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HOW WAS THE METAZOA THRESHOLD CROSSED: THE URMETAZOA

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ABSTRACT

The transition from unicellular to multicellular organisms occurred in three kingdoms of life: in Fungi, Plantae and in Metazoa. Sponges [Porifera] as "living fossils" positioned at the base of multicellular animals have been used to answer basic questions in metazoan evolution by molecular biological techniques. During the last few years cDNAs/genes coding for proteins have been isolated and characterized from sponges, especially from the marine demosponges *Suberites domuncula* and *Geodia cydonium*. The analyses of their deduced amino acid sequences allowed a molecular biological resolution of the monophyly of Metazoa. The hypothetical ancestral animal, the URMETAZOA from which the metazoan lineages diverged (600 MYA) may have had the following characteristics: Cell adhesion molecules with intracellular signal transduction pathways - morphogens/growth factors forming gradients - a functional immune system - a primordial nerve cell/receptor system.

Key words: evolution; Metazoa; Urmetazoa; molecular phylogeny.

KAKO JE PREĐEN PRAG METAZOA: PRAMETAZOA

REZIME

Prelaz od jednoćelijskih do višećelijskih organizama desio se u tri carstva: Kod Fungi, Plantae i Metazoa. Sunđeri [Porifera] kao "živi fosili" smešteni su na osnovi višećelijskih organizama, korišćeni su da se odgovori na osnovna pitanja evolucije metozoa tehnikama molekularne biologije. Tokom poslednjih nekoliko godina izolovani su i okarakterisani iz sunđera, pre svega morskih demospongia *Suberites domuncula* i *Geodia cydonium,* cDNA/geni koji kodiraju proteine. Hipotetički predak životinja PRAMETAZOA, od kojeg su divergirale loze metazoa (600 MYA) mogao je imati sledeće karakteristike: molekuli ćelijske adhezije sa intracelularnim putevima transdukcije signala, morfogeni/faktori rasta koji prave gradijente - funkcionalni imuni sistem - prvobitna nervna ćelija - receptroski sistem.

Ključne riječi: evolucija, Metazoa, Prametazoa, molekularna filogenija

INTRODUCTION

It had been proposed that multicellularity has arisen several times in evolution and occurred independently in all major eukaryotic kingdoms; in Viridiplantae, Fungi, and Metazoa. Evidence has been presented indicating that multicellular plants, the red algae, the brown algae, the land plants and the yeasts arose separately from different unicellular ancestors. This view had been adopted also for the origin of Metazoa which is thought to lie in the colonies of Choanoflagellata. This conclusion based primarily on palaeontological, morphological and embryological data.

Applying molecular biological techniques and studying nucleotide sequences from sponges, coding for informative molecules (in contrast to rDNA sequences), it was possible to establish experimentally that all metazoan phyla, including the sponges [Porifera], evolved from a common ancestor. This conclusion, the monophyly of Metazoa, which resolved one major enigmatic problem in phylogeny was supported subsequently also by other groups. Since the data compiled in the last few years contributed already to a close insight into the functions of signal transduction pathways, cell adhesion molecules and immune molecules in Porifera – which showed close homology/similarity with the related molecules of higher metazoan phyla – we propose a new name for the common ancestor of all Metazoa, the URMETAZOA (Figure 1).

GEOLOGY OF EARLY METAZOA

Multicellularity of Viridiplantae, Fungi and Metazoa arose in the Proterozoic approximately 1,000 million years ago [MYA]. Focusing on the earliest Metazoa, the sponges, it is documented that the major poriferan taxa, the Hexactinellida, Demospongiae and Calcarea, existed already since the Early Cambrian (Atdabatian). Even though it might be assumed that Porifera are not the first metazoan phylum which evolved, they are witnesses of an evolutionary step that occurred during the maturation of the Metazoa near the Proterozoic-Phanerozoic boundary, close to 1,000 MYA. In this respect they can be considered as living fossils. In the last years our group has analyzed genes of sponges in order to obtain an insight into the genome organization as well as the function of those genes coding for functional proteins. Genes from Demospongiae, *Suberites domuncula* and *Geodia cydonium*, from Calcarea, *Sycon raphanus*, as well as from Hexactinellida, *Aphrocallistes vastus*, have been analyzed in detail, also with the aim to describe the hypothetical ancestral Metazoa, the URMETAZOA.

A COMMON EARLY MULTICELLULAR ANCESTOR? THE EVOLUTIONARY NOVELTIES

We attempted to select those molecules for phylogenetic analyses which seem to be restricted to multicellularity, and to evolutionary novelties, which had to be invented during the transition of uni- to multicellular organisms. Such novelties comprise those molecules which protect cells of multicellular organisms against the extracellular milieu, composed of the extracellular matrix [ECM] and/or adjacent cells: *(i)* extracellular structures to allow the organization of individual cells in a complex organism, *(ii)* cell adhesion to facilitate contact to the environment and *(iii)* intracellular signaling for a tuned communication between the individual cells and between the cells and their matrix environment.

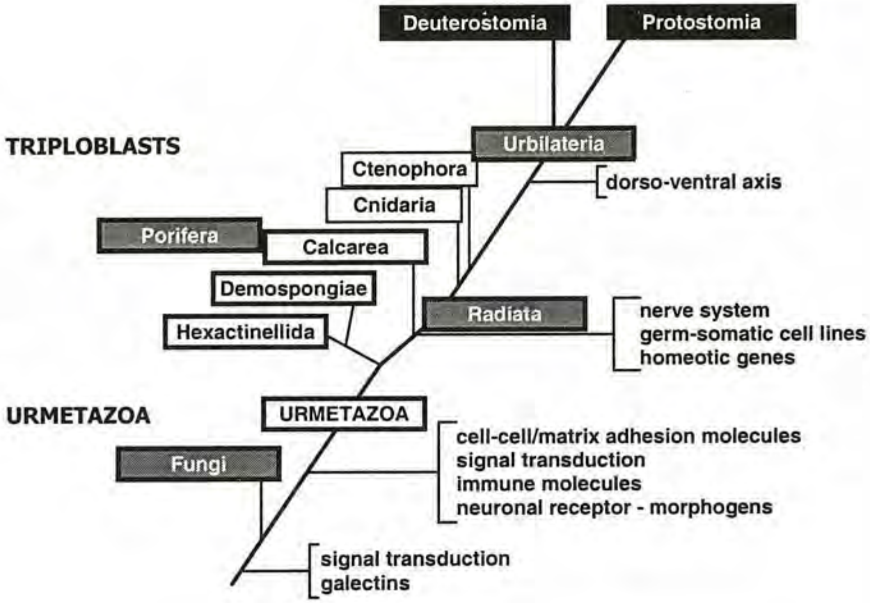

Figure 1. Postulated evolution of Urmetazoa from a common ancestor with Fungi. It is proposed that the multicellular Fungi/Urmetazoa evolved after the establishment of signal transduction- and adhesion molecules. The invention of new proteins, novelties, including cell-cell/matrix-, signal transduction-, immune-, neuronal- and morphogenetic molecules, which is proposed to be present in the hypothetical metazoan ancestor, the Urmetazoa, allowed the branching into the Porifera and later to the

Radiata and the Urbilateria, a lineage which further split into the Protostomia and Deuterostomia.

A series of molecules has been analyzed by us from the three poriferan classes and detailed analyses of housekeeping proteins, e.g. heat shock protein or ß-tubulin, and proteins involved in signal transduction, e.g. Ser/Thr kinase or calmodulin, revealed that among the three classes of Porifera the Hexactinellida are the phylogenetically oldest taxon, while Calcarea is the class closest related to higher metazoan phyla.

Based on calculations described earlier, it has been outlined that the transition to multicellularity took place about 1,000 MYA; later in evolution the green algae evolved, 700 MYA, while sponges, with the first metazoan fossils, have been dated back at least to 580 MYA. Hence, sponges lived more than 30 to 50 million years before the Cambrian Explosion, the time of main divergence of metazoan phyla. This conclusion is supported by calculations based on the extent of aa substitutions of two galectins from *G. cydonium*. It had been found that these molecules diverged from the galectin isolated from the nematode *C. elegans* 800 MYA. Further on, data, especially from studies with a series of Ser/Thr kinases suggested that the Calcarea might be sister groups to higher metazoan phyla. The branching order originating from ancestral unicellular eukaryotes via Viridiplantae-Fungi to Porifera, the simplest metazoans, follows both the published fossil data and the sequence data obtained (Figure 1).

MONOPHYLY OF FRESHWATER SPONGES

The phylogenetic position of the sponges [Porifera] with respect to other metazoan taxa could be resolved by the study of genes encoding informative proteins which established a monophyletic origin. Focusing on freshwater sponges a similar clarification of the evolutionary relationship remains to be determined. An interesting approach to solve the evolutionary origin of the freshwater sponges are studies on endemic freshwater sponges especially from geologically old biotopes. The Lake Baikal is famous because of its high biodiversity, especially with respect to its freshwater sponges. This lake is the oldest (>24 million years), deepest (1,637 m) and most voluminous lake on earth, comprising one fifth of the world's unfrozen freshwater; it homes more than 1,500 endemic species. Recently, we have undertaken a clarification of the phylogenetic relationship of selected species from the two Baikalian sponge families Lubomirskiidae (Genus Lubomirskia: *Lubomirskia baicalensis*, Genus Baikalospongia: *Baikalospongia intermedia*, *Baikalospongia recta* and *Baikalospongia bacillifera*, Genus Swarchewskia: *Swarchewskia papyracea*) and Spongillidae (Genus Spongilla: *Spongilla lacustris*). Two new initiatives have been made to resolve the phylogenetic relationship; analyses of *(i)* nucleotide sequences from one mitochondrial gene, the cytochrome oxidase subunit I (COI), and of *(ii)* one selected intron of the tubulin gene. The sequence comparison of the mitochondrial COI gene revealed a monophyletic grouping of the endemic Baikalian sponges with *S. lacustris* as the most related species to the common ancestor. The sequences of the COI gene from *B. recta*, *B. intermedia*, *B. bacillifera* and *L. baicalensis* were found to be identical and separated from that from *S. lacustris* and *S. papyracea* (Figure 2A). In the second study the exon/intron sequences framing the intron-2 of the sponge tubulin gene were chosen for the phylogenetic analysis. The intron sequences were aligned and used to construct a phylogenetic tree. This analysis revealed again a monophyletic grouping with *S. lacustris* as the closest related species to the common ancestor (Figure 2B). It is concluded that the Baikalian sponges are of monophyletic origin; the data suggest that the endemic species *S. papyracea* is the phylogenetic oldest still extant endemic Baikalian sponge species. At present we are working on the phylogenetic relationship of another interesting freshwater sponge, *Ochridaspongia rotunda* (Walter Arndt, 1937), from Lake Ohrid (Figure 3).

CONCLUSION: SPONGES ARE REFERENCE ANIMALS FOR THE URMETAZOA

With the establishment of the monophyly of all Metazoa it appears to be timely to formulate the common features of the metazoan ancestor. The diploblastic Metazoa – phyla Cnidaria and Ctenophora – from which the triploblastic Metazoa, the URBILATERIA evolved, are characterized by true epithelial layers. Besides on expanding systems of signaling proteins and transcription factors, e.g. the *Para/Hox* clusters (homeobox genes) that pattern the main body axis, the organization of these animals bases on those building blocks, which are already present in Porifera, e.g. cell surface receptors. One further feature of the diploblastic Metazoa is the novel acquisition of developmental characters that distinguish between germ- and somatic cells.

The major novelties that characterize the hypothetical ancestral animal of Metazoa, the URMETAZOA, from which first the Porifera and subsequently the diploblastic- and triploblastic Metazoa diverged (1,000 to 600 MYA), are the following: extracellular matrix molecules, transmembrane adhesion molecules, the G-protein linked transmembrane receptors, receptor tyrosine kinases, morphogens, extracellular matrix, neuronal receptors and immune molecules. Taken together, the overwhelming molecular evidence indicates that during the transition to Metazoa a qualitative set of novelties has emerged which were used as a repertoire for the evolution into the different metazoan phyla including sponges; these novelties are characteristics of the metazoan animal ancestor, the URMETAZOA.

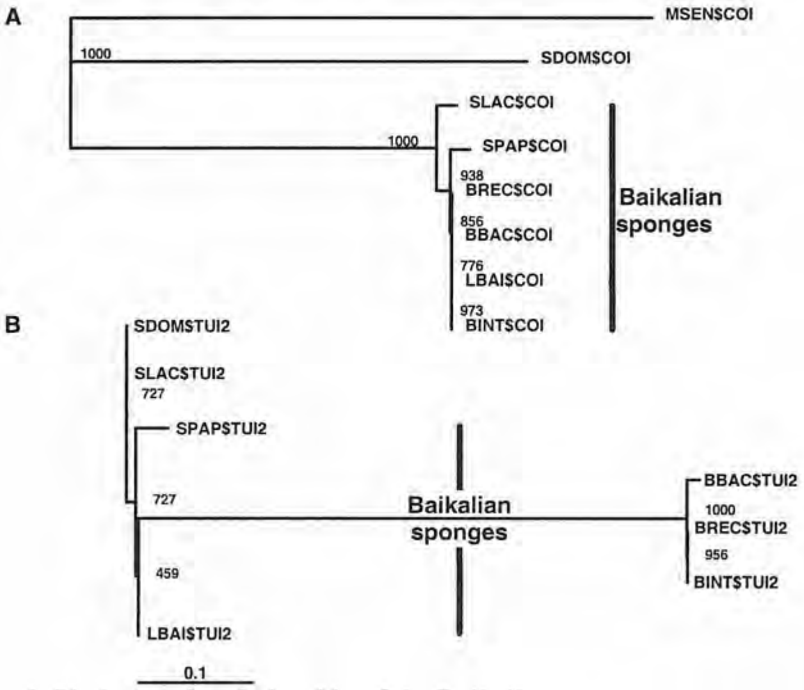

Figure 2. Phylogenetic relationship of the Baikalian sponges.

A. Relationship based on the nt sequence comparison of the mitochondrial cytochrome oxidase subunit I (COI) gene. The 676 nt long sponge segments of the COI from *(i)* the freshwater sponges *S. lacustris* (SLAC$COI), *L. baicalensis* (LBAI$COI), *B. intermedia* (BINT$COI), *B. recta* (BREC$COI), *B. bacillifera* (BBAC$COI), *S. papyracea* (SPAP$COI), *(ii)* the marine demosponge *Suberites domuncula* (SDOM$COI) and from *(iii)* the brown sea anemone *Metridium senile* [Cnidaria; Anthozoa] (MSEN$COI; U36783 were aligned. The phylogenetic tree was constructed and the coral sequence from *M. senile* was used as an outgroup.

B. Relationship based on the nt sequence of the partial tubulin genes, comprising intron-2 from *S. domuncula* (SDOM$TUI2) in comparison with the corresponding sequences from *S. lacustris* (SLAC$TUI2), *L. baicalensis* (LBAI$TUI2), *B. intermedia* (BINT$TUI2), *B. bacillifera* (BBAC$TUI2), *B. recta* (BREC$TUI2) and *S. papyracea* (SPAP$TUI2). The tree was rooted using the sequence from *S. domuncula* (SDOM$TUI2) as outgroup.

Figure 3. Skeleton from the freshwater sponge, *Ochridaspongia rotunda*, from Lake Ohrid; collected at Struga. Size of the animal: 5 cm.

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