<arttitle> Animal emergence during Snowball Earths by thermosynthesis in submarine hydrothermal vents

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<abs> Darwin already commented on the lateness in the fossil record of the emergence of the animals, calling it a valid argument against his theory of evolution[1]. This emergence of the animals (metazoans: multicellular animals) has therefore attracted much attention[2-5]. Two decades ago it was reported that extensive global glaciations (Snowball Earths) preceded the emergence[6-7]. Here we causally relate the emergence and the glaciations by invoking benthic sessile[8-11] thermosynthesizing[12-13] protists that gained free energy as ATP while oscillating in the thermal gradient between a submarine hydrothermal vent[14] and the ice-covered ocean. During a global glaciation their size increased from microscopic to macroscopic due to the selective advantage of a larger span of the thermal gradient. At the glaciation's end the ATP-generating mechanisms reversed and used ATP to sustain movement. Lastly, by functioning as animal organs, these protists then through symbiogenesis[15-17] brought forth the first animals. This simple and straightforward scenario for the emergence of animals accounts for their large organ and organism size and their use of ATP, embryo and epigenetic control of development. The scenario is extended to a general model for the emergence of biological movement[18]. The presented hypothesis is testable by collecting organisms near today's submarine hydrothermal vents and studying their behaviour in the laboratory in easily constructed thermal gradients.

<p> In *On the Origin of Species* Darwin remarked on the lateness and abruptness of the emergence of the animals: "To the question of why we do not find records of the vast primordial periods, I can give no satisfactory answer." and "The case at present must remain inexplicable; and may be truly urged as a valid argument against the views here entertained." The emergence was later dated near the Varanger-Ediacaran boundary of the late Proterozoic,

with the Varanger being one out of a series of intense global glaciations called ‘Snowball Earths’ [6-7] (Fig. 1).

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<p> Due to the free energy available in their gradients, submarine hydrothermal vents can have played an important role in evolution. A complete ice-cover of the ocean may have forced life on Earth to retreat to these submarine hydrothermal vents, which therefore may have constituted a refugium[19], and we relate the emergence to benthic sessile thermosynthesizing protists that lived there.

<p> Thermosynthesis[12-13] refers to a theoretical free energy gain mechanism that worked on thermal cycling using a thermal variation of the binding change mechanism[20] of today’s ATP Synthase (Fig. 2a-b). A single thermosynthesizing ‘First Protein’ (FP) has been applied in a model for the origin of life that accounts for the emergence of the hereditary machinery, including the genetic code, and the emergence of bacterial photosynthesis, including the emergence of the chemiosmotic machinery. Suspension in convecting volcanic springs brought about the thermal cycling (Fig. 2c).

<p> In sunlight thermosynthesizers lose the competition to photosynthesizers. Intermittent scarcity of light during global glaciations or large volcanic eruptions must however often temporarily have favoured thermosynthesis. Short-time presence during evolution of sessile thermosynthesizers is therefore plausible. They can have sustained major evolutionary

advances, and may in particular have brought about novel methods to implement biological movement (Table 1).

<p> During evolution active biological movement cannot have started very small, say on the scale of proteins or bacteria, since viscosity dominates inertia on this length scale. It is inefficient to put small objects in motion. The domination is characterized by the Reynolds number *Re*, the ratio of inertial and viscous (frictional) forces:

<fd> $Re = v\ L\ \rho\ /\ \eta = v\ L\ /\ \upsilon$,

<p> where v is the speed of the fluid, L a relevant length, ρ the fluid density, η the fluid viscosity, and $\upsilon = \eta\ /\ \rho$ the dynamic viscosity of the fluid[3,18]. At low Reynolds number repetitive movement such as swimming cannot cause displacement[18]: the organism remains at the same spot (although rotation such as by the bacterial flagellum can cause displacement[18]). When a small biological object changes its shape after a temperature change, this change is not hampered by the high viscosity of a low Reynolds number. The origin of biological movement is attributed to such thermal shape changes (just recently, small engineered devices that similarly change shape by thermal cycling have been reported[23]).

<p> By attaching FPs to objects oscillating due to cyclic thermal expansion and contraction, ATP or other high free energy compounds were generated. Extensive global glaciations lasted tens of millions of years. There was therefore enough evolutionary time for the size of sessile thermosynthesizers to increase: the larger span of the thermal gradient gave the selective advantage of a higher energy gain through a higher amplitude of the thermal cycle. Some oscillations were preadaptations for ATP driven movement after the reversal at the end of the glaciation, when regular ATP sources again became available.

<p> To the ~ 2900 Mya glaciation[22] the emergence of a thermotether is attributed (Fig. 2d), a filamentous protein that oscillated in the thermal gradient by cold denaturation[24-25] (Table 1). Connection to the thermotether turned the planktonic thermosynthesizer of Fig. 2c into a sessile thermosynthesizer. Emergence of the bacterial flagellar motor from the thermotether has been modeled, with a progenitor pumping protons across the cell membrane during thermal cycling[11]. In the model protons pumped by photosynthesis or respiration at the end of the glaciation reversed the process and caused the thermotether to function as the flagellum of the bacterial flagellar motor.

<p> The acritarchs found after the next global glaciation of ~ 2200 Mya are commonly held to be eukaryotes. Similar to Margulis[15], we assume their emergence by endosymbiosis of prokaryotes; we also assume that acquired histones present in prokaryotes such as *Thermoplasma* yielded chromatin. This chromatin permitted epigenetic control[23] that facilitated symbiogenesis by enabling gene silencing in the chimera genome obtained from merged symbionts.

<p> We propose the emergence of the eukaryotic cell from a symbiont[15-17] consisting of a thermotether/prokaryote combination that functioned inside a sessile thermosynthesizing prokaryote. The thermotether was a progenitor of filamentous proteins such as microtubule and actin, and the First Protein the common progenitor of kinesin, dynein and myosin, which emerged upon the reversal; this reversal became possible after endosymbiosis[15-17] with the progenitors of today's mitochondria had added an ATP source. Symbiosis is common in protists[15-17].

<p> In the late Proterozoic, during the Varanger Snowball Earth, the three major emerged preadaptations were: (1) a larger thermotether (Fig. 2f-j), (2) the thermopharynx (Fig. 2k), and (3) the thermotentacle (Fig. 2m). The enlarged thermotether eventually formed a symbiont with the thermopharynx, that after the reversal became the frond of Fig. 1b. The

thermopharynx contained a cyclically opening and closing entry to the same body cavity as present in the sponge. Addition of thermotentacles (Fig. 2l) yielded the progenitors of the Ediacaran anemones. Figure 3 gives an overview of the emergence of the macroscopic Ediacaran animals.

<p> The preadaptations that emerged during the short glaciation at the start of the Cambrium were (1) the thermoprecipitate and (2) the use of the thermal diffusion potential. The thermoprecipitate involved a high temperature precipitation of $CaCO_3$ and Ca-phosphate that yielded protons[27] which generated ATP by chemiosmosis, using an ATP Synthase in the membrane of the body cavity of a thermopharynx organism. The precipitate would have been removed together with the ocean water heated during the working cycle of the thermopharynx.

<p> The use of the thermal diffusion potential[28] was the most complex of the proposed preadaptations, and concerns a little known phenomenon (see Supplementary Information). A thermal gradient generates in solution an electric potential, which in general has only a small value, ~ 0,5 mV/°C that varies with the ionic composition of the medium (H^+ and OH^- give relative large values). Where a macroscopic filamentous membrane spans a large temperature difference and the medium composition at the two sides of the membrane differs strongly, an electrical voltage difference of ~ 40 mV seems feasible that could suffice for chemiosmosis by ATP Synthase at a high H^+/ATP ratio.

<p> The reversal yielded the skeleton and the nerve. The single-organ protists that had emerged in the Ediacaran and Cambrian then formed by symbiogenesis[15-17] the numerous multicellular and multiple-organ animals (metazoans) of the Cambrian explosion (Fig. 4). Note that Empedocles already proposed such a mechanism for the formation of large organisms from independently living smaller ones.

<p> This symbiogenesis was complex: first the symbiont was formed from rightly positioned independent protists. Next their genomes merged. The cells of the chimera functioned as embryonic stem cells that by epigenetic control differentiated into the many cell types of an animal and then migrated to the position held in the progenitor symbiont. During symbiogenesis the external functionality of the animal did not change but the internal functionality with the acquired development capability became much more complex. The first embryo fossils are dated at the end of the Marinoan and Varanger Snowball Earths[29].

<p> Thereafter the animals obviously continued to evolve, but because of its complexity, the macroevolutionary symbiogenesis was a non-repeatable one-off on the path from root to branch on the evolutionary tree. In the descendants in addition to microevolution only other types of macroevolution were possible, and change was gradual. As a result the proposed contributors to animal symbiogenesis still are recognizable in today's four types of animal tissue: epithelium, connective tissue, muscle, and nerve. The collagen of epithelium relates to the thermotentacle, although connective tissue of course contains collagen as well. The skeleton can be related to a combination of the thermotentacle and the thermoprecipitate. Clearly, muscle would have the contracting thermotether as progenitor, and the nerve with is action potential would descend from a protist that made use of the thermal diffusion potential.

<p> We conclude that a consistent and comprehensive scenario can be formulated based on macroevolution through symbiogenesis for the emergence during global glaciations of the multicellular animals from protists near submarine hydrothermal vents. Their large size was the result of its advantage during thermosynthesis. Emergence of the embryo and epigenetics is accounted for. Darwin's problem of the late emergence of animals is explained by a requirement for global glaciations, which occurred only late in Earth's history. More generally, previously proposed environmental fluctuations that can accelerate evolution[30] are associated with the Earth's global glaciations. Emergence of biological movement, i.e. the bacterial

flagellar motor, and the movement of ATPases along filamentous proteins, is also explained by reversal of preadaptations that emerged in sessile thermosynthesizers during these global glaciations. Thermosynthesizers were suitable hosts for endosymbiosis. The contribution of symbiosis to evolution[17] seems just as important as the contribution of natural selection; these two disparate and complementary notions would have equal standing, being comparable to Empedocles' love and strife. In addition to the origin of life, thermosynthesis can also explain the emergence of the animals by a simple and robust scenario.

<bibcit> 1. Darwin, C. *On The Origin of Species* (John Murray, 1859).

<bibcit> 2. Signor, P.W. & Lipps, J. H. in *Origin and Early Evolution of the Metazoa* (eds Lipps, J.H. & Signor, P.W.) 3-23 (Plenum Press, 1992).

<bibcit> 3. Valentine, J.W. *On the Origin of Phyla* (University of Chicago Press, 2004).

<bibcit> 4. Narbonne, G.M. The Ediacara biota: Neoproterozoic origin of the animals and their ecosystems. *Ann. Rev. Earth Planet. Sc.* **33**, 422-442 (2004).

<bibcit> 5. McCall, G.J.H. The Vendian (Ediacaran) in the geological records. Enigmas in geology's prelude to the Cambrian explosion. *Earth-Science Rev.* **77,** 1-229 (2006).

<bibcit> 6. Kirschvink, J.L. in *The Proterozoic Biosphere: A Multidisciplinary Study* (eds Schopf, J.W. & Klein, C.) 51-52 (Cambridge University Press, 1992).

<bibcit> 7. Hoffman, P.F. & Schrag, D.P. Snowball Earth. *Sc. Am.* **282**, 68-75 (2000).

<bibcit> 8. Schulze-Makuch, D. & Irwin, L.N. Energy cycling and hypothetical organisms in Europa's ocean. *Astrobiology* **2**, 105-121 (2002).

<bibcit> 9. Muller, A.W.J. & Schulze-Makuch, D. Thermal energy and the origin of life. *Origins of Life Evol. Biospheres* **36**, 77-189 (2006).

<bibcit> 10. Muller, A.W.J. in *ISSOL 2008. Program and Book of Abstracts* (eds Branciamore, S., Fondi, M. & Graziani, L.) 184 (ISSOL, 2008).

<bibcit> 11. Muller, A.W.J. Emergence of Animals from Heat Engines. Part 1. Before the Snowball Earths. Preprint at: <http: //www. arxiv.org/abs/0811.1375> (2008).

<bibcit> 12. Muller, A.W.J. Were the first organisms heat engines? A new model for biogenesis and the early evolution of biological energy conversion. *Prog. Biophys. Mol. Biol.* **63**, 193-231 (1995).

<bibcit> 13. Muller, A.W.J. Thermosynthesis as energy source for the RNA world: A model for the bioenergetics of the origin of life. *BioSystems* **82**, 93-102 (2005).

<bibcit> 14. Baross, J.A. & Hoffman, S.E. Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. *Origins of Life* **15**, 327-345 (1985).

<bibcit> 15. Margulis, L. *Symbiosis in Cell Evolution* (Freeman, 1981).

<bibcit> 16. Margulis, L. *Origin of Eukaryotic Cells* (Yale University Press, 1970).

<bibcit> 17. Sapp, J. *Evolution by Association* (Oxford University Press, 1994).

<bibcit> 18. Purcell, E.M. Life at low Reynolds number. *Am. J. Physics* **45**, 3-11 (1977).

<bibcit> 19. Moczydlowska, M. The Ediacaran microbiota and the survival of Snowball Earth conditions. *Precambrian Res.* **167**, 1-15 (2008).

<bibcit> 20. Boyer, P.D. The ATP synthase — a splendid molecular machine. *Ann. Rev. Biochem.* **66**, 717-749 (1997).

<bibcit> 21. Young, G.M., von Brunn, V., Gold, D.J.C. & Minter, W.E.L. Earth's oldest reported glaciation; physical and chemical evidence from the Archean Mozaan Group (~2.9 Ga) of South Africa. *J. Geol*. **106**, 523-538 (1998).

<bibcit> 22. Evans, D.A., Beukes, N.J. & Kirschvink, J.L. Low-latitude glaciation in the Palaeoproterozoic era. *Nature* **386**, 262-266 (1997).

<bibcit> 23. Leong, T.G. *et al*. Tetherless thermobiochemically actuated microgrippers. *Proc. Natl Acad. Sci. USA* **106**, 703-708 (2009).

<bibcit> 24. Brandts, J.F. in *Thermobiology* (ed. Rose A.H.) 25-72 (Academic Press, 1967).

<bibcit> 25. Todgham, A.E., Hoaglund, E.A. & Hoffman, G.E. Is cold the new hot? Elevated ubiquitin-conjugated protein levels in tissues of Antarctic fish as evidence for cold-denaturation of proteins in vivo. *J. Comp. Physiol. B* **177**, 857-866 (2007).

<bibcit> 26. Casadesus, J. & Low, D. Epigenetic gene regulation in the bacterial world. *Microbiol. Mol. Biol. Rev.* **70**, 830-856 (2006).

<bibcit> 27. McConnaughey, T.A. & Whelan, J.F. Calcification generates protons for nutrient and bicarbonate uptake. *Earth-Science Rev.* **42**, 95-117 (1997).

<bibcit> 28. Agar, J.N. Thermogalvanic cells. *Adv. Electrochem. Electroeng.* **3**, 31-121 (1963).

<bibcit> 29 . Yin, L. *et al*. Doushantuo embryos preserved inside diapause egg cysts. *Nature* **446**, 661-663 (2007).

<bibcit> 30. Kashtan, N., Noor, E. & Alon, U. Varying environments can speed up evolution. *Proc. Natl Acad. Sci. USA* **104**, 13711-13716 (2007).

<supp>**Supplementary Information** accompanies the paper on **www.nature.com/nature**.

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<LEGEND> **Figure 1 | The Snowball Earths of the late Proterozoic and the Ediacaran organisms. a,** Photosynthesis prefers the faster diffusing ^{12}C isotope of carbon in CO_2. Subduction of the ocean floor removes the ^{12}C enriched remains of organisms from the biosphere, leaving the ^{13}C. During a low global photosynthesis rate, such as during a global glaciation, less ^{12}C is removed and carbonate precipitated on the ocean floor then contains a lower ^{13}C /^{12}C ratio. Global glaciations inferred from isotope ratios in carbonate deposits are named the Sturtian, Marinoan and the Varanger. At the beginning of the Cambrian a global glaciation occurred as well. The fossils of the first animals are found right after the Varanger; fossils resembling animal embryos are found at the end of the Varanger but also already at the end of the Marinoan. **b,** Macroscopic animal fossils found in the Ediacaran comprise the fern-resembling fronds and anemones. The sizes of the fossils range from a few cm to almost 2 m.

<LEGEND> **Figure 2** | **Thermosynthesis mechanisms. a**, Thermosynthesis[12] is based on a thermal variation of the binding change mechanism of today's ATP Synthase[20]. Its β-subunit binds ADP and phosphate, and forms ATP that remains bound until free energy generated by a charged membrane opens the enzyme and enables the ATP to leave. **b**, In the proposed First Protein (FP) bound ATP was similarly formed but it was instead released by thermal unfolding, either at high or at low temperature (hot or cold denaturation). During the origin of life this condensation was non-specific, and also yielded peptide and phosphodiester bonds[12,13]. **c**, The required repeated thermal cycling was effected by suspension in a convecting volcanic hot spring. In this cartoon the FP unfolds by hot denaturation. **Sessile thermosynthesizers.** Oscillation in a thermal gradient instead of convection drove thermal cycling. **d, Operating cycle of the thermotether.** Attached to the hydrothermal vent, the thermotether at high temperature folded into a compressed state, expanded, and made thermal contact with the cold ocean water, whereafter it denatured again and contracted. **e** Connection of a cell containing FPs to the thermotether yielded a thermally cycled, and thermosynthesizing, cell. **Emergence, at increasing size, of the Ediacaran frond. f,** Addition of a calcified holdvast to the thermotether. **g,** Addition of a bending stalk yielded a larger and stronger connection between the holdfast and the main body, permitting a wider range of oscillating movement. **h,** Connection of the thermotether to the stalk further enhanced the movement range. **i,** Addition of a fractally grown structure yielded a larger organism, the 'thermofrond'. **j,** The Ediacaran frond emerged when the thermal cycling requirement became superfluous at the end of the glaciation and movement became ATP driven. Small symbiotic sponges —derived from the thermopharynx— had been added. **k, Operating cycle of the thermopharynx.** At the start the entry to the body cavity or sac was closed after the protein in the thermopharynx had contracted by cold denaturation. The cold water in the cavity warmed up, where after the thermopharynx also warmed, the entry protein folded and the entry opened. The warm water in the cavity was replenished by cold ocean water, and the entry closed as the entry protein cooled, unfolded and contracted. The organism resembled a sponge. **l, Addition of thermotentacles. m, Operating**

cycle of the thermotentacle. The hanging thermotentacle worked on *hot* denaturation (collagen seems suitable protein). In the cold ocean it folded and extended. When extended it made contact with the warm site, unfolded and contracted again.

<LEGEND> **Figure 3 | Animal emergence in the Ediacaran (overview).** Microscopic protists emerged during the Varanger that oscillated in the thermal gradient above a submarine hydrothermal vent. The *thermotether, thermopharynx* and *thermotentacle* resulted in thermal cycling of First Proteins that sustained thermosynthesis in the temperature gradient. A larger span of the thermal gradient is beneficial for thermosynthesis: this yielded the evolutionary driving force for larger, macroscopic organisms, which in turn evolved into the sponge, polyp and anemone of the Ediacaran, after sunlight, photosynthesis and respiration had returned. Some of the emerged organisms consisted of symbionts: the polyp resembles a combination of a thermofrond and multiple thermopharynx-like sacs.

<LEGEND> **Figure 4 | Animal emergence in the Cambrian.** Emergence of a Cambrian animal by symbiogenesis of macroscopic progenitors that constituted its organs and that previously, during the early Cambrian or the Ediacaran, had lived on thermosynthesis; at the time of the emergence these progenitors lived however on respiration and used mitochondria. The numerous possible combinations for symbiogenesis yielded the large number of animal species that emerged during the Cambrian explosion.

<TBLTTL> Table 1 | Correlation of extensive glaciations, thermosynthesis-based preadaptations and following emerged movement capabilities.

<TBLROW>

time of glaciation	Geological period	Thermosynthesis-based preadaptations	Emerged organisms with novel movement capabilities

<TBLROW>

2900 Mya[21]	Archean	external thermotether	Prokaryotes with bacterial flagellar motor

<TBLROW>

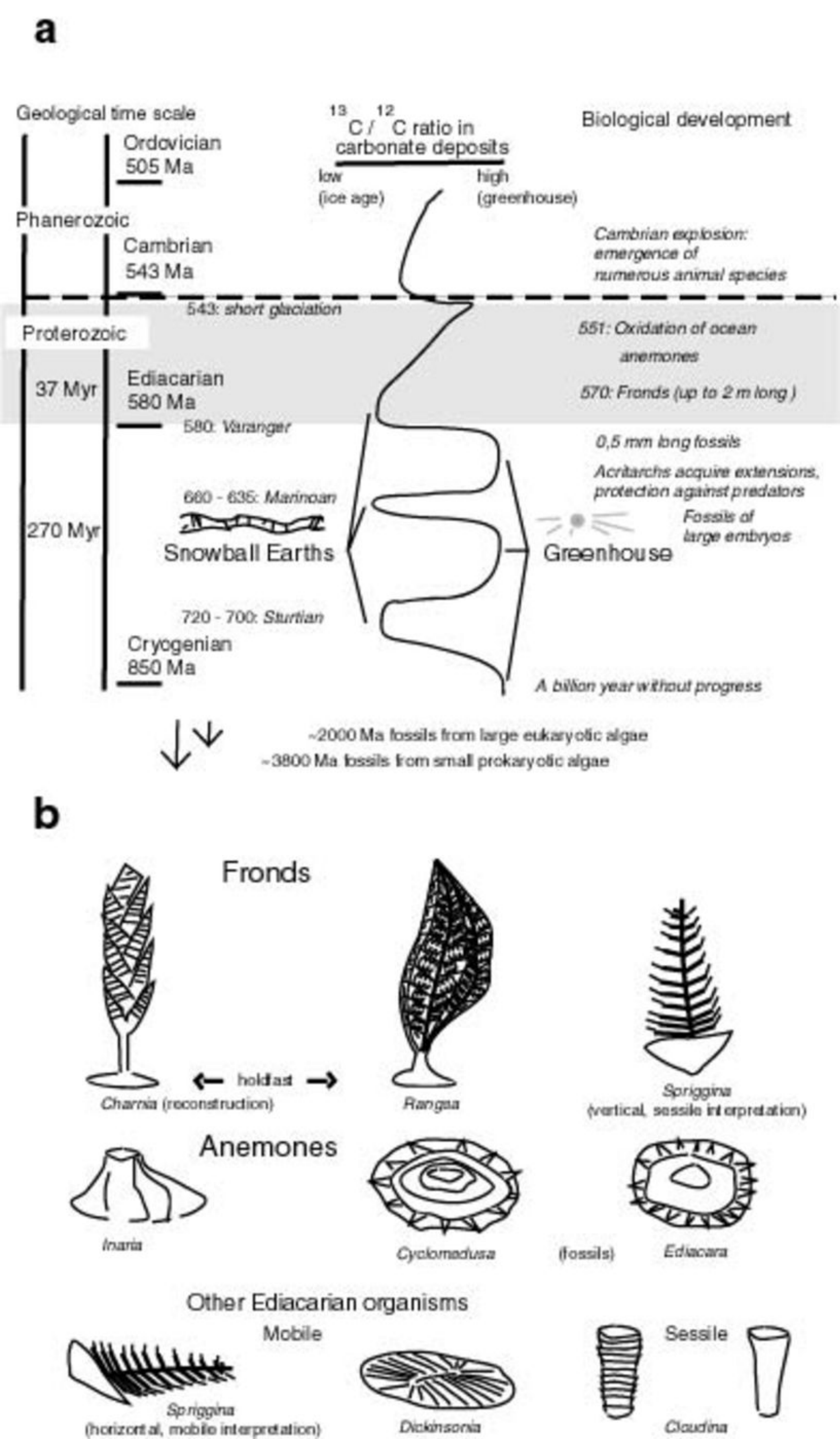
2900 Mya[21]	Archean	external thermotether	Prokaryotes with bacterial flagellar motor

<TBLROW>

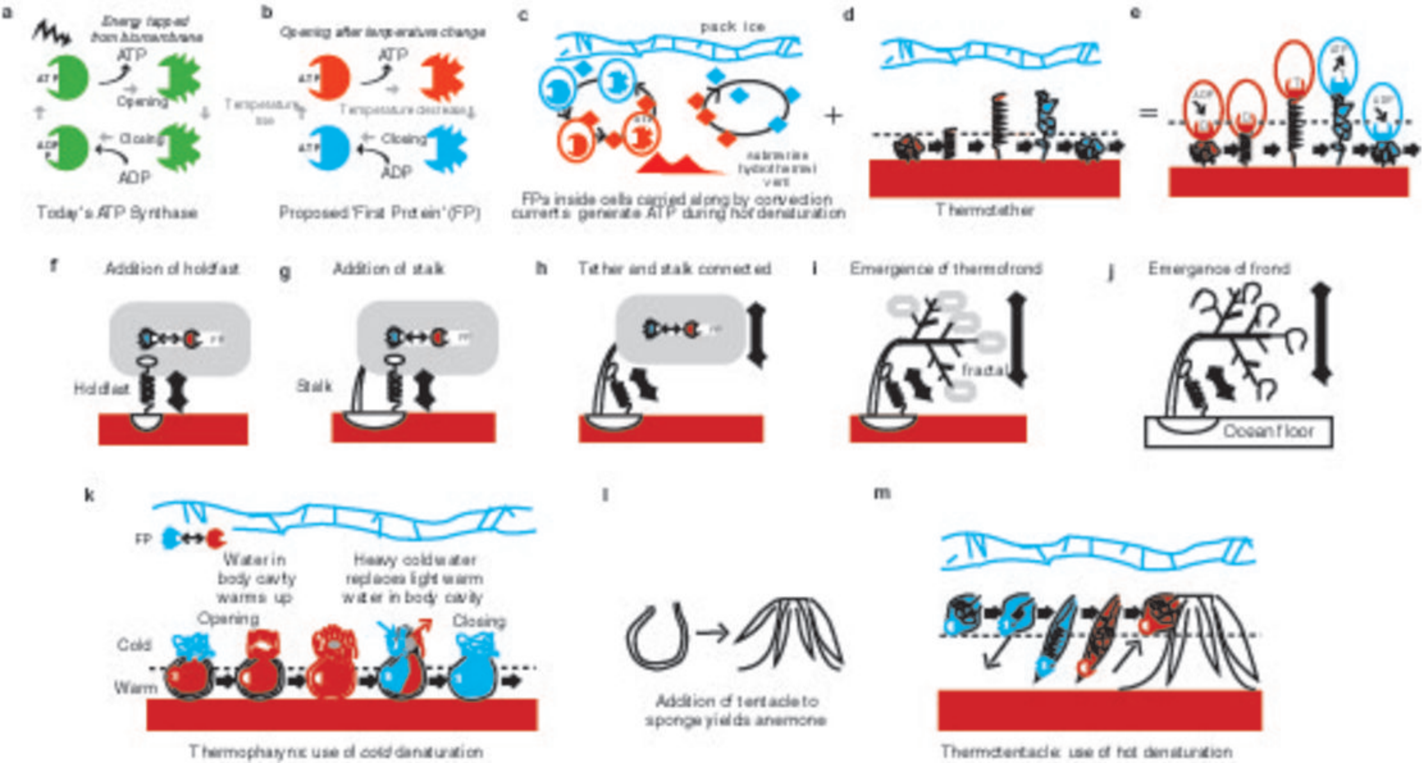
720-580 Mya	Late Proterozoic	thermofrond, thermopharynx and thermotentacle	Coelenterates with large body cavity and tentacles

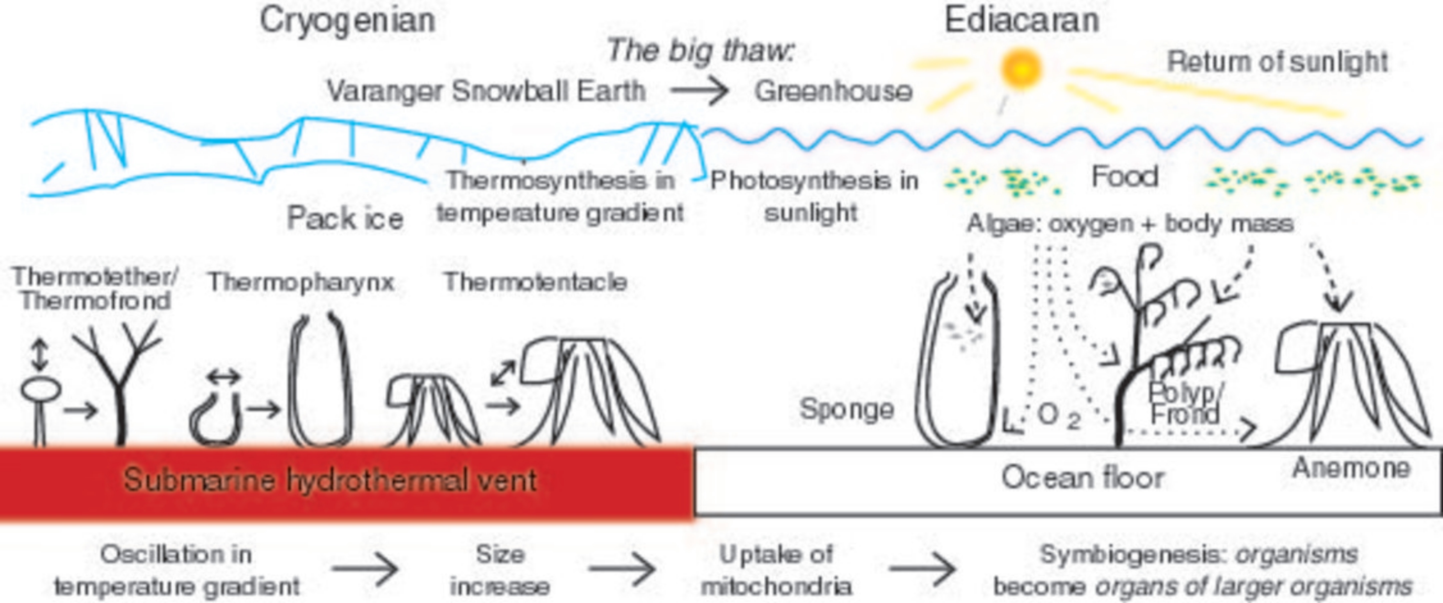
<TBLROW>

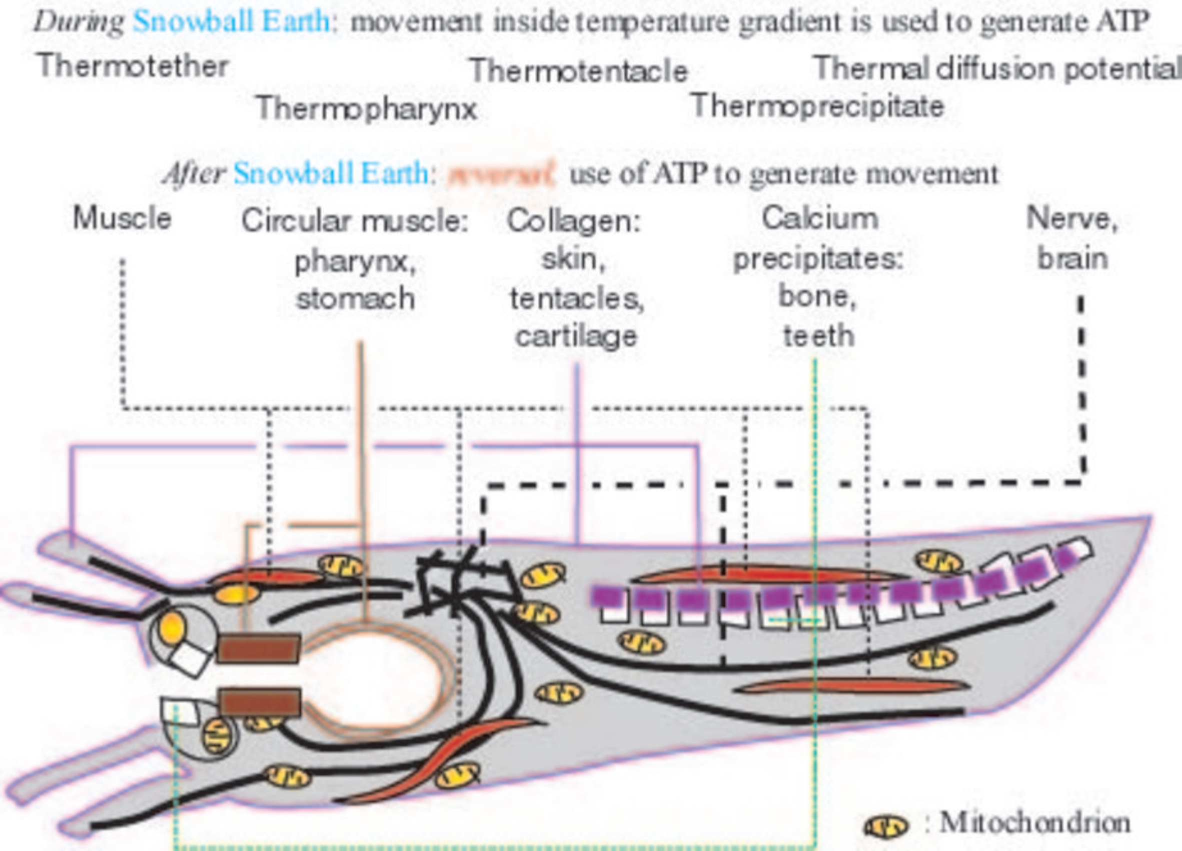
543 Mya	Cambrian	thermoprecipitate and use of thermal diffusion potential	Large animals with large moving organs: ‘The Cambrian explosion’



a
Geological time scale
Ordovician
505 Ma
Phanerozoic
Cambrian
543 Ma
Proterozoic
37 Myr
Ediacarian
580 Ma
270 Myr
Cryogenian
850 Ma
13 C / 12 C ratio in carbonate deposits
low (ice age)
high (greenhouse)
543: short glaciation
580: Varanger
660 - 635: Marinoan
Snowball Earths
Greenhouse
720 - 700: Sturtian
Biological development
Cambrian explosion: emergence of numerous animal species
551: Oxidation of ocean
anemones
570: Fronds (up to 2 m long)
0,5 mm long fossils
Acritarchs acquire extensions, protection against predators
Fossils of large embryos
A billion year without progress
~2000 Ma fossils from large eukaryotic algae
~3800 Ma fossils from small prokaryotic algae
b
Fronds
holdfast
Charnia (reconstruction)
Rangea
Spriggina (vertical, sessile interpretation)
Anemones
Inaria
Cyclomedusa
(fossils)
Ediacara
Other Ediacarian organisms
Mobile
Sessile
Spriggina (horizontal, mobile interpretation)
Dickinsonia
Cloudina


a
Energy tapped from biomembrane
ATP
Opening
Closing
ADP
Today's ATP Synthase
b
Opening after temperature change
ATP
Temperature rise
Temperature decrease
Closing
ADP
Proposed 'First Protein' (FP)
c
pack ice
submarine hydrothermal vent
FPs inside cells carried along by convection currents generate ATP during hot denaturation
+
d
Thermotether
=
e
f
Addition of holdfast
Holdfast
FP
g
Addition of stalk
Stalk
h
Tether and stalk connected
i
Emergence of thermofrond
fractal
j
Emergence of frond
Ocean floor
k
FP
Water in body cavity warms up
Heavy cold water replaces light warm water in body cavity
Opening
Closing
Cold
Warm
Thermopharynx use of cold denaturation
l
Addition of tentacle to sponge yields anemone
m
Thermotentacle use of hot denaturation


Cryogenian
Ediacaran
The big thaw:
Varanger Snowball Earth → Greenhouse
Return of sunlight
Pack ice
Thermosynthesis in temperature gradient
Photosynthesis in sunlight
Food
Algae: oxygen + body mass
Thermotether/ Thermofrond
Thermopharynx
Thermotentacle
Sponge
O 2
Polyp/ Frond
Anemone
Submarine hydrothermal vent
Ocean floor
Oscillation in temperature gradient → Size increase → Uptake of mitochondria → Symbiogenesis: organisms become organs of larger organisms


During Snowball Earth: movement inside temperature gradient is used to generate ATP
Thermotether
Thermotentacle
Thermal diffusion potential
Thermopharynx
Thermoprecipitate
After Snowball Earth: *reversal* use of ATP to generate movement
Muscle
Circular muscle: pharynx, stomach
Collagen: skin, tentacles, cartilage
Calcium precipitates: bone, teeth
Nerve, brain
: Mitochondrion