

The origin of Metazoa: a transition from temporal to spatial cell differentiation

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Dedicated to the 60th year of A. A. Zakhvatkin's Synzoospore hypothesis

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For over a century, Haeckel's Gastraea theory remained a dominant theory to explain the origin of multicellular animals. According to this theory, the animal ancestor was a blastula-like colony of uniform cells that gradually evolved cell differentiation. Today, however, genes that typically control metazoan development, cell differentiation, cell-to-cell adhesion, and cell-to-matrix adhesion are found in various unicellular relatives of the Metazoa, which suggests the origin of the genetic programs of cell differentiation and adhesion in the root of the Opisthokonta. Multicellular stages occurring in the complex life cycles of opisthokont protists (mesomycetozoeans and choanoflagellates) never resemble a blastula. Here, we discuss a more realistic scenario of transition to multicellularity through integration of pre-existing transient cell types into the body of an early metazoan, which possessed a complex life cycle with a differentiated sedentary filter-feeding trophic stage and a non-feeding blastula-like larva, the synzoospore. Choanoflagellates are considered as forms with secondarily simplified life cycles.

Keywords: Ichthyosporea; molecular phylogenetics; origin of Metazoa; Rel/NF kappa B; T-box family

Introduction

The origin of multicellular animals and the nature of their ancestor are long-debated and challenging questions in both

classical and modern biology.^(1–3) The advent of large-scale sequencing approaches and comparative genomics provides a chance to unveil the past by reading and putting together vestiges left in the genomes of extant metazoans and their close relatives. Two conceptual approaches are integrated in this analysis: phylogenomic reconstructions of relationships between animals (Metazoa) and their unicellular relatives,^(4–7) and identification of genes previously considered as Metazoa-specific in the genomes of unicellular protists and other eukaryotes. Homologs of many metazoan genes involved in cell interaction and differentiation have now been discovered in various lineages of unicellular organisms, thus dating their origin before the emergence of Metazoa.^(5,7–10) Here, we discuss alternative scenarios of the transition to multicellularity that are required to account for the emerging molecular data.

The Gastraea theory meets new challenges

Historically, Haeckel's Gastraea theory⁽¹¹⁾ was the first widely accepted model of the origin of animals. According to Ernst Haeckel, the transition from unicellularity to multicellularity proceeded through two consecutive stages (Fig. 1A). First, unicellular flagellates aggregated to form a hollow ball-shaped floating colony of identical cells. This hypothetical stage was named the Blastaea to reflect its similarity with the blastula, an early stage in animal embryogenesis. Outside the Metazoa, ball-shaped colonies of flagellated cells are observed in the green algae *Volvox* and the

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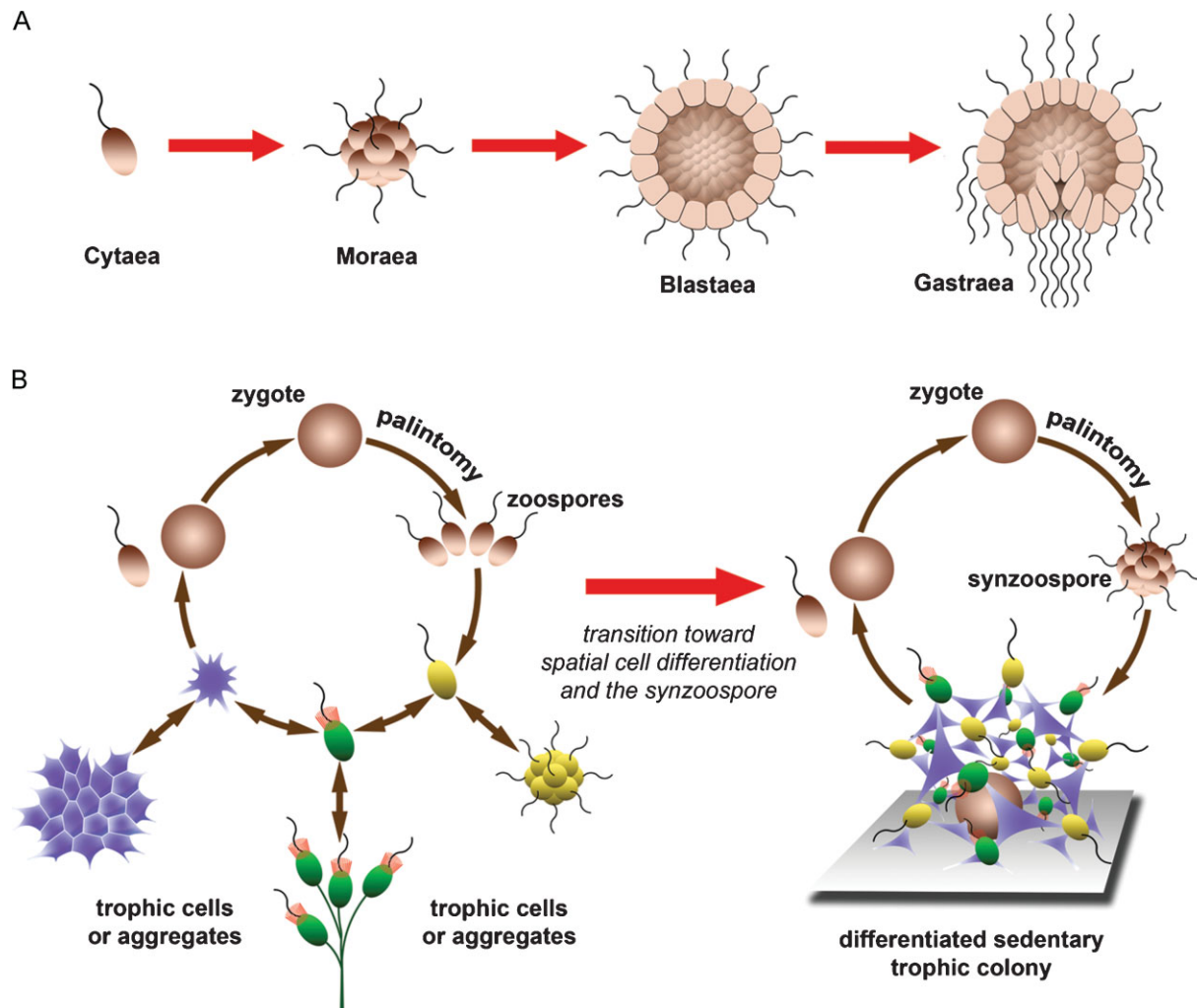


Figure 1. The Gastraea and Synzoospore scenarios. **A:** The Gastraea theory assumes gradual modification of a colony of uniform cells. Primary cell differentiation occurs with the formation of functional primary gut, the evolutionary precursor of endoderm. **B:** The Synzoospore theory envisions the metazoan ancestor as a protist with a complex life cycle that includes monotomously dividing trophic cells (or cellular aggregates), hypertrophic growth of gametes, and their subsequent palintomic cleavage producing non-feeding dispersal zoospores. The transition to multicellularity occurs with (i) integration of trophic cells into a differentiated colonial body and (ii) integration of zoospores into the uniform synzoospore, the primary lecithotrophic dispersal larva of the animals. Red arrows mark hypothetical evolutionary transitions, brown arrows designate the life cycle.

choanoflagellate *Sphaeroeca volvox*, to name a few. At the second stage, the homogenous cellular wall of the Blastaea invaginated to form a second layer, the precursor of the primary gut. After this important event the Blastaea acquired primary cell differentiation (=spatial cell differentiation).

Haeckel named this hypothetical ancestral form the Gastraea, again to indicate its similarity with the gastrula stage in animal embryogenesis. According to Haeckel, extant cnidarians and sponges are the first direct descendants of the ancestral Gastraea,⁽¹¹⁾ because their body plans derive from two embryonic layers. The presence of a uniform blastula and gastrula with differentiated ectoderm and endoderm were

postulated by Haeckel to be the characteristic of all Metazoa. This definition has led to the understanding that the unity of animal organization, and not the discrete embranchments paradigm, is the rightful outcome of a major debate in comparative anatomy of the 19th century held between Étienne Geoffroy Saint-Hilaire and George Cuvier.⁽¹²⁾ In other words, it led to the recognition that the diversity of animal organization is derived from a common ancestor.

The general acceptance and appeal of the Gastraea theory stimulated development of its numerous successors. For instance, Otto Bütschli hypothesized the metazoan ancestor to be a single-layered benthic colony of protists

with functional differentiation of individual cells into feeding (basal) and locomotory (apical) parts. The subsequent horizontal cell division (delamination) led to the formation of a two-layered multicellular plaque, the Placula.⁽¹³⁾ Its bending resulted in a gastrula-like stage with the blastopore and a cavity for large food particles. According to Bütschli, inflation of the Placula with liquid and its bending explain the emergence of the true invaginating gastrula in the same way as the Gastraea theory does.

The Bütschli's hypothesis gains support from the discovery of the plate-like multicellular organism *Trichoplax adhaerens*, which possesses several cell types with no defined organs, and whose bauplan can be described as a two-layered cellular plate.^(14–19) Despite the temptation to derive *Trichoplax* from the Placula, the question whether its simplicity is primary or secondary is highly controversial in the light of both morphological and molecular^(20–25) evidence. Phylogenetic analyses of 104 genes from the recently published *Trichoplax* genome convincingly suggest its derived nature compared to more basal sponges.^(26,27) Therefore, we do not consider Placozoa here as a living relict⁽²⁸⁾ when discussing the origin of Metazoa. Relevant theories are outlined in several reviews.^(16–19) Instead, we juxtapose available evidence to suggest that cell differentiation might have preceded the emergence of multicellularity, and not vice versa.

Phylogenetic studies based on rDNA,^(20,29–33) mitochondrial,⁽³⁴⁾ and nuclear protein-coding genes^(4,6,7) have reliably identified Choanoflagellata as the extant sister lineage of Metazoa. Choanoflagellata together with Ichthyosporea, fungi, and several small protist taxa (such as *Ministeria vibrans*, *Capsaspora owczarzaki*, *Corallochytrium limacisporum*, and nucleariid amoebae) form a large monophyletic “superclade” named the Opisthokonta (Fig. 2). Interestingly, all Opisthokonta display three groups of traits, which are not predicted by the Gastraea theory:

- (i) A complex life cycle with multiple highly differentiated cell types.
- (ii) Frequent occurrence of cellular aggregates bearing no resemblance to a blastula.
- (iii) Widespread occurrence of genes that are homologous to “true” metazoan genes known to be involved in cell adhesion, differentiation, and signal transduction across animal phyla.

None of these facts alone falsifies the Gastraea hypothesis. However, alternative scenarios can be proposed to integrate the emerging molecular data and hypothesize the presence of different cell types (temporal cell differentiation) already in the life cycle of the common ancestor of Metazoa. In our opinion,

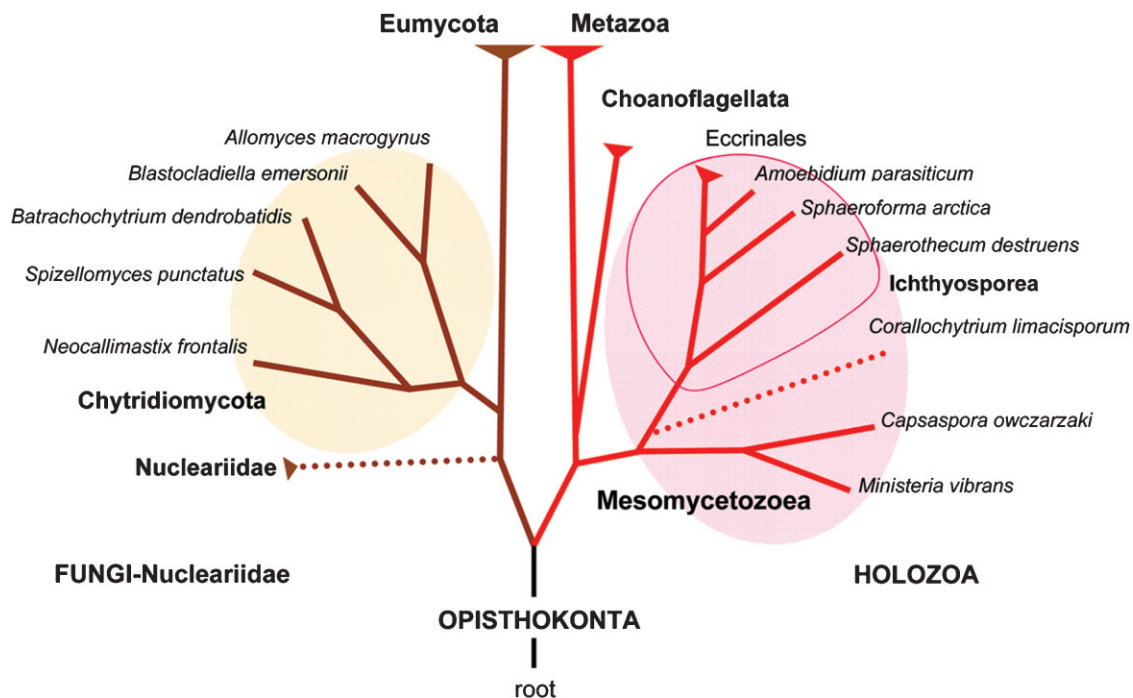


Figure 2. A phylogenomic tree of the Opisthokonta. Monophyly of the Holozoa is well supported by individual and multiple gene-based phylogenetic analyses.^(4–7,10,31–34) Phylogenomic data alone produce statistically significant support for the monophyly of Metazoa + Choano-Choanoflagellata,^(4–7,10,34) Ichthyosporea + *C. owczarzaki*,⁽⁶⁾ *C. owczarzaki* + *M. vibrans*,⁽¹⁰⁾ and Chytridiomycota.⁽⁶⁾ Sister positions of Nucleariidae to fungi and *Co. limacisporum* to Mesomycetozoea are suggested by analyses of individual published genes.⁽⁴⁾

the pre-existence of temporal differentiation in complex life cycles of unicellular and colonial pre-metazoans was a platform for the origin of cell differentiation during the emergence of early animals – in contrast to the Gastraea hypothesis, which postulates cell differentiation *de novo*.

Table 1 presents a collection of hypotheses of the origin of multicellular organization. Some of these hypotheses share an important difference from the Gastraea theory: the assumption that cell differentiation preceded the formation of colonies. The most elaborated of these is the “Synzoospore theory” proposed by Alexey Zakhvatkin in 1949 (Fig. 1B).^(35–37) Here, we discuss this hypothesis in the light of emerging molecular evidence and advocate that temporal cell differentiation preceded spatial differentiation in the origin of animals.

Outline of the Synzoospore theory

The hypothesis of Synzoospore was proposed solely on the basis of evidence from protistology and embryology.^(35,36) The major generalization made by Zakhvatkin is the alternation of three types of the cell cycle in metazoan ontogenesis: (i) monotomy, (ii) hypertrophic growth, and (iii) palintomy.

Monotomy refers to alternating events of cellular growth and division – the process that is typical for somatic cells. Hypertrophic growth is observed in the female germ cells.

During this process the oocyte actively feeds and grows but does not divide. Such growth is necessary to enter the third type of the cell cycle, the palintomic phase, which occurs during ontogenesis of all animals; the egg ceases to feed and undergoes a series of successive divisions utilizing the resources built up during hypertrophic cell growth. Non-feeding cells that are produced during palintomic cleavage can form the classical blastula. Juxtaposing known life cycles of unicellular protists, Zakhvatkin realized that some protozoans do show alternation of different cell cycle types. Furthermore, he hypothesized that the unicellular ancestor of Metazoa already possessed differentiated cells as a result of complex life cycles in the ancestral forms. Zakhvatkin refrained from naming the closest protist relative of Metazoa. He, however, identified two important features of complex life cycles.

- (1) Monotomic cells are usually sedentary, or at least less mobile, and can change their phenotype (flagellated to amoeboid, *etc.*) depending on the environment.
- (2) The process of palintomy is required to produce swarmer cells (spores or zoospores). Spores are usually morphologically identical and carry protective and dispersing functions. Particularly in animals, these cells remain attached to each other forming a primary flagellated larva, the blastula (synzoospore).

Table 1. A selection of hypotheses of the origin of Metazoa

Hypothesis name	Ancestral body plan	Lifestyle	Transition to cell differentiation	Cell types
Gastraea (Fig. 1)	Blastula-like colony	Pelagic	Invagination	Ectoderm and endoderm ⁽¹¹⁾
Parenchymella or phagocytella	Blastula-like colony	Pelagic	Immigration	Ectoderm and endoderm ^(81,82)
Planula	Morula-like colony	Pelagic	Delamination	Ectoderm and endoderm ⁽⁸³⁾
Genitogastrula	Stomoblastula-like or blastula-like colony	Pelagic	Immigration or invagination	Ectoderm and gonocytes ^(78,84)
Gallertoid	Blastula-like colony with extracellular matrix and basal lamina	Pelagic	Immigration	Outer epithelial, inner mesenchymal cells ^(16,85)
Placula	Single-layered colony arranged in a flat plate	Near-bottom swimmer	Delamination	Ectoderm and endoderm ^(13,15–19)
Bilaterogastraea	Blastula-like colony with gonocytes	Near-bottom swimmer	Immigration and invagination	Ectoderm, endoderm, and gonocytes ^(86,87)
Primary colony	<i>Proterospongia</i> -like asexual colony	Benthic, sedentary	Cell types predated multicellularity	Various somatic cell types and gonocytes ⁽⁶⁸⁾
Synzoospore (Fig. 1B) and related hypotheses	Composite life cycle with primary larva	Benthic-pelagic with sedentary benthic stage	Cell types predated multicellularity	Trophozoites, zoospores, and gonocytes ^(35–37,79,88–90)
Contact aggregation	Optional or temporary <i>Dictyostelium</i> -like aggregates	Benthic, slow moving	Cell types predated multicellularity	Cell types as in extant Porifera ^(91,92)
Cellularization	Non-colonial polyenergic ciliate-like protist	Near-bottom swimmer	Compartmentalization of the multinucleate unicellular protist	All necessary cell types ^(93,94)

Thus, according to Zakhvatkin, the blastula had never existed as a separate species (=the postulated Haeckel's Blastaea). In contrast, the blastula might, instead, represent a pelagic, dispersing, and primarily non-feeding larva of a benthic sedentary ancestor of animals. At the time of publication (1949), this scenario seemed too audacious, but today we find diverse evidence supporting it.

Metazoa-specific genes in unicellular relatives of animals

According to the Synzoospore theory, multicellularity in Metazoa emerged through integration of different cell types already present in the life cycle of the ancestor. One of the major predictions of this theory is that programs of cell differentiation must have been present in the metazoan ancestor, and at least some extant unicellular opisthokonts should possess these as well.

Choanoflagellates are the Metazoa's closest unicellular relatives.^(5–7,10,34) Their genomes contain a multitude of genes originally considered exclusively metazoan,^(7–9,38) with many of these comprising the genetic toolkit for multicellular body development.^(3,8,39) Among the examples are tyrosine kinases – proteins involved in the regulation of cell proliferation, differentiation, adhesion, and motility in multicellular animals. This protein family was suggested to have emerged before the divergence of choanoflagellates and metazoans and to have diversified within the two groups.^(40–42) The rich tyrosine kinase variety is observed in the genomes of *Monosiga brevicollis*, *Monosiga ovata*, *Stephanoecca diplocostata*, and *Codosiga gracilis*, but does not, however, comprise many orthologs in animals.^(40,42) Given the simplicity of choanoflagellates' life cycle, such a variety might be explained by inheritance from ancestors with a more complex life cycle with multiple cell types.

Is there any evidence of “traditional Metazoa-specific” genes outside the Metazoa + Choanoflagellata clade? Yes, such genes were recently reported from the mesomycetozoean *M. vibrans*:⁽¹⁰⁾ the tyrosine kinase receptor (a homolog of animal protooncogene Ros1), homologs of Notch receptor and ligand, and hedgehog Hint domain.⁽¹⁰⁾ *M. vibrans* was also found to possess cadherins (mediators of Ca²⁺-dependent cell-to-cell adhesion in animals) and components involved in cell-matrix adhesion, such as focal adhesion kinase, the Crumbs protein, and integrin beta, the latter two lacking in the genome of the choanoflagellate *Mo. brevicollis*.^(3,8,10)

The opisthokonts outside Metazoa and Choanoflagellata included in the multitaxon genome initiative^(5,43) are *C. owczarzewski* and *Amoebidium parasiticum*, which were identified as mesomycetozoeans in molecular studies.^(6,7,30,44–46) The NCBI Trace Archive contains about 400 000 whole-genome shotgun reads of both organisms.

Like *M. vibrans*, they appear to possess “Metazoa-specific” genes, with some of them lacking in the genome of the choanoflagellate *Mo. brevicollis*. One of these genes in *C. owczarzewski* encodes a membrane-associated guanylate kinase-like protein involved in the assembly of multiprotein complexes at regions of cell-to-cell contacts called “tight junctions.”⁽⁷⁾ Others encode selectins (transmembrane proteins of cell-to-cell adhesion involved in lymphocyte homing in animals) and tetraspanins (transmembrane proteins involved in various processes such as cell activation, proliferation, differentiation, adhesion, and motility).⁽¹⁰⁾ We found tetraspanins in choanoflagellates as well, although their homologs are much more similar between metazoans and mesomycetozoeans.

At least two more families of transcription factors “lost” their Metazoa-specific status during our BLAST⁽⁴⁷⁾ analyses in the NCBI Trace Archive (see Supporting Information). One is the T-box factor, a large family of morphogenes involved in the specification of mesodermal, ectodermally and endodermally derived structures, particularly in the differentiation of the posterior mesoderm in coelomic animals.⁽⁴⁸⁾ This family occurs throughout triploblastic and diploblastic animals and was thought to have evolved in the last common ancestor of Metazoa.^(49–51) Now, at least three members of this family have been found in the genome of the amoeba *C. owczarzewski*, and one in the genome of the fungus-like mesomycetozoean *A. parasiticum*, suggesting an origin for T-box genes in basal unicellular opisthokonts. Interestingly, neither of the T-box members is found in choanoflagellates,⁽³⁾ which appears to be an example of gene loss and a result of general reduction of the transcription factor diversity in this group.⁽⁵²⁾

Another family of transcription factors, Rel/NF-kappa B, which was also considered to be Metazoa-specific,⁽⁵³⁾ is nevertheless found in *C. owczarzewski* but absent from the choanoflagellate *Mo. brevicollis*, thus further suggesting gene loss in this lineage. In Metazoa, NF-kappa B is part of the Toll-signaling cascade involved in the development of inflammatory responses, mitosis regulation, and specification of dorso-ventral polarity in animal embryogenesis. The elements of the Toll cascade, including homologs of the Toll-interacting protein and Evolutionarily Conserved Signaling Intermediate in Toll pathways (ECSIT) that link the animal Toll pathway to MAP kinase, are as well found in *C. owczarzewski* and choanoflagellates.

The major corollary that follows from expanding the taxonomy of “Metazoa-specific” genes beyond Metazoa is that a multitude of genes involved in cell differentiation and adhesion had indeed been present already in the common ancestor of the Metazoa + Choanoflagellata + Mesomycetozoea group. Therefore, the gap in structural and genetic complexity separating multicellular and unicellular organisms might be just a bit less striking than the Gastraea theory predicts.

The Synzoospore theory and the variety of life cycles

How do life cycle strategies of extant choanoflagellates, mesomycetozoeans, and metazoans actually fit the Synzoospore hypothesis? Our knowledge about the biology of unicellular animal relatives is scarce, and soon we will know more about their genomes than about their physiology or even general biology. However, processes such as temporal cell differentiation, occurrence of sedentary trophic stages, and a tendency toward cell aggregation are commonly present in these organisms.

For example, in the life cycle of the choanoflagellate *Proterospongia choanojuncta* at least four cell types can be morphologically recognized: aflagellar sessile cells and three types of flagellated cells.⁽⁵⁴⁾ The origin and fate of some of these cell types remain unclear. It was suggested that a variety of currently described choanoflagellate species in fact represent mobile and sessile stages of life cycles of a smaller number of species.⁽⁵⁵⁾ Radial symmetry of the choanoflagellates' collar of microvilli and the array of microtubules around the flagellum basal body⁽⁵⁵⁾ suggest a primarily sedentary lifestyle. Some choanoflagellate species form colonies of monotonously dividing cells.

Complex life cycles with a variety of cell types are typical for Ichthyosporea. At certain stages they may form colonies of morphologically identical cells (*Sphaeroforma arctica*)⁽⁵⁾ or develop obligatory trichomes (Eccrinales).⁽³²⁾ These structures consist of uniform monotonously dividing cells (trophozoites) like those comprising the body of adult animals. The majority of ichthyosporeans also exhibit cellular hypertrophic growth, the phase that is obligatory for metazoans but lacking in choanoflagellates. In ichthyosporeans this phase is necessary to form sporangia and produce endospores. The moderately hypertrophied "spore" of the rosette agent *Sphaerothecum destruens* undergoes complete palintomic cleavage to form small flagellated zoospores.⁽⁵⁶⁾ Sexual reproduction is unknown in mesomycetozoeans; therefore, an asexual "spore," not a zygote, enters the palintomic phase. Similar processes can be observed in animals, e.g., during the development of parthenogenetic eggs in aphids or asexual agametes in Orthonectida and Dicyemida.

A rich variety of genetic mechanisms of cell stress responses and differentiation are critical to inhabit dynamically changing environments or respond to potentially dangerous stimuli. The majority of currently studied mesomycetozoeans are parasitic species that need to develop rapid cell responses in their interaction with the host. Saprophytic mesomycetozoeans like *Co. limacisporum*⁽⁴⁾ must be able to find rich substrates and develop dormant stages under adverse conditions. Saprophytic and parasitic fungi from the Chytridiomycota utilize fine or quickly depleting

organic matter and must dynamically respond to changing food resources.

Different cell types are formed during the chytridiomycote life cycle: trophic somatic cells, hypertrophied sporangia, and non-feeding palintomic zoospores. Related to fungi, nucleariid amoebae^(4,5) utilize different strategies to feed on bacteria and algae.⁽⁵⁷⁾ In their life cycle they develop different cell types during floating and surface-dwelling phases and can form temporary multinucleate trophic syncytia in response to environmental conditions.⁽⁵⁷⁾ In summary, the observed diversity of biology and recent phylogenetic reconstructions suggest that a complex life cycle is likely to be ancestral for all Opisthokonta, and, consequently, the temporal differentiation of cells in various life cycles and the underlying molecular machinery of cell differentiation preceded the origin of Metazoa.

The origin of Metazoa: a suggested scenario

The latest modification of the Gastraea theory, the Choanoblastaea hypothesis,⁽¹⁾ envisions the emergence of Metazoa through aggregation of individual uniform choanoflagellates by means of cadherins, attachment to extracellular matrix with integrins and establishment of cell-to-cell signaling with tyrosine kinases. The resulting Choanoblastaea is a blastula-like floating colony. However, cadherins, integrins, and numerous tyrosine kinases were reported not only from choanoflagellates,^(8,9,58) but can also be found in mesomycetozoeans.⁽¹⁰⁾ Indeed, complex life cycles of Ichthyosporea with well-differentiated cell types, quorum-sensing behavior, and a diverse collection of responses to environmental factors and extracellular stimuli^(59,60) require the presence of complex genetic programs underlying these processes, and the origin of cadherins, integrins, and tyrosine kinases can be traced back to the basal opisthokonts.

Large-scale phylogenomic analyses (Fig. 2) suggest that the Metazoa + Choanoflagellata stem branch is short compared to the stem branch of the Holozoa.⁽⁶⁾ This provides additional evidence to expect major evolutionary innovations to have taken place at the latest along the Metazoa + Choanoflagellata + Mesomycetozoea branch, i.e., in the common ancestor of this group. We suggest that metazoans are, therefore, unlikely to have evolved genetic programs of cell differentiation *de novo* during transition to multicellularity, but instead used pre-existing mechanisms to integrate different cell types occurring at subsequent stages of the ancestral life cycle into one multicellular body.

In contrast to the Gastraea hypothesis, the Synzoospore hypothesis implies the emergence of a whole array of intermediate multicellular stages with heterogeneous cell content in a complex life cycle of the pre-metazoon, which

makes this hypothesis a more likely theoretical explanation of the transition to multicellularity in the light of new genomic evidence. Under this Synzoospore scenario, the origin of Metazoa in fact corresponds to *the evolutionary transition from temporal cell differentiation to spatial cell differentiation*.

This proposed scenario also gains support from our knowledge of marine biology and comparative anatomy. Several selective factors could drive this major transition. Multicellularity enables the development of novel advantageous feeding strategies based on filtration. Some extant nucleariids⁽⁵⁶⁾ and mesomycetozoeans (*M. vibrans*) utilize filtration at particular stages of the life cycle, while choanoflagellates became specialized filtrators. The effectiveness of filtration, however, is greatly enhanced by cooperation of many cell types. Thus, it is well known that effective filtration in sponges is in many respects facilitated by the Bernoulli's force,⁽⁶¹⁾ which accelerates water currents flowing through the body at no expense of the flagellar beating simply due to the properties of the sponge chambers' dimensional architecture. Aggregation of different cell types is required to construct such feeding architectures, which might have been critical to improve the trophic function of the sedentary colonial stage of the ancestral macroscopic metazoan. Effectively feeding colonies are likely to be more effective in producing dispersal stages and supplying them with nutrients.

Dispersal cells produced by the sedentary filtrating stage, the zoospores, in early metazoans remained attached together as a synzoospore to form a primary larva, the blastula. At first glance, this transition seems to reduce the efficiency of larval dispersion. On the other hand, however, it increases larval survival: a multicelled larva has a larger size and possesses an advantage in escaping larger unicellular and, later, multicellular predators,⁽⁶²⁾ which under this scenario were likely to have been adult sedentary microphage filtrators. Furthermore, such a larva is more competitive in establishing a new colony by bringing along primary building materials and occupying a larger area of the settlement site as fast as possible. The young colony then quickly recovers the adult ability to utilize Bernoulli's force for filtration. This strategy is very commonly utilized by lecithotrophic larvae of extant sponges,^(63,64) commonly considered the earliest metazoans.

Development of the entire colony from a multicelled larva proceeds through differentiation of genetically identical cells, the zoospores (animal blastomeres). In the course of metazoan evolution this had likely been critical to establish true multicellularity with the maintenance of long-term cell adhesion and cooperation. A genetically uniform population of cells has a greater potential to maintain complex multilevel interactions of trans-differentiated cells and, therefore, to develop greater stability by avoiding the emergence of selfish cell lineages.^(2,65,66) Outside opisthokonts, complex multi-

cellular organisms, such as higher plants, brown and red algae, develop their bodies from genetically identical cells. Our current knowledge suggests that colonies and aggregations composed of genetically heterogeneous cells, such as differentiated fruit bodies of slime molds (*e.g.*, *Dictyostelium*) and acrasid social amoebae, are always temporary and do not contain true tissues. Genetically polymorphic cell aggregations are also predicted to be evolutionarily unstable by the kinselection theory.⁽⁶⁷⁾

Problems and perspectives of the Synzoospore theory

Many predictions of the Synzoospore theory are in accordance with growing evidence from evolutionary genomics and comparative biology. It may also provide surprising answers to key questions about early metazoan evolution, such as ancestral life cycles of animals and the origin of Eumetazoa.

Thus, interesting implications of this theory can be drawn in relation to extant Ctenophora (comb jellies). Zoological textbooks and a series of more recent molecular studies place ctenophores in a close position to Bilateria and Cnidaria. Adhering to the traditional view would not require to specifically address the fact that ctenophores possess a truly pelagic, not benthopelagic, life cycle. However, phylogenetic analyses of small subunit and large subunit rDNA^(20,68) and conserved elements in the rRNA secondary structure,⁽²¹⁾ a large number of protein-coding genes,^(69,70) as well as morphological^(71,72) and embryological^(21,37) evidence challenged the tradition and suggested that Ctenophora are an ancient lineage that diverged before the emergence of Cnidaria and Bilateria. If ctenophores are considered a basal animal group, in the light of the Synzoospore theory they must have lost the primary benthic phase from their life cycle, which implies the neotenic origin of the whole group.^(21,73) There is no direct evidence to prove or disprove this inference. However, one characteristic property of ctenophore development in this respect is encouraging: the formation of larval gonads and their subsequent involution (dissogeny),⁽⁷⁴⁾ which has already been interpreted as the ability of early larvae to perform reproductive functions in lower Metazoa.^(1,21)

Another set of inferences relate to the fact that larvae of the majority of marine invertebrates are not lecithotrophic. Instead, they are predacious and formed with monotonously dividing differentiated cells. A feeding primary larva with a gut is expected under the Gastraea theory and its derivatives such as Trochaea theory,⁽¹⁾ but is to be considered as a derived larval state under the Synzoospore theory. Reviewing this situation more closely suggests that the Synzoospore model might in fact propose an evolutionary scenario with far-reaching implications to our understanding of early animal evolution and the emergence of higher Metazoa.

1. Intercalation of the blastula stage in the life cycle
2. Filter-feeding specialization (sponges)
3. Planktotrophic specialization (eumetazoans): loss of the primary benthic filtrator stage

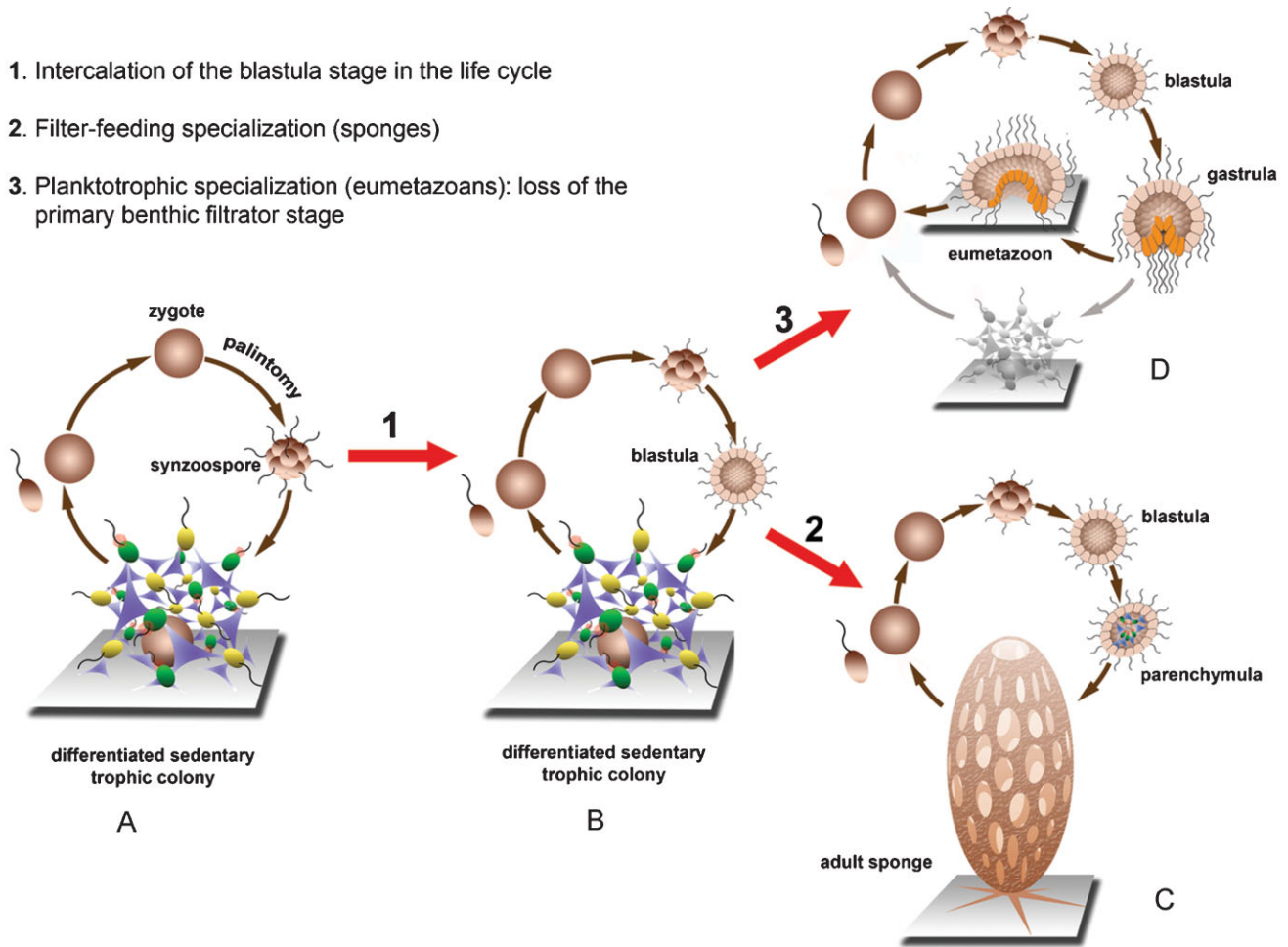


Figure 3. Evolutionary scenarios in early Metazoa. **A:** The ancestral state with a complex life cycle. **B:** Emergence of a larger larva capable of carrying primary building material – a multicelled synzoospore, the blastula. **C:** Development of large lecithotrophic larvae in the evolution of sponges – emergence of the parenchymula carrying different cell types of the adult sponge. **D:** Emergence of feeding larvae in the evolution of Eumetazoa. Development of the primary gut (colored in orange) as an adaptation to catch the multicelled prey. Transition toward neoteny and loss of the sedentary filtrator stage.

Thus, assuming the microphage filtrator as the ancestral form of animal life implies that the ecological niche of the planktonic predator was vacant and early plankton gradually became rich with lecithotrophic larvae. Occupying this niche would trigger evolutionary transitions toward a predatory lifestyle: the acquirement of an internal cavity to digest the multicelled prey, which led to the formation of the gut (Fig. 3)⁽⁷⁵⁾ and development of locomotion and coordination systems, which led to the formation of muscles and a nervous system, the latter being associated with ciliary bands and sensory tufts in extant predatory larvae. Interestingly, extant pelagic ctenophores are active predators but utilize their muscular system for catching prey, not for locomotion, which remains ciliar. This observation might indicate the primary function of muscles. Secondarily benthic platyctenid ctenophores develop their creeping sole through eversion of the

stomodeum, and muscles used for their locomotion are in fact a part of the gastral system.

In higher Metazoa the gut is formed in early embryogenesis during gastrulation, which leads to separation of two embryonic layers, the ectoderm and endoderm. While gastrulation also occurs in sponges, their larvae are always lecithotrophic and thus are not expected to undergo processes required to develop macrophagy under the Synzoospore theory. However, homology of sponge embryonic layers with the ectoderm and endoderm of higher Metazoa was always (and still is) a subject of debate.^(76,77) According to contemporary views, gastrulation in sponges is related to the ingression of gametocyte precursors, rather than to the formation of the gut,^(77–79) which is in agreement with the hypothesis of different larval origin in sponges and higher Metazoa.

Conclusions

In an age when genomic data became easily accessible and available from organisms across the tree of life, our understanding of fundamental events in the course of natural history is often reshaped. Putting together pieces of evidence from evolutionary genomics, comparative anatomy, marine biology, and ecology allows us to revisit the major question of the origin of multicellularity and propose a new evolutionary scenario, which fits emerging data better than widely accepted classic paradigms and offers a number of intriguing predictions. One such scenario, filled with novel molecular information, recapitulates the Synzoospore hypothesis by Zakhvatkin formulated in the middle of the last century. We argue that multicellularity was not a trigger to the emergence of cell differentiation in a multicelled ancestor but, inversely, emerged as a result of integration of different cell types. In fact, the origin of Metazoa corresponds to the major evolutionary transition from temporal or transient cell differentiation occurring at different stages of the life cycle to spatial permanent differentiation forming the trophic body of the ancestor. In the ancient ocean, dispersal stages of this sedentary trophic body, the primary blastula-like larvae, acquired adaptations toward a predatory lifestyle to utilize ample resources of their lecithotrophic kin, which triggered the development of primary gut, muscular, and nervous systems of multicellular animals. The “true” gastrula, an attribute of all eumetazoans, should be considered as a derived larval state that emerged secondarily in early plankton as a potential predator and became a predecessor of the variety of true planktonic larvae of marine invertebrates. Studies of larval physiology, morphogenesis, and their underlying genetic mechanisms will be crucial to understand the origin and early evolution of key functional and structural systems in Metazoa. These studies might also shed light on the origin of Eumetazoa⁽⁸⁰⁾ as neotenic descendants of early planktonic larvae, which lost the primary benthic stage of microphage filtrators and developed fundamental elements of the higher metazoan body plan as evolutionary adaptations to predatory lifestyle and macrophagy.

Acknowledgments: The authors are grateful to V. V. Malakhov, I. A. Kosevich, and T. G. Simdyanov for helpful discussions, J. Netherton for helpful comments on the manuscript, M. Nickel and the porifera.net lab (www.porifera.net) for access to rare old publications. This work was supported by the Russian Foundation for Basic Research (06-04-49288, 08-04-00244, 08-04-00478, 08-04-01746, 09-04-01150), RFBR-DFG 09-04-92741, DFG RECESS (to VVA), MCB RAS (to YVP), UK Biotechnology and Biological Sciences Research Council grant BBSB14418 (to PVT), National Institute of Health, National Science Foundation, McKnight Brain Research Foundation, and University of Florida Opportunity funds (to LLM).

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