doi:10.20944/preprints202008.0302.v1

Dining on DOM: Stimulus for the Origin of Metazoan Life?

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ABSTRACT:
The most widely accepted scientific theory for the origin of life on Earth is that prokaryotic microbes evolved from simple organic compounds in seawater under anoxic conditions. For about 1 billion years thereafter, these microbes consumed the same dissolved organic matter (DOM) from which they had evolved before scarcity of DOM forced the evolution of cyanobacterial photosynthesis followed by eukaryosis. Could the more efficient consumption of DOM have also stimulated the subsequent origin of multicellular animal life? In this report, we synthesize past and recent evidence to propose the "DOM uptake hypothesis" for the origin of metazoans. A choanoflagellate-like protozoan was the likely ancestor of the first sponge-like metazoan to evolve on Earth. Choanoflagellates have outwardly facing flagellae that are subject to viscous water movement, while sponges have choanocytes in chambers with flagellae directed to pump water with greater fluidity across an aquiferous system with a huge cellular surface area. While generally considered particle feeders, both choanoflagellates and sponges absorb DOM, with some sponges relying on DOM for as much as 90% of their diet. We propose that the earliest metazoans may have evolved to survive the dire nutritional conditions of the Cryogenian "snow-ball Earth" period (~700 million years ago) by developing a body plan with the enhanced ability to absorb low concentrations of DOM in seawater from sources such as the viral lysis of microbes, exudates of benthic stromatolites, or refractory DOM compounds. Additionally, species of extant sponges that have a high abundance of microbes living in their bodies consume the greatest amounts of DOM, suggesting that the DOM uptake hypothesis may also be dependent on microbial symbiosis.

Keywords: origin of life, DOM, DOC, choanoflagellate, sponge, Porifera, Neoproterozoic, Cryogenian

1. Introduction

The most common scientific narrative for the origin of life on Earth is that prokaryotic microbes evolved from simple organic compounds that formed in seawater under anoxic conditions ~4.0 billion years ago (Ga) [1, 2]. Prokaryotes then used the same dissolved organic matter (DOM) for nutrition for ~1 Ga before scarcity of DOM stimulated change with the evolution of photosynthesis. The eventual, and then increasing, production of free oxygen from photosynthesis beginning ~500 million years (Ma) later stimulated considerable additional change, including the evolution of oxidative metabolism, eukaryosis and multicellularity. The earliest animal life arose independently among multicellular taxa about mid-way through the Neoproterozoic Era (1000-540 Ma), with current majority scientific opinion that choanoflagellates were the closest common protistan ancestor with the earliest metazoan [3-5].

Although still controversial, consensus is returning to support sponges (Phylum Porifera) as the sister group to all other metazoans [6-8]. This relationship is parsimonious from the standpoint of morphology, because choanoflagellates and sponges share a cell type that features a collar of microvilli surrounding a beating flagellum that induces flow through the collar and along the axis of the flagellum [4, 9]. Further, living choanoflagellates can form multicellular colonies that are evocative of a transition to a sponge-like form, a relationship that has been recognized since the 19th century [reviewed in 9] and is the subject of active genomics research today [5]. The oldest steroid biomarker molecules from the fossil record attributable to metazoans are for sponges, and have been dated to the late Cryogenian [10]. While there have been many ideas about the environmental conditions of the early Earth that may have *permitted* the rise of the first multicellular metazoans, including thresholds in oxygen concentrations, salinity and temperature, few have addressed the conditions that may have *stimulated* this transition [10-12].

1.1 What was different about the first metazoans?

Multicellularity arose independently in several taxa, including red and brown algae and fungi [13]. Metazoans are unique among multicellular groups because they lack cell walls, thereby allowing nutritional uptake across the cell membrane in two ways: phagocytosis of particles or direct absorption of DOM [11]. Of these two methods, phagocytosis of particles by collar cell microvilli has been the focus of cellular nutrition in hypotheses of animal origins [4, 9, 14]. Direct absorption of DOM has mostly been ignored, perhaps because it has been generally accepted that the prokaryotic microbes found in high abundance in seawater are competitively superior at absorbing DOM [15].

In addition to the molecular genetic evidence supporting their association with sponges, choanoflagellates are a particularly intriguing model for the origin of metazoan multicellularity. As recently reviewed by Koehl [14], many species of choanoflagellates have the ability to alter their morphologies, from unicellular to colonial, swimming to attached (sessile), and with variation in the relative size and shape of both the collar and flagellum. Comparisons across stages and morphologies of swimming speed, particle capture, and susceptibility to predation have revealed trade-offs that are advantageous under different environmental conditions. For example, the formation of multicellular colonies does not improve swimming performance, but does result in greater flux of particles to some cells than for a single cell; however, colonies are easier targets for protozoan predators than single cells. Morphological plasticity has likely been preserved among extant choanoflagellates because of its selective advantage in highly variable aquatic environments. Choanoflagellate swimming and feeding performance has also been modeled to assess the effects of changes to cell morphology [16], but again, particle capture has been the sole nutritional mode considered in these studies.

1.2 DOM as a food source for extant choanoflagellates and sponges.

DOM in seawater is a complex and poorly understood mixture of small and large molecules that range from labile (small, building-block molecules like simple sugars, amino acids, etc.) to refractory (large, complex molecules that are resistant to microbial consumption; also called recalcitrant DOM) with the latter constituting the greatest proportion of the huge reservoir of carbon in the biosphere that exists dissolved in the ocean [17, 18]. Extant choanoflagellate species are known to consume DOM, and in coastal Antarctic waters reach their peak abundance in response to high seawater concentrations of semi-refractory DOM compounds produced by blooms of the haptophyte alga *Phaeocystis* [19]. Feeding by sponges, as for choanoflagellates, has mostly been considered a matter of particle uptake [20], despite decades-old evidence of DOM consumption [21]. The importance of DOM feeding by sponges became topical with the “sponge-loop hypothesis” [22], which proposed that coral reef sponges remove as much DOM from the water column in 30 min as water column microbes remove in 30 days. Recent evidence suggests that many extant sponge species rely on DOM for >60%, and as much as 90%, of their nutrition [23-26]. While these and other studies have quantitatively determined uptake of bulk DOM (as dissolved organic carbon, or DOC), the metabolites involved remain unknown, as does their relative position on the spectrum from labile to refractory. Perhaps access to DOM as a food resource was the stimulus for the emergence of sponge-like metazoans from colonial choanoflagellate ancestors?

1.3 New ideas: Nutritional limitation and symbiosis

The history of life on Earth is thought to be marked by a series of catastrophes that stimulated major evolutionary advances. The origin of metazoans in the Cryogenian ~700 Ma was coincident with dramatic environmental changes brought on by increasing oxygen levels and plate tectonics that led to “snowball Earth” conditions detrimental to eukaryotic life, as ice-covered seas experienced reductions in gas exchange, light available for photosynthesis, and access to essential nutrients (N, P, Fe) from terrestrial sources. The resulting decline in food availability to choanoflagellate ancestors may have been a critical stimulus. The Holobiont Theory of metazoan emergence [12] proposes that colonies of choanoflagellate-like ancestors formed symbioses with anaerobic or autotrophic prokaryotes to mitigate starvation, with subsequent horizontal gene transfer from prokaryotic symbionts to eukaryotic host speeding the evolution of the earliest metazoan. Among the discoveries that support this theory: induction of choanoflagellate colony formation triggered by secondary metabolites produced by associated bacterial symbionts [27, 28], and the very high abundances, up to 40% by volume, of sponge-specific prokaryotic symbionts in the tissues of some extant sponge species [29]. While the formation of a holobiont may have been critical to setting the stage for rapid evolution by gene transfer with symbionts [12], it doesn’t explain the transition from colonies of choanoflagellate-like cells with flagellae and collars directed outward [the choanoblastea; 30], to the sponge-like condition of flagellae and collars directed inward, and ultimately, lining chambers connected to cell-lined tubes inside a sponge-like body.

2.0 The DOM uptake hypothesis

Building on a wealth of recent theories [4, 9, 11, 12], we propose that the evolution of the earliest sponge-like metazoans from choanoflagellate-like ancestors was driven by nutritional limitation from Cryogenian “snowball Earth” conditions, resulting in the internalization of flagellated collar cells into chambers and cell-lined tubes (the aquiferous system) that greatly enhanced food uptake (Fig. 1). In particular, the evolution of flagellated chambers transformed the kinetics of DOM uptake, because the outward-facing flagellae of unicellular and colonial choanoflagellates are subject to the molasses-like conditions faced by microscopic cells under high Reynolds numbers [16, 31], while the flow of water induced by collar cells through the aquiferous system of the sponge-like ancestor increased the fluidity of seawater as it passed across the enormously high surface area of the cell-lined tubes and collar cells, enhancing the uptake of food and oxygen and the release of CO_2 and waste metabolites. While collar microvilli are important for particle capture, the surface area of microvilli are also available for DOM uptake, and the flow of seawater through the collar microvilli, and past the absorptive cells lining the aquiferous system, allowed sponge-like ancestors (Fig. 1G) to out-compete water column microbes for labile DOM. Sources of labile DOM in Cryogenian seas would have included the viral lysis of microbes in the water column and the photosynthetic exudates of autotrophic eukaryotes and cyanobacteria, including benthic stromatolites (Fig. 2). Additionally, this more efficient feeding mechanism may have permitted sponge-like ancestors to absorb and consume more refractory forms of DOM, which likely dominated the DOM pool then as now [18]. Indeed, the dominant refractory DOM compounds in modern oceans are humic substances, which may have newly appeared in Cryogenian seas as run-off from the earliest terrestrial soils [32]. Consumption of DOM may have been advanced by the symbiotic association of the earliest metazoan with microbes, either through direct uptake of DOM by symbiont cells associated with the aquiferous system, or by the provision of genes involved in DOM uptake from prokaryotic symbionts to the metazoan cells through horizontal gene transfer [12].

3.0 Discussion

In biology as in geology, “the present is the key to the past.” As previously mentioned, extant species of choanoflagellates are known to consume DOM, and in some cases are dependent for their nutrition on refractory forms of DOM [19]. Analogous to “snowball Earth” conditions, modern tropical coral reef ecosystems are nutrient-poor, having stimulated the evolution of diverse symbioses to overcome nutritional limitations, with the zooxanthellae of reef-building corals a prime example. Sponge communities on coral reefs are highly diverse, with extant species rooted in the oldest known metazoan clades [33, 34]. Recent research suggests that sponges on contemporary oligotrophic coral reefs face severe nutritional limitations [24], and many species rely on DOM for most of their nutrition [23, 35]. On reefs in the Caribbean, cyanobacterial mats produce high concentrations of DOM [36] that are likely to contribute to sponge nutrition, analogous to the provision of DOM from the exudates of benthic cyanobacteria and stromatolites at the time of the evolution of the earliest metazoans.

The overwhelming proportion of DOM compounds in natural waters are refractory [18], and this was likely also true in Cryogenian seas. Refractory DOM is produced through both abiotic and biotic means, the former including interactions of more labile molecules with sources of energy (uv radiation, heat, electrical discharge; Fig. 2). Cyanobacteria may also produce refractory analogs to building-block metabolites, such as D-enantiomers and non-protein amino acids [37], which may be consumed by sponges. The most abundant refractory DOM in modern oceans are humic substances, which are largely derived from the decomposition of terrestrial vascular plants, but Cryogenian seas may have received humic substances as run-off from the earliest terrestrial soils [32].

3.1 Morphological transitions that enhance DOM uptake

The novelty of the DOM uptake hypothesis is two-fold: (1) the recognition that DOM could play as important, if not a more important, role than particle capture in the nutrition of the choanoflagellate-like ancestor that evolved into the earliest metazoan, and (2) that the morphological transition of colonies of collar cells from flagellae and collars directed outward to inward, and subsequent development of the cell-lined tubes of the aquiferous system, may have improved the hydrodynamics of feeding (both DOM uptake and particle capture; Fig.1). In addition to many extant choanoflagellate species exhibiting multicellularity [14], a multicellular, sheet-forming, unattached species was recently described (*Choanoeca flexa*) that contracts in response to changing light levels to form a cup-shape with the collars and flagellae facing inward [38]. Colonies with flagellae-out swim rapidly, but those with flagellae-in do not; however, the latter captured particles more efficiently than the former (75% vs. 10% of beads captured, respectively). The authors speculated that this adaptation served as a mechanism for both predator escape (flagellae-out, fast swimming) and as a phototactic response to enhance feeding (flagellae-in, higher particle uptake). Although not sessile (colonies are unattached), the association between living *C. flexa* with the hypothetical transition shown in Fig. 1 is striking, and demonstrates the ability of extant choanoflagellate colonies to contract [38]. Equally intriguing is the discovery that one species of choanoflagellate, *Salpingoeca rosetta*, has colonial cells that have enhanced macropinocytotic activity that is not evident in the solitary form of the same species, and that this activity is shared to some degree with the collar cells of a sponge [39]. It was speculated that macropinocytotic activity may be involved in the detection of environmental chemical signals [39], but another likely function is the enhanced uptake of DOM.

3.2 The role of microbial symbiosis

It has long been suspected that DOM uptake by sponges is mediated by microbial symbionts [40], but some recent studies have suggested otherwise. Analyses of seawater processing by nine Caribbean sponge species clearly separated those with microbial symbionts (high microbial abundance, or HMA species) as net consumers of DOM, and those without symbionts (low microbial abundance, or LMA species) as net producers of DOM [23]. Yet, others have reported net DOM uptake by LMA sponge species [26, 41], and studies of the sponge microbiome across the LMA-HMA spectrum have not revealed a clear connection between symbiont community structure and DOM uptake [42]. Further, tracer experiments with isotopically enriched DOM using a combination of transmission microscopy and mass spectrometry have linked DOM uptake to sponge cells rather than microbial symbionts in one coral reef sponge species [43] and to both sponge cells and microbial symbionts in two temperate sponge species [44]. It is likely that all possible scenarios can be found among extant sponge species, given the great diversity of the phylum, and the range of symbiosis that can be found among species, from LMA to HMA.

Is the stimulus of DOM uptake required for the evolutionary progression shown in Figure 1? An alternative trajectory could be based primarily on particle feeding, with the earliest metazoan similar to an extant LMA sponge that produces, rather than absorbs, DOM. The acquisition of microbial symbionts to form an HMA sponge, and nutritional reliance on DOM, could have come thereafter. However, this alternative is a less parsimonious explanation for the evolution of the cell-lined tubes of the aquiferous system (Fig. 1G) in response to the Cryogenian nutritional crisis that plays a pivotal role in the DOM uptake hypothesis. These cell-lined tubes are not specialized for particle capture, but do have a high surface area for absorption of DOM. For HMA sponge species, the high surface area of these cell-lined tubes is surrounded by a matrix filled with microbial symbionts. Morphological comparisons have also revealed that HMA sponge species tend to be larger and more robust (tougher consistency), pump water more slowly, have smaller choanocyte chambers, and a narrower aquiferous system than LMA sponge species [45, 46]. Microbial symbioses impose a cost on the host, and it has been suggested that exploitation of the host may drive the origin of microbial mutualisms [47]. The LMA condition, with a smaller, more fragile form, higher choanocyte chamber density, and higher pumping rates, may have evolved secondarily when the host sponge shed its microbiome and associated costs to accentuate particle capture as a primary nutritional mode [48].

3.3 The rise of other animal phyla

One hypothesis proposes that the *planktonic larva* of the earliest sponge-like ancestor gave rise to all other metazoan clades, including the Ctenophora, Cnidaria and bilaterian animals [30]. This may explain why sponges have a distinctly different mechanism for feeding, that, while offering a highly successful nutritional strategy then as now, was left behind by all other animal groups. Collar cells were retained in other animal groups, but are no longer used for feeding, and instead are employed in sensory, excretory, or other functions [39]. Filter-feeding using an enclosed body space re-evolved, most notably in the molluscs (bivalves) and chordates (tunicates), but without the huge surface area of the sponge aquiferous system, and with multi-ciliated epithelial cells instead of collar cells providing water flow.

3.4 New directions for research

Without time travel, full knowledge of the evolutionary process leading to the rise of the metazoa is unlikely, but several lines of research may be fruitful in testing aspects of the

DOM uptake hypothesis using extant taxa to advance our understanding of what may have occurred. As indicated above, nutritional and behavioral studies of the uptake of DOM by choanoflagellates is surprisingly lacking, despite evidence that it occurs under natural conditions [19]. Better controlled laboratory experiments of choanoflagellate feeding on DOM, both with and without particles, would be useful in determining the importance of DOM to the diet of choanoflagellates, and how DOM uptake changes as a function of coloniality and across species. Models of choanoflagellate feeding that have considered particle capture [16] could be reconfigured for diffusive flux of DOM in assessing the efficiencies of the transition from unicellular to multicellular, from swimming to attached, and, following the proposed evolutionary progression, from outward- to inward-facing collars and flagellae and the formation of an aquiferous system (Fig. 1).

On the other end of the proposed evolutionary progression, studies of sponge feeding may offer important insights into components of the DOM uptake hypothesis. Analogous to Cryogenian seas, modern coral reef ecosystems are oligotrophic, and in some locations, sponges are living at or below their nutritional limits [24]. Comparable to ancient stromatolites, cyanobacterial mats are common on many modern reefs and are prodigious sources of DOM [36]. We have much to learn about the composition of DOM in seawater, including the sources and identities of constituent compounds, their relative microbial lability, and whether sponges can consume them. Finally, sponges provide us with a rich diversity of species along a spectrum of symbiosis, from those that appear to lack a microbiome, to those whose internal microbial biomass nearly rivals their overall sponge cell biomass [29]. Comparisons of sponge feeding along this spectrum may inform the link between the uptake of DOM and microbial symbiosis, although the possibility of horizontal genetic transfer [12] may thwart any definitive conclusions. Application of ever-advancing technologies for characterizing seawater chemistry and the feeding capabilities of extant organisms in modern environments may tell us much about the rise of metazoan life.

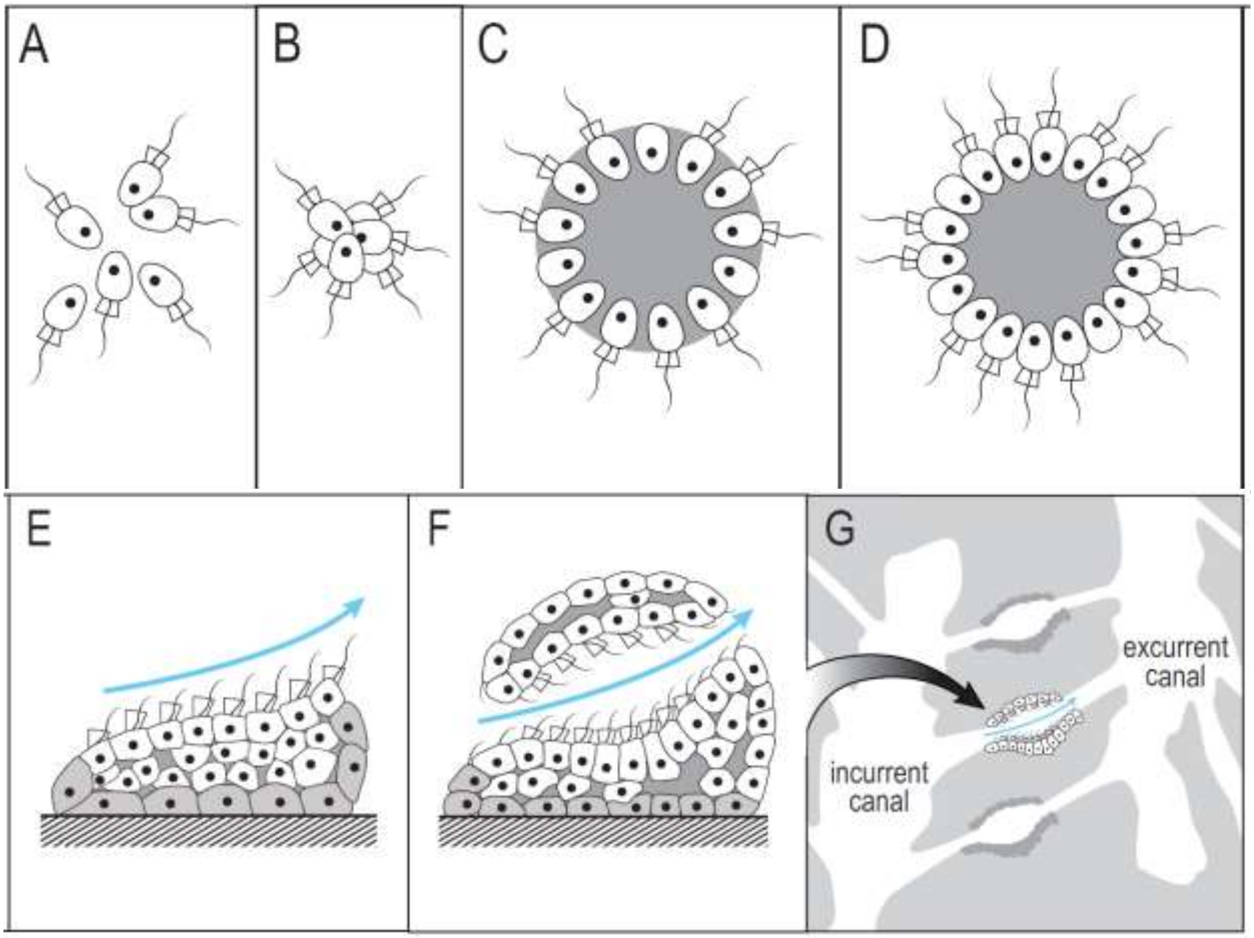

Figure 1. Evolutionary progression from single-celled choanoflagellate ancestor to earliest metazoan. Increasing colonial complexity (A-D), transition to benthic form (E), formation of enclosed flagellated chamber with directed water flow (F), aquiferous system of cell-lined channels to and from flagellated chambers in a sponge-like ancestor (G). Collar cell uptake shifted from high to lower Reynolds number flow as the body plan became enclosed and flow directed (E, F). Surface area for DOM uptake increased substantially with the evolution of the aquiferous system (G). C-F adapted from Nielsen [30].

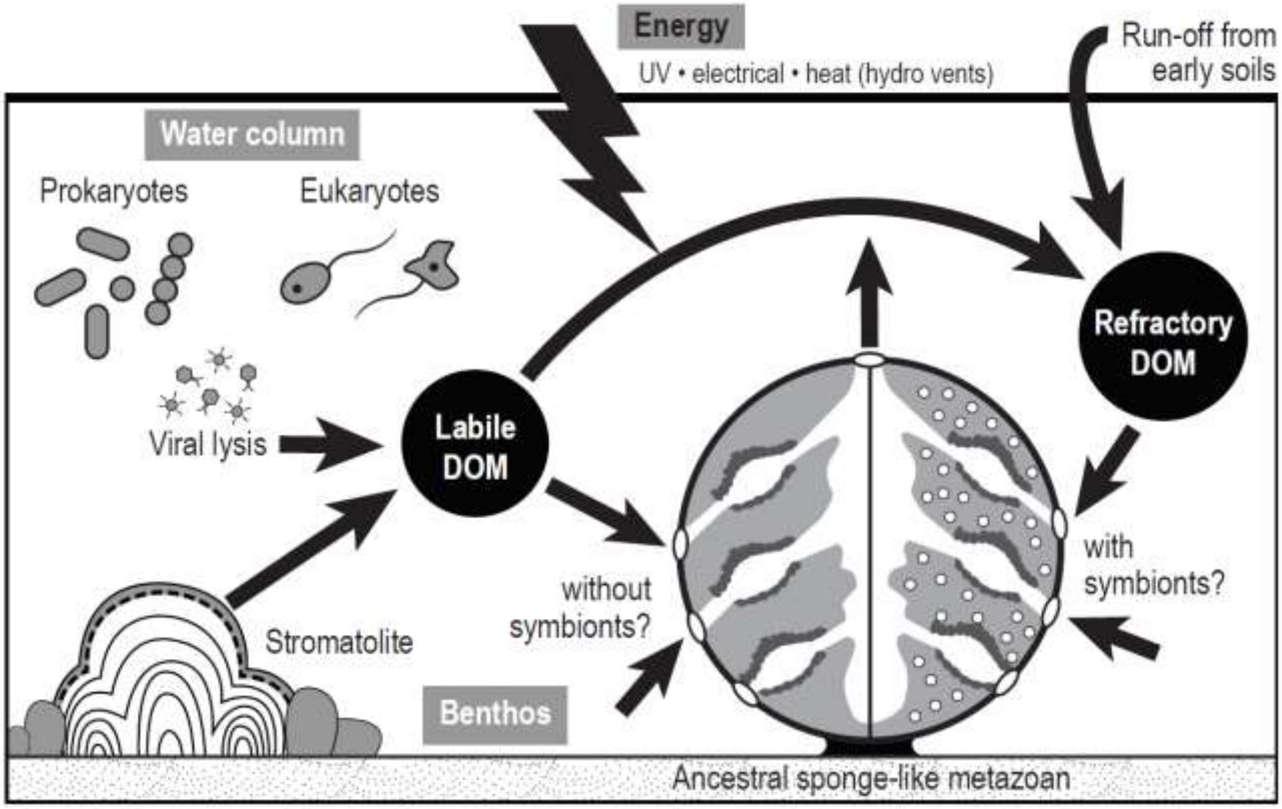

Figure 2. Cryogenian ancestral sponge-like metazoan consuming DOM. Labile DOM derived from viral lysis and exudates from autotrophs, including stromatolites. Abiotic reaction of labile DOM produced refractory DOM, which may also have run-off of early soils. Consumption of DOM may or may not have been dependent on symbiotic prokaryotes.

doi:10.20944/preprints202008.0302.v1

Data accessibility. Not applicable.

Authors' contributions. J.R.P. developed the initial concepts and wrote the first draft of the manuscript with subsequent discussion and revision contributed by the other authors.

Competing interests. We declare we have no competing interests.

Funding. This research was supported in part by NSF grant OCE 1558580.

Acknowledgements. We thank Melissa Smith for helping with the illustrations.

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