Opinion

(Why) Does Evolution Favour Embryogenesis?

Stefan A. Rensing

Complex multicellular organisms typically possess life cycles in which zygotes (formed by gamete fusion) and meiosis occur. Canonical animal embryogenesis describes development from zygote to birth. It involves polarisation of the egg/zygote, asymmetric cell divisions, establishment of axes, symmetry breaking, formation of organs, and parental nutrition (at least in early stages). Similar developmental patterns have independently evolved in other eukaryotic lineages, including land plants and brown algae. The question arises whether embryo-like structures and associated developmental processes recurrently emerge because they are local optima of the evolutionary landscape. To understand which evolutionary principles govern complex multicellularity, we need to analyse why and how similar processes evolve convergently – von Baer’s and Haeckel’s phylotypic stage revisited in other phyla.

Haeckel’s Theory, and Embryogenesis in Animals and Plants

The ‘Biogenetic Law’

Around 150 years ago [1], Ernst Haeckel described that vertebrate embryos (see Glossary) resemble each other at a certain stage of development, and that features such as gills come and go during ontogenesis, apparently recapitulating phylogenesis scripted by the evolutionary history of the lineage. The ‘biogenetic law’ Haeckel described was later transferred into the recapitulation theory or Biogenetische Grundregel. Embryos are known from multicellular animals (Metazoa), red and brown algae, and land plants. Such embryos arise from a zygote after fertilisation and possess a 3D body plan characterised by polarity and symmetry breaking. I will use this definition of an embryo throughout, and refer to embryogenesis as the early developmental progression from zygote to embryo, typically until developmental arrest or birth. It should be noted that formation of a zygote is not sufficient for embryogenesis, but apparently a necessary prerequisite. Embryogenesis apparently evolved several times independently (convergently), but we do not yet understand the underlying molecular evolutionary principles. After introducing animal and plant embryogenesis, I will discuss the (convergent) evolution of multicellularity and embryogenesis, eventually coming back to Haeckel’s ideas to see whether they have a molecular foundation and whether such applies to more than vertebrate embryos.

Animals

Embryogenesis of animals comprises all developmental steps from zygote (generated via gamete fusion) to larval or foetal stages until metamorphosis and birth. After fertilisation, the zygote starts to divide and forms a hollow sphere or disc, the blastula or blastoderm. This process is called cleavage (because the cells divide without increasing their size until the 254-cell stage); translation during this phase is mainly determined by maternal (egg) transcripts. Activation of transcription of the novel diploid genome, created by the fusion of the haploid parental gamete nuclei, as well as cell fate determination, occur only later. At the onset of gastrulation, the primary axis is already established and initially two so-called germ layers form: ecto- and endoderm. Future endodermal cells migrate as single cells or sheets inside the hollow blastula.

1Plant Cell Biology, Faculty of Biology, University of Marburg, Karl-von-Frisch-Str. 8, D-35043 Marburg, Germany
2BIOSS Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany
*Correspondence: stefan.rensing@biologie.uni-marburg.de (S.A. Rensing).
@Twitter: @RensingStefan
resulting in a two-layered embryo. In Bilateria (encompassing vertebrates), the third germ layer, the mesoderm, forms either from early set-aside cells or following inductive processes from the endomesoderm. Concomitant with induction and the establishment of secondary polarity axes, organogenesis starts. Postembryonic ontogenesis primarily serves organ and thus body growth.

The last common ancestor of the Eumetazoa, unifying the Cnidaria (e.g., sea anemones) with the Bilateria, possessed at least the primary oral–aboral axis, and two germ layers [2,3]. Its gene repertoire already comprised the genes required to establish three germ layers as well as the primary anterior–posterior (head to tail) and secondary dorsal–ventral (back to belly) and left–right axes in the animal lineage crown group Bilateria (Figure 1) [3]. Embryonic development of the Eumetazoa is regulated by the homeodomain (HD) transcription factors (TFs) of the HOX family that interact with TALE (three amino acid length extension) HD TFs such as PBC (Table 1 and Figure 1). The HOX hexapeptide (HX) motif, which interacts with TALEs, probably evolved in the last common ancestor of the Eumetazoa [4].

At the basis of the Eumetazoa, the Placozoa already show evidence of germline regulation by HD proteins, for example, by POU (Figure 1) [5]. The next sister lineage are the Porifera (sponges; it should be noted, though, that the basal metazoan branching order is still debated [6]). Sponges possess an apico–basal axis in embryos and larvae, thus axis formation apparently is a synapomorphy of the Metazoa (Figure 1). No canonical gastrulation or germ sensu stricto are observed, but cells are able to migrate and, in some cases, to form epithelia [7,8]. It has been argued from the expression pattern of Wnt and TGF-β (Table 1) along the embryonic axis that the ability for complex patterning was already present in the last common ancestor of the Metazoa [9,10].

Metazoa, that is, multicellular animals, are thought to have evolved from single-celled organisms akin to Choanoflagellida via colonial forms. The rosette-forming Salpingoeca rosetta makes use of conserved Septins to control cytokinesis [11]; a C-type Lectin probably involved in cell adhesion allows this organism to form multicellular colonies [12]. Based on the information derived from the above-mentioned genomes via comparative genomics approaches, animal science has made big steps forward in recent years in unravelling evolution of morphogenesis and thus of body plans.

Plants

The canonical embryogenesis of flowering plants (angiosperms or Magnoliophyta) describes the ontogenesis of the diploid zygote until an arrest (dormancy) occurs that is broken once the seed germinates. The embryo progresses through a globular stage, followed by polarised growth, eventually leading to the heart stage in dicotyledonous plants (possessing two embryonic leaves or cotyledons). During the globular stage, shoot and root apical meristems (SAM/RAM) – and thus stem cells – are formed in the Arabidopsis (Arabidopsis thaliana) embryo [13]. Several HD TFs are involved in the asymmetric division leading to the globular stage. The expression of WOX HD TFs [14] is already different in the cells of the octant (eight-celled) stage. The embryo suspensor connects the embryo with the maternal (sporophytic) tissues of the developing seed, representing a nutritional (trophic) link.

One could be tempted to compare the progression of the animal zygote to blastula to gastrula with the one of the plant zygote to globular stage to heart stage, and protoderm, root, and shoot apical meristem with the three germ layers of gastrulation. However, shape comparisons are evidently flawed since, for example, monocotyledonous plants do not possess a heart stage. Nutrition of the embryo is taken care of by parental tissue in both lineages (placenta/yolk, endosperm). In contrast to many animals, however, the plant zygote polarises, that is, forms an
axis, even before its first asymmetric cell division. Animal embryos rely on egg cell transcripts typically up to the 254-cell stage. Plant embryos are able to develop for a limited time without the need for transcription [15] – early asymmetric division and transcription are activated within a few hours after fertilisation. In plants, some paternal (sperm cell) transcripts are clearly important for zygote development [15], and cell lineage does not determine embryo form in plants, but rather vice versa. Animal embryogenesis usually has a defined end (birth), while in plants this is not the case; dormancy can occur at different stages or it might not happen at all (e.g., in viviparous mangroves).

As Kaplan and Cooke stated in [16]: plant embryogenesis is not a separate stage, but is the initiation of an iterative process of meristematic activity that will continue throughout the life of the plant (yet, axis formation and parental nutrition are not continuing). While all plants are able to recurrently form meristems, not all plants have unlimited regeneration potential from differentiated cells (as, e.g., mosses do) – and by contrast some animals do possess regenerative processes forming, for example, appendices such as the tail of the lizard or the antlers of the deer, or the ability to regenerate the whole organism from pieces such as in sponges or hydra. Apparently, regeneration capability even was an ancestral feature of tetrapods and was lost only secondarily [17]. In the remainder, I will ponder the questions how and why multicellularity and embryogenesis arise, and whether and how different flavours of embryogenesis are evolutionary related.

**From Single Cells to Multicellular Organisms**

Multicellularity is necessary, but not sufficient, for embryogenesis. During ontogeny, complex multicellular organisms arise from single cells, and sexually reproduce via single cells. This developmental progression probably reflects the evolution of multicellularity from single-celled ancestors. The unicellular ‘bottleneck’ of multicellular organisms is even evident without sexual (meiotic) reproduction [18], for example, in parthenogenesis, single-celled vegetative propagules or vegetative buds. Clonal multicellular organisms derive from division of a single cell, while aggregation of genetically distinct cells can also lead to multicellularity (e.g., in the slime mould *Dictyostelium discoideum*; Figure 1). On top of that, there are multinucleate syncytia in which many nuclei (either genetically identical or distinct) form a complex body (e.g., siphonous algae or non-septate fungi). Here, I will mainly talk about clonal multicellularity, which arguably has led to the highest levels of morphological complexity. Such multicellularity requires a highly orchestrated and differential cell division to ensure proper division planes and tissue determination.

Selection acts on functional traits rather than on their underlying mechanisms, hence multicellularity evolved by different routes [19]. However, multicellular organisms rarely evolve back to unicellularity. One of the rare cases are yeasts with their bacterial-like life strategy [20]; they are also a nice example for recurrent evolutionary trajectories: deletion of an important polarisation gene leads to reproducible ‘repair’ of the module within 1000 generations by the very same genes [21]. Yeasts probably went back to unicellularity from filamentous forms; they form zygotes, but do not perform embryogenesis. Yet, clonal multicellularity can be found in yeast [22] and also in Choanoflagellates, the sister lineage to Metazoa [23]. Unicellular species can even be placed under selection, resulting in the evolution of multicellularity [18].

So how do unicellular organisms evolve clonal multicellularity? Genes for cell adhesion, cell to cell signalling and cell lineage-specific transcriptional control were already present in the unicellular ancestor of animals [7,24,25], and the situation in plants is similar [26,27]. Confusingly, cell–cell connections of aggregative multicellularity in *D. discoideum* are similar to those in multicellular opisthokonts, although they evolved independently [28,29] – and, for example, Catenins, involved together with Cadherins in animal cell junctions, have Armadillo homologs in plants [30] (Figure 1). Recently, cell surface Arabinogalactan proteins (AGPs), important for plant
development, have also been found in the brown alga *Fucus serratus*, where they are important for embryogenesis [31].

**Convergence or Conservation of Gene Functions?**

Some of the genes underlying embryogenesis are clearly homologous, for example, HD TFs. The question, however, remains whether they were convergently co-opted, following a recurring pattern, or whether they already shared an ancestral function in the last eukaryotic common ancestor (LECA). Vision, as an example, has been proposed to have evolved only once, and subsequently transmitted vertically or horizontally [32]. In a more narrow setting, the HD gene *PAX6*, which controls eye formation, is clearly a synapomorphy of the Bilateria [32].

Protein kinases have been argued to be instrumental for the establishment of complex signalling networks in multicellular animals [33], fungi [34], plants [35], and brown algae [36]. Expansion of...
the tyrosine kinase network has been shown to be correlated with increasing organismal complexity in animals [37,38], while genes encoding SHAGGY-like kinases expand with plant evolution [39], due to retention after genome duplications [40].

Land plant body plan determination was hypothesised to have evolved from **HD-TALE** KNOX/ **BELL-like** sexual determination/meiosis control genes of algae [41], similar to the control of animal embryogenesis by HD interactions (Figure 2). Indeed, KNOX and BELL homologs

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Table 1. Proteins Involved in Embryogenesis

<table>
<thead>
<tr>
<th>Family</th>
<th>Subfamily</th>
<th>Metazoa</th>
<th>Choanoflagellates</th>
<th>Fungi</th>
<th>Plants</th>
<th>Green Algae</th>
<th>LECA</th>
<th>Comment/Function</th>
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<tr>
<td>Non-TALE HD</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td>[4,90]</td>
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<td>[2,4]</td>
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<tr>
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<td>–</td>
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<td>[32]</td>
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<td>[5]</td>
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<td>WOX</td>
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<td>x</td>
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<td></td>
<td>– WUS related homeobox, with loop extensions</td>
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<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>x</td>
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<td>BELL-like</td>
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<td>x</td>
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<td>–</td>
<td>Involved in embryo patterning</td>
<td>[2,9]</td>
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<tr>
<td>TGF-β</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>Transforming growth factor</td>
<td>[9]</td>
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<td><strong>Cell-Cell Interaction Proteins</strong></td>
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<td>x</td>
<td>x</td>
<td>β-Catenin is the canonical WNT mediator</td>
<td>[30]</td>
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<tr>
<td>Cadherins</td>
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<td>x</td>
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<td>–</td>
<td>Cell–cell connection</td>
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<td><strong>Other Proteins</strong></td>
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<td>Septins</td>
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<td>GTP binding</td>
<td>[11]</td>
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<td>–</td>
<td>–</td>
<td>Carbohydrate binding</td>
<td>[12]</td>
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<td>x</td>
<td>x</td>
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<td>x</td>
<td>x</td>
<td>Histone mark modification</td>
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<td>Calpain</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>Protease</td>
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<td>Protein kinases</td>
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<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
<td>Many subfamilies</td>
<td>[33,37,39,40]</td>
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<td>x</td>
<td>x</td>
<td>GTPase</td>
<td>[61,69–72]</td>
</tr>
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</table>

Abbreviations: bHLH, basic helix–loop–helix; TALE, three amino acid length extension.

*aList is not comprehensive, but based on proteins mentioned in the main text.

*bThe homeobox (HB) DNA sequence encodes a 60–66 amino acid residue DNA-binding domain, the homeodomain (HD).

*x denotes in which lineages a particular protein, respectively the gene encoding it, is present. If present in the last eukaryotic common ancestor (LECA), it is supposed to be present in all lineages shown and therefore marked as such (but note that secondary loss might have occurred).
regulate meristem maintenance in flowering plants, and are upregulated in moss sporophytes [42]. It has been hypothesised that the land plant life cycle (with its alternating multicellular gametophyte and sporophyte; Figures 2 and 3) evolved from charophytes, in which the diploid stage was represented solely by the zygote. Interestingly, diploid zygotes in the genus Chara are dormant, that is, meiosis is delayed until conditions are suitable for meiosis and subsequent germination of the haploid gametophyte. In some Coleochaete species, the zygote is retained on the female gametophyte, to which it has a nutritional link. By intercalation of mitotic divisions prior to meiosis, the multicellular sporophyte probably evolved [43] – and with it maybe the need for embryogenesis. Class 2 KNOX genes prevent the sporophytic moss embryo from developing a gametophytic body plan [44], while the polycomb repressive complex 2 (PRC2) represses the formation of a sporophytic body plan in the gametophyte [45,46], and zygote and/or embryo formation is enabled by a BELL TALE HD TF [47]. In all cases, action with other (TALE) HD TFs as a heterodimer is probable.
The heterodimeric interaction of non-tale HD proteins with TALE HD proteins, which is important for animal and plant development, apparently was already present in the LECA [4]. Moreover, plant TALE KNOX/BELL proteins contain the MEINOX interaction domain also known from animal TALE proteins [50]. Evolution has certainly added motifs and combinatorial complexity via gene duplication to the ancestral scheme, some of them most probably convergent developments. However, regulation by HD/TALE was already present in the LECA, probably to control plants [14,48], are also involved in moss gametophytic stem cell formation [49], suggesting a conserved role in cell fate determination.

Figure 3. Comparison of Life Cycles of Lineages that Perform Embryogenesis. Schematic representation as in Figure 2; the moss scheme from Figure 2 is compared with the life cycles of brown algae (such as from the genus Fucus or Laminaria), seed plants (such as Arabidopsis), and multicellular animals (such as mouse). The fusion of two haploid gametes to form a diploid zygote is the unifying (necessary, but not sufficient) element of the lineages that perform embryogenesis. However, the dormant stage in mosses is the haploid spore, which will develop into the dominant (haploid) form without performing embryogenesis, while in the other lineages a diploid structure, which is the product of embryogenesis, will be the dormant/dominant form. All land plants perform mitotic division after meiosis, thus generating a haploid, multicellular generation, the gametophyte. Therefore, they perform an alternation of generations and have a haplodiplontic, sometimes even diplobiontic (e.g., ferns) life cycle. The same is true in some brown algae, such as, from the genus Laminaria. While algae from the genus Fucus perform mitosis after meiosis, the resulting gametes are unicellular. It is to be considered a haplodiplont as well, although possibly on the evolutionary road to becoming a diplontic haplobiont. Multicellular animals are diplontic haplobionts. Note that animal egg cells are typically formed earlier than sperm cells – egg cells during embryogenesis, sperm at maturity.
meiosis – which was a feature of the LECA used for DNA recombination and ploidy reduction [51–54] – and has subsequently been co-opted to control the escape from the unicellular bottleneck of multicellular organisms. Hence, meiosis – as well as multicellularity and a zygote – are necessary but not sufficient prerequisites for embryogenesis. Other important developmental regulators exist across eukaryotes, for example, MADS-box or basic helix–loop–helix (bHLH) TFs [55,56], controlling developmental state transitions. The question arises why some of these ancestral regulatory network nodes are used for what we call animal and plant embryogenesis, and whether other multicellular lineages make similar use of them. If homologous, sometimes even orthologous, genes recurrently evolve to be instrumental in embryogenesis, does that suggest an as yet not comprehended evolutionary principle?

Why Embryogenesis?

Embryogenesis without meiosis is possible, and genetic or epigenetic body plan determination can be overcome [57]. For example, apomictic parthenogenesis is known in animals as well as in plants (where it is usually referred to as apomixis), and describes the formation of an embryo from an unfertilised egg cell that has not gone through meiosis. In plants, however, clonal (asexual) reproduction via an embryo can also occur from other cells, for example, by somatic embryogenesis [58]. Moreover, plant gametophytic (haploid) cells can form haploid sporophyte-like bodies (apogamy), and sporophytic (diploid) cells can form diploid but gametophyte-like bodies (apospory). However, the apomictic initial cell differs from the megaspore mother cell in its expression profile [59], including TALE (BELL-like) TFs, suggesting that non-meiotic embryo development is different. In moss, ectopic expression of a BELL TF leads to the formation of somatic embryos from endoreduplicated cells (Figure 2) [47].

The upright body plan derived from plant embryogenesis probably was an evolutionary advantage on land. Parental nutrition might constitute a fitness advantage, since a complex 3D structure with specialised tissues can be maintained much better if parental nutrition is provided. Axes and symmetry breaking are probably necessary for complex tissue and organ formation. Yet, does selection drive embryo evolution, or does the neutral evolution of an embryo constitute a secondary advantage, or even a feature that cannot be reversed (an evolutionary one way street)?

Asymmetry, Polarisation, Uniparental Effects, and Positional Signalling

Axis establishment and symmetry breaking are hallmarks of embryogenesis. During embryo development, plants frequently undergo asymmetric cell divisions to form new cell types [60]. Similar to plants, the zygote of brown alga of the genus Fucus initiate a growth axis within hours after fertilisation. The first division is already asymmetric, and the daughter cells will establish the body plan precursor (one cell becoming a rhizoid and later the holdfast, the other developing into a thallus cell and later into photosynthetic stipe and reproductive fronds) [61]; very similar development occurs in the red alga Ceramium, in the green lineage (Streptophyta + Chlorophyta) sister group. Brown algae belong to the stramenopiles, a eukaryotic lineage that is diverse from opisthokonts/animals and Archaeplastida/plants (Figure 1), suggesting convergent evolution of embryos in red and brown algae as well as in animals and plants.

The sperm entry site defines zygote polarity, and hence the embryonic axis, in some animals (e.g., frogs, insects), some plants [e.g., arabidopsis but not maize (Zea mays) and rice (Oryza sativa)], and some brown algae, for example, of the genus Fucus. This polarisation is dependent on environmental cues in Fucus spiralis and thus can be altered using external stimuli [62,63]. In arabidopsis, the polarisation signal is translated via a sperm-delivered kinase transcript [64], representing a so-called parent-of-origin effect, that is, the inheritance of genetic variation from one parent. Epigenetic silencing of a parental allele is called imprinting; interestingly, PRC2 (mentioned earlier for repressing the moss sporophyte body plan) is involved in regulating
imprinted genes in the plant endosperm as well as in the animal placenta \([15,65]\). Alternatively, uniparental transcripts can be delivered by only one gamete, as outlined earlier. In effect, both genetic and epigenetic effects will lead to only one parental allele being expressed. Such effects only come into being after meiotic zygote formation.

The Streptophyta comprise the land plants (Embryophyta) and the charophyte freshwater algae, from which the land plants evolved. Interestingly, polarized cell growth in the multicellular charophyte alga \textit{Coleochaete scutata} depends on neighbouring cells \([66]\), that is, on positional signalling, potentially laying the ground for complex body plan development. Several plant homologs of animal genes have been found that are important in that regard. The Calpain Dek1 is important for embryonic epidermal cell fate determination in arabidopsis \([67]\); in the moss \textit{Physcomitrella patens}, the orthologue encoding Dek1 is crucial for establishment of the correct division plane when switching from 2D to 3D growth \([68]\). Heterotrimeric G proteins were already present in the LECA \([69]\) – in particular the Ras superfamily, to which Rho belongs \([70]\). Rho GTPases regulate polarity in polarized cell growth of land plants \([71,72]\). Moreover, prenylation is required for polar cell elongation, cell adhesion, and differentiation in the moss \textit{P. patens} \([73]\).

Based on building blocks present in the LECA (Figure 1), axis formation and symmetry breaking apparently evolved independently, but following similar patterns.

**Embryogenesis and Mutational Burden**

Embryos evolved several times, suggesting an evolutionary advantage. One possibility is that embryogenesis may reduce mutational load, perhaps by limiting the number of cell divisions during organ development. The generation time hypothesis assumes that short generation time will lead to a high overall rate of genome replication through more mitoses per time (and thus more replication errors and hence more fixed mutations). This seems to hold well for animals with their determinate development, where a fixed rate of mitosis correlates with generation time, such as in rodents and primates \([74]\). The number of cell divisions needed to form female gametes is less than to form male gametes, which is mirrored by the higher male mutation rate. Consequently, the female to male ratios of both mutations and divisions to form germ cells are larger in animals with longer generation times \([75]\). This is mainly due to the fact that egg cells are formed during embryogenesis, while sperm is formed by the adult (Figure 3). In plants, however, all germline cells are formed late in individual development. Scofield and Schultz found that populations of small-statured plants exhibit a significantly lower mutation rate, while large-statured plants exhibit very high mutation rates \([76]\). They concluded that the per-generation mutation rate of such plants is a function of the number of mitoses (smaller plants = lower number of mitoses) that occur from zygote to gamete – similar to animals. By contrast, mutation rate was found to be negatively correlated with organism height in plants in another study \([77]\). A possible explanation for this phenomenon is the ROM (rate of mitosis) hypothesis: such mutations that occur during mitosis in the apical meristem have a good chance to make it to the germline. The slower growth of taller plants therefore means a lower chance of germline mutations. Annual plants have been described to have higher mutation rates than perennials \([78]\); the lifestyle of an annual plant, as compared to for example a tree, is akin to the rodent–human comparison.

In all cases, the number of cell divisions needed to form gametes appears to be critical. Might embryogenesis indeed be advantageous because it allows reduction of the mutational load? Could the number of mitoses be significantly lower when going through embryogenesis, as compared with starting from scratch (i.e., a single cell, such as moss spores), each time a germline shall be established?

**The Evolutionary Hourglass**

The hourglass model describes a phylotypic stage in which animal embryos show a large resemblance within their phylum \([79]\), while during early and late embryogenesis morphology is
diverse. It has been first described by Karl Ernst von Baer in 1828 (his ‘third law’), and is at the core of Haeckel’s recapitulation theory. It has been shown that genes expressed in the phylotypic stage are most conserved in the case of Drosophila [80], and oldest in the case of zebrafish [81], mirroring the morphological hourglass pattern. During the phylotypic stage it is hypothesised that much constraint is placed on interaction partners of gene regulatory networks. Potentially, the body plan of a given phylum is defined during the phylotypic stage. The finding that a molecular hourglass pattern also exists in arabidopsis, although no morphological phylotypic stage is discernible, suggests recurrent evolution of similar patterns [82], and thus further similarities of plant and animal embryogenesis. Interestingly, an evolutionary model has recently been put forth that is able to explain the emergence of hourglass patterns [83] – such patterns might even represent a general emerging feature not only in biology but also in technology. Strikingly, a molecular hourglass pattern could recently also be demonstrated during development of a mushroom-forming fungus [84]. Here, a potential phylotypic stage was detected after the onset of meiosis – however, the mushroom does not develop from a zygote and neither via embryogenesis. Taken together, the hourglass model provides the molecular basis for Haeckel’s recapitulation theory (in particular, von Baer’s third law describing the phylotypic stage), that is, a link between phylogeny as scripted by sequence evolution [85] and the embryonic development of the individual organism. The phylotypic stage of the molecular hourglass, in which the oldest and most conserved genes are expressed, is the stage of highest morphological resemblance, in which ontogenetic recapitulation of the phylogeny is evident. Haeckel was referring to vertebrate embryos; however, the molecular hourglass pattern holds true for other phyla as well, as pointed out earlier.

**Concluding Remarks and Future Directions**

If we ponder the vision example again, fairly astonishingly complex structures can be achieved, for example, the camera-like vision of bacteria [86], or the usage of endosymbiotic organelles as building blocks to form the look-alike of a multicellular eye in certain protists [87]. Yet, complexity without multicellularity is limited. Clonal multicellularity evolved several times across the eukaryotic tree of life, and with it the complex organisms that macroscopically dominate land and water, such as mammals, trees, and kelps. The LECA already possessed many genes (such as those encoding HD TFs) that recurrently became important for different evolutionary implementations of complex multicellularity (Figure 1), the majority involving embryogenesis. However, molecular homology does not necessarily imply functional/morphological homology [19], a fact that is known as the molecular homology–analogy paradox.

Yet, many ancestral key regulatory genes carry out conserved functions and can complement proteins in lineages that are separated by hundreds of millions of years of evolution. As an example, orthologues encoding the bHLH TF controlling root hair development in the seed plant sporophyte also control rhizoid formation in the moss gametophyte [88]. The gene itself is homologous, while the generation and the tissue would usually be considered analogous. Yet, they carry out the same function (rooting/nutrition) and are controlled by orthologous genes.

Regardless, however, of whether we term it developmental homology if the underlying genes are homologous, we have to wonder why similar control and development is recurring, although it evolved independently. In the case of the hourglass, as stated earlier, there seems to be a recurring emerging pattern. Is the same true for complex clonal multicellularity and, in particular, embryogenesis? In other words, are there optima in the evolutionary landscape that lineages will eventually end up in, like an evolutionary one way street? Is embryogenesis an advantage and why? Are genes co-opted for embryogenesis present in non-embryogenic lineages and what is their function there? Which features are necessary and sufficient for embryogenesis? How do complex body plans develop without embryogenesis, like in mushrooms and moss gametophytes? (See Outstanding Questions.)
To answer these questions, we need to understand the underlying principles that drive apparently convergent evolution. And to do so, we must broaden our view that is currently mainly dominated by (vertebrate) animals and (flowering) plants. We should look into other lineages, such as charophytes, red and brown algae or fungi, and analyse them in terms of genetic and epigenetic control of zygote/embryo development. Such analyses might teach us a great deal about evolutionary principles driving complexity.

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