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Evolutionary relationship of Porifera within the eukaryotes

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Abstract

Molecular data, gathered in our laboratory, strongly support the view that all metazoan phyla, including Porifera, are of monophyletic origin. More recently, a hypothetical common ancestor for all Metazoa, the Urmetazoa, had be formulated. The Urmetazoa possessed besides the key structures of an extracellular matrix also immune molecules and signal transduction molecules that are characteristic to Metazoa. In addition, we could establish that all Metazoa share a common ancestry with the Fungi and Plantae, while the unicellular eukaryotes are only distantly related. With respect to the origin of freshwater sponges, especially the species found in the Lake Baikal as a representative for an "old" lake, have been investigated. Sequence comparisons were performed with the ubiquitously distributed freshwater sponge *Spongilla lacustris* (family Spongillidae) as well as with one marine sponge, *Suberites domuncula*. 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 from *Baikalospongia recta, B. intermedia, B. bacillifera* and *Lubomirskia baicalensis* were found to be identical and separated from that of *Swartschewskia lacustris* and *S. papyracea*. In a further approach the exon/intron sequences framing the intron-2 of the sponge tubulin gene were chosen for the phylogenetic analysis. Data analyses revealed again a monophyletic grouping with *S. lacustris* as the closest related species to the common ancestor. It is concluded that the baikalian sponges, which have been studied here, are of monophyletic origin. Furthermore, the data suggest that the endemic species *S. papyracea* is the phylogenetic oldest still extant endemic baikalian sponge species.

Introduction

With the introduction of molecular data based on analyses of nucleotide [nt] sequences, coding for proteins, it became evident that the phylum Porifera (sponges) is with the other metazoan phyla of monophyletic origin. The molecules which most strongly suggest that Porifera have to be included in the Metazoa are those which are constituent elements (i) of the basal lamina, e.g. integrin receptor (Pancer et al., 1997a), fibronection (Pahler et al., 1998) and polypeptides, rich in scavenger SRCR and SCR modules (Pahler et al., 1998), (ii) of neuronal transmission, e.g. metabotropic glutamate receptor (Perovic et al., 1999), as well as (iii) homologs/modules of an primordial immune system, e.g. immunoglobulin-like molecules (Schäcke et al., 1994a), SRCR- and SCR-repeats (Pancer et al., 1997b) or the Rhesus system (Seack et al., 1997), and (iv) cell surface receptor molecules, e.g. receptor tyrosine kinases (Schäcke et al., 1994b). The cDNAs have been isolated – and some of them also expressed – from all classes of Porifera, mainly from the Demospongiae *Geodia cydonium* and *Suberites domuncula*, but also from Calcarea, *Sycon raphanus* and from Hexactinellida, *Rhabdocalyptus dawsoni*. Based on these molecular data it is reasonable to the accept that multicellular animals

evolved only once (monophyly), as first published in 1994 (Müller et al., 1994) and later in detail (Müller, 1995, 1998). This conclusion drawn from analyses of protein-coding nt sequences has been recently supported also by rRNA ribosomal data (Cavalier-Smith et al., 1996).

Unlike with Metazoa the monophyletic origin of Plantae appears to be established since a longer time; they can be traced back to the Chlorophyta (reviewed in: Margulis & Schwartz, 1995). The ancestry of the third group of multicellular eukaryotes, the Fungi, is less clear; monophyletic and polyphyletic evolution of these spore forming and amastigote organisms are discussed (in Margulis & Schwartz, 1995).

We addressed for the first time the phylogenetic relationship of the three multicellular eukaryotic subkingdoms to unicellular eukaryotes, putting the main emphasis on the position of sponges. Deduced amino acid [aa] sequences of cDNAs from sponges coding for proteins, found in all representatives of these subkingdoms have been analyzed. The data presented (Schütze et al., 1999) show that the sponge molecules, especially those obtained from the classes Demospongiae and Calcarea form the basis of the metazoan branch. Furthermore, the results reveal that the Metazoa appeared later during evolution from the unicellular eukaryotes than the Fungi and the Plantae (Viridiplantae).

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. Lake Baikal is famous because of its high biodiversity, especially with respect to its freshwater sponges (Rezvoi, 1936). This lake is the oldest (> 24 million years), deepest (1637 m) and most voluminous lake on earth, comprising one fifth of the world's unfrozen freshwater; it contains more than 1500 endemic species (Stewart, 1990).

Phylogenetic position of sponges: the Urmetazoa

The aim of a previous study (Schütze et al., 1999) was the evaluation of the phylogenetic relationship of the three multicellular eukaryotic subkingdoms to unicellular eukaryotes, with the main interest in the position of sponges. Amino acid [aa] sequences found in all representatives of these subkingdoms, one heat shock protein, the serine/threonine (Ser/Thr) kinase domain of protein kinases, β-tubulin and calmodulin were analyzed together with those deduced from sponge cDNAs.

First, polypeptides grouped to the heat shock proteins [HSP] of the 70-kDa class, the HSP70, were investigated. Heat shock proteins are highly conserved throughout living kingdoms. These molecules act as molecular chaperones under physiological and stress conditions; the chaperones of prokaryotic organisms, which are related to the eukaryotic HSP70 multigene family are termed DnaK. They are divided according to their different inducibility into (i) the group of constitutively expressed heat shock proteins, which are also present under nonstressed conditions, and (ii) the group of HSP70 polypeptides, which are induced under temperature shock and other specific stress situations. The inducible, cytoplasmic HSP70 amino acid (aa) sequences were selected for the analysis (Koziol et al., 1996, 1998).

Second, the serine/threonine (Ser/Thr) kinase domain found in protein kinases from Fungi and Metazoa, but not in Plantae or unicellular eukaryotes (Hardie & Hanks, 1995; Kruse et al., 1997) was used for the analyses. These enzymes are essential for fungal or metazoan organisms to recognize extracellular signals and to initiate intracellularly appropriate adaptive biological responses.

Third, sequences of β-tubulin, one major element of microtubuli (intracellular structures that are employed for a number of functions in eukaryotes, including flagellar motility and chromosome aggregation as well as cell maintenance of cellular morphology) were analyzed (Schütze et al., 1999).

Finally, calmodulin, a protein ubiquitous in eukaryotes, was selected for phylogenetic analysis. It is a Ca^{2+} binding protein of approximately 150 aa residues that is involved in a wide range of intracellular signaling pathways. The molecule binds four Ca^{2+} ions in a cooperative fashion during which it undergoes a conformational change. The calmodulin sequence from *G. cydonium* was identified for the purpose of the phylogenetic analysis (Schütze et al., 1999).

The data on β-tubulin are summarized. Homology searches of the sponge deduced aa β-tubulin sequence TBB_GEOCY revealed highest similarity to the sequence from *Caenorhabditis elegans* (96% similar aa). Phylogenetic analysis was performed which showed that the β-tubulin molecules from Metazoa are more closely related to the tubulin sequences from Fungi or Plantae than to unicellular eukaryotes (Schütze et al., 1999); Figure 1. The rooted tree strikingly shows that the sponge sequence forms with the other metazoan molecules one branch (Fig. 1). The robust grouping separates the Metazoa from the Viridiplantae, the Fungi and the unicellular eukaryotes. As an outgroup for this analysis the bacterial cell-division protein FtsZ was used.

In conclusion, phylogenetic analyses of the four deduced aa sequences from sponges [HSP70, Ser/Thr kinase domain, β-tubulin and calmodulin], (i) support the findings indicating that all metazoans including the phylum Porifera are of monophyletic origin (Müller, 1995, 1998) and (ii) extend the view that the Metazoa are more closely related to other multicellular eukaryotes (Fungi) than to unicellular eukaryotes.

The formation of the extracellular matrix [ECM] is highly complex. Even though, only a few structural molecules have been identified until now in sponges, e.g. collagen, dermatopontin, and mucus-like protein, these examples are sufficient to document that sponge cells are embedded in a matrix which allows a reversible stabilization within the organism and provides the basis for an integrated cell communication (Müller, 2001). These fibrous proteins interact with cell surface receptors, e.g. the receptor tyrosine kinase(s), or integrins, cell surface molecules, which are restricted to Metazoa. The complex network of the secreted proteins that builds the extracellular space between the sponge cells is also a reservoir for morphogens, controlling cell growth and differentiation. Thus the sponges are provided with key molecules allowing the evolution of multicellularity. Even more, since the molecules display high sequence similarity/homology to the related molecules in other metazoan phyla, these facts also support the view that sponges have a common evolutionary origin together with the more complex metazoan animals. The hypothetical common ancestor for all Metazoa has been termed Urmetazoa (Müller, 2001). The major novelties, which can be attributed to the Urmetazoa, have been summarized (Müller, 2001).

The phylogenetic position of the three sponge classes

Until our studies the phylogenetic position of the class Hexactinellida within the phylum Porifera [sponges] could not be resolved by molecular data, because no cDNA encoding a protein from the sponge class Hexactinellida was available. We have isolated and characterized the cDNA encoding a protein kinase C belonging to the C subfamily (cPKC) from the hexactinellid sponge *Rhabdocalyptus dawsoni* (Kruse et al., 1998).

There have been several attempts to separate the Hexactinellida from other sponges at the subphylum (classes) level based on their remarkably different tissue structures. The most recent proposal, which resulted from extensive ultrastructural studies and the discovery that unlike cellular sponges *R. dawsoni* could propagate behaviourally meaningful events, suggested dividing the Porifera into the subphylum Cellularia, which includes the classes Demospongiae and Calcarea, and the subphylum Symplasma, with only one class, the Hexactinellida (Reiswig & Mackie, 1983). The fundamental structural difference between these sponges raises the question whether the ancestors of the Metazoa in general, and the Porifera in particular, were colonial flagellates (Hyman, 1940) or syncytial ciliates (Hanson, 1977). Two alternative hypotheses have been proposed to explain the relationships between the major sponge classes. One groups the Porifera into the adelphotaxa Hexactinellida and Demospongiae/Calcarea based on the gross difference in tissue structure and on differences in the structure of the flagella, whose beating generates the feeding current through sponges (Mehl & Reiswig, 1991). The other hypothesis assumes that the Demospongiae are more closely related to the Hexactinellida based on presumed larval similarities (Böger, 1988). There is, however, very little information available on hexactinellid larvae.

The rational solution was possible on the basis of aa sequence data for the protein kinase C. Prior

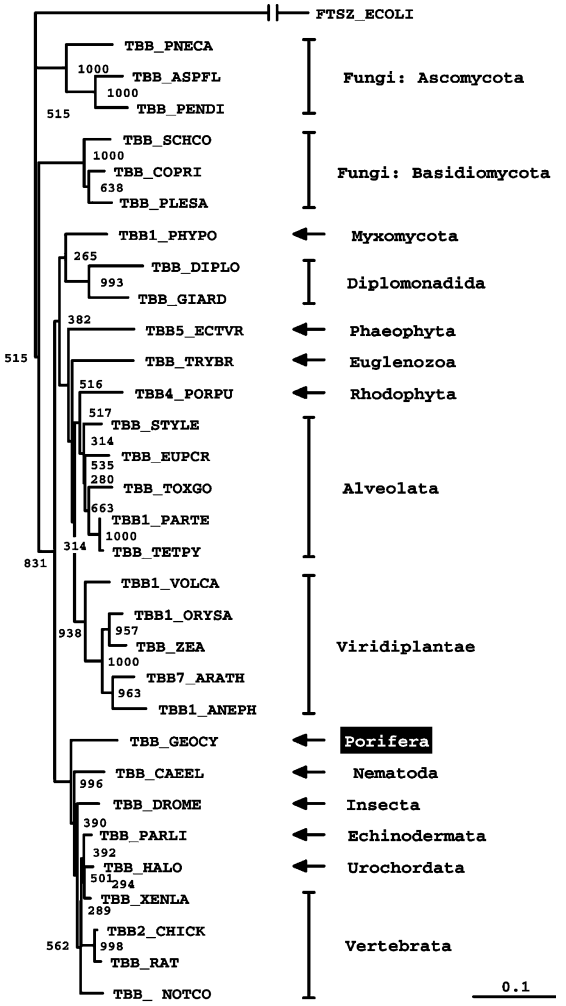

Figure 1. Phylogenetic analysis of deduced aa sequences of β-tubulin. The deduced aa sequence of the sponge β-tubulin from *G. cydonium* [TBB_GEOCY] was compared with the sequences from *I. unicellular eukaryotes:* (i) *Diplomonadida: Diplomonad* ATCC 50330 [TBB_DIPLO], *Giardia intestinalis* [TBB_GIARD], (ii) *Euglenozoa: Trypanosoma brucei* [TBB_TRYBR], (iii) *Alveolata: Stylonychia lemnae* [TBB_STYLE], *Euplotes crassus* [TBB_EUPCR], *Toxoplasma gondii* [TBB_TOXGO], *Paramecium tetraurelia* [TBB_PARTE], *Tetrahymena pyriformis* [TBB_TETPY], *II. Fungi:* (i) *Ascomycota: Pneumocystis carinii* [TBB_PNECA], *Aspergillus flavus* [TBB_ASPFL], *Penicillium digitatum* [TBB_PENDI], (ii) *Basidiomycota: Schizophyllum commune* [TBB_SCHCO], *Coprinus cinereus* [TBB_COPRI], *Pleurotus sajor-caju* [TBB_PLESA], *III. Myxomycota: Physarum polycephalum* [TBB_PHYPO], *IV. Plantae:* (i) *Phaeophyta Ectocarpus variabilis* [TBB_ECTVR], (ii) *Rhodophyta Porphyra purpurea* [TBB_PORPU], (iii) *Viridiplantae: Volvox carteri* [TBB_VOLCA], *Oryza sativa* [TBB_ORYSA], *Zea mays* [TBB_ZEA], *Arabidopsis thaliana* [TBB_ARATH], *Anemia phyllitidis* [TBB_ANEPH], *V. Metazoa:* (i) *Nematoda: Caenorhabditis elegans* [TBB_CAEEL], (ii) *Insecta: Drosophila melanogaster* [TBB_DROME], (iii) *Echinodermata: Paracentrotus lividus* [TBB_PARLI], (iv) *Urochordata: Halocynthia roretzi* [TBB_HALO], (v) *Vertebrata: Xenopus laevis* [TBB_XENLA], *Gallus gallus* [TBB_CHICK], *Rattus norvegicus* [TBB_RAT], *Notothenia coriiceps* [TBB_NOTCO]. The related sequence from the bacteria *Escherichia coli* [FTSZ_ECOLI] was used as outgroup. The numbers at the nodes refer to the level of confidence as determined by bootstrap analysis [1000 replicates]. Scale bar indicates an evolutionary distance of 0.1 aa substitutions per position in the sequence.

to our studies no cDNA coding for a protein had been cloned from the Hexactinellida, hence no phylogenetic analysis based on such molecules was possible. We have isolated a cDNA encoding one key enzyme involved in signal transduction pathways, a protein serine/threonine kinase (Nishizuka, 1992), from the hexactinellid sponge *R. dawsoni*. The analysis of the deduced aa sequence of this protein kinase C (PKC) belonging to the subfamily cPKC, using both the catalytic domain and the regulatory part of the enzyme, composed of the pseudosubstrate site, the Cys/His effector binding domains (zinc-fingers) and the C2 domain (Ca^{2+} specific domain), indicates that the Hexactinellida diverged earlier than either the Demospongiae or the Calcarea, from a common ancestor. In addition, it is shown that according to this analysis the cPKC from the Calcarea shares a common ancestor with protostome and deuterostome cPKCs.

Previous extensive comparative work with sequences of cPKC from Bacteria, Protozoa, Metazoa, and Planta (Hardie & Hanks, 1995) has shown that both regions of the kinase – the highly conserved part of the catalytic domain and the N-terminal end with the highly conserved regulatory blocks, which are interspersed by less conserved sequences – can be used separately for phylogenetic analyses. The data of alignment of the catalytic domains were compiled in a construction of (un)rooted trees. They included sequences for cPKC Ser/Thr-protein kinases from metazoans and from the yeast *Saccharomyces cerevisiae*. The metazoan sequences comprise sponge sequences isolated from (i) the demosponges *G. cydonium* and *S. domuncula*, (ii) the calcareous sponge *S. raphanus*, (iii) the hexactinellid sponge *R. dawsoni*,

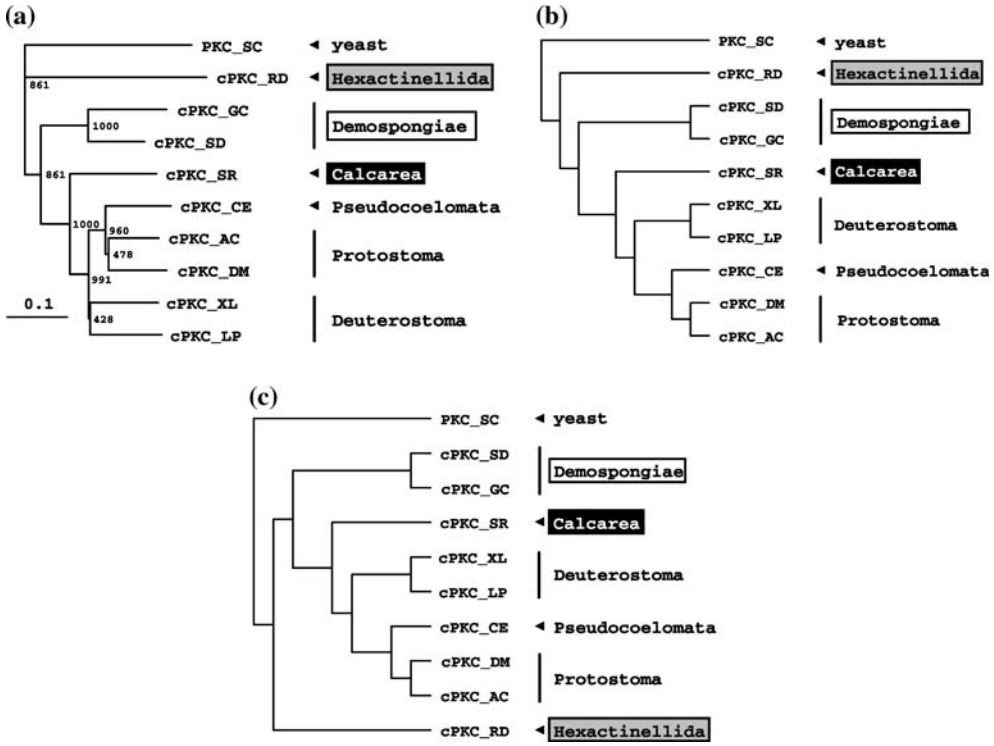

Figure 2. Trees computed from the ten PKC sequences (catalytic domain): I. Metazoa, cPKC from the deuterostomes *Xenopus laevis* [frog – cPKC_XL] and *Lytechinus pictus* [sea urchin – PKC_LP], from the protostomes cPKC from *Drosophila melanogaster* [fruit fly – PKC_DM] and *Aplysia californica* [mollusc, PKC_AC] and those from the sponges of the classes (i) Demospongiae, *G. cydonium* [CPKC_GC] and *S. domuncula* [CPKC_SD], (ii) Calcarea, *S. raphanus* [CPKC_SR], and (iii) the Hexactinellida, *R. dawsoni* [CPKC_RD] as well as from II. Yeast *Saccharomyces cerevisiae* [PKC_SC]. (a) Phylogenetic trees were constructed on the basis of aa sequence alignment by using the CLUSTAL-W program (Higgins & Sharp 1988). The degree of support for internal branches was further assessed by bootstrapping. The distance matrix was calculated as described (Dayhoff et al., 1978). Scale bar indicates an evolutionary distance of 0.1 aa substitutions per position in the sequence. (b) Computing of the same sequences by using the procedure of neighbour-joining applying the "Neighbor" program from the PHYLIP package PROTPARS [Protein-Parsinomy] (Felsenstein 1993). (c) Computing of the same sequences by using the algorithm described by Fitch & Margoliash (1967).

and from higher metazoan phyla, from (i) the pseudocoelomate [Nematoda] *C. elegans*, from (ii) two protostomes, *Aplysia californica* and *Drosophila melanogaster*, and from (iii) two deuterostomes *Lytechinus pictus* and *Xenopus laevis*.

Phylogenetic trees were constructed and statistical bootstrap probabilities were calculated (Fig. 2). The earliest offshoot was found to be the yeast *S. cerevisiae*. In this multifurcational tree the hexactinellid sponge *R. dawsoni* is separated from both the calcareous sponge *S. raphanus* and the demosponges *G. cydonium* and *S. domuncula* with high statistical significance, and the evolutionary distance between *R. dawsoni* and the demosponges is greater than that between *R. dawsoni* and the Calcarea. Furthermore, later in the phylogram (Fig. 2a) the pseudocoelomate [Nematoda] *C. elegans* branches off together with the two protostomes, *A. californica* and *D. melanogaster*, as well as with the two deuterostomes, *L. pictus* and *X. laevis*. The robustness of this alignment is seen in the fact that three programs used for the construction of the tree – CLUSTAL-W program (Fig. 2a), PROTPARS Protein-Parsinomy program (Fig. 2b) and the algorithm of Fitch and Margoliash (Fig. 2c) – resulted in the same relationships.

In conclusion, within the phylum Porifera, the class Hexactinellida diverged first from a common ancestor, while Calcarea and Demospongiae appeared later (Fig. 3). Furthermore, the cPKC se-

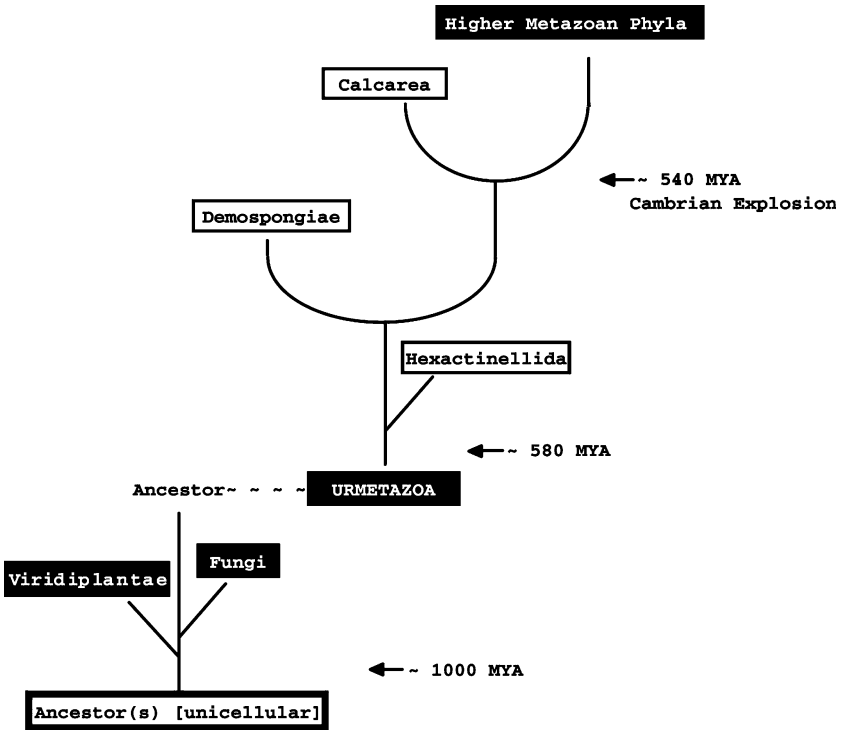

Figure 3. Proposed branching order of the three major subkingdoms, Viridiplantae, Fungi and Metazoa evolving from ancestral unicellular eukaryotes. The basis of the Metazoa form the Porifera, with the classes Hexactinellida, Demospongiae and Calcarea, which have a common ancestor with the higher metazoan phyla (Urmetazoa). The dates of the approximate divergence are indicated.

quences from higher invertebrates are more closely related to the cPKC from Calcarea.

The freshwater sponges: Lake Baikal

Operationally the baikalian Porifera, freshwater sponges, have been subdivided into two families, Lubomirskiidae and Spongillidae (Masuda et al., 1999). Fossil freshwater sponges are well known since the Lower Cretaceous (Ott & Volkheimer, 1972) or Middle Eocene (Müller et al., 1982). However, the taxonomy of the baikalian Porifera remains obscure in spite of intense morphological, cytological, and embryological studies in the past (Kozhov, 1963; Swartschewsky, 1902; Annandale, 1913, 1914). In a recent approach, by applying molecular sequence data of 18S rDNA (Itskovich et al., 1999) it was shown that Lubomirskiidae and Spongillidae are closely related. However, the question could not be solved unequivocally if the baikalian sponges originated *polyphyletically* (Dybowsky, 1882; Swartschewsky, 1902; Annandale, 1913; Rezvoi, 1936; Martinson, 1940) and perhaps from different marine sponge taxa, or *monophyletically* (Efremova, 1994) during the history of Lake Baikal.

Two approaches have been undertaken in a recent study to solve the problem of potential monophyly of baikalian sponges (Schröder et al.; in press): Cloning (partial) of the mitochondrial cytochrome oxidase and one selected exon/intron sequence of tubulin.

Approximately 676 nt long fragments encoding the mitochondrial cytochrome oxidase subunit I (COI) gene have been isolated by PCR from DNA of the freshwater sponges from Lake Baikal as well as from the marine sponge *S. domuncula*. The alignment of the sequences was performed and a phylogenetic tree was constructed. The tree showed that the sequence from the marine sponge *S. domuncula* forms the basis from which the branch to *S. lacustris* originates. The sequence from this freshwater sponge is significantly differ-

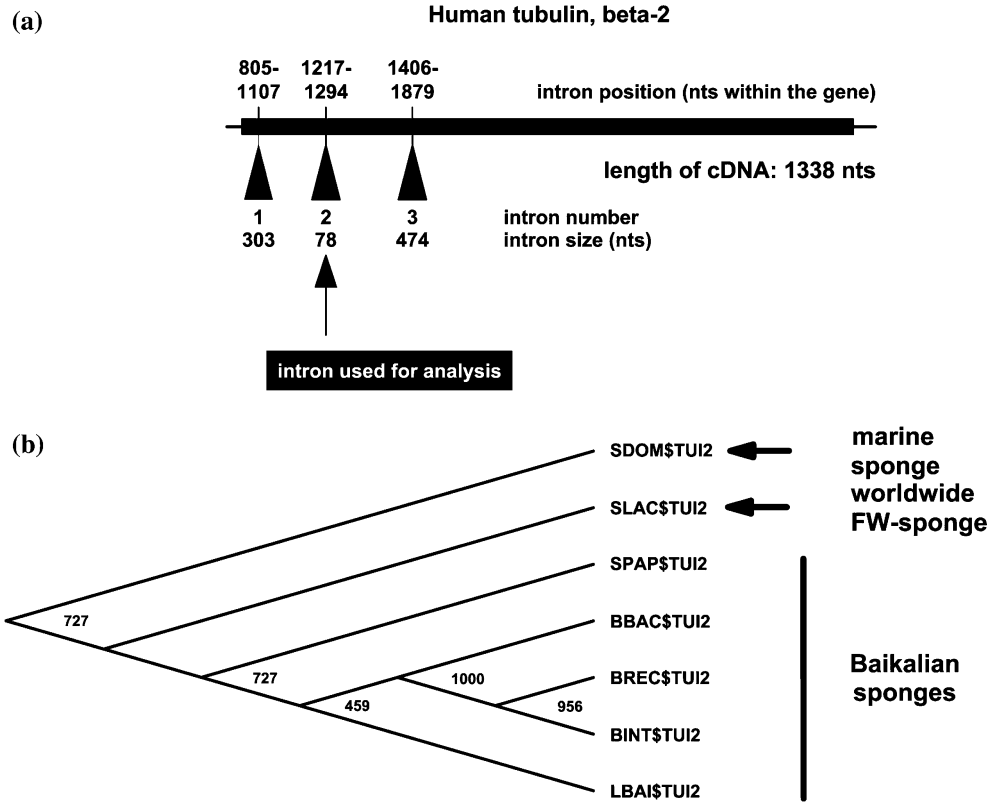

Figure 4. Phylogenetic relationship of the intron 2 sequences from tubulin gene of the freshwater sponges in relation to the marine sponge *S. domuncula*. (a) Intron/exon organization of the human tubulin beta 2 gene (accession number X02344). The coding region of the tubulin gene is interspersed with (at least) three introns; their number, size (in nucleotides) as well as the position within the human tubulin is indicated; the numbers refer to those within the corresponding cDNA (accession number NM_006088) which comprises a size of 1338 nt. (b) Phylogenetic tree, constructed after the alignment of the intron-2 sequences from the tubulin genes of the freshwater sponges *S. lacustris* (SLAC$TUI2), *L. baicalensis* (LBAI$TUI2), *B. intermedia* (BINT$TUI2), *B. recta* (BREC$TUI2), *B. bacillifera* (BBAC$TUI2) and *S. papyracea* (SPAP$TUI2); in addition the corresponding sequence from *S. domuncula* (SDOM$TUI2) was used. The slanted cladogram was constructed from the sequences after alignment. The analysis was performed by neighbour-joining. The numbers at the nodes are an indication of the level of confidence – given in percentage – for the branches as determined by bootstrap analysis [1000 bootstrap replicates]. The sequence from *S. domuncula* (SDOM$TUI2) was used as outgroup.

ent (≈94 bootstrap percentage) from the endemic baikalian sponges.

It is concluded from these analyses that the baikalian sponges derived from one common ancestor from which also the cosmopolitan species *S. lacustris* derived. A further resolution between the different baikalian sponge species is not possible using this gene segment.

In a second approach the exon/intron sequences framing the intron-2 of the sponge tubulin gene were chosen for the phylogenetic analysis. This intron is located close to the N-terminus of the translated protein (Fig. 4a) within the human beta 2 tubulin gene (NM_006088, X02344 and T08726) at aa position 93 (Gly). The ≈515 nt long fragments obtained comprise the second intron, which had sizes from 50 nt (*L. baicalensis*) to 89 nt (*B. recta*, *B. intermedia* and *B. bacillifera*). The intron sequences were aligned and the data used for construction of the phylogenetic tree; it was rooted with the intron isolated from the sequence of the *S. domuncula* gene. This tree shows that *S. domuncula* as well as *S. lacustris* branch off at the same position (Fig. 4b), while all baikalian sponges branch off from these two species. As the first baikalian species *S. papyracea* splits off from the other species under investigation [. . . . ?]. With a low significance, the *L. baicalensis* sequence is

separated from *S. papyracea*. Finally, the three Baikalospongia *B. bacillifera*, *B. recta*, and *B. intermedia* emerge in one cluster.

The robustness of the phylogenetic analysis, given here, was confirmed by a parallel application of the PROTPARS Protein-Parsinomy program (Felsenstein, 1993) as well as by the algorithm of Fitch & Margoliash (1967). The trees which have been constructed resulted in the same relationship with respect to the baikalian sponges (not given).

Conclusions

The phylogenetic position of Porifera within the metazoan animals has been well established in the past years based on molecular cloning of those molecules, which allow communication of cells with their environment and with each other. Because some of these molecules especially the receptors and their ligands of the adhesion and signal transduction system, are found only in Metazoa, they cannot be used for the elucidation of the phylogenetic relationship of the multicellular eukaryotes, Metazoa, Planta, Fungi, and Algae, to the unicellular eukaryotes. To solve this question data from nt sequences coding for those molecules from sponges have been used, which exist also in the other subkingdoms. While (i) the HSP70 chaperone proteins of eukaryotes are distantly related to the prokaryotic DnaK molecules (Koziol et al., 1998) and the Ser/Thr kinase domain is found in enzymes both from eukaryotes and from prokaryotes (Kruse et al., 1996), (ii) the families of calmodulin and β-tubulins are present only in eukaryotes.

Based not only on nt sequence data but also on fossil records the transition to multicellularity has been calculated to have taken place about 1000 million years ago [MYA] in red algae (Knoll, 1992; Kumar & Rzhetsky, 1996). Later in evolution the green algae evolved, 700 MYA (Knoll, 1992; Kumar & Rzhetsky, 1996), while the first metazoan fossils, the sponges, have been dated back to 580 MYA (Li et al., 1998). These sponges were provided with siliceous spicules and have been classified to the class of Demospongiae (Li et al., 1998). Hence, sponges lived 40 to 50 million years before the Cambrian Explosion (Valentine et al., 1996), the time of main divergence of metazoan phyla (Valentine, 1994). Based on the extent of aa substitutions of two galectins from *G. cydonium* it had been calculated that these molecules have diverged from the galectin, isolated from the nematode *C. elegans*, 800 MYA (Hirabayashi & Kasai, 1993; Pfeifer et al., 1993), supporting the conclusion that sponges existed prior to the Cambrian Explosion. The branching order originating from ancestral unicellular eukaryotes via Viridiplantae-Fungi to the simplest metazoans, the Porifera, follows both the published fossil data (see above) and the sequence data given here (Fig. 3). In addition the data reported here support recent findings which indicate that among the three classes of Porifera, the Hexactinellida, the Demospongiae, and the Calcarea, the Hexactinellida are the phylogenetically oldest taxon, while the Calcarea are that class which is most closely related to the higher metazoan phyla (Koziol et al., 1997, 1998); Figure 3.

In conclusion, phylogenetic analyses of the four deduced aa sequences from sponges [HSP70, Ser/Thr kinase domain, β-tubulin and calmodulin], (i) support earlier findings which indicated that all metazoans including the phylum Porifera are of monophyletic origin (Müller, 1995, 1998) and (ii) extend the view that the Metazoa originated from multicellular eukaryotes as ancestor and not from unicellular eukaryotes.

Focusing on freshwater sponges, which are present in Lake Baikal, the data presented in this and the previous study (Schröder et al., in press) show that there is evidence for a monophyletic origin of baikalian sponges. The molecular data analysis applied in the present study, using the sequences of one intron from the tubulin gene and of a part of the mitochondrial cytochrome oxidase gene, indicate that the baikalian sponges selected here, and the ubiquitously distributed freshwater sponge *S. lacustris* (Penney & Racek, 1968) possess a common ancestor. Moreover, it can be deduced that the baikalian sponge *S. papyracea* branches off first among the different baikalian sponges. A further resolution of the other taxa cannot be given using the approach applied.

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