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Review

Why are there eggs?

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ABSTRACT

A description and update of the "egg-as-novelty" hypothesis is presented. It is proposed that the major animal phylum-characteristic suites of morphological motifs first emerged more than a half-billion years ago in multicellular aggregates and clusters that did not exhibit an egg-soma divergence. These pre-metazoan bodies were organized by "dynamical patterning modules" (DPMs), physical processes and effects mobilized on the new multicellular scale by ancient conserved genes that came to mediate cell–cell interactions in these clusters. "Proto-eggs" were enlarged cells that through cleavage, or physical confinement by a secreted matrix, served to enforce genomic and genetic homogeneity in the cell clusters arising from them. Enlargement of the founder cell was the occasion for spontaneous intra-egg spatio-temporal organization based on single-cell physiological functions – calcium transients and oscillations, cytoplasmic flows – operating on the larger scale. Ooplasmic segregation by egg-patterning processes, while therefore not due to adaptive responses to external challenges, served as evolutionarily fertile "pre-adaptations" by making the implementation of the later-acting (at the multicellular "morphogenetic stage" of embryogenesis) DPMs more reliable, robust, and defining of sub-phylum morphotypes. This perspective is seen to account for a number of otherwise difficult to understand features of the evolution of development, such as the rapid diversification of biological forms with a conserved genetic toolkit at the dawn of animal evolution, the capability of even obligatory sexual reproducers to propagate vegetatively, and the "embryonic hourglass" of comparative developmental biology.

Contents

1. Introduction

A publication several years ago suggested that the animal egg originated later in evolution than the main morphological features of animal body plans and that it was retained by virtue of its unique capacity to stabilize developmental pathways [1]. The implication of this hypothesis is that while an egg-stage characterizes most present-day animal life cycles, the respective eggs, and the transient and persistent heterogeneities that appear within them before and after fertilization, play a much more limited role in embryogenesis than would be implied by their position at the initiation of development.

Several puzzles are resolved by this concept, if true. These include the capability of some metazoan organisms to circumvent the egg-stage and develop normally as asexual propagules or monozygotic multiples, the ability of embryo-cell chimeras of phylogenetically divergent members of some phyla, whose development is otherwise egg-dependent, to develop into healthy, though evolutionarily unprecedented, representatives of the

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phylum, and the observation that species within a given phylum can exhibit very different egg types, pass through a common intermediate morphology (the phylotypic stage), only to diverge again, exemplifying the "embryonic hourglass."

The "egg-as-novelty" hypothesis was based on the earlier recognition that animal development is mediated by physical forces, processes, and effects acting on multicellular clusters [2,3]. These clusters arise part-way during development, and are variously termed (depending on the species) blastula, blastoderm, or inner cell mass. The physical effects that mold and pattern these clusters are mobilized by the products of certain ancient, conserved genes (the "interaction toolkit"; [1]), forming a collection of "dynamical patterning modules" (DPMs). Since different animal clades have different subsets of interaction toolkit (IT) genes, not every DPM is manifested in all embryo types [1,4]. Indeed, it was proposed that the approximately three-dozen animal phyla are defined by their DPMs, which in turn are specified by the presence or absence of the various IT genes [1].

The hypothesis has several components, each with a set of assumptions:

(i) The distinct metazoan body plans arose in populations of unicellular organisms that were the common ancestors of the present-day multicellular animals and single-celled choanoflagellates. The ancestral cells contained genes (inferred by their presence in descendants) for cadherins and other cell surface proteins. These molecules acquired homophilic adhesive properties at some point, giving the cells the capability to aggregate.

(ii) The ancestral cells also contained a selection of additional IT genes, so when a critical cell number was reached in the aggregates, combinations of DPMs were brought into play and primitive versions of phylotypic body plans took form. (The species-characteristic cluster of dozens to hundreds of equal-sized cells at which this occurred is termed the "morphogenetic stage" [1].) The cells of these populations, while generally uniform regarding their complements of IT genes, were otherwise genetically variable.

(iii) Cells in these ancient aggregates had, it was argued, less stringent size regulation than present day cells. "Proto-eggs" were cells in these aggregating populations that were larger than typical. When released from an aggregate and induced to divide, their large size was an impediment to complete cytokinesis. This had the effect of producing morphogenetic-stage clusters in which the cells were clonal and thus of uniform genotype. These clusters were the first *multicellular individuals*, and were new units of selection. Alternatively, proto-eggs that produced a confining matrix – a primitive zona pellucida – would foster non-dispersal of daughter cells, and thus clonal cell clusters, even if not produced by cleavage.

(iv) The interiors of proto-eggs were loci of a variety of "egg-patterning processes" (EPPs). These derived from previously established physiological phenomena in single cells – calcium ion transients and oscillations, cytoplasmic flows – that came to operate at a larger scale than that of the cells in which they had originally functioned. The effect of EPPs was to cause the egg cytoplasm to become spatially nonuniform, and this, in turn, led to morphogenetic stages in which different cells (despite being genetically identical) had different molecular compositions.

(v) EPPs, therefore, were not *adaptations*, i.e., phenotypic characters that arose by natural selection in response to external challenges, but rather side-effects [5] or "spandrels" [6], inexorable consequences of other changes (in this case, enlargement of the founder cell), with indeterminate evolutionary consequences.

(vi) The phylum-associated DPMs that were subsequently (at the embryo's morphogenetic stage) mobilized in cell clusters earlier acted upon by EPPs were implemented with pre-set boundary and initial conditions, making their morphological outcomes more reliable and stereotypical than they would be with random starting conditions. Furthermore, because cell clusters of a given phylotype (i.e., multicellular individuals with a phylum-characteristic array of IT genes) could sustain different EPPs and therefore generate distinct spatially nonuniform morphological-stage spatiotemporal patterns, stable subclades (subphyla, classes, and so forth) could arise within each phylum. In this sense, EPPs were often pre-adaptations or "exaptations" [7] that ultimately contributed to stability and diversification of taxonomic identity.

This scenario for the origination of eggs implies that true eggs were late arrivals in the evolution of animal development. They would have arisen over time from proto-eggs, which themselves succeeded the emergence of the characteristic morphological motifs of the Metazoa. Following the appearance of proto-eggs, the evolution of oogenesis, i.e., the pre-release specialization of these organism-founding cells, including introduction of maternal factors, would have prepared them for increasingly determinate and reliable transformation by subsequently activated EPPs. Such activation could (at different evolutionary time points), have been spontaneous, caused by specific environmental cues or by other specialized cells, proto- and definitive male gametes. This implies that the egg cell had an independent evolutionary path from the sperm cell and from sexual differentiation itself.

My objective here is to establish the plausibility that all animal body plans, even those that in present-day forms obligatorily arise from sexual reproduction, were primitively independent of an egg stage. In the absence of experimental access to extinct organisms, the most decisive tests of this hypothesis are those that relate to the autonomy, in extant organisms, of multicellular development from any process involving an egg, or where that is not possible to demonstrate, of the establishment of the major phylum-specific features of body plans independently of the details of pre- or post-fertilization intra-egg patterning. Such findings would be most persuasive if independence from the egg could be demonstrated in situations never plausibly encountered in phylogeny, and therefore representing primitive capabilities rather than evolved mechanisms compensating for developmental perturbation. The following section contains several such examples.

2. Evidence for the egg-as-novelty hypothesis

2.1. Eggs are not needed for complex, multicellular development

In a critique of "adultocentric" concepts of development, the evolutionary-developmental biologist Alessandro Minelli provides numerous examples of organisms whose development does not begin with, or pass through, an egg stage [8]. Marine and freshwater sponges, as well as colonial cnidarians such as corals, release multicellular propagules which develop into organisms indistinguishable from the originating ones [9]. Other cnidarians, such as members of the freshwater genus *Hydra*, reproduce instead by an intraorganismal vegetative process. These small, tubular animals produce featureless buds that extend laterally from the body stalk. The primordium develops in an apicobasal sequence into a fully mature individual before detaching from the parental organism at its newly formed basal disc, the last structure to differentiate [10].

Some species of sponges and *Hydra* are also capable of forming gametes and reproducing sexually. When germ cells form in these animals, however, rather than being pre-specified in the parental organism as they are in nematodes, mollusks, chordates, etc., they are recruited epigenetically from a population of somatic cells. According to Extavour et al. who studied this process in the sea anemone *Nematostella*, "preformation in germ cell development might have evolved from [an] ancestral epigenetic mechanism that was probably used by a metazoan ancestor" [11]. This implies that the distinct germ line seen in most animal phyla evolved by the loss of gametogenic potential that was exhibited by the majority of cells in more primitive organisms [12].

Vegetative propagation is the primary means of reproduction in some organisms, such as tunicates, with even more complex body plans than the sponges or cnidarians. These ascidians have sophisticated digestive, circulatory and nervous systems. *Botryllus schlosseri* is a colonial tunicate which is capable, through sexual reproduction, of forming the characteristic swimming tadpole larvae that mark acsidians as chordates [13], but more typically propagates vegetatively [14]. Interestingly, its long-lived germ line precursor cells share determinants with stem cells that contribute to asexual development [15,16], placing its mode of germ line determination intermediate on the hypothesized preformation-epigenetic evolutionary axis [11].

Minelli also aptly points to polyembryony, the production of multiple offspring from a single divided embryo, as an example of vegetative reproduction [8,17]. This is well-known in mammals and other vertebrates, but occurs as well in most other animal phyla, including arthropods [18], flatworms [19] and bryozoans [20]. Although the initial embryo is formed from a fertilized egg, this phenomenon demonstrates that the capability for generating new embryos and individuals resides in blastomeres or cells from more advanced stages.

2.2. Cell originating from different species' eggs can cooperate to form healthy, evolutionarily unprecedented organisms

In the above examples animals were seen to be capable of developing vegetatively, without passing through an egg stage. But since the organisms in question exhibit sexual as well as asexual modes of development, in each case the propagules or primordia plausibly descended from an "original" that did develop directly from an egg. It is possible that the early egg-dependent events (at least in present-day forms) were imprinted on the derived cells and re-evoked when each episode of vegetative development got underway. However, if coherent development could be demonstrated to occur from mixed populations of cells from different species (considering that intra-egg processes can vary extensively in phylogenetically related organisms, see next section), the decisive role of specific events at the egg stage in generating phylotypic body plans would be further called into question.

It is therefore notable that embryonic cells from members of distinct subfamilies of the mammalian order *Artiodactyla*, goats and sheep, can be mixed together, after which they develop into a composite organism with a variable phenotype with features of each species [21,22]. The 1984 studies described three methods for producing chimeric embryos: (i) single blastomeres from four-cell goat embryos were combined with single blastomeres from four-cell sheep embryos, or with single blastomeres from eight-cell sheep embryos in evacuated zonae pellucida; (ii) an eight-cell goat embryo from which the zona pellucida had been removed was surrounded with the dissociated blastomeres of three eight-cell sheep embryos or reciprocally, with the species reversed; (iii) the inner cell mass and polar trophectoderm from day eight goat blastocysts were inserted into day-eight sheep blastocysts, or vice versa. The embryos resulting from these procedures were then transferred to the uteri of recipient ewes or female goats where they typically developed to full term.

These experiments demonstrated that sheep and goat blastomeres or inner cell mass cells can signal to each other, via a commonly decipherable interaction toolkit, despite the divergence of these groups 5–7 million years ago [23], forming fully mature animals (termed "geeps;" [21]). The adult animals were often fertile, showing that the "normal" outcome even extended to the soundness of the reproductive system. But since these animals are mosaics of cells of the two originating species (that is, not hybrids, in which the genetic material of each cell would be bi-specific), the offspring of mated geeps are goats, sheep, or hybrids between the two, not themselves geeps.

While the structure and physiology of goat and sheep eggs are likely to be similar, the variability in rates and waveforms of post-fertilization calcium transients, for example, across different mammalian species [24] (see below), suggest that early egg-dependent events in the two types of animal probably differ in detail. The cells of their embryos that demonstrably cooperate to generate coherent (though evolutionary unprecedented) organisms are thus likely to have arrived at the chimeric morphogenetic stage with different EPPs in their ontogeny.

More recently, another interordinal chimera, this one between the teleost fishes medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*), was produced by mixing long-term cultivated medaka embryo stem (ES) cells with blastomeres from early-stage zebrafish embryos [25]. These two species diverged from one another ~320 million years ago, have more extensive differences between them in their EPPs than goats and sheep (see next section), and differ by more than twofold in their speed of development, with zebrafish being faster. In the chimeras, the medaka cells were found to accommodate to the developmental rate of the zebrafish.

As described in the previous section, the vegetative reproduction of optionally sexual animals from populations of isogenomic cells not directly derived from an egg counts against the recurrent necessity for embryogenesis of an egg stage in these species. Here, the development of embryo chimeras from species which under normal circumstances are obligatorily egg-dependent occurs by cooperation of cells that experience different egg-stage determinants. This gives further credence to the idea that however important the egg stage and its internal patterning processes may have become for present-day animal development, neither was likely to be intrinsic to the evolutionary establishment of phylum-specific body plans.

2.3. Different species in a given animal phylum can have different egg-patterning processes

The previous two subsections demonstrated that groups of cells can be developmentally distant from the influence of their species' egg stage and still give rise to an organism indistinguishable from a sexually produced member of the species, or even be millions of years distant from the egg stage of a common ancestor and nonetheless be capable of cooperating to form a biologically coherent member of their phylum. However, events that occur pre- and post-fertilization at the egg stage in sexual reproduction are clearly not inconsequential; disrupting them will typically derail development [24,26–28]. A possible conclusion from this is that while some intra-egg arrangement is essential for normal species-specific development that begins with an egg or a sperm–egg pair, the specific character of such arrangements is nonetheless arbitrary, or at least highly variable, between species in a given phylum. I will explore this possibility in this subsection.

The vertebrates provide some relevant examples. In a comparison of cytoplasmic events during early development in medaka and zebrafish, Webb et al. [29] note that the egg stage processes

occur in eggs that are anatomically different to begin with. In particular, the medaka egg has a yolk membrane and lipid globules that are absent in the zebrafish. The two species share common features, e.g., Ca^{2+} activation waves associated with cytokinesis, but they also differ in significant ways, e.g., the presence in medaka (but not zebrafish) of a Ca^{2+} hot spot at the vegetal pole of the zygote. As development proceeds, there are Ca^{2+}-driven rhythmic contractions in the blastoderm of medaka that are not seen in zebrafish. The activation Ca^{2+} signal is consistently measured to be faster in zebrafish (~9–10 μm/s) than in medaka (12.5–15 μm/s) by several different techniques.

Shortly following fertilization and activation, medaka embryos undergo episodes of ooplasmic segregation, accompanied by additional calcium transients, in which there is simultaneous movement of most of the peripheral cortical cytoplasm to the animal pole and of the lipid droplets toward the vegetal pole [29]. This produces a distinct convex region of cytoplasm (the blastodisc) at the animal pole, with most of the lipid droplets coalescing at the vegetal pole [30]. Zebrafish embryos also undergo ooplasmic segregation post-fertilization, although the details differ from that in medaka. In zebrafish there is large-scale streaming movement of cytoplasm from the vicinity of yolk granules to the animal pole [31,32]. The increase in volume that generates the blastodisc is due to this animal pole-directed cytoplasmic streaming and separation from the yolk, not, as in medaka, to countercurrent movement of lipid droplets, which, as mentioned, are not present in zebrafish. In both species, ooplasmic segregation ensures that the cells of the morphogenetic-stage cluster (the blastula, in teleosts), contain distinct, species-characteristic cytoplasmic determinants.

In mammals, post-fertilization Ca^{2+} transients are similarly essential for egg activation and subsequent development [24,26], but there is little evidence for associated ooplasmic segregation. Calcium transients in mammalian eggs have broadly similar properties [24], but even in these organisms, reproducible species-specific differences in the details, coupled with the ability of evolutionarily diverged mammals to form healthy chimeras, discussed above, suggest that however important these signals may be for specific developmental pathways, their precise character is irrelevant to phyletic identity.

In mammals with relatively large eggs, such as human, cow and pig, there is a periodic train of Ca^{2+} signals at fertilization, with one transient about every 30 min [33–35]. In species with smaller eggs, such as mouse and hamster, calcium concentration rises about a minute after sperm–egg membrane fusion and lasts one or several minutes, followed by a series of further oscillations with a period of about 10 min, over several hours [36–38]. The initial calcium signals in mouse and hamster — two rodents — differ, however. In the mouse egg the first Ca^{2+} transient occurs in two distinct steps over ~10 s, whereas the ones that follow show a more rapid rate of rise, on the order of 1 s or less [24,38]. In contrast, the calcium concentration in the eggs of hamsters rises monotonically during the first and all subsequent spikes, with decreasing periods [39].

While the vertebrate examples demonstrate conservation of subphylum clade identity (teleost, rodent) in the face of moderate differences in intra-egg patterning events, the nematodes are the prime example of morphological conservation despite extreme variation in egg-patterning processes. This phylum is represented by as many as a million species. Apart from their size, however, which can range in body length from a few millimeters to a few centimeters, the anatomy of these worms (except for a very small number of species with diversely divergent morphology [40]), is nearly indistinguishable. In spite of this morphological conservation, postfertilization events, and specifically the deployment of EPPs, differ dramatically within this group. A survey of nematode EPPs is presented in [1]. Here I will provide a brief summary.

The egg of the nematode *Caenorhabditis elegans*, the most popular model system of the phylum, is unpolarized before it is fertilized. Upon sperm entry the egg's cortical cytoplasm becomes reorganized by cytoplasmic flows, resulting in an asymmetrical distribution of various factors before the first cleavage. This polarity is required for the establishment of the anteroposterior (A–P) axis during embryogenesis [41]. The cytoplasmic flows depend on both the contractile protein complex actomyosin and the activity of sperm-contributed microtubules, causing the local enrichment of an initially uniformly distributed enzyme complex. Thus the sperm entry point, though not pre-specified, thereby becomes the future embryo's posterior pole [42–44].

Other nematodes whose final forms are essentially identical to *C. elegans* break their embryonic symmetry in very different ways from that organism and from one another. In *Bursaphelenchus xylophilus*, for example, the sperm entry point becomes the future *anterior* pole of the embryo and the patterns of cortical flow and its relation to the sperm microtubules are entirely different from that in *C. elegans* [45]. In *Romanomermis culicivorax*, the first cleavage is symmetrical rather than asymmetrical, and the pattern of asymmetrical cleavages and alternative assignment of cell fates indicate that A–P polarity is determined in a still different fashion from the other two species [46].

For parthenogenetic species of nematodes, where there is obviously no role for sperm entry in the assignment of A–P polarity, this feature is acquired in ways that not only differ from the other instances mentioned, but also from one another, on the basis of interaction of intrinsic and extrinsic factors [47]. Finally, in the freshwater nematode *Tobrilus diversipapillatus*, no asymmetrical cleavages and no distinct cell lineages are generated until the morphogenetic (i.e., blastula) stage [48].

Thus, whereas A–P polarity is clearly an essential aspect of nematode anatomy, the way that it is acquired during development, sometimes via EPPs and sometimes not, seems to have little impact on the final morphological outcome, which is always essentially the same. This is not to suggest that the effects of the EPPs are a matter of complete indifference: the cytoplasmic patterns in a given nematode species are reproducible and precise, and their experimental disruption can be inimical to successful development [27,28].

3. Is the mammalian egg an atavism?

A major role of egg-patterning processes (EPPs) is to establish (often in conjunction with asymmetrical effects of oogenesis), embryo polarities, and more generally, positional differences between individual cells of the early embryo at the very beginning of development. If the unequal distribution of determinants in the egg is irreversible, the embryo is termed "mosaic," and to the extent that it is reversible the embryo is "regulative" [49]. As we have seen, both modes can exist within a single phylum, exemplified by the contrast between early development in the nematode worms *C. elegans* and *T. diversipapillatus*. Among the vertebrates, blastomere fates are irreversibly set in fish and amphibians by egg-stage allocation of ooplasmic determinants, causing isolated cleavage products to develop in an abortive fashion.

In mammals, however, insofar as pre-cleavage polarities exist [50], they are labile, with blastomeres of most species being capable of developing into normal individuals up till the 8-cell stage. This corresponds to the ready capacity of mammalian embryos to generate monozygotic twins and other multiples, as described above.

In light of the egg-as-novelty hypothesis [1] it is interesting to speculate that the mammalian egg, relieved by virtue of internal fertilization and gestation of the need for founder cell-based

mechanisms of progeny sequestration that the innovation of an egg stage of development is proposed to have solved (see point (iii) of Section 1), has reverted to a more primitive state of plasticity. In particular, the much smaller eggs resemble, in some ways, the ancient cells that (according to the hypothesis) first aggregated to give rise to proto-phyletic body plans (see points (i) and (ii) of Section 1). Clusters of lineage-uncommitted cells, generated by cleavage (as in the embryos of mice, humans, etc.) or by physical confinement without strong cell–cell attachment (as in opossum embryos [51]), by mixing of the blastomeres of dizygotic twins [52], or even of different species [21,22] (see Section 2, above), so long as they contained the vertebrate set of IT (interaction toolkit) genes and the associated DPMs, could generate viable and entirely functional members of this clade.

Because (according the egg-as-novelty hypothesis), EPPs originated as side-effects of enlargement of the founder cell of proto-embryonic multicellular animals (see points (iv) and (v) of Section 1), secondary reduction of egg size in mammals would be expected to suppress some of these processes. In particular, this simplification entailed the loss of early lineage specification via positional allocation of ooplasms. Instead, polarization came to be based on 8–16 cell stage self-organizational effects related to differential adhesion [53].

This does not imply that what remains of EPPs is unimportant in the mammalian egg. Calcium transients may not be needed for ooplasmic reorganization as in annelids [54], other vertebrates [55], and invertebrate chordates such as ascidians [56,57], but, as mentioned above, they are essential for egg activation [26], and their perturbation leads to impaired developmental outcomes in terms of implantation rate, and pre- and post-implantation embryo growth rates [58–60]; see also discussion in [61]. Perhaps the calcium transient-based mechanism of intra-egg reorganization was carried over in vertebrates from chordate ancestors and recruited to other precision-ensuring functions in mammals when the ooplasmic segregation functionality was dispensed with in this group.

4. Discussion

The multiple lines of evidence presented here are fully consistent with the egg-as-novelty hypothesis. To summarize, animals of some groups (e.g., sponges, cnidarians) can develop naturally independent of fertilization and an egg stage, and those of other groups (fish, mammals) which are normally entirely egg-dependent can develop in a fully coherent fashion from post-cleavage cells, from fused cleavage-stage twins, and from aggregated cells of embryos of orders evolutionarily separated by several or many millions of years.

In addition, anatomically nearly identical organisms (different species of nematodes) can exhibit extremely dissimilar or no active ooplasmic organization processes at the beginning of their embryogenesis, even though those processes are essential to further development in species that have them. Finally, mammals, in which the original role of the egg stage of development (protection against dispersal of cleavage products) has been rendered moot by internal gestation, have reverted to producing eggs that are smaller than in other vertebrates, and internally uniform, with only remnants of the zygote-stage ooplasmic organizing processes of their chordate ancestors.

While the hypothesis presented here (see also [1]), like most proposed evolutionary scenarios, is not susceptible to direct experimental tests, it can be evaluated by the coherence that it, and the allied conceptual framework for body plan determination of DPMs [2,3,62], bring to the understanding of early evolution of animals, and with some adjustments [63,64], multicellular plants. These ideas help explain the explosive radiations of animal forms that occurred in the Ediacaran and early Cambrian periods [65] and the fact that these episodes of rapid morphological change occurred with a highly conserved interaction toolkit of gene products [66]. They also provide a basis for resolving the apparent paradox of the embryonic hourglass of comparative developmental biology [1,67], and the more general puzzle of why egg-stage spatial and lineage determinants seem so arbitrarily related to later developmental processes and why they, and even the egg stage itself, are so commonly dispensable. Since no other models have yet been advanced that account for all these phenomena in such a parsimonious fashion, further progress will depend on formulation of alternatives against which it could be evaluated as new phenomena of evolutionary developmental biology come to light.

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