FROM CELLS TO PRIMMORPHS AND ADULT SPONGES: AN APPROACH TO UNDERSTAND THE BAUPLAN OF DEMOSPONGIAE

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ABSTRACT

Molecular data suggest that the phylum Porifera with its three classes, Hexactinellida, Demospongiae and Calcarea consists of two groups, sponges with a siliceous skeleton (Hexactinellida and Demospongiae) and those possessing a calcareous skeleton (Calcarea). Recent data indicate that all Metazoa, including the Porifera evolved from one common ancestor, the Urmetazoa. The Demospongiae are provided not only with the basic molecules for cell-cell and cell-matrix interactions but also with elements allowing a distinct pattern formation. The studies reviewed have been primarily performed with intact demosponges (*Suberites domuncula*) but also with primmorphs, a special form of 3D aggregates derived from them.

KEY WORDS

Sponges, Porifera, *Suberites domuncula*, homeobox genes, LIM-homeobox protein, channel formation, morphogenesis, primmorphs, functional molecular evolution, Bauplan.

INTRODUCTION

During the last 10 years a strong support for the monophyly of all multicellular animals has been provided by sequence data of informative molecules and analyses of their functional roles in sponges (MÜLLER *et al.,* 1994; MÜLLER, 1998a,b). At present it is well established that sponges possess the major extracellular matrix molecules, *e.g.* collagen (EXPOSITO *et al.,* 1991; SCHRÖDER *et al.,* 2000), as well as signal transduction elements, *e.g.* receptor tyrosine kinases (SCHÄCKE *et al.,* 1994), which are representatives of the characteristic metazoan-specific gene families (Fig. 1). Therefore Porifera are considered to be the still extant earliest common ancestor of all metazoan phyla, that branched off first from the hypothetical Urmetazoa (MÜLLER, 2001; MÜLLER *et al.,* 2001a).

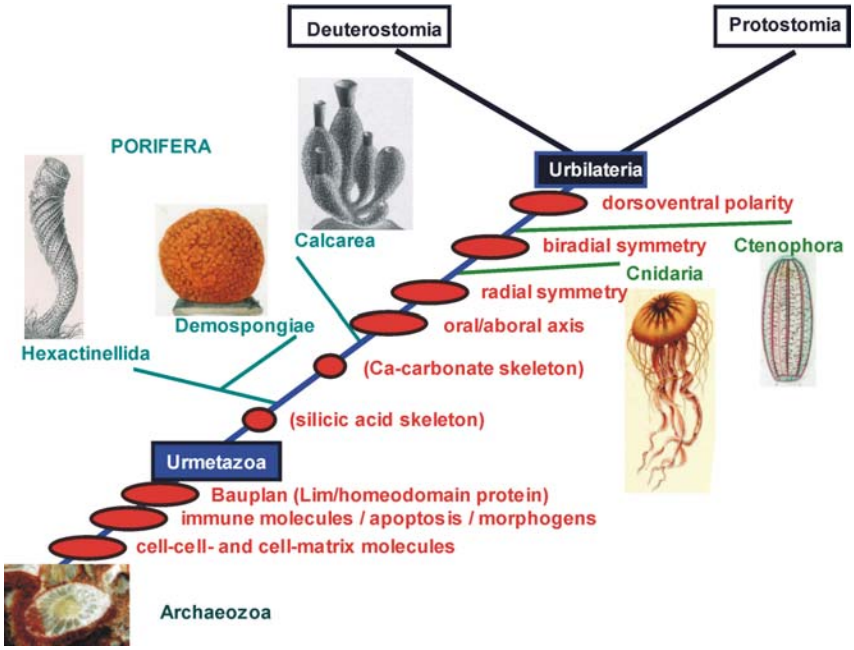

Fig. 1. Proposed relationships of the multicellular animals. The hypothetical Urmetazoa, which is evolutionary related to the extinct taxon the Archaeozoa (archaeocyathans), comprises the receptors as well as the interacting ligands required for cell-cell and cell-matrix interaction. The immune molecules together with the genes controlling apoptosis allowed the establishment of the individuality of the Urmetazoa. Key elements required for the Bauplan formation had been the morphogens and the homeobox genes. First the siliceous sponges (Hexactinellida and Demospongiae) evolved followed by the Calcarea which have a skeleton made of Ca-carbonate. Between the Calcarea and the Urbilateria the Cnidaria, with the radially symmetrical diploblastic Ctenophora, exist which show an increasing Bauplan complexity. Finally, the dorsoventral polarity is established in the Urbilateria, the basis for the Protostomia and Deuterostomia.

Porifera are themselves also of monophyletic origin and have been subdivided into three classes; the most ancient Hexactinellida and Demospongiae, as well as the younger Calcarea, the taxon closest related to the non-sponge metazoans (MÜLLER, 1998b). This grouping was first established by molecular studies analyzing evolutionarily novelties of Metazoa, *e.g.* receptor tyrosine kinases (KRUSE *et al.,* 1997, 1998) and later confirmed (BORCHIELLINI *et al.,* 2001; MEDINA *et al.,* 2001). Hence the elucidation of the Bauplan (bodyplan) in Demospongiae will provide a closer understanding of the original metazoan Bauplan. For most of our studies the demosponges *Suberites domuncula* and *Geodia cydonium* have been selected. Especially *S. domuncula* has proven to be very suitable since *in vitro* as well as *ex situ* culture conditions have been well defined for this species (MÜLLER *et al.,* 1999a; LE PENNEC *et al.,* 2003). Here we give a review of the recent achievements in the understanding of the Bauplan-formation in sponges.

DIFFERENTIATION CAPACITY

In sponges in general and in *S. domuncula* in particular the differentiation of stem cells into somatic cell types, including germ cells, may start from the archaeocytes, one of only a few cell types that sponges possess (BOROJEVIC, 1970; KOZIOL *et al.,* 1998). However, it is also possible, especially for the male lineage, that the germ cells originate from choanocytes (SARÀ, 1992), suggesting that these cells are the source for both female and male germ cells. Nevertheless, sponges are – like all other metazoans – provided with a defined Bauplan starting from a defined basis (KAANDORP & KÜBLER, 2001). The morphogenetic processes in Metazoa are controlled by extracellular signals and ligands which interact with their corresponding receptors, that in turn trigger signal transduction cascades, as well as by regulatory genes. Those tuned interactions specify the spatial distribution of cells during embryogenesis. In sponges the extracellular adhesion molecules are lectins (galectins) (PFEIFER *et al.,* 1993; SCHÜTZE *et al.,* 2001) that interact with their adhesion receptors composed of scavenger receptor cysteine-rich domains (BLUMBACH *et al.,* 1998) as well as collagens and the responding integrins (WIMMER *et al.,* 1999); such systems are crucial for specific cell adhesion and represent metazoan autapomorphies.

Among the regulatory genes the homeobox genes are responsible for the establishment of cell proliferation, differentiation and pattern formation. Five scientific groups have isolated homeobox genes from sponges (see: SEIMIYA *et al.,* 1994; HOSHIYAMA *et al.,* 1998; RICHELLE-MAURER & VAN DE VYVER, 1999; MANUEL & LE PARCO, 2000; WIENS *et al.,* 2003c). While most of the sponge homeobox genes could be grouped to defined classes, *e.g.* to the *POU*-, *Pax*-, *Cad*-, *En*-, *Msx*- or *NK-2* family (MANUEL & LE PARCO, 2000), they could not be assigned to a specific function. It has been proposed that in *Ephydatia muelleri* EmH-3 controls cell proliferation and differentiation (RICHELLE-MAURER & VAN DE VYVER, 1999). Recently, a homeobox gene has been identified in *S. domuncula* which comprises high sequence similarity to the Lim-class homeobox genes (WIENS *et al.*, 2003c). Besides the Paired-class and the Antennapedia-class factors the Lim-class molecules are responsible for the establishment of anterior patterning (GALLIOT & MILLER, 2000).

BAUPLAN

In metazoans the Bauplan formation can operationally be divided into two steps: *(i)* pattern formation and *(ii)* axis formation. At present, experimental evidence is lacking that in Porifera and in Cnidaria transcription factors, primarily the characteristic bilaterian *Hox* clusters, exist that might induce axis formation (PETERSON & DAVIDSON, 2000). Until now only a very distantly related *Hox*-like gene has been isolated from sponges (SEIMIYA *et al.,* 1994). In Cnidaria two Cnox homeodomains (HDs) exist which are involved in head formation (GAUCHAT *et al.,* 2000); the *Hox* clusters appeared with the Bilateria. Consequently studies in these two phyla focus on the molecular elucidation of those transcription factors which cause pattern formation through regional specification. In sponges attempts have been undertaken to understand the regulatory mechanisms causing the construction of the Bauplan through regional expression of morphogens (SCHRÖDER *et al.,* 2000)

and transcription factors (SEIMIYA *et al.,* 1994; RICHELLE-MAURER & VAN DE VYVER, 1999; MANUEL & LE PARCO, 2000).

The recently described cDNA encoding a LIM-homeobox protein in *S. domuncula* (WIENS *et al.,* 2003c) is a further step towards an elucidation of the genetic basis of Bauplan formation in Demospongiae. The molecule comprises high sequence similarity to the related LIM-HD proteins in its LIM as well as in its HD domains. The expression of this gene is upregulated after attaching *S. domuncula* primmorphs (3D-cell aggregates) to the homologous galectin matrix; this finding suggests that the galectin matrix induces major morphogenetic processes. In *S. domuncula* more transcription factors have been isolated which play important roles in morphogenesis. One set belongs to the T-domain transcription factors, the two T-box genes *Brachyury* (*Sd-Bra*) and *Tbx2* (*Sd-Tbx*). The other set of transcription factors existing in Demospongiae are the forkhead molecules which, together with the T-box factors, are involved in the differentiation of tissue layers (ADELL *et al.,* 2003) (Fig. 2).

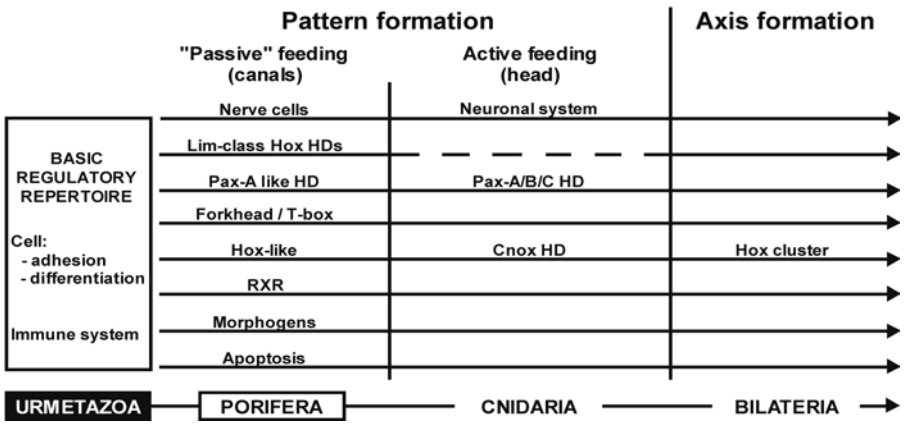

Fig. 2. Evolutionary inventions, including morphogens and transcription factors, which allow pattern formation in Porifera. These genes, together with the existence of neuronal receptors and apoptotic molecules as well as the basic regulatory repertoire present in the hypothetical Urmetazoa, enabled the sponges to a ("passive") feeding behavior. An active feeding became possible after the development of a head as in Cnidaria, based on a functional neuronal system; in Bilateria axis differentiation became possible, due to sequential expression of *Hox* gene cluster(s).

It is generally agreed that sponges lack a nervous system; a functional nerve system is present in phyla evolutionarily younger than sponges such as Cnidaria. These animals have an active oral pole allowing them an active feeding behavior (GALLIOT & MILLER, 2000). However, neuronal receptors, such as the metabotropic glutamate (GABA-like) receptor, have already been identified in sponges (PEROVIC *et al.,* 1999). Consequently, it can be assumed that sponges are able to respond to external stimuli via these receptors and are provided with the property for an efficient, perhaps more "passive", feeding through a controlled opening of the incurrent and excurrent channels (see: SIMPSON, 1984) (Fig. 2). In this context it is

interesting to note that in triploblasts, Protostomians and Deuterostomians, the Lim-class HD proteins have been implicated in the differentiation of specific neurons and in axon guidance (see: BACH, 2000). These pieces of evidence, the presence of neuronal-like cells in sponges and the existence of at least one Lim-class HD protein, led us to propose that in primmorphs the increased expression of the HD protein in response to and during the galectin-caused development of canals is one prerequisite for the differentiation of pluripotent cells to channel-forming epithelial (pinacoderm) cells. The Lim-class HD proteins needs still to be identified in Cnidaria.

As summarized in Fig. 2, the hypothetical ancestor of Metazoa, the Urmetazoa (MÜLLER, 2001; MÜLLER *et al.,* 2001a) was already provided with the basic repertoire of regulatory factors known for metazoans.

INDIVIDUALITY IN SPONGES: IMMUNE SYSTEM

Demospongiae possess effective defense systems against microbes and parasites which comprise engulfment of bacteria into specific cells, but also signal transduction pathways which actively kill bacteria. Among those is the LPS-mediated pathway, with the stress-responsive kinases (BÖHM *et al.,* 2001). In addition, sponges are provided with an interferon-related system, with the (2-5)A synthetase as controlling enzyme (WIENS *et al.,* 1999; GREBENJUK *et al.,* 2002). Transplantation studies have been performed on tissue, as well as on cellular level ("mixed sponge cell reaction assay") which demonstrate the complex molecular strategy by which sponges respond to allogeneic- and/or autogeneic signals (BLUMBACH *et al.,* 1999; MÜLLER *et al.,* 1999a; MÜLLER *et al.,* 2002).

Among the molecules involved in histo(in)compatibility response of sponges, cytokines e. g. the allograft inflammatory factor 1, have been identified which transmit extracellularly signals to control rejection of allografts (see: MÜLLER *et al.,* 1999b). Furthermore, transcription factors, with Tcf-like factor as an example, have been identified which very likely control gene expression during histocompatibility reactions. Interestingly enough the immune reactions in sponges can be modulated by FK506, a drug which has been successfully used as immunosuppressant in humans (MÜLLER *et al.,* 2001b). One further surprising finding was the fact that *G. cydonium* has several molecules which contain polymorphic Ig-like domains of the variable type (PANCER *et al.,* 1996; BLUMBACH *et al.,* 1999). Hence, it can be concluded that the successful evolutionary transition to the Metazoa, with the sponges as the oldest still extant phylum, and the subsequent rapid radiation into the other metazoan phyla, became possible because of the acquisition of modular molecules involved in cell adhesion and immune system.

INDIVIDUALITY IN SPONGES: APOPTOSIS

Until the recent discoveries (WIENS *et al.,* 2000a,b) it was proposed that the physiological cell death is restricted to multicellular organisms, which have separate germ and somatic cells. Two lines of evidence led us to assume that also sponges are provided with complex apoptotic pathways. As a first gene potentially involved in apoptosis of sponge cells the *MA-3* gene from *S. domuncula* was identified (WAGNER *et al.,* 1998); the corresponding mouse *MA-3* cDNA is assumed to encode an apoptotic molecule.

SPONGE PRO-APOPTOTIC MOLECULES

As the most promising segment to screen for a pro-apoptotic molecule, we selected the death domain part which is found in the mammalian apoptosis controlling proteins, Fas, tumor necrosis factor-α or its receptor, and FADD; this protein is absent in the nematode *Caenorhabditis elegans*. Our approach was successful; the molecule isolated from *G. cydonium* comprises even two death domains (WIENS *et al.,* 2000a) whose comparisons revealed that they are to be grouped within the death domain family. It was claimed before that the death domain found in humans has a relationship to ankyrin motifs, an assumption which could be substantiated also experimentally. We performed functional assays with allografts from *G. cydonium* and found in rejecting tissue a strong increase of the expression of the death domain-comprising gene *GCDD2* (WIENS *et al.,* 2001).

CASPASES

In vertebrates the death domain containing receptors and adapter molecules interact intracellularly with the caspase-8 proenzyme through the death-effector domain with a similar region in the caspase. An adapter-mediated oligomerization causes an activation of the procaspase(s) which undergo cleavage and finally heterodimerization. Finally, upstream caspase(s) activate pro-caspase-3 which in turn is split into the large and small subunits that activate after heterodimerization a factor necessary for the DNase activity to degrade chromatin into the nucleosomal fragments the hallmark of apoptosis.

It is known that in Bilateria a series of caspases are involved in the tuned control of apoptosis, starting with caspase-8 in the cascade and ending with caspase-3. Interestingly enough, until now only one gene has been identified in *G. cydonium* which encodes two transcript forms, for caspase-8 and for -3 equivalents (WIENS *et al.,* 2003a). Functional studies indicated that the two forms of the sponge caspases act in *G. cydonium* in the apoptotic pathway.

EXAMPLE: EXPRESSION OF CASPASE DURING ALLOGENEIC REJECTION

Sponges possess remarkable regeneration capabilities (WILSON, 1907; GALTSOFF, 1925; HUMPHREYS, 1963). This regeneration capacity in combination with inflammatory responses to injury are essential components of their ability to survive. In addition, sponges have developed mechanisms to distinguish between self and non-self. Only little is known about natural challenges to self integrity in sponges, most of it is available from experimental transplantation studies. SMITH & HILDEMANN (1986) in their extensive review have grouped sponge alloimmune responses in experimental transplantations into two major rejection processes. Some species may form barriers to separate from non-self tissue; *e.g.* the marine sponge *Axinella verrucosa* (BUSCEMA & VAN DE VYVER, 1983), while others may react by cytotoxic factors which destroy the transplant; *e.g.* the marine sponges *G. cydonium* (PFEIFER *et al.,* 1992).

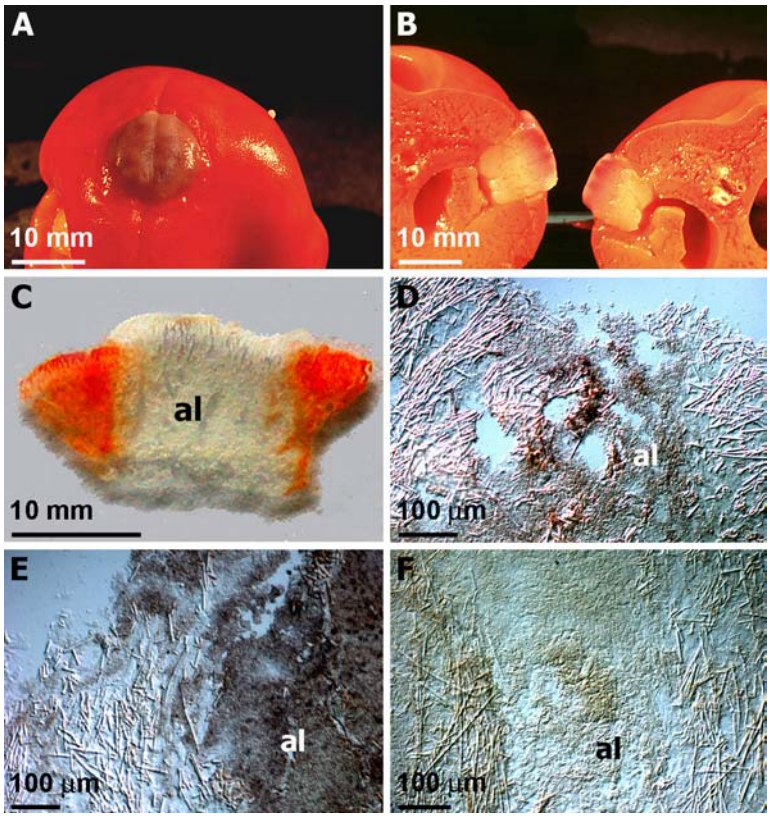

Fig. 3. Tissue recognition in the sponge *S. domuncula*. Application of the insertion technique for grafting experiments: tissue pieces were removed with a cork drill from one specimen and inserted into holes in the recipients, which had a slightly narrower diameter (**A** and **B**). **C,** Allografts initially fused together but underwent already necrotic degeneration after 3 days a process which ultimately resulted in resorption. The allogeneic tissue was taken from a white specimen (al). **D-F,** Cryosections through a tissue region shown in Fig. 3C which has been analyzed by *in situ* hybridization of mRNA encoding for *caspase* . **D** and **E,** *in situ* hybridization of sections using a DIG-labeled antisense-*caspase*-probe. The cells in the allografts (al) are highlighted by positive hybridization signals, which are shown in dark brown. **F,** In one control experiment it is demonstrated that no signal is seen, if hybridization is performed with a DIG-labeled sense-*caspase*-probe.

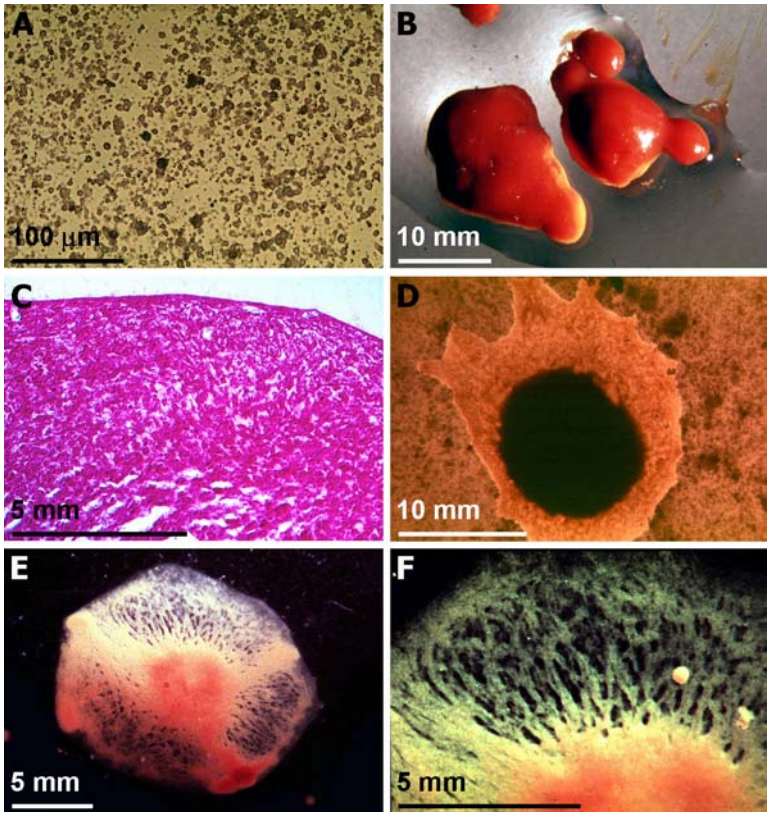

Fig. 4. Formation of primmorphs using the *S. domuncula* model. **A,** Single cell suspension. **B,** Primmorphs formed after 5 days. **C,** Cross section through a primmorph which has been stained with Ziehl's fuchsin. **D-F,** Primmorphs formed on a galectin matrix. **F,** Higher magnification from **E** of the channel system in primmorphs.

In the experiments documented here with *S. domuncula* the insertion technique was applied. Tissue pieces were removed with a cork drill from one specimen (diameter of ≈1 cm; approximate length of 4 cm) and inserted into holes in the recipients (see: MÜLLER *et al.,* 1999b) (Figs 3A, B). All autografts fused and no boundary line is seen finally. Allografts initially fused together (Fig. 3C); after approximately 2 to 3 days the rejected graft tissue formed a pronounced demarcation boundary and underwent necrotic degeneration (Figs 3D-F) and finally resorption. *S. domuncula* occurs in nature in red, orange, whitish, blue or a mixture of these colors; these color differences could be used to distinguish between graft and host (Fig. 3C).

We have used the *S. domuncula* cDNA encoding a caspase enzyme in order to demonstrate its expression during allogeneic rejection. This cDNA was cloned and found to be differentially expressed after exposure of the tissue to the toxic okadaic acid (WIENS *et al.,* 2003b). The technique of *in situ* localization was used as described before (LE PENNEC *et al.,* 2003; PEROVIC *et al.,* 2003). In brief; after partial removal of the siliceous spicules with HF-NH_4F the sponge pieces were embedded in Tissue-Tek. Cryosections (8 μm) were performed which were fixed for 30 min with 4 % paraformaldehyde. After fixation the cryosections were incubated with 1 μg per ml proteinase K (with 1 mM $MgCl_2$) for 30 min. After post-fixation and prior to hybridization the color of the sponge cells was removed by an ethanol treatment. Hybridization was performed in 2x SSC and 50 % formamide. An activated digoxigenin-labeled probe was used which was processed as described (10 pmol per ml) (LE PENNEC *et al.,* 2003; PEROVIC *et al.,* 2003). The sections were incubated with Tris-buffer, supplemented with the dye reagents NBT-X-Phosphate (Roche) for 1 hr at 37° C in the dark. After washing the sections for 5 min in 1x PBS, they were covered in Phytohisto and analyzed under the microscope.

The DNA probe was constructed based on the *S. domuncula caspase* cDNA (WIENS *et al.,* 2003b). The *caspase* probe spanned the segment 5'-TCGAGGTGGATCACCTGCA-3' (nt_{703} to nt_{721}) corresponding to the aa stretch LEVDHLH (aa_{222} to aa_{228}) to 5-CATCTGCATACAGACACAATT-3 (nt_{1255} to nt_{1265}) [HLHTDTI; aa_{403} to aa_{409}] and isolated by PCR technique. Both an antisense and a sense (negative control) was used for the *in situ* hybridization of mRNA encoding for *caspase*. It is seen that cryosection performed through an allograft (Fig. 3C) stains strongly with an antisense-*caspase*-probe in the region at which the allogeneic tissue is present (Figs 3D, E). In a control series of experiments a sense-*caspase*-probe was used. Under those conditions no reaction (staining) was seen in the allogeneic tissue region (Fig. 3F).

METAZOAN ANTI-APOPTOTIC CELL SURVIVAL PROTEINS

Besides the activation of the apoptotic process through TNF and TNF-receptor additional pathways have been described in Bilateria, which include activation through growth factor deprivation, heat shock or bacterial infection, that have also been described in sponge systems (WAGNER *et al.,* 1998). The signal transduction pathway initiated by those factors can be blocked by the function of molecules belonging to the Bcl-2 family. After having established that both in *S. domuncula* and in *G. cydonium* apoptosis can be initiated by environmental stress factors (WAGNER *et al.,* 1998) an intense screening for members of the Bcl-2 family (anti-apoptotic

molecules) was started. This effort resulted in the cloning of the anti-apoptotic cell survival proteins from these two sponge species (WIENS *et al.,* 2000a,b, 2001). The proof that the sponge gene product acts as a cell survival protein was performed by transfection studies using mammalian cells; it could be shown that in stably transfected mammalian cells the sponge *Bcl-2* related gene confers resistance against heat shock and growth factor deprivation (WIENS *et al.,* 2001).

Taken together, this bulk of evidence shows that the sponges are provided with a complex apoptotic machinery which allows the elimination of unwanted tissue, e. g. in allo-transplantation, and very likely also in the establishment of a controlled Bauplan.

PRIMMORPHS, A SYSTEM TO STUDY THE SPONGE BAUPLAN

The first successful demonstration of large scale and long-term proliferation of sponge cells *in vitro* was achieved with the establishment of the primmorph system (CUSTODIO *et al.,* 1998; MÜLLER *et al.,* 1999a). The successful strategy for the cultivation of primmorphs was based upon the observation that sponge cells lose the ability to divide if they are dissociated to single cells. It was discovered that sponge cells are provided with extracellular matrix molecules which interact with their cell surface receptors (reviewed in: MÜLLER, 1997). After ligand : receptor-interactions, intracellular signal transduction pathways are activated which result in the control of gene expression. Our rational conclusion was that only in the aggregated state the sponge cells retain the ability to grow; a view which is hitherto correct.

The marine demosponge *S. domuncula* served as starting material to obtain the cells which subsequently assembled to cell aggregates and finally to primmorphs (CUSTODIO *et al.,* 1998; MÜLLER *et al.,* 1999a). This technique was also successfully applied for the sponge *Dysidea avara* (MÜLLER *et al.,* 2000); it could be demonstrated that primmorphs from *D. avara* are even able to produce relatively high amounts of the bioactive low-molecular weight compound avarol. The detailed procedure of primmorph formation has been additionally outlined recently (LE PENNEC *et al.,* 2003). The single cells (Fig. 4A) were resuspended in seawater enriched with 0.1 % of RPMI1640–medium and silicate (60 µM) together with Fe(+++) (60 µM) (KRASKO *et al.,* 2000, 2002). Under these conditions round-shaped primmorphs are formed which display a compact smooth, waxy, brightly coloured surface (Fig. 4B). The primmorphs are characterized by the presence of proliferating cells as well as by a characteristic histology. The diameters of these 3D-cell aggregates vary from 3 to 8 mm. Microscopic analysis of cross sections through primmorphs showed an almost complete single-cellular layer of epithelial-like cells surrounding the primmorphs (CUSTODIO *et al.,* 1998; MÜLLER *et al.,* 1999a) (Fig. 4C). The cells in the squamous epithelium of the primmorphs are pinacocytes, as judged from their flattened, fusiform extensions and their prominent nucleus. The cells inside the primmorphs are primarily spherulous cells while the others may be termed amoebocytes and archaeocytes.

A different reorganization pattern of the primmorphs is seen if the cells are plated after dissociation onto poly-L-lysine (M_r 30,000 - 70,000) or onto galectin-coated tissue culture test plates (WIENS *et al.,* 2003c) (Fig. 4D-F). The homologous galectin (rGALEC1SUBDO) was obtained in a recombinant manner from a

complete cDNA, termed *SDGALEC1* (accession number AJ493055; WIENS *et al.,* 2003c). For coating 500 µl of a poly-L-lysine solution (10 µg per ml) or of a recombinant galectin solution (10 µg per ml) were added per well. After 3 hrs at room temperature the wells were washed and the dissociated cells were added. After 5 – 8 days primmorphs had formed. In contrast to primmorphs that developed on non-coated plates primmorphs grown on galectin- or poly-L-lysine-coated tissue culture test plates showed canal-like structures. The canal-containing primmorphs had as size of approximately 10 mm.

EXPRESSION OF THE GENE ENCODING THE LIM-HOMEOBOX PROTEIN

Under conditions during which the primmorphs form canal-like structures the expression of the gene encoding the LIM-homeobox protein is strongly upregulated (WIENS *et al.,* 2003c). Competition experiments with galactosylceramides isolated from *S. domuncula* were performed which revealed that a ß-galactosylceramide, named Sdgal-1, prevented the expression of the LIM gene on the galectin matrix, while Sdgal-2, a ceramide with a terminal α-glycosidically linked galactose caused no effect on the formation of channels in primmorphs nor on LIM expression. This study demonstrates for the first time, that an extracellular matrix molecule, galectin, induces a morphogenetic process in sponges which is very likely caused by a LIM-homeobox protein.

EFFECT OF RETINOIC ACID

In a second series of experiments we could show that retinoic acid causes a tissue regression in intact individuals of the demosponge *S. domuncula* and also in primmorphs (WIENS *et al.,* unpubl. data). The primmorphs used were cultivated on a galectin or poly-L-lysine matrix in order to induce canal formation. In the presence of 1 or 50 µM the canals formed underwent regression, a process which is however reversible. This effect is not caused by apoptosis as measured by the determination of liberated nucleosomes. Next we cloned the cDNA coding for the retinoid X receptor (RXR) from *S. domuncula*; it comprises the two characteristic motifs, the DNA-binding domain, including the zinc fingers (C4 type) and the ligand-binding domain of nuclear hormone receptors. Phylogenetic analysis revealed that the sponge putative RXR belongs to the 2B group receptors. Expression studies using Northern blotting revealed that the expression of RXR undergoes strong upregulation in response to retinoic acid treatment, while the expression of the sponge caspase shows no increase. Likewise, the gene encoding the LIM homeodomain (HD) protein (tetra-Lim-protein; *SDLIM4*) was found to be strongly upregulated in response to retinoic acid treatment. In contrast, the expression of the *S. domuncula* genes encoding one caspase, *SDCASPR*, and a member of the cytochrome P450 superfamily, *CYP4SD*, remained constant. These data indicate that also in sponges the RXR and its ligand retinoic acid play a role in the control of morphogenetic events.

CONCLUSION

Taken together, it can be assumed that the hypothetical ancestor of Metazoa, the Urmetazoa (MÜLLER, 2001; MÜLLER *et al.,* 2001a), was already provided with the basic regulatory repertoire, like cell adhesion molecules [like the aggregation factor or galectin] and cell differentiation factors [involved in collagen synthesis] as well as with a highly elaborated immune system (MÜLLER *et al.,* 1999b) which allowed a pattern formation due to an expression of the already published morphogens, a Pax-A like HD protein, Hox-like molecules or a Lim-class HD protein. In addition, recent studies in our laboratory demonstrated that *S. domuncula* expresses *Forkhead* and *T-box* genes and also a retinoic acid receptor (ADELL *et al.,* 2003). Furthermore, apoptotic genes are expressed (*e.g.* WIENS *et al.,* 2001) which are considered to be involved in the formation of the sponge body cavities. Based on these recently gathered data it can be deduced that sponges have amazingly rich and diversified regulatory molecules allowing pattern formation. Like the Cnidaria they are lacking those gene clusters allowing a vectorial patterning mechanism. The establishment of the primmorph system was very favorable for the further understanding of the morphogenetic processes in sponges.

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