrates from chordates and invertebrates? How early in development do the first NMPs appear in different lineages of vertebrates?).

The last question relates to the parts of the axis that are affected by NMP activity. Linde-Medina alludes to 'mainly the tail' but it is not clear what is the evidence for that, apart of selective attention by some authors to tail formation; for all we know NMPs may be present from the very start of gastrulation and thus condition the whole floorplate of the brain and the whole underlying notochord (head to tail), NMPs thus becoming an integral part (submechanism) of gastrulation. I recommend examining Lwoff's 1894 data on maximal proliferative activity at the primitive line in Amphioxus development, which would cause according to that author caudalwards elongation of the embryo (see also Ferran et al. 2022); these results are much earlier than the literature cited by Linde-Medina; there is additional experimental labelling literature on notochordal gastrulation than reveals a notochordal proliferative center within the primitive line – that is, within gastrulation; the node is also an integral part of the primitive line and gastrulation, not an extra, later detail, as suggested subliminally by the author; its caudalwards regress directly reflects the gradual rostrocaudal end of gastrulation, which eventually reaches the tail tip. If the described 'notochord proliferative center' in the primitive line contains the NMPs, then NMPs can be seen as a partial mechanism of gastrulation, not a separate process.

Answers to my other questions might also illuminate the potential evolutionarily emergent profile of the NMP cells. At first sight one would expect no true NMPs in invertebrates, because they do not have either a neural plate/tube which would need a floor plate or a notochord/somite system. However, McClay et al. 2021 do affirm that sea urchins have an early group of NNE cells (neural/notochordal/endodermal cells as possible as yet undecided fates) which might be their ancestral equivalent. See comments below on confusing terminology.

I was also surprised to see in the cited review that invertebrates generate invaginating non-skeletal mesoderm from the large micromeres (I had long thought that invertebrate gastrulation was purely endodermal; for me this raised the theoretic possibility that the vertebrate gastrulation homolog process might be fulfilled once the endoderm invaginates -which reportedly occurs earliest-, so that ulterior invaginated mesoderm and neural floorplate formation might be parallel epiphenomena related to NMPs). Surprisingly, the sea urchin non-skeletal mesoderm invaginates before the endodermal Veg2 and Veg1 cells (McClay et al. 2021). This contrasts with the contrary sequence in chordates (and obviously these cells do not form a notochord). Unfortunately, I did not see described the precise fate of that 'non-skeletal mesodermal tissue' (maybe involved in the new mouth area in deuterostomes?).

This issue relates to another problem in the field, not commented by the author, namely that sometimes the meaning of terms used are tendentious, imprecise or confusing. Variability in terms or concepts used easily is presented illogically as intrinsic variability in the process discussed. For instance, should the sea urchin cells invaginating at the advance of gastrulation be really called 'mesoderm'? If the molecular profile does suggest 'mesoderm' identity (or partial similarity), we urgently need an evolutionary developmental hypothesis on how this invertebrate primordium evolves through emergence of Nieuwkoop's centre into the topologically different and heterochronic chordate primordium for mesoderm.

The related terms 'endomesoderm' and 'mesendoderm' (assumed here at face value by the author) refer to cells still uncommitted to these fates, and thus, even when the terms are qualified as 'conditional', are tendentious (McClay et al. 2021). This tissue contains simply transient 'non-ectodermal cells' or 'undecided vegetal cells', since they are still on their way to differentiate either as endodermal or mesodermal elements. No adult cell type has both characters, so that a true 'endomesoderm' does not exist ever (is a fiction). McClay et al. (2021) make it clear that partial conditional co-expression of some endodermal or mesodermal gene markers does not represent either of the final profiles. Thus, for scientific clarity, the names should not characterize a transient cell state by the sum of the future relevant alternative fates (apply this to 'neuromesodermal' NMPs). Moreover, the literature abounds in false references to 'mesendoderm tissue' identified after the end of gastrulation, disregarding the fact that the distinct fates are then fully established and the transient earlier cells no longer exist.

The term gastrulation/gastrula itself appears variably referred to as a thing (a double- or triple-layered structure), a process (cell invagination by diverse mechanisms, which do not exclude NMPs) or a time period (a ‘gastrulation stage’ with an arbitrary end before the tail is formed). Authors often are not careful to dissociate or consider these different ontological meanings in their arguments about how similar the gastrulation process is in different animals. I remind everybody that homology does not require either apparent similarity or identity of function (this now requires analysis in terms of GRPs; note that crucial genes may change if the attractors still achieve their adaptative objective). What we need is a well-documented notion of topologic and causal invariance in gastrulation, irrespective of the flurry of accompanying irrelevant variations.

References:

Ferran JL, Irimia M, Puelles L. 2022. Is there a prechordal region and an acroterminal domain in Amphioxus? Brain Behav Evol DOI: 10.1159/000521966.

Lwoff B. 1894. Die Bildung der primären Keimblätter und die Entstehung der Chorda und des Mesoderms bei den Wirbelthieren. Moskau: Universitätsbuchdruckerei.

McClay DR, Croce JC, Warner JF. 2021. Conditional specification of endomesoderm. Cells & Development 167:203716