REVIEW

Porifera a reference phylum for evolution and bioprospecting: the power of marine genomics

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Abstract. The term Urmetazoa, as the hypothetical metazoan ancestor, was introduced to highlight the finding that all metazoan phyla including the Porifera [sponges] derived from one common ancestor. Analyses of sponge genomes, from Demospongiae, Calcarea and Hexactinellida have permitted the reconstruction of the evolutionary trail from Fungi to Metazoa. This has provided evidence that the characteristic evolutionary novelties of Metazoa existing in Porifera share high sequence similarities and in some aspects also functional similarities to related polypeptides found in other metazoan phyla. It is surprising that the genome of Porifera is large and comprises substantially more genes than Protostomia and Deuterostomia. On the basis of solid taxonomy and ecological data, the high value of this phylum for human application becomes obvious especially with regard to the field of chemical ecology and the hope to find novel potential drugs for clinical use. In addition, the benefit of efforts in understanding molecular biodiversity with focus on sponges can be seen in the fact that these animals as "living fossils" allow to stethoscope into the past of our globe especially with respect to the evolution of Metazoa. (Keio J Med 53 (3): 159–165, September 2004)

Key words: evolution, metazoa, Urmetazoa, bioprospecting, bioactive compounds

Introduction

In the last decade the phylogenetically oldest metazoan phylum, the Porifera (sponges) gained special interest. Mainly due to the introduction of molecular biological techniques solid evidence was elaborated which indicated that this phylum provides a cornucopia of new information which allows a grasping for the understanding of the dynamics of evolutionary processes occurring during the Earth period of Ediacara, the time prior to the Cambrian Explosion which can be dated back approximately 540 million years ago. Furthermore, the species of this phylum are rich and valuable sources for bioprospecting, the translation of life-science discoveries into practical products or processes for the benefit of the society.

Palaeontology

The so far oldest isolated skeletal elements of sponges, the spicules, were found in China in the Hubei Province.[1] These spicules are monaxones or triaxones from hexactinellids (glass sponges). These specimens are dated to the Late Proterozoic age which is stratigraphically equivalent with the Ediacara (South Australia). Since the Early Cambrian (Atdabatian) the major poriferan taxa, Hexactinellida, Demospongiae, and Calcarea, have been existent as documented by isolated spicules as well as by more or less completely preserved sponge fossils, *e.g. Solactiniella plumata*. The earliest evidence of the Demospongiae and the Calcarea is the presence of isolated spicules in thin sections of rocks from Early Cambrian (Atdabatian).

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History

The elucidation of the evolutionary origin of metazoans in general and of sponges in particular was problematic since the beginning of modern biology in the 18th century. First Oken (1805) suggested that Metazoa originated from an ''Urschleim'' which is composed of Infusoria;[2] the unicellular organisms are created by spontaneous generation. Then the ''Gastraea''-theory was formulated by Haeckel (1874) who postulated that Monera are (again) the result of a spontaneous generation from which Amoeba derive and aggregate to Moraea which then develop to Blastaea and finally to pelagic Gastraea.[3] With respect to Porifera, James-Clark (1867) proposed the ''Choanoflagellate''-theory which assumes that flagellated Protozoa aggregate to a colonial sponge.[4] Even 10 years ago it was still outlined that sponges evolve from biofilms, accumulations composed of bacteria, choanoflagellates and their secreted mucus;[5] as still cited in modern textbooks sponges are assumed to be composed of functionally independent cells.[6]

The enigmatic relationship of Porifera to other multicellular animals was frequently studied but could only be resolved by application of gene cloning. Earlier, these species were grouped to plants or to animals; as ''Zoophyta'' or ''Thierpflanzen''.[7,8] Not knowing the luxury of sponge genes and their cell-cell-, cell-matrix receptors and the signal transduction pathways originating from these receptors the sponges were grouped to the Parazoa [''almost'' or ''near'' animals].[9]

The introduction of molecular cloning of genes, coding for informational proteins increased in a rapid, self-accelerating manner our knowledge on the evolution to the Porifera. It furthermore facilitated the subdivision of this phylum into the three classes, the Hexactinellida, the Demospongiae and the Calcarea. The first report in line with this new strategy was on the cloning of a galectin from the demosponge *Geodia cydonium*.[10,11] This protein is known to be crucially important for cell-cell interactions;[12] the deduced sponge protein showed high sequence similarity to the corresponding galectins of other metazoan taxa and no relationship to proteins from other kingdoms. Following this strategy, a number of subsequent publications[13,14] clearly demonstrated that the Porifera have to be grouped together with the other metazoan phyla into one monophyletic unit.[15,16] The hypothetical ancestor of all metazoan phyla was finally named Urmetazoa (Fig. 1).[17,18]

Molecular Evolution

It is generally agreed that multicellularity in Plantae, Fungi and Metazoa arose in the Proterozoic approximately 1,000 million years ago. The Porifera evolved as the earliest Metazoa, with the three major taxa: Hexactinellida, Demospongiae and Calcarea. Our group has analyzed genes of sponges in order to obtain an insight into the genome organization as well as the function of genes coding for functional proteins. In detail, genes from Demospongiae, *Suberites domuncula* and *Geodia cydonium*, from Calcarea, *Sycon raphanus*, as well as from Hexactinellida, *Aphrocallistes vastus* and *Rhabdocalyptus dawsoni*, have been analyzed and the results were used to describe the hypothetical ancestral Metazoa, the Urmetazoa (Fig. 1). The study of several sequences of informative molecules, as housekeeping proteins, *e.g.* heat shock protein or β-tubulin,[19] proteins involved in signal transduction, *e.g.* Ser/Thr kinase[20] or calmodulin,[19] from the three poriferan classes, revealed that the Hexactinellida is the phylogenetically oldest taxon, while Calcarea is the class closest related to higher metazoan phyla (Fig. 1).

Based on calculations described earlier it has been outlined that the transition to multicellularity took place about 1,000 million years ago.[19] Later in evolution, 700 million years ago, the green algae evolved, while the first sponge fossils have been dated back at least to 580 million years ago. 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*, approximately 800 million years ago.[10,11] Furthermore, data especially from studies with a series of Ser/Thr kinases suggested that the Calcarea might be a sister group to higher metazoan phyla (Fig. 1).[21] 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.

Structural Features of the Sponge Bauplan

Sponges, as the oldest still extant metazoan phylum are characterized by a simple Bauplan. They are filter-feeding organisms that are usually sessile. Their body is composed of an epithelial layer which surrounds a mesogleal compartment, the mesohyl; this is reticulated in a highly organized manner by a canal system.

Molecules involved in cell-cell interaction

Sponges have become a classical model for basic studies to understand metazoan cell-cell adhesion. It was Wilson[22] who introduced this system in experimental biology which then became a traditional model to study both cell-cell- and cell-matrix adhesion.[23] With

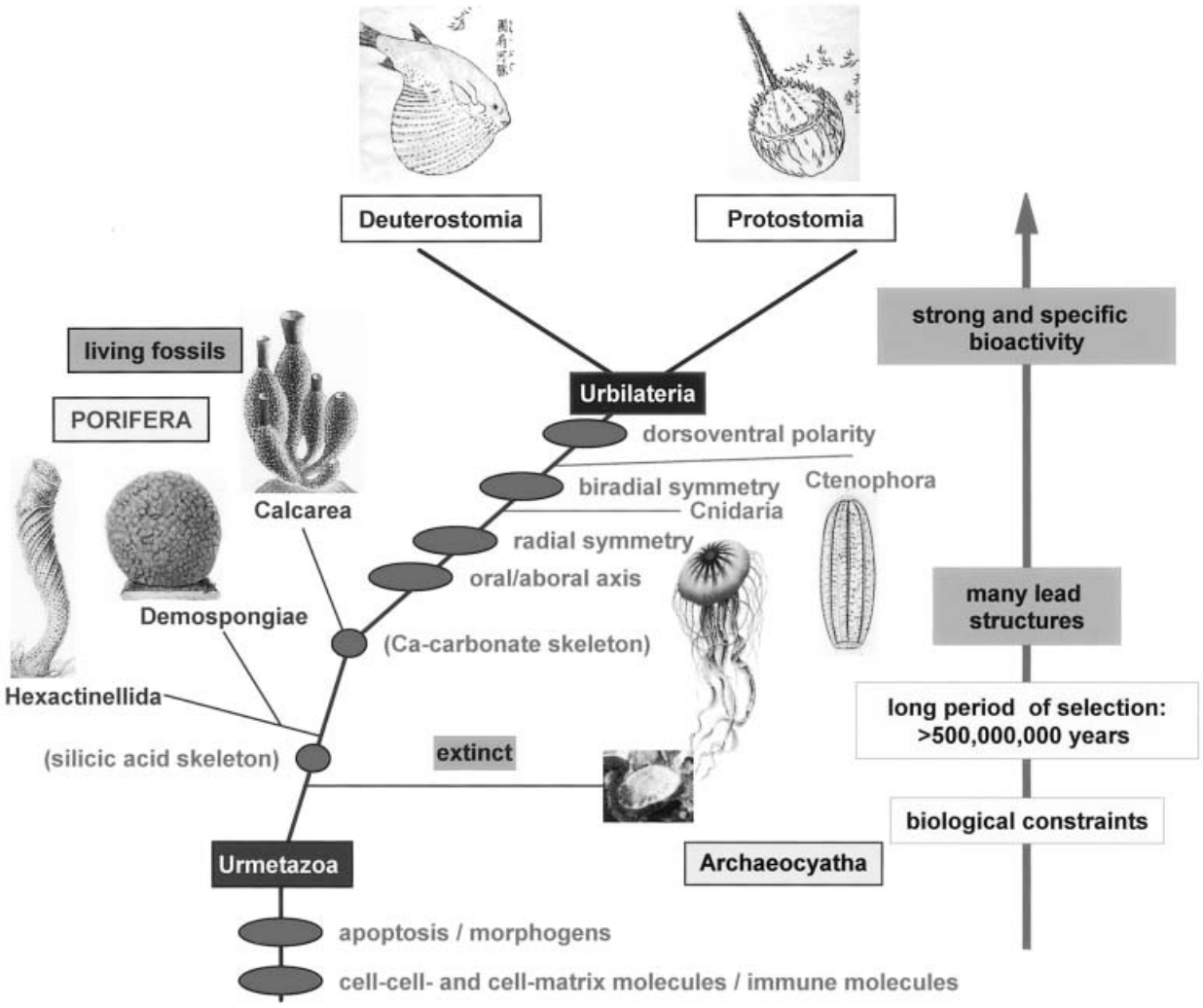

Fig. 1 Phylogenetic position of the Porifera, as "living fossils" between the Urmetazoa and the Urbilateria which gave rise to the Protostomia (example: horseshoe crab [K. Takizawa (1879)]) and Deuterostomia (example: fugu fish [K. Takizawa (1879)]). The major evolutionary novelties which have to be attributed to the Urmetazoa are those molecules which are highlighted. Sponges use secondary metabolites for their protection; these compounds became shaped and hence more potent and more specific during their long evolutionary history.

the isolation of a galectin sequence as the first cell-cell adhesion molecule,[10] and with the integrin sequence as the first cell-matrix adhesion receptor in the demosponge *G. cydonium*,[24,25] it became overt that sponges contain highly related molecules known to also promote adhesion in Protostomia and Deuterostomia. Sequence analyses of the galectin cloned from *G. cydonium* revealed that those aa residues which are involved in binding of galectins from mammalians to galactose are also conserved in the sponge sequence.[10] The putative aggregation receptor was cloned from *G. cydonium* and found to comprise fourteen scavenger receptor cysteine-rich domains, six short consensus repeats, a C-terminal transmembrane domain and a cytoplasmic tail.[26]

Molecules involved in cell-substrate interaction

The dominant molecule present in the extracellular matrix of sponges which functions as a cell-matrix adhesion molecule is collagen. The corresponding gene was cloned both from the freshwater sponge *Ephydatia muelleri*[27] as well as from the demosponge *S. domuncula* (Schröder *et al.*, 2000).[28] Cell surface-spanning receptors, in particular the single- as well as the seven-pass transmembrane receptor proteins, serve as receivers for extracellular signaling molecules. Also these molecules could be identified in sponges. The first, 1-transmembrane receptor (1-TMR) which represents an autapomorphic character for Metazoa, was the receptor tyrosine kinase.[29] Interestingly the tyrosine dephos-

phorylating enzymes, the protein-tyrosine phosphatases, exist already in yeasts.[30] As a 7-transmembrane receptor (7-TMR) the metabotropic glutamate/GABA-like receptor was characterized in *G. cydonium*;[31] it was found to respond to extracellular agonists as well as antagonists known from metabotropic glutamate/GABA-like receptors present on nerve cells from mammals.[31,32]

Molecules involved in morphogenesis

Two classes of proteins are addressed here, the morphogens and the transcription factors. In the extracellular space of sponges morphogens have been identified, *e.g.* the endothelial-monocyte-activating polypeptide or myotrophin.[28,33] Both are very likely involved in the organization/differentiation of cells within the sponge body.

Transcription factors

During development of animals, a set of genes, most of them transcription factors, that are responsible for cell fate and pattern determination, is expressed. Among them, T-box, Forkhead and Homeobox gene families have been found to be extremely conserved, on sequence and functional level. Among the T-box family, *Brachyury* is involved in the formation and differentiation of the third germ layer, the mesoderm, in tripoblastic animals. Recently, two T-box genes have been isolated from the sponge *S. domuncula*; a *Brachyury* gene and a homologue of the Tbx2-3-4-5 genes from chordates, which, interestingly, are involved in the formation of the limbs.[34] Functional studies demonstrated that the expression of the *Brachyury* gene is upregulated in differentiating sponge cells during formation of canal-like structures.[34] Based on this observation we postulate that already in sponges a primordial axis formation is genetically fixed. This assumption was subsequently confirmed by the isolation and phylogenetic characterization of five members of the forkhead gene family from the sponge *S. domuncula*.[35,36] Forkhead proteins form a subfamily within the large group of helix-turn-helix proteins. They are responsible for a wide range of functions and key roles in early developmental processes, during organogenesis and also for the function of the major organs and tissues in the adult animals.

Transcription factors: homeodomain molecules

The developmental processes resulting in the formation of a body axis require a head center; *e.g.* in bilaterians, the Spemann's organizer.[37] The genes which are involved in the establishment of the head organizer during embryogenesis have been grouped into three classes of homeobox genes, the Paired-class, the Antennapedia-class and the Lim-class genes.[38] Also in this area a strong progress was made in sponges in the last few years. A paired-class (*Pax-2/5/8*)-gene had been isolated from the freshwater sponge *Ephydatia fluviatilis*,[39] which encodes a complete although substantially degenerated homeodomain. In *S. domuncula* a cDNA encoding a LIM/homeobox protein has been isolated which comprises high sequence similarity to the related LIM homeodomain proteins in other animals.[40]

Genes in S. domuncula indicative for Wnt signaling

The Wnt signaling pathway is a cell communication system which regulates cell-fate decisions, tissue polarity and morphogenesis. The Frizzled protein is the membrane receptor for the Wnt secreted glycoproteins. Through the canonical Wnt signaling pathway, the activated Frizzled binds to Dishevelled (Dsh), which leads to the stabilization and accumulation of β-catenin in the nucleus, where it activates the TCF/LEF transcription factor. Very recently we isolated the gene encoding the Frizzled receptor from *S. domuncula*.[41] *In situ* hybridization analysis in adult specimens demonstrated its expression in the cortex region and in the epithelial layer of the aquiferous canals. The findings suggest that a Wnt-pathway involved in cell-fate determination and morphogenesis was already established in the phylogenetically oldest metazoans, the sponges.

Molecules present in tight junctions

Our screening for a gene encoding a tight junction scaffold protein from a sponge, here *S. domuncula*, was successful; the scaffold protein membrane-associated guanylate kinase with inverted arrangement (MAGI) had been identified.[34] In addition, the existence of one tetraspan receptor, tetraspanin, in *S. domuncula* has been reported. The tetraspanins belong to a group of hydrophobic proteins, comprising four transmembrane domains with a series of conserved aa residues in the extracellular loops.

Bioprospecting

The field of bioprospecting of Porifera may be of tremendous potential benefit for humans from the applied point of view. Taking into account that the chemical diversity of natural bioactive compounds is much higher than that of compounds synthesized in a standard combinatorial chemistry approaches, and that natural compounds display an impressive selectivity, the high value of the secondary metabolites from natural resources in general and from sponges in particular

can only be roughly imagined.[42] It is also conceivable that especially during first screening programs aimed to discover new lead molecules which are directed against newly discovered targets, the natural compounds are superior to those generated by combinatorial chemistry.[43] Evolution has designed during 800 million years the optimal bioactive compound against a particular organismic disorder. On this background the highly diverse chemical compounds from sponges which are likewise provided with a diverse bioactivity are extremely valuable. This exiting research field was pioneered by Bergmann and Scheuer.[44,45]

The diversity of secondary metabolites produced in sponges has been highlighted in a large number of reviews.[46,47] They range from derivatives of amino acids and nucleosides to macrolides, porphyrins, terpenoids to aliphatic cyclic peroxides and sterols. This diversity reflects the efficient mechanisms of combinatorial biochemistry which the animals have acquired during their evolutionary history. The question arises if sponges being the host of associated/symbiotic bacteria are the real producers or whether the microorganisms which they harbor produce these substances. Recent data strongly favor the view that the microorganisms are the main producers of the natural products which are subsequently stored and accumulated in the sponge as chemical defense, although sponge metabolites can also be produced by specific sponge cells. As an example the phosphatase inhibitor okadaic acid can be cited.[48] This compound was first isolated from the sponge *Halichondria okadai* and later found to be produced by the free-living microalgae *Prorocentrum lima* and perhaps even by bacteria which are associated with them.[49]

Very promising is the potential application of bioactive compounds from Porifera in human therapy, however, their use is limited by the difficulty to obtain sufficient supply of the respective sponge species. Until now only in one case a bioactive compound from sponges is applied in clinics, arabinofuranosyladenine (ara-A) as antiviral drug;[50] ara-A is a derivative of a lead structure isolated from a sponge.[44] This drug is used as an anti-Herpes Simplex virus drug; *e.g.* in Japan it is the most applied ointment against this disease [Arasena-A® (Mochida Pharmaceutical Co; Tokyo)].

The Future: Evochemistry

Thanks to the progress initiated by the pressure of the society for a sustainable use of natural resources for human benefit, the exploitation of natural biodiversity became possible through the application of the techniques of molecular biology and modern cell biology. It appears to be enigmatic why Porifera and also other phylogenetically old taxa, all of them being sessile filter feeders, have been so successful during the long evolutionary period of 800 million years. Answers have been given in the last two years. As summarized the expected number of genes in the sponge genome might be >100,000.[51] This number is comparatively large; the human genome contains ≈ 35,000 genes; *Caenorhabditis elegans* (nematode [worm]) ≈ 19,000, the insect *Drosophila melanogaster* ≈ 14,000 and the yeast *Saccharomyces cerevisiae* 6,000 genes (Fig. 2). Two major conclusions must be drawn: FIRSTLY, during the transition from Yeast to the Urmetazoa a period of dramatic gene duplication must have occurred and SECONDLY, during the period of evolution from the Urmetazoa to the Protostomia [*C. elegans* and *D. melanogaster*] and the Deuterostomia [*Homo sapiens*] a reduction of the number of genes must have taken place. In parallel with the metazoan *evolution by loss of genes* [*domains and families*] a second evolutionary progress, which occurred after the Urmetazoa, must be postulated for the metazoan phyla, an *evolution by diversification of gene families*. The hypothetical Urmetazoa has been proposed as the last common ancestor for all Metazoa.

The finding that the number of sponge genes is higher than that in the ''crown'' phyla allows also an explanation why the diversity of the secondary metabolites in sponges and other filter feeders is so comparably high. These animals had – in order to survive the environmental threats – to form bioactive compounds from the ''regular'' metabolites, the secondary metabolites. During their long evolutionary history the biological/defensive/pharmacological effectiveness of these compounds were trimmed for higher potency and selectivity (evochemistry). In consequence, the richness of the sessile filter feeders in bioactive compounds is due to their growth and nutrition characteristics and their comparably large genetic repertoire (Fig. 2).

Conclusion

It is a fortune that, according to the fossil records, the phylogenetic oldest metazoan phylum, the Porifera did not become extinct during the last 800 million years. By analyzing their genetic diversity it became obvious that all Metazoa originated from one common ancestor, the Urmetazoa. With respect to the functional aspect it could be demonstrated that the degree of complexity of the Porifera is beyond any expectations. It becomes now increasingly obvious that they are provided with most of the metabolic pathways and genetic elements required for constructing a metazoan Bauplan.[52] Finally it should be stressed that the potential commercial value of sponge biodiversity is of an unpredictable scale. A new application field which very likely will

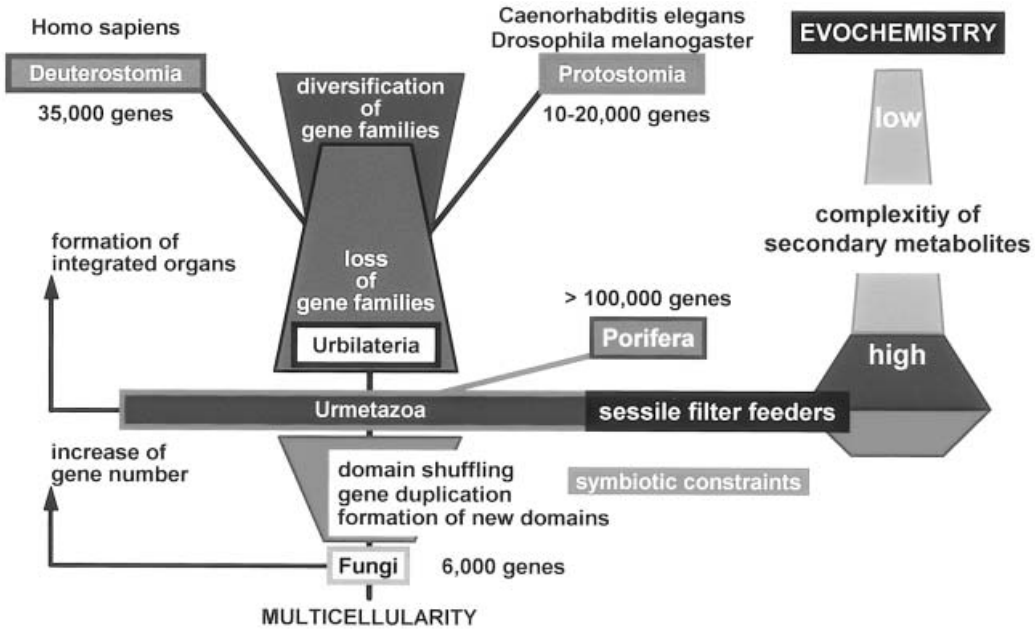

Fig. 2 Proposed evolutionary processes proceeding from Yeast to Urmetazoa and subsequently from this hypothetical ancestor of Metazoa to the Deuterostomia [*Homo sapiens*] and Protostomia [*Caenorhabditis elegans* and in *Drosophila melanogaster*]. It is proposed that the number of genes, calculated to be present in sponges and – very likely also in Urmetazoa – has been created by gene duplication. This dramatic increase in gene number was presumably caused by symbionts (*e.g.* bacteria) which put evolutionary pressure on the host to diversify its gene complexity. During this process also new domains were formed which gave rise to novel mosaic proteins. In the metazoan phyla, derived from the Urmetazoa, the number of genes decreased by elimination of gene families and in parallel the remaining gene families diversified. Based upon the higher gene complexity in the lower metazoan phyla also their potency to synthesize a more complex spectrum of bioactive compounds became possible.

have a considerable impact in biotechnology is the question of the synthesis of bioactive, low-molecular weight compounds in a recombinant manner. To reach this goals both the gene cluster required for the synthesis of these compounds from the sponge host and the associated symbiotic bacteria have to be isolated and expressed in a heterologous system.

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