

# Evolution of Metazoan Cell Junction Proteins: The Scaffold Protein MAGI and the Transmembrane Receptor Tetraspanin in the Demosponge *Suberites domuncula*

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**Abstract.** Until recently the positioning of the sponges (phylum Porifera) within the metazoan systematics was hampered by the lack of molecular evidence for the existence of junctional structures in the surface cell layers. In this study two genes related to the tight junctions are characterized from the demosponge *Suberites domuncula*: tetraspanin (*SDTM4SF*), a cell surface receptor, and MAGI (*SDMAGI*), a MAGUK (membrane-associated guanylate kinase homologue) protein. Especially the MAGI protein is known in other metazoan animal phyla to exist exclusively in tight junctions. The characteristic domains of MAGI proteins (six PDZ domains, two WW domains, and a truncated guanylate kinase motif) are conserved in the sponge protein. The functional analysis of *SDMAGI* done by *in situ* hybridization shows its expression in the surface epithelial layers (exopinacoderm and endopinacoderm). Northern blot studies reveal that expression of *SDMAGI* and *SDTM4SF* increases after formation of the pinacoderm layer in the animals as well as in primmorphs. These results support earlier notions that sponges contain junctional structures. We conclude that sponges contain epithelia whose cells are organized by cell junctions.

**Key words:** Sponges — Porifera — *Suberites domuncula* — Cell junctions — MAGI — Scaffold protein — Tetraspanin — Epithelial polarity — Axis formation

## Introduction

It is now well established that all metazoan phyla, including sponges (phylum Porifera), evolved from one common ancestor, the Urmetazoa (Müller 2001). Data from molecular biological studies with the demosponge *Suberites domuncula* revealed that sponges have characteristic cell–cell and cell–matrix adhesion molecules, such as integrins (Pancer et al. 1997; Müller 1997) and collagens (Exposito et al. 1991; Schröder et al. 2000), in common with the “higher” metazoan phyla. The arrangement of cells within a sponge organism is genetically controlled by the same evolutionary conserved genes found also in higher metazoans; homeodomain proteins (reviewed by Wiens et al. 2003) and T-box transcription factors (Adell et al. 2003) have been also found to be responsible for the bodyplan formation in Porifera. The sponge body is surrounded by a pinacoderm, which has been described as an epithelium-like layer of cells; however, these cells are not connected by cell junctions according to the literature (Mackie 1984;

The sequence from *Suberites domuncula* reported here, the protein membrane-associated guanylate kinase with an inverted arrangement (MAGI), is deposited in the EMBL/GenBank database under accession number AJ580406.

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reviewed by Nielsen 2001). The apical cell contacts observed in the ciliated cells of sponge larvae have been looked upon as cell junctions, but not related to metazoan cell junctions (Rieger 1994). “Septate” junctions, similar to those found in some invertebrate taxa (see Knust 2000), were visualized in some sponge species between choanocytes and sclerocytes (Ledger 1975). Until now, no gene presumably involved in the formation of cell junctions has been identified in sponges.

The knowledge on the degree of differentiation of the pinacoderm, the sealing cell layer in sponges, is crucial for its classification as a “porous” cell layer or as epithelium. Three forms of pinacoderm layers are distinguished in *Calcarea* and *Demospongiae*; the exopinacoderm facing the external milieu, the endopinacoderm surrounding the aquiferous canal system, and the basopinacoderm, which attaches the animal to the substratum (Garrone 1978). The first molecular evidence indicating that in some sponges a basement membrane exists was the discovery of type IV collagen in the demosponge *Pseudocortidium jarrei* (Boute et al. 1996). A confirmation that the pinacoderm is really composed of cells with a polarized phenotype in an asymmetric distribution of organelles and their polarized orientation of the cytoskeleton (Knust 2000) can be expected from the isolation of genes controlling this pattern. Polarization of epithelial cells occurs at the tight junctions by the assembly of protein scaffolds that cluster transmembrane proteins and anchor them to the underlying cytoskeleton (see: Yeaman et al. 1999). Especially in vertebrates tight junctions regulate the passage of water and ions as well as of organic molecules through the paracellular pathway.

Among the dominant transmembrane proteins of the tight junctions, the receptors occludin, claudin, and other junctional adhesion molecules should be mentioned; they are linked to characteristic classes of scaffold molecules, the intracellular effector molecules/receptors (Yeaman et al. 1999). Occludin and claudin form the backbone of the tight junction strands; like tetraspanin they have four transmembrane regions (González-Mariscal et al. 2003). The scaffold molecules are subdivided into proteins containing the PDZ motif and others that lack this motif (González-Mariscal et al. 2003). Among the PDZ-containing proteins, the role of the membrane-associated guanylate kinases (MAGUK) in the function of tight junctions is well understood (González-Mariscal et al. 2000). A homologue of a MAGUK protein, a zonula occludens homologue, was identified in the Cnidaria *Hydra vulgaris* (Fei et al. 2000). It is well established that Cnidaria possess functional epithelia that define distinct tissue layers (Sarras et al. 1991); therefore tight junction proteins might be predicted. In sponges, where the existence of true epithelia is still under dispute (see: Pechenik 2000),

the proof of the existence of scaffold proteins characteristic for tight/cell junctions would change the view on the quality of the pinacoderm layer as epithelial layer. Since no approach to identify potential junctions in *S. domuncula* has been performed microscopically, we term the linkages between the cells operationally cell junctions.

In the present study the successful screening for a gene encoding a cell junction scaffold protein from a sponge, *S. domuncula*, is reported. After domain analyses of the 15,000 ESTs in our database from *S. domuncula* (Chevreux et al. 2004) a cell junction scaffold protein has been identified. The gene encodes the scaffold protein membrane-associated guanylate kinase with inverted arrangement (MAGI). MAGI proteins are characterized by a unique arrangement of the functional domains: six PDZ domains, two WW domains, and a truncated guanylate kinase motif that, in contrast to other MAGUK proteins, is located at the NH<sub>2</sub>-terminus rather than at the COOH-terminus (Dobrosotskaya et al. 1997). MAGI proteins function as scaffold molecules, linking the membrane-associated receptors, the signaling cortical proteins, and the actin cytoskeleton (Shoji et al. 2000; Dobrosotskaya and James 2000). Some isoforms from alternative splicing have been also found to localize in the nucleus, which supports their role in the intracellular communication pathways (Dobrosotskaya et al. 1997; Dobrosotskaya and James 2000). In this report, *in situ* hybridization studies demonstrate that the MAGI gene from *S. domuncula*, termed *SDMAGI*, is highly expressed at the surface of the animal and in cells of the pinacoderm lining the canals.

In addition we describe the existence of one tetraspan receptor, tetraspanin (*SDTM4SF*), in *S. domuncula*; this molecule has been described earlier (Müller et al. 1999b). The tetraspanins are a huge superfamily, with more than 37 members described alone in *Drosophila melanogaster* and numerous more in other organisms. Together with occludin and claudin, they belong to a group of hydrophobic proteins with four transmembrane domains and a series of conserved aa residues in the extracellular loops (Yunta and Lazo 2003; González-Mariscal et al. 2003). Tetraspanin forms complexes with other cell surface receptors, e.g., integrin, and is involved in regulation of proliferation and migration of cells (Tiwari-Woodruff et al. 2001).

The regeneration capacity of sponge tissue is high. After ablation of tissue from the body surface a series of genes is upregulated, i.e., the integrin receptor (Wimmer et al. 1999) and the allograft inflammatory factor in *S. domuncula* (Kruse et al. 1999). The primmorph system from sponges, which represents special 3D-cell aggregates composed of proliferating and differentiating cells, has successfully been applied to investigate the role of cell–cell and cell–matrix

adhesion molecules as well as of transcription factors (Wiens et al. 2003; Perović et al. 2003). After approximately 5 days in culture the aggregates/primmorphs are surrounded by a surface layer composed of cells reminiscent of pinacocytes (Müller et al. 1999a). Expression of *SDMAGI* (encoding MAGI scaffold protein) as well as of *SDTM4SF* (*S. domuncula* tetraspanin [Müller et al. 1999b]) is highly upregulated after regeneration of the epithelial layer of the sponge and after formation of the pinacoderm layer in primmorphs.

## Materials and Methods

### Chemicals and Enzymes

The sources of chemicals and enzymes used were given previously (Kruse et al. 1997; Krasko et al. 2000).

### Sponges

Specimens of *S. domuncula* (Demospongia, Hadromerida, Subritidae) were collected from the Adriatic Sea, close to Rovinj (Croatia), and then kept in aquaria in Mainz for more than 3 years, as described by Le Pennec et al. (2003). For the regeneration studies the 2-mm-thick surface layer, including the pinacoderm, was ablated from the test animal. Then this surface sample and tissue from the interior of the specimen were taken and used for the Northern blot experiments.

### Formation of Primmorphs

The procedure described for the formation of primmorphs from single cells was applied (Müller et al. 1999a). Starting from single cells, primmorphs of approximately 5 mm formed after 5 days. They were cultivated in natural seawater, supplemented with 0.2% of RPMI-1640 medium and with the optimal concentration of 60  $\mu\text{M}$  silicate and 30  $\mu\text{M}$   $\text{Fe}^{3+}$  (added as ferric citrate) (Krasko et al. 2000).

### cDNA for the Putative MAGI Scaffold Protein

A fragment of the cDNA encoding the putative MAGI protein was identified in the *S. domuncula* EST database after domain analysis (Chevreux et al., submitted); the cDNA library of *S. domuncula* had been constructed as described (Kruse et al. 1997). The fragments obtained were used to screen the cDNA library (Wiens et al. 2000); the cDNA, termed *SDMAGI*, was completed using the RACE technique (Invitrogen GeneRacer Kit; Invitrogen, Groningen, The Netherlands). DNA sequencing was performed with an automatic DNA sequenator (Li-Cor 4000S). As determined by Northern blotting the complete sequence *SDMAGI* was obtained; it comprises 3271 nucleotides (nt). The phylogenetic tree was constructed on the basis of aa sequence alignments by neighbor-joining; degree of support for internal branches was further assessed by bootstrapping (Felsenstein 1993).

### In situ Localization Studies

The method applied bases on the procedure described by Polak and McGee (1998) with modifications described recently (Perović et al.

2003). In brief, sections were obtained at  $-30^{\circ}\text{C}$  and fixed with paraformaldehyde. The cuts were incubated with proteinase K and subsequently fixed again with paraformaldehyde. To remove the sponge color the cuts were incubated with ethanol. After rehydration with  $1 \times \text{PBS}$ , digoxigenin (DIG)-labeled DNA probes were added to the hybridization solution. Hybridization was performed overnight in a glass chamber at  $45^{\circ}\text{C}$ ; the subsequent washes were performed at  $50^{\circ}\text{C}$  as described (Perović et al. 2003). After blocking the sections were incubated with an anti-DIG antibody conjugated with alkaline phosphatase. NBT/X-phosphate was used for the visualization of the signals. In all cases reported here positive signals were obtained with the antisense probes, while the sense probes (controls) showed no staining.

*In situ* hybridization was performed with DIG-labeled ssDNA probes. The probes were labeled with the PCR DIG Probe synthesis Kit (Roche). DNA probes were constructed, basing on the *S. domuncula* cDNA sequences, and spanned the region of amino acid 131 (aa<sub>131</sub>) to aa<sub>363</sub> of the deduced MAGI\_SD sequence or aa<sub>86</sub> to aa<sub>160</sub> of TM4SF\_SD. Both antisense and sense probes were prepared by PCR, using the linearized cDNA as a template. The sense probes were obtained by applying a forward primer in the 5'-to-3' sense direction; the complementary antisense probes were obtained by using a reverse primer in the 3' to 5' orientation. The probes had lengths of 693 bp (*SDMAGI*) and 225 bp (*SDTM4SF*), respectively.

The sections were inspected with an Olympus VANOX AHB T3 microscope; the images were recorded with a ColorView 12 camera and were composed applying the Soft Image System analySIS 3.0 (Soft Image System GmbH, Münster; Germany).

### Northern Blotting

Total RNA was extracted from liquid nitrogen-pulverized primmorphs with TRIzol reagent (Gibco-BRL, Grand Island, NY). An amount of 5  $\mu\text{g}$  of total RNA was electrophoresed through a 1% formaldehyde/agarose gel and blotted onto a Hybond-N<sup>+</sup> nylon membrane following the manufacturer's instructions (Amersham; Little Chalfont, Buckinghamshire, UK) (Wiens et al. 1998). Hybridization was performed with the 693-nt-large part of the *SDMAGI* cDNA and the 225-nt-long *SDTM4SF* (tetraspanin; CAA77026.1) probe. The probes were labeled with the PCR-DIG-Probe-Synthesis Kit according to the instruction manual (Roche). After washing, DIG-labeled nucleic acid was detected with anti-DIG Fab fragments (conjugated to alkaline phosphatase; dilution of 1:10,000) and visualized by chemiluminescence technique using CDP, the chemiluminescence substrate alkaline phosphatase, according to the instructions of the manufacturer (Roche). For the Northern blot studies the cDNA of the housekeeping gene  $\beta$ -tubulin, *SDTUB* (AJ550806), of *S. domuncula* was used as an internal standard.

## Results and Discussion

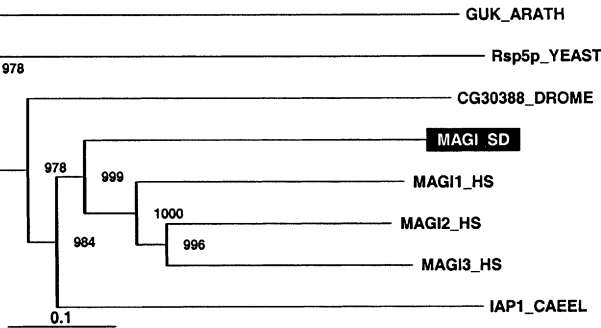
### Identification of a MAGI-Related Gene in *S. domuncula*

The cDNA, encoding the complete MAGI scaffold protein, *SDMAGI*, was isolated. The nucleotide sequence is 3271 residues long, with the open reading frame ranging from nt<sub>7-9</sub> to nt<sub>3241-3243(stop)</sub> (accession number AJ580406). The deduced amino acid (aa) sequence, MAGI\_SD, with 1078 residues, represents a calculated 119,042-Da polypeptide (Fig. 1A). MAGI\_SD shares the highest similarity to the related

A

MAGI_SD	MSKSHKRSDSLFRKHWHSIACEISLVCRNADGFCFLNVEGGABSCLPFFVGETRQDKRHYQNG-KIHHGDIITLVNKRVPGLK	84
MAGI2_HS	MSKSLK-----KSHHTYSKWHESVGRNPPFCQLGFLVGGAGNQPVYLGVEVRCQKVAVBSGSKIVSERLLLVNTPVAGLITR	80
	[ = PDZ0 = ]	
MAGI_SD	DVIALITRSADDPVLLVYKQANLTKKDVRCYLAIRPKKNSVDSLEQCHIRENHLRVVPCITTRPQPCGRCRQIDYAFISVETSKR	169
MAGI2_HS	DLVAVIHKCKDPLKCKVKGGLVDRKDIRVYLNTRFKQKSVDELEQCHIRNRYNLRVAVPCITTRPKHKGVEVGVYDFITIVEDGME	165
	[ = PDZ0 = ] (- GuK -)	
MAGI_SD	MRKQALLESVYVYGTGPKPPSDPPSSTIPAYSRSATATGYSKEAIIPLSDATASRPGGRGPAHLMPKTV-----	244
MAGI2_HS	LRKQALLESVYVYGTGPKPPAPLPLNVTDQILPQATPSAEGKRKRKNSVSMKASIEPEEEEEERPVVNGVGVVTV	250
	[ = PDZ0 = ] (- GuK -)	
MAGI_SD	-----CPLEANWEIATYENNENKVFITDHCCTFHHMLDPLR	278
MAGI2_HS	PESEEHDKSAGASGEMPSQYPYAPVYSQPEELKEQMDDTKPTKPEDNEEPPDLSDNWEIAYTEKQGVFIDH--KQKSSULDPLR	335
	[ = WW1 = ]	
MAGI_SD	AKRHGHMMECAENELVYGWKRVNDRVYGVYITDVRHNRVQYENPVYAKRRFAGSCLEQRVPHQEPFVHLLHLENHFLYSLQL	363
MAGI2_HS	AKRARP-PPCEKNELVYGWKIDDDIVYGVYVDHNRVQYENPVYAKRRVQQRNMDHTELOTKLQAPGRF-----	408
	[ = WW2 = ] (- WW2 -)	
MAGI_SD	PLRNNVRLKLLPCGLTQVSADNPDSYFYPYQQQQHHQQCCQENHDLAEVFCGVYHVGLOKTKASGFGFTTIGGDRFGLLQI	448
MAGI2_HS	-----EKLFLTRDASQKCFKTFSTTKKSNMGFGFTTIGGDRFGLLQV	452
	[ = PDZ1 = ]	
MAGI_SD	KSVIRGSDVDRDGRLLQVDVLRVINGHSVPTYSHRKVVDFQSLFENSEVSEVVRGVPVDFRQD-----	514
MAGI2_HS	KGVIPDGPAAQDKKMEAGDVIYVNEVYVLCVCHNHADVVKLQSVPIQGSVAVLQGVYVDFPDPFANSMPVPLAIMERPPVVM	537
	[ = PDZ1 = ]	
MAGI_SD	-----EIPSYNESHGQNGDYPPQDQYNNPQAQQQPEKVSVMIVKCPILGFGFSLGGS	566
MAGI2_HS	VNGRHNYEYTYLEYSRTSQQVVDITDRPHSLHSMPPDQCLDGYPPPVHDDVMSASSGATQALDTLITVTKCAQGFGTIADS	622
	[ = PDZ2 = ]	
MAGI_SD	EMCPVVKQIMDHPKCAQLCEGDVLMQVNGSRVSYAHSQIVYVLRKCEKCNQALFVVR-----	625
MAGI2_HS	PTGQRVQIHLTQQSPGLCEGDIVFVINGQVONLSHVMVLLKQCHTSSETSLIHRGGFSPWKTKPKIMDRWENQSPQTS	707
	[ = PDZ2 = ]	
MAGI_SD	-----CPVZEPYSYSGEGYPPFQMGSDGR-FTIMVVRVLRQVSGFGPRI	670
MAGI2_HS	LSAPAIPQNLPPFPPALHRSSFPDSTEAFDPRKDPYELYKSRALVYESRQVPPRTSFRVDSGSDKCLDWHLRBESGGRPRI	792
	[ = PDZ3 = ]	
MAGI_SD	IGGEPEESQANVGAIVAGCAADLDRVQIGDEITHINGCSVYNASHRDVILGCPAAAGSEVVICIRKRMPTDTSVPP-----	748
MAGI2_HS	IGGEPEEQPILIGAVAVGSAADLDRVHFDDELVVDSPVAGKTRHVVLDLHPAARNGQVALVRRKVLGGGPECEENGRSPG	877
	[ = PDZ3 = ]	
MAGI_SD	-----PGSYGGVPVNYQQYQHQGHDMLELEPPQSGLPQGRNIVYVDRPNIQSFGFVLSNTILRAG-----CMTQRLVPS	819
MAGI2_HS	SVSTHSSPPSDYATTNSNAAPSSMASPPECFASHLSLQTSDVVLRH-KENEGGFVHISLNRPSGSGTITVXHKIKRITDGS	961
	[ = PDZ4 = ]	
MAGI_SD	PAEKNOIYVYDELLAVNCKIVSRMDFHGDIVATKSSGDTDFHVAQQPDDLELNRMQMTEDQ-----	882
MAGI2_HS	PADRCARVQDRI LAVNGQSHIMPHADIVRLTKDAKLSVTRHITPQEBNSPTSAPSSKQSPMAQQSPLAQQSPLQPSPAT	1046
	[ = PDZ4 = ]	
MAGI_SD	-----QQQTSVGGQQCPQGGYQEDRGNFDQYFGMEDDGAQIIS	922
MAGI2_HS	PNSPIAQFAPPQPLQLQGHENSYSRSEVKARQDVVKFDTRQPPFTYRQPFLLVTRQPPGQDYQQPPFLDYRQPFLLDYRQHSPTDTRQ	1131
	[ = PDZ5 = ]	
MAGI_SD	ADTSEIAS-DEEIHHDVFRISGGEGFSIRGGAIVNSPFCVLRITDCAVADGRLRVGDLELITNGSSTESVLSHSDATLTKHK	1006
MAGI2_HS	YPLSEYRQPDVYFVDMDEKAKGEGSIRGGRVMDLVLRLELDCPAINRGRMRVGDQIIEINGSTPDLTHARATPLKKS	1216
	[ = PDZ5 = ]	
MAGI_SD	GDVVKLITRR-----VDEPFIIGSGKSGHQDQGTFSPTAVQTKDRYNOEYLNLSRNRDRSYSGCAEHRSPFNE-----	1078
MAGI2_HS	GRVRVLLKRGTOQVQVDEPAPWSSFAAAAPCPPEVGVSLDDGLAPFSPSHAPPDPSHQISPEFTWDLKRRKHDVVRKPKELS	1301
	[ = PDZ5 = ]	
MAGI2_HS	ACGQKQRLGEGREERSASPPRAARPRLEEAQGGQRPSEAGRFASEARAPGLAAADAADAARAGGKEAPRAAAGSELCRREGLGAA	1386
MAGI2_HS	PASADPDGSGGALAAEGRAGARAAFRSVRPPFAGAAARRAAGAPGXWVVRGSDKLPVSLKPGASAAAR	1455

B

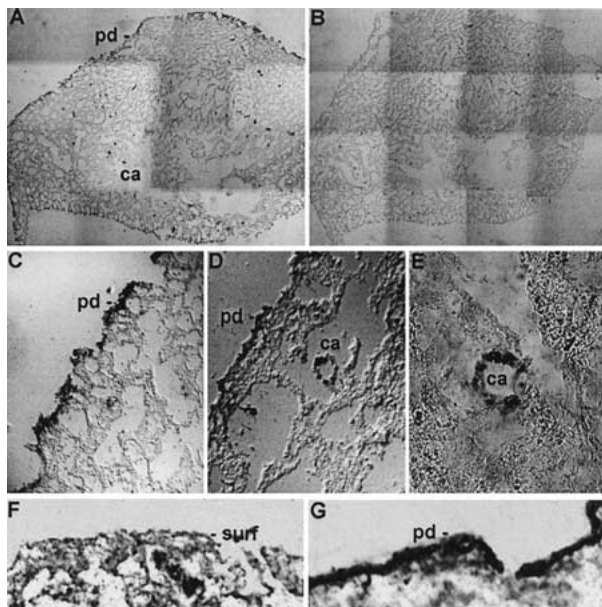


**Fig. 1.** *S. domuncula* MAGI-related scaffold protein in *S. domuncula*. **A** The deduced sponge MAGI protein was aligned with the human membrane-associated guanylate kinase 2 (MAGI2\_HS; AAC05370 [Wood et al. 1998]). Amino acids, similar among the sequences, are in inverted type. The characteristic conserved regions of the MAGI scaffold proteins, the six PDZ domains (= PDZ =), the guanylate kinase motif (- GuK -) and the two WW domains (~ WW ~) are marked. **B** Rooted phylogenetic tree constructed from the above-mentioned sequences as well as the other human sequences, MAGI-1B alpha beta (MAGI1\_HS; AAK94064 [Laura et al. 2002]) and MAGI-3 (MAGI3\_HS;

NP\_690864 [Thomas et al. 2002]), as well as two distantly related sequences from *Caenorhabditis elegans*, interacting protein 1 (IAP1\_CAEL; NP\_502221), *Drosophila melanogaster*, the hypothetical protein CG30388-PA (CG30388\_DROME; NP\_611551), and the yeast (*Saccharomyces cerevisiae*) protein involved in ubiquitin-mediated protein degradation, Rsp5p (Rsp5p\_YEAST; NP\_011051) together with the plant (*Arabidopsis thaliana*) guanylate kinase (GUK\_ARATH; NP\_566276). The tree was rooted using the plant sequence as an outgroup. The scale bar indicates an evolutionary distance of 0.1 aa substitution per position in the sequence.

metazoan scaffold proteins with an “expect value” ( $e$ ; Blast-NCBI [Coligan et al. 2000]) of  $\approx e^{-1.49}$ . As shown in Fig. 1A, the sponge MAGI protein displays the six characteristic PDZ domains: at aa<sub>22</sub> to aa<sub>105</sub> (domain 0 [according to González-Mariscal et al. 2003]), aa<sub>422</sub> to aa<sub>504</sub> (domain 1), aa<sub>549</sub> to aa<sub>612</sub> (2), aa<sub>656</sub> to aa<sub>738</sub> (3), aa<sub>785</sub> to aa<sub>868</sub> (4), and aa<sub>937</sub> to aa<sub>1019</sub> (5). The PDZ domains are conserved structural elements of approximately 80 aa which are involved in the formation of protein-protein interactions (Ponting et al. 1997; Ranganathan and Ross 1997).

Among the six PDZ domains in MAGI proteins, PDZ-2 has been shown to bind to the signaling molecule GEP (for MAGI-1 in mammals [Mino et al. 2000]) and to PTEN, a tumor suppressor gene (for MAGI-2 in humans [Wu et al. 2000b; Tolkacheva et al. 2001]). Human MAGI-3 binds through PDZ-2 to PTEN and in the synaptic junctions through the PDZ-5 to the NMDA receptor (Hirao et al. 1998; Wu et al. 2000a,b). MAGI proteins, including that from *S. domuncula*, possess additionally two WW domains that bind to proline-rich peptide motifs



**Fig. 2.** Expression of *MAGI* in tissue from *S. domuncula*, determined by *in situ* hybridization. Tissue slices through the surface of a specimen were prepared and hybridized with antisense (A, C–G) as well as sense (B) *SDMAGI* probe. The antisense *MAGI* reacted with the pinacoderm layer (pd), which seals the animals (A, C, D), and also with the cells which surround the canals (ca) (D, E). F Low expression of *MAGI* at the surface (surf) of the sponge tissue immediately after ablation of the pinacoderm layer. G After a regeneration period of 6 days a strong signal is seen at the newly formed pinacoderm (pd). Original magnifications: A and B,  $\times 40$ ; C to E,  $\times 400$ ; F and G,  $\times 100$ .

(Sudol et al. 1995; Ermekova et al. 1998) of those proteins that are involved in signal transduction processes (Bork and Sudol 1994). Finally, the conserved guanylate kinase (GuK) motif is found from aa<sub>109</sub> to aa<sub>189</sub> of the sponge *MAGI* protein (Fig. 1A). The conserved guanylate kinase (GuK) motif in *MAGUK* proteins obtained its name because of its sequence similarity to the yeast GuK, an enzyme which synthesizes GDP from GMP under consumption of ATP (Berger et al. 1989). However, none of the GuK domains in *MAGUK* are enzymatically active (Dobrosotskaya et al. 1997).

### Phylogenetic Relationship

In vertebrates three members of the *MAGI* scaffold proteins have been identified, which localize in the tight junctions of epithelial cells and also in the synaptic junctions (Dobrosotskaya et al. 1997; Wood et al. 1998; Wu et al. 2000a; Laura et al. 2002; Thomas et al. 2002; reviewed by González-Mariscal et al. 2003). All three *MAGI* proteins represent different isoform products of alternative splicing. For the present alignment the longest forms of the proteins have been included. We selected the three human *MAGI* scaffold proteins, *MAGI*-1

(Laura et al. 2002), *MAGI*-2 (Wood et al. 1998), and *MAGI*-3 (Dobrosotskaya et al. 1997; Thomas et al. 2002), that displayed the highest sequence similarity to the *S. domuncula* protein. They share 45% similar aa residues and 28% identical residues with the *S. domuncula* protein. It is striking that the highest similarity between the sponge and the human molecules is seen within the conserved regions of the polypeptide (Fig. 1A). In contrast, the sequence similarity to the two *MAGI* sequences from invertebrates (from *Drosophila melanogaster*, the hypothetical protein CG30388-PA, and from *Caenorhabditis elegans*, the interacting protein 1 molecule) is considerably lower (31% similarity/19% identity to these invertebrates). Only distantly related are the molecules from the yeast *Saccharomyces cerevisiae*, a protein involved in ubiquitin-mediated protein degradation, and the plant *Arabidopsis thaliana*, a guanylate kinase (<10% similarity/<10% identity). These relationships are also reflected by the rooted tree, constructed from these sequences (Fig. 1B). The sponge *MAGI* protein forms the base of the branch, including the three human *MAGI* forms, while the insect and nematode polypeptides branch off earlier. The plant and yeast sequences show only a distant relationship.

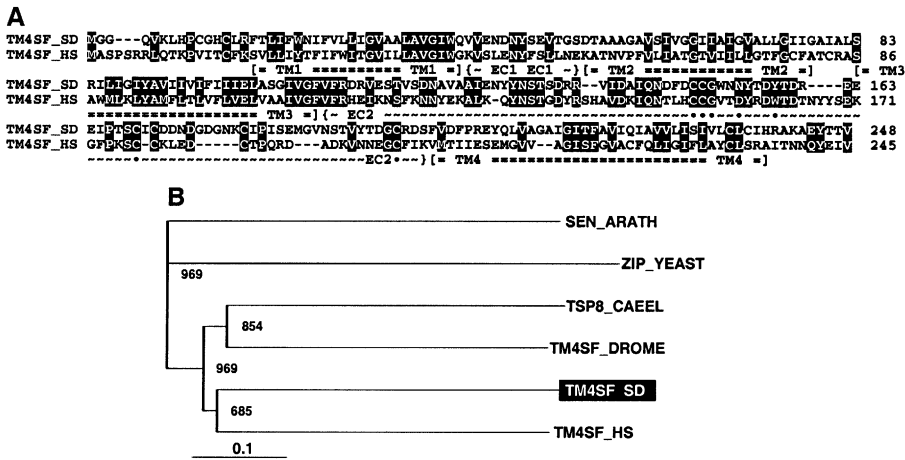
### Identification of Cells Expressing the *MAGI* Gene

Since the technique of *in situ* hybridization was established in sponges on the cellular level (Perović et al. 2003), this method became a powerful tool to learn more about the function of the expressed genes. Both antisense and sense *SDMAGI* probes were applied for the hybridization procedure, in tissue of *S. domuncula* specimens. While the sense probe did not show any signal (Fig. 2B), the antisense *SDMAGI* reacted with cells lining the pinacocyte layer, the pinacoderm, and some canals within the mesohyl, the internal part of the sponge (Fig. 2A). A higher magnification of the sections showed that especially the surface cell layer of the pinacoderm reacted positively with the antisense *SDMAGI* probe (Figs. 2C and D), likewise only one cell sheet, surrounding the canals reacted with the probe (Fig. 2D and E).

From these data we conclude that the sponge *SDMAGI* gene is expressed in the surface/epithelial layers in sponges, primarily in the pinacoderm and also frequently around the canals.

### The *S. domuncula* Tetraspanin

To further support the existence of *MAGI* and its selective expression in the surface cellular layers another putative receptor found in cell junctions of higher metazoans was analyzed in *S. domuncula*.

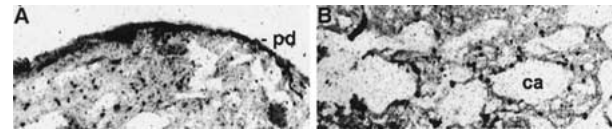


**Fig. 3.** *S. domuncula* tetraspanin. **A** The deduced protein TM4SF\_SD was aligned with the human tetraspanin (TM4SF\_HS; NP\_003261 [Berditchevski 2001; Maeda et al. 1998; Todd et al. 1998]). The characteristic segments within the receptor, the four transmembrane regions (TM1–TM4) and the two extracellular loops (EC1 and EC2) are marked together with the conserved amino acids in EC2 (●). **B** The rooted phylogenetic tree was

constructed together with the sequences from *C. elegans*, a tetraspanin family member (TSP8\_CAEEL; NP\_510445), *D. melanogaster*, tetraspanin 74F (TM4SF\_DROME; NP\_524132), *S. cerevisiae*, ZIPpering protein (ZIP\_YEAST; NP\_011265), and *A. thaliana*, the senescence-associated protein (SEN\_ARATH; NP\_176515). The plant sequence was used as outgroup.

Until now only a sequence for tetraspanin was identified; no other junctional proteins, e.g., occludin, claudins, or junctional adhesion molecules, have been identified yet. The identification of the cDNA encoding the *S. domuncula* tetraspanin (*SDTM4SF*; accession number CAA77026.1) was given previously (Müller et al. 1999b). The deduced protein of 248 aa has a calculated  $M_r$  of 26,701 and shows four putative transmembrane segments as concluded from the hydrophobicity plot (Kyte and Doolittle 1982) (Fig. 3A). At the NH<sub>2</sub>-terminus, the first cytoplasmic domain, and at the COOH-terminus, the third domain, is found, while the second domain is located between the second and the third transmembrane domain (Fig. 3A); especially in these regions the similarity to the other metazoan molecules is high. The characteristic aa residues within the tetraspanins are also found in the *S. domuncula* molecule (Fig. 3A), especially the conserved residues in the second extracellular loop (see Yunta and Lazo 2003).

A comparison to the human tetraspanin (Berditchevski 2001; Maeda et al. 1998; Todd et al. 1998) reveals a strong relationship (Fig. 3A); the polypeptides share 42% similar aa and 25% identical aa with each other. Again, the sponge polypeptide clusters in the rooted phylogenetic tree with the human tetraspanin (grouped with the transmembrane 4 superfamily which interacts with integrins; [Berditchevski 2001]; NP\_003261). The tetraspanins from the two selected invertebrates, *D. melanogaster* (a deduced protein obtained from random sequencing [Adams et al. 2000]; NP\_524132) and *C. elegans* (a tetraspanin family member, tsp-8, that is orthologous to the human gene KANGAI 1 [NP\_510445;



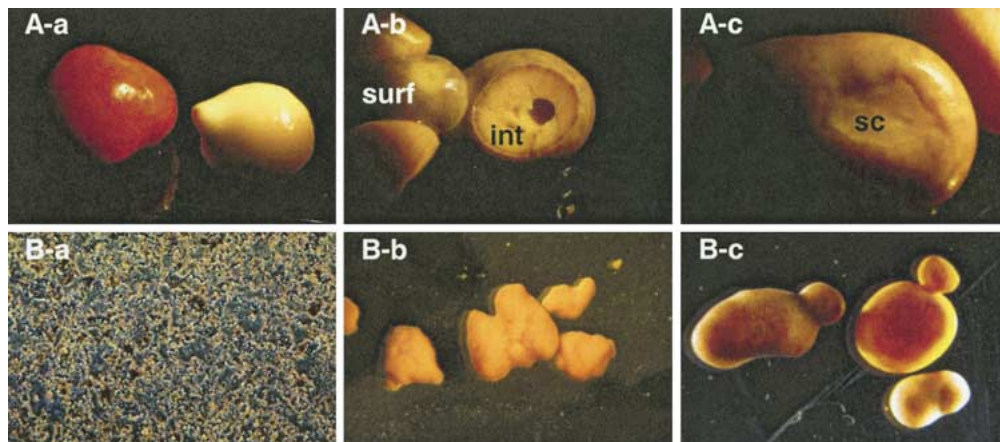
**Fig. 4.** Localization of cells within sponge tissue, expressing *tetraspanin*. Cryosection through tissue was prepared and subjected to *in situ* hybridization using the *SDTM4SF* antisense probe. **A** Area of the exopinacoderm; the pinacoderm layer (pd) is marked. **B** Cut through the internal body of the sponge specimen; one canal is marked (ca). Original magnification: A,  $\times 100$ ; B,  $\times 250$ .

sequence source, NM 078044.1]), form a separate branch (Fig. 3B).

The nonmetazoan polypeptides from *S. cerevisiae*, the ZIPpering protein (presumably involved in segregation of chromosomes [NP\_011265]), and from *A. thaliana*, the senescence-associated protein (cDNA, obtained by random sequencing of chromosome 1 [Tettelin et al. 1997]; NP\_176515), have only a weak similarity.

#### Localization of Cells Expressing Tetraspanin Within Sponge Tissue

The *in situ* hybridization technique was applied to localize the cells in the sponge tissue which highly express *tetraspanin*. The sections through the outer surface of the specimens revealed that the *tetraspanin* gene is expressed especially in the pinacoderm layer (exopinacoderm) (Fig. 4A). The cells of the endopinacoderm which surround the canals are highlighted to a lower degree (Fig. 4B). From these data we conclude that the expression pattern of the *tetraspa-*



**Fig. 5.** *S. domuncula* animals and primmorphs. **A-a** Sponge specimens. Magnification,  $\times 1$ . **A-b** From the surface of a specimen a 2-mm-thick layer was ablated (surf), and the interior of the animals became visible (int);  $\times 1$ . **A-c** After a period of 6 days the

surface of the animal regenerated and almost no scar (sc) was seen;  $\times 2$ . Primmorph formation. Dissociated cells (magnification,  $\times 20$ ; **B-a**), after 3 days, form compact aggregates ( $\times 5$ ; **B-b**), which develop further to primmorphs after 6 days ( $\times 3$ ; **B-c**).

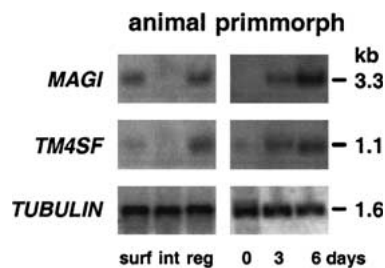
*nin* gene is very similar to that seen for the *MAGI* gene.

#### Expression of tetraspanin and MAGI in Sponge Tissue and Primmorphs

Two biological systems were used to determine the expression of the two cell junction proteins semi-quantitatively; (i) the regeneration of the pinacoderm layer in adult animals and (ii) the formation of primmorphs from cells of *S. domuncula*.

The surface layer of intact animals (Fig. 5A-a) was ablated (Fig. 5A-b). A sample each from the surface as well as from the interior tissue of the animal was immediately used for the preparation of the RNA. Six days after regeneration of the ablated tissue had started, another sample was taken from the regeneration zone (Fig. 5A-c). Northern blot analysis was performed using the labeled probes from *SDMAGI* (encoding MAGI), *SDTM4SF* (tetraspanin), and *SDTUB* ( $\beta$ -tubulin). The results revealed that the expression of the 3.3-kb *SDMAGI* transcript was detectable in the surface tissue which had been removed from the specimen, while the RNA from the interior part of the animal gave no signal (Fig. 6; left). After 6 days of regeneration the *SDMAGI* transcripts could be identified again in the RNA sample (Fig. 6; left). A similar expression pattern is seen for the tetraspanin (*SDTM4SF*) gene. The intensities of the *tubulin* gene were almost identical in the different samples, indicating that the same amount of RNA was loaded on the gels (Fig. 6; left).

In dissociated cells (Fig. 5B-a) obtained from the sponge almost no transcripts could be resolved on the blots for *SDMAGI* and for *SDTM4SF* (Fig. 6; right). Expression started, however, already 3 days after reaggregation (Fig. 5B-b) and increased during the subsequent 3 days of incubation (Fig. 6; right), the

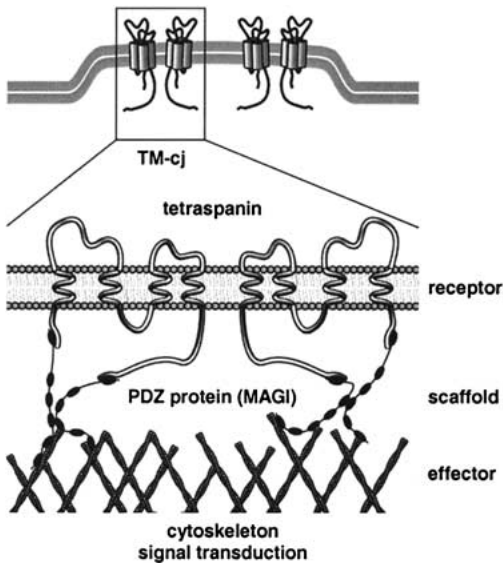


**Fig. 6.** Expression of *SDMAGI* (the cell junction scaffold protein) and *SDTM4SF* (the tetraspanin receptor). **Left:** A surface sample (surf) as well as tissue from the interior of the individual (int) was collected immediately after cutting. In parallel, RNA was extracted from a tissue sample of the surface of the animal 6 days after regeneration (reg) and processed likewise. Five micrograms of total RNA per each slot were size-separated; after blot transfer hybridization was performed with either *SDMAGI*, *SDTM4SF*, or  $\beta$ -*tubulin*. **Right:** Detection of transcripts for the genes *SDMAGI*, *SDTM4SF*, and  $\beta$ -*tubulin*. RNA samples were obtained from single cells (time: 0 days), from aggregates (3 days after reaggregation was started), or from primmorphs (after 6 days).

period during which the primmorphs with their pinacoderm layer are formed (Fig. 5B-c). Again the levels of the *tubulin* transcripts remained the same (Fig. 6; right).

From these data we conclude that the expressions of the genes coding for *S. domuncula* MAGI and tetraspanin proteins are high in tissue samples which contain epithelial surface cells, the pinacoderm. Focusing on the primmorphs it became evident that *MAGI* and *tetraspanin* are expressed during formation of the smooth cell surface layer.

To monitor the expression of *SDMAGI* during restoration of the pinacoderm after ablation of a part of the surface tissue from an individual sponge, *in situ* hybridization analysis was performed with the anti-sense *SDMAGI* probe. The data prove that immediately after the removal of the surface tissue, the cells



**Fig. 7.** Schematic representation of the molecules involved in the formation of cell junctions in sponges, here from *S. domuncula*. Epithelial cells are sealed together using specific receptors/molecules clustered in cell junctions (cj). The adjacent plasma membranes are held together by strands of transmembrane cellular (junctional) proteins/receptors (TM-cj). In *S. domuncula*, data suggest that the cell membrane-spanning receptor tetraspanin associates with the scaffold protein, the PDZ protein MAGI. MAGI, as a scaffold protein, plays a crucial role in the organization of the membrane receptor molecules and the effector molecules. The latter ones compose the cytoskeleton and the signal transduction molecules.

at the border did not show high expression levels of *MAGI* (Fig. 2F). However, if the surface of regenerating tissue is analyzed after 6 days a strong signal is seen in the newly formed cell layer, covering the specimen (Fig. 2G).

## Conclusion

It was already assumed, but it could not be proven, that the cells of the outermost sealing layer of sponges (Porifera) are tightly attached to each other; therefore the epithelial layers were termed “ectoderme” and “endoderme,” and their cells “cellules conjonctives” (Lieberkühn 1857; DeLage 1892). Only a few electron microscopic studies showed that the cell–cell contact of the cells in the epithelial layer is firm; therefore the contact zones between the cells were cautiously termed membrane bridges in the demosponge *Microciona prolifera* (Evans and Bergquist 1974), attachment plaque in the demosponge *Ephydatia muelleri* (Pavans de Ceccatty 1981), desmosome-like in *Ephydatia fluviatilis* (Pottu-Boumendil 1975), and septate junctions in *E. fluviatilis* (De Vos 1977) and in the calcareous sponge *Sycon ciliatum* (Ledger 1975). In addition, it could be shown, also by electron microscopy, that the intracellular fibers are connected

with intercellular bridges (Pavans de Ceccatty 1989). In spite of these data a convincing demonstration of the existence of junctions in sponges will remain incomplete and not generally accepted (Nielsen 2001), without the demonstration that also the junctional molecules connecting the cells are present. The first molecular data came from the demonstration that in sponges genes exist indicative of the presence of a basement membrane, like type IV collagen in the demosponge *P. jarrei* (Boute et al. 1996). Furthermore, it can be hypothesized that asymmetrically arranged cells might be present on the surface of sponge specimens. Polarized cells are required for the establishment of epithelia, sheets which have been considered fundamental features of multicellular organisms (Perez-Moreno et al. 2003). Without the demonstration of the existence of the characteristic junctional proteins and the proof of their localization/function in sponges, their cellular contacts were termed, in the past, “almost” junctions (Westheide and Rieger 1996; Nielsen 2001).

The proof of the junctional molecules in *S. domuncula* by molecular biological methods gave us sufficient evidence that in demosponges the underlying receptors and their organizing scaffold proteins are present. The demonstration that the gene for a tetraspanin receptor in *S. domuncula* with its characteristic functional amino acids exists was not surprising, since this type of receptor is not exclusively found in tight/cell junctions but is functionally present also in other microdomains of metazoan cells, where they interact with integrin receptors (Yunta and Lazo 2003). However, the result that the gene coding for a scaffold protein, MAGI, exists in *S. domuncula* as well and is even expressed in the surface layers, both the exopinacoderm and the endopinacoderm, provides compelling evidence for the presence of cell junctions.

It cannot be ignored that other molecules related to the tight junctions, such as the zonula occludens proteins, already found in cnidarians (Fei et al. 2000), could be present also in sponges. A schematic model for the organization of the junctions in sponges can be proposed (Fig. 7), in which the *S. domuncula* tetraspanin interacts with SD\_MAGI, through its PDZ modules. The complexes formed are able to cover spatial cues at the cell surface, originating functionally distinct membrane domains that are characteristic for (postulated polarized) epithelia.

In summary, the identification of cell junction proteins, the tetraspanin receptor and the scaffold protein MAGI, confirms that during the evolutionary transition from the fungi, as the closest related kingdom to the Metazoa (Schütze et al. 1999), to the multicellular animals, with the sponges as the oldest phylum, epithelial layers with cell junctions had been formed.



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## References

- Adams MD, Celniker SE, Holt RA, Evans CA, 191 others (2000) The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–2195
- Adell T, Grebenjuk VA, Wiens M, Müller WEG (2003) Isolation and characterization of two T-box genes from sponges, the phylogenetically oldest metazoan taxon. *Dev Genes Evol* 213:421–434
- Berditchevski F (2001) Complexes of tetraspanins with integrins: More than meets the eye. *J Cell Sci* 114:4143–4151
- Berger A, Schiltz E, Schulz GE (1989) Guanylate kinase from *Saccharomyces cerevisiae*. Isolation and characterization, crystallization and preliminary X-ray analysis, amino acid sequence and comparison with adenylate kinases. *Eur J Biochem* 184: 433–443
- Bork P, Sudol M (1994) The WW domain: A signalling site in dystrophin? *Trends Biochem Sci* 19:531–533
- Boute N, Exposito JY, Boury-Esnault N, Vacelet J, Noro N, Miyazaki K, Yoshizato K, Garrone R (1996) Type IV collagen in sponges, the missing link in basement membrane ubiquity. *Biol Cell* 88:37–44
- Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Müller WEG, Wetter T, Suhai S (2004) Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genomics*, in press
- Coligan JE, Dunn BM, Ploegh HL, Speicher DW, Wingfield PT (2000) Current protocols in protein science. John Wiley and Sons, Chichester, UK
- De Vos L (1977) Morphogenesis of the collagenous shell of the gemmules of a fresh-water sponge *Ephydatia fluviatilis*. *Arch Biol* 88:479–494
- DeLage Y (1892) Embryogénie des éponges. *Arch de Zool Exp [Ser 2]*(10):345–498
- Dobrosotskaya I, James GL (2000) MAGI-1 interacts with  $\beta$ -catenin and is associated with cell-cell adhesion structures. *Biochem Biophys Res Commun* 270:903–909
- Dobrosotskaya I, Guy RK, James GL (1997) MAGI-1, a membrane-associated guanylate kinase with a unique arrangement of protein-protein interaction domains. *J Biol Chem* 272:31589–31597
- Ermekova KS, Zambrano N, Linn H, Minopoli G, Gertler F, Russo T, Sudol M (1998) The WW domain of neural protein FE65 interacts with proline-rich motifs in Mena, the mammalian homolog of *Drosophila* enabled. *J Biol Chem* 272:32869–32877
- Evans CW, Bergquist PR (1974) Initial cell contact in sponge aggregates. *J Microsc* 21:185–188
- Exposito JY, Le Guellec D, Lu Q, Garrone R (1991) Short chain collagens in sponges are encoded by a family of closely related genes. *J Biol Chem* 266:21923–21928
- Fei K, Yan L, Zhang J, Sarras MP (2000) Molecular and biological characterization of a zonula occludens-1 homologue in *Hydra vulgaris*, named HZO-1. *Dev Genes Evol* 210:611–616
- Felsenstein J (1993) PHYLIP, ver. 3.5. University of Washington, Seattle
- Garrone R (1978) Phylogenesis of connective tissue. Morphological aspects and biosynthesis of sponge intercellular matrix. S. Karger, Basel
- González-Mariscal L, Betanzos A, Avila-Flores A (2000) MAGUK proteins: Structure and role in the tight junction. *Semin Cell Dev Biol* 11:315–324
- González-Mariscal L, Betanzos A, Nava P, Jaramillo BE (2003) Tight junction proteins. *Prog Biophys Mol Biol* 81:1–44
- Hirao K, Hata Y, Ide N, Takeuchi M, Irie M, Yao I, Deguchi M, Toyoda A, Sudhof TC, Takai Y (1998) A novel multiple PDZ domain-containing molecule interacting with *N*-methyl-D-aspartate receptors and neuronal cell adhesion proteins. *J Biol Chem* 273:21105–21110
- Knust E (2000) Control of epithelial cell shape and polarity. *Curr Opin Genet Dev* 10:471–475
- Krasko A, Batel R, Schröder HC, Müller IM, Müller WEG (2000) Expression of silicatein and collagen genes in the marine sponge *Suberites domuncula* is controlled by silicate and myotrophin. *Eur J Biochem* 267:4878–4887
- Kruse M, Müller IM, Müller WEG (1997) Early evolution of metazoan serine/threonine and tyrosine kinases: Identification of selected kinases in marine sponges. *Mol Biol Evol* 14:1326–1334
- Kruse M, Steffen R, Batel R, Müller IM, Müller WEG (1999) Differential expression of allograft inflammatory factor 1 and of glutathione peroxidase during auto- and allograft response in marine sponges. *J Cell Sci* 112:4305–4313
- Kyte J, Doolittle RF (1982) A simple method for displaying the hydrophobic character of a protein. *J Mol Biol* 157:105–132
- Laura RP, Ross S, Koeppen H, Lasky LA (2002) MAGI-1: A widely expressed, alternatively spliced tight junction protein. *Exp Cell Res* 275:155–170
- Le Penec G, Perovic S, Ammar MSA, Grebenjuk VA, Steffen R, Brümmer F, Müller WEG (2003) Cultivation of primmorphs from the marine sponge *Suberites domuncula*: Morphogenetic potential of silicon and iron, A review. *Mar Biotechnol* 100:93–108
- Ledger PW (1975) Septate junctions in the calcareous sponge *Sycon ciliatum*. *Tissue Cell* 7:13–18
- Lieberkühn N (1856) Zur Entwicklungsgeschichte der Spongillen (Nachtrag). *Arch Anat Physiol*, pp 399–414
- Mackie GO (1984) Introduction to the diploblastic level. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds) *Biology of the integument*. Springer-Verlag, Berlin, Vol 1, pp 907–920
- Maeda K, Matsuhashi S, Hori K, Xin Z, Mukai I, Tabuchi K, Egashira M, Niikawa N (1998) Cloning and characterization of a novel human gene, TM4SF6, encoding a protein belonging to the transmembrane 4 superfamily, and mapped to Xq22. *Genomics* 52:240–242
- Mino A, Ohtsuka T, Inoue E, Takai Y (2000) Membrane-associated guanylate kinase with inverted orientation (MAGI-1)/brain angiogenesis inhibitor 1-associated protein (BAP1) as a scaffolding molecule for Rap and small G protein GDP/GTP exchange protein at tight junctions. *Genes Cells* 5:1009–1016
- Müller WEG (1997) Origin of metazoan adhesion molecules and adhesion receptors as deduced from their cDNA analyses from the marine sponge *Geodia cydonium*. *Cell Tissue Res* 289:383–395
- Müller WEG (2001) How was metazoan threshold crossed: The hypothetical Urmetazoa. *Comp Biochem Physiol [A]* 129:433–460
- Müller WEG, Wiens M, Batel R, Steffen R, Borojevic R, Custodio MR (1999a) Establishment of a primary cell culture from a sponge: Primmorphs from *Suberites domuncula*. *Mar Ecol Progr Ser* 178:205–219
- Müller WEG, Schatton W, Wimmer W, Bohm M, Batel R, Filic Z (1999b) Initiation of an aquaculture of sponges for their sustainable production of bioactive metabolites in open systems: example *Geodia cydonium*. *Mol Mar Biotechnol* 1:569–579
- Nielsen C (2001) Animal evolution. Oxford University Press, Oxford
- Pancer Z, Kruse M, Müller I, Müller WEG (1997) On the origin of adhesion receptors of metazoa: Cloning of the integrin  $\alpha$  subunit cDNA from the sponge *Geodia cydonium*. *Mol Biol Evol* 14:391–398

- de Pavans Ceccatty M (1981) Demonstration of actin filaments in sponge cells. *Cell Biol Int Rep* 5:945–952
- de Pavans Ceccatty M (1989) Les éponge, à l'aube des communication cellulaires. *Pour Sci* 142:64–72
- Pechenik JA (2000) *Biology of the invertebrates*. McGraw–Hill, Boston
- Perović S, Schröder HC, Sudek S, Grebenjuk VA, Batel R, Štufanić M, Müller IM, Müller WEG (2003) Expression of one sponge *Iroquois* homeobox gene in primmorphs from *Suberites domuncula* during canal formation. *Evol Dev* 5:240–250
- Polak JM, McGee JD (1998) *In situ* hybridization. Oxford University Press, Oxford
- Ponting CP, Philips C, Davies KE, Blake DJ (1997) PDZ domains: Targeting signalling molecules to sub-membranous sites. *Bio-Essays* 19:469–479
- Perez-Moreno M, Jamora C, Fuchs E (2003) Sticky business: orchestrated cellular signals at adherens junctions. *Cell* 112:535–549
- Pottu-Boumendil J (1975) Ultrastructure, cytochemie, et comportements morphogénétique des cellules de l'éponge *Ephydatia muelleri* (Lieb). Thèse Univ. Claude Bernard, Montpellier, pp 1–101
- Ranganathan R, Ross EM (1997) PDZ domain proteins: Scaffolds for signaling complexes. *Curr Biol* 7:R770–R773
- Rieger RM (1994) Evolution of the “lower” Metazoa. In: Bengtson S (ed) *Early life on earth*
- Sarras MP, Madden ME, Zhang X, Gunwar S, Huff JK, Hudson BG (1991) Extracellular matrix (Mesoglea) of *Hydra vulgaris*. I. Isolation and characterization. *Dev Biol* 148:481–494
- Schröder HC, Krasko A, Batel R, Skorokhod A, Pahler S, Kruse M, Müller IM, Müller WEG (2000) Stimulation of protein (collagen) synthesis in sponge cells by a cardiac myotrophin-related molecule from *Suberites domuncula*. *FASEB J* 14:2022–2031
- Schütze J, Reis Custodio M, Efremova SM, Müller IM, Müller WEG (1999) Evolutionary relationship of Metazoa within the eukaryotes based on molecular data from Porifera. *Proc R Soc Lond B* 266:63–73
- Shoji H, Tsuchida K, Kishi H, Yamakawa N, Matsuzaki T, Liu Z, Nakamura T, Sugino H (2000) Identification and characterization of a PDZ protein that interacts with activin type II receptors. *J Biol Chem* 275:5485–5492
- Sudol M, Chen HI, Bougeret C, Einbound A, Bork P (1995) Characterization of a novel protein-protein module—The WW domain. *FEBS Lett* 369:67–71
- Tettelin H, Agostoni Carbone ML, Albermann K, Albers M, 21 others (1997) The nucleotide sequence of *Saccharomyces cerevisiae* chromosome VII. *Nature* 387:81–94
- Thomas M, Laura R, Hepner K, Guccione E, Sawyers C, Lasky L, Banks L (2002) Oncogenic human papillomavirus E6 proteins target the MAGI-2 and MAGI-3 proteins for degradation. *Oncogene* 21:5088–5096
- Tiwari-Woodruff SK, Buznikov AG, Vu TQ, Micevych PE, Chen K, Kornblum HI, Bronstein JM (2001) OSP/claudin-11 forms a complex with a novel member of tetraspanin super family and beta 1 integrin and regulates proliferation and migration of oligodendrocytes. *J Cell Biol* 153:295–305
- Todd SC, Doctor VS, Levy S (1998) Sequences and expression of six new members of the tetraspanin/TM4SF family. *Biochim Biophys Acta* 1399:101–104
- Tolkacheva T, Boddapati M, Sanfiz A, Tsuchida K, Kimmelman AC, Chan AML (2001) Regulation of PTEN binding to MAGI-2 by two putative phosphorylation sites at threonine 382 and 383. *Cancer Res* 61:4985–4989
- Westheide W, Rieger R (1996) *Spezielle zoologie; Part 1*. Gustav Fischer Verlag, Stuttgart
- Wiens M, Koziol C, Hassanein HMA, Batel R, Müller WEG (1998) Expression of the chaperones 14–3–3 and HSP70 induced by PCB 118 2,3',4,4',5-pentachlorobiphenyl in the marine sponge *Geodia cydonium*. *Mar Ecol Prog Ser* 165:247–257
- Wiens M, Krasko A, Müller CI, Müller WEG (2000) Molecular evolution of apoptotic pathways: Cloning of key domains from sponges (Bcl-2 homology domains and death domains) and their phylogenetic relationships. *J Mol Evol* 20:520–531
- Wiens M, Mangoni A, D'Esposito M, Fattorusso E, Korchagina N, Schröder HC, Grebenjuk VA, Krasko A, Batel R, Müller IM, Müller WEG (2003) The molecular basis for the evolution of the metazoan bodyplan: Extracellular matrix-mediated morphogenesis in marine demosponges. *J Mol Evol* 57:S60–S75
- Wimmer W, Blumbach B, Diehl-Seifert B, Koziol C, Batel R, Steffen R, Müller IM, Müller WEG (1999) Increased expression of integrin and receptor tyrosine kinase genes during autograft fusion in the sponge *Geodia cydonium*. *Cell Adhes Commun* 7:111–124
- Wood JD, Yuan J, Margolis PL, Colomer V, Duan K, Kushi J, Kaminsky Z, Kleiderlein JJ, Sharp AH, Ross CA (1998) Atrophin-1, the DRPLA gene product, interacts with two families of WW domain-containing proteins. *Mol Cell Neurosci* 11:149–160
- Wu Y, Dowbenko D, Spencer S, Laura R, Lee J, Gu Q, Lasky LA (2000a) Interaction of the tumor suppressor PTEN/NMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. *J Biol Chem* 275:21477–21485
- Wu X, Hepner K, Castelin-Prabhu S, Do D, Kaye MB, Yuan XJ, Wood J, Ross C, Sawyers CL, Whang YE (2000b) Evidence for regulation of the PTEN tumor suppressor by membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. *Proc Natl Acad Sci USA* 97:4233–4238
- Yeaman C, Grindstaff KK, Hansen MDH, Nelson WJ (1999) Cell polarity: versatile scaffolds keep things in place. *Current Biol* 9:R515–R517
- Yunta M, Lazo PA (2003) Tetraspanin proteins as organisers of membrane microdomains and signalling complexes. *Cell Signal* 15:559–564