Ethylene Modulates Gene Expression in Cells of the Marine Sponge *Suberites domuncula* and Reduces the Degree of Apoptosis

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Sponges (phylum Porifera) live in an aqueous milieu that contains dissolved organic carbon. This is degraded photochemically by ultraviolet radiation to alkenes, particularly to ethylene. This study demonstrates that sponge cells (here the demosponge *Suberites domuncula* has been used), which have assembled to primmorphs, react to 5 μM ethylene with a significant up-regulation of intracellular Ca\(^{2+}\) concentration and with a reduction of starvation-induced apoptosis. In primmorphs from *S. domuncula* the expression of two genes is up-regulated after exposure to ethylene. The cDNA of the first gene (SDERR) isolated from *S. domuncula* encodes a potential ethylene-responsive protein, termed ERR_SUBDO; its putative M\(_s\) is 32,704. Data bank search revealed that the sponge polypeptide shares high similarity (82% on amino acid level) with the corresponding enzyme from *Hewa brasiliensis*. Until now no other metazoan ethylene-responsive proteins have been identified. The second gene, whose expression is up-regulated in response to ethylene is a Ca\(^{2+}\)/calmodulin-dependent protein kinase II. Its cDNA, *SDCCdPK*, encodes a M\(_s\) 54,863 putative kinase that shares 69% similarity with the corresponding enzyme from *Drosophila melanogaster*. The expression of both genes in primmorphs from *S. domuncula* is increased by ~5-fold after a 3-day incubation period with ethylene. It is concluded that also metazoan cells, with sponge cells as a model, may react to ethylene with an activation of cell metabolism including gene induction.

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Sponges (phylum Porifera) represent the phylogenetic oldest Metazoa that share a common ancestor with all multicellular animals; they lived already before the “Cambrian Explosion” at least 580 million years ago (for review, see Refs. 1–4). Sponges possess most of the structural elements known from more complex Metazoa, e.g. adhesion molecules (galectin), adhesion receptors (receptor tyrosine kinase, integrin receptor, receptor(s) featuring scavenger receptor cysteine-rich domains) or elements involved in signal transduction pathways (G-proteins, Ser/Thr protein kinases) (for review, see Ref. 5). Additionally sponges display simple elements of an immune system related to that found in vertebrates, e.g. macrophage-derived cytokine-like molecules, the (2’-5’)oligoadenylate synthetase system, or a molecule very similar to the mammalian T-cell receptor (6) (for review, see Ref. 7).

Ethylene is one major alkene produced in seawater from oceanic dissolved organic carbon by photochemical reactions initiated especially by sunlight (ultraviolet radiation) (8–10). The concentration of ethylene in filtered seawater was determined to be close to 100 pm (11) and remains almost constant during a period of 8 days in the dark (11). The concentration of dissolved organic carbon is similarly high (12) in the habitats of sponges. Because sponges are efficient benthic filter-feeders, some of which filter 24,000 liters kg\(^{-1}\) of sponge (13) every day, they are likely to take up large amounts of ethylene.

There have been only a few successful approaches to define the energy sources of sponges. It is generally assumed that sponges ingest particulate nutrients by phagocytosis (for review, see Ref. 14) or accumulate amino acids from the marine environment (15). However, until recently it was not possible to cultivate sponge cells by feeding them with particulate nutrients, e.g. bacteria or subcellular particles from them, and/or in media composed of amino acids or vitamins, only. Recently, a technique was established that allows single sponge cells to associate to “organ-like” aggregates, termed primmorphs; in this state the cells are able to divide (16). Evidence has been presented that some cells within the primmorphs undergo apoptotic death; subsequently the resulting cell fragments are very likely taken up via phagocytosis by those cells that express on their surface receptors composed of scavenger receptor cysteine-rich repeats (7, 17). So far, the volatile products in the marine environment including ethylene have not been experimentally studied as a potential energy source for sponges.

The rationale of this study was to investigate the effect of ethylene on sponge metabolism. Ethylene is known to serve as an energy source for some bacteria, e.g. *Paracoccus denitrificans* (18), and to contribute to plant growth (for review, see Ref.19). Recently we showed that starvation of *Suberites domuncula* as a whole and also of single cells results in the induction of apoptosis (20). To determine the effect of ethylene on sponge cells, primmorphs from *S. domuncula*, containing proliferating cells (16), were kept under pressure either in the absence or presence of ethylene to imitate natural conditions. It is striking that most sponges live in a species-specific depth in the marine environment. As an example, *S. domuncula* lives preferentially in a depth of 20–25 m (21), suggesting that the pressure of the surrounding water is one important factor for its growth.

Here we show that ethylene reduces the extent of apoptosis caused by starvation. Two molecular markers for the effect of ethylene were chosen. As a first molecule, the sponge gene, related to the plant stress-induced gene *HEVER*, which was...
found to be ethylene-responsive (22), was selected. This sponge gene was cloned; its expression is strongly induced after exposure of cells to ethylene. The second gene selected was the Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaM kinase II),\(^1\) which plays a central role in transduction of Ca\(^{2+}\) signals in cells. In previous studies (23) it had been demonstrated that the intracellular concentration of Ca\(^{2+}\), [Ca\(^{2+}\)]\(_i\), changes rapidly in response to exposure of agonists for the metabotropic glutamate receptor. The CaM kinase II is activated by an increase of [Ca\(^{2+}\)]\(_i\), (24) and also causes gene expression (25). Calmodulin has been shown to play a crucial role in integrin-mediated signal transduction in sponges (26) via activation of CaM kinase II.\(^2\) The expression of the CaM kinase II gene is up-regulated after exposure to ethylene.

The results presented in this study show for the first time that, among Metazoa, sponges are provided with a signaling cascade in which ethylene activates cell metabolism and gene expression.

EXPERIMENTAL PROCEDURES

Materials and Solutions—The sources of chemicals and enzymes used were given previously (26, 27). The composition of Ca\(^{2+}\) - and Mg\(^{2+}\)-free artificial seawater was described earlier (28). Natural, Ca\(^{2+}\)-, and Mg\(^{2+}\)-containing seawater (SW) was obtained from Sigma (Deisenhofen, Germany).

Sponges—Specimens of the marine sponge S. domuncula (Porifera, Demospongiae, Hadromerida) were collected in the northern Adriatic near Rovinj (Croatia) and then kept in aquaria in Mainz (Germany) at a temperature of 17 °C.

Dissociation of Cells and Formation of Primmorphs—The procedure for dissociation of sponge cells was described previously (16, 29). Primmorphs, special aggregates, reassOCIATED from single cells after transferring them into medium composed of SW (16, 29), supplemented with 0.1% (v/v) of Marine broth 2216 (Difco). A suspension of 10\(^6\) cells/ml is adjusted; after two days, primmorphs at least 1-mm in diameter (average, 2-3-mm) are formed. After 5 days, the primmorphs were used for the incubation experiments.

The primmorphs were kept under pressure of 1 physical atmosphere (atm) (Fig. 1). The pressure in the culture chamber was generated by air. In the studies using ethylene, reservoir I was filled with medium (SW containing Marine broth) and 5 \(\mu\)l ethylene (adjusted from a stock solution of 1 nM ethylene). The solution was pumped at a rate of 1 ml/h through the culture chamber; the extruded medium was collected in reservoir II (Fig. 1A). The culture chamber (3.5 × 1-cm) contained the primmorphs in 6 ml of medium (Fig. 1B).

Loading of S. domuncula Cells with Fura-2-AM and Measurement of Intracellular Calcium—Chambered coverglass incubation chambers (Lab-Tek, Nunc) were coated with poly-l-lysine (M\(_g\) > 300,000, 0.1 mg/ml) as described (23, 26). Loading was performed in the dark for 120 min in Ca\(^{2+}\)- and Mg\(^{2+}\)-free artificial seawater containing 10–12 \(\mu\)M Fura-2-AM and 1% bovine serum albumin. To determine the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), cells on coverglass were transferred into the pressure chamber (Fig. 1C). After exposure for 3 h to 1 atm the cells were illuminated with 340- and 380-nm light from a mercury source (23, 28). Ratios of sequential 340/380-nm excitation image pairs were compared with a standard curve for free Ca\(^{2+}\) (30). Measurements were performed in a Ca\(^{2+}\)-containing (10 mM) modified Locke’s solution adjusted to seawater osmolality (500 mOsm NaCl, 5.6 mM KCl, 3.6 mM NaHCO\(_3\), 5.6 mM glucose, and 10 mM HEPES, pH 7.4). Calibration was performed with the Fura-2 calcium imaging calibration kit according to the manufacturer’s instructions (Molecular Probes, Leiden, The Netherlands). One ratio unit 340/380-nm corresponds to 143 nM [Ca\(^{2+}\)]\(_i\). For the incubation experiments with ethylene, the culture medium in the chamber (diameter of the glass plates was 1 cm) was adjusted to 5 \(\mu\)M ethylene.

Cell Death Assay—DNA fragmentation (TUNEL staining) was determined using the in situ cell death detection kit (Roche Molecular Biochemicals). Dissociated cells were air-dried and fixed using 4% paraformaldehyde following the manufacturer’s protocol. The TUNEL-stained cells were counterstained with propidium iodide (5 \(\mu\)g/ml) and visualized by fluorescence microscopy.

Polymerase Chain Reaction (PCR) Cloning of the Sponge Ethylene-responsive Protein—The complete sponge cdNA, encoding the related ethylene-responsive protein, termed SDERR, was isolated from the S. domuncula cdNA library by PCR (27). The degenerate forward primer, directed against the conserved aa segments found in the sequences from

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\(^1\) The abbreviations used are: CaM kinase II, Ca\(^{2+}\)/calmodulin-dependent protein kinase II; DIG, digoxigenin; SW, Ca\(^{2+}\)- and Mg\(^{2+}\)-containing seawater; TUNEL, terminal dUTP nick-end labeling; aa, amino acid(s); PCR, polymerase chain reaction; bp, base pair(s); nt, nucleotide(s).

\(^2\) A. Krasko, H. C. Schröder, S. Perovic, R. Steffen, M. Kruse, W. Reichert, I. M. Müller, and W. E. G. Müller, submitted for publication.
the ethylene-inducible protein from *Hevea brasiliensis* (aa143, and aa152, GenBank™ accession number Q39963) (22), the hypothetical 31.4-kDa protein C29B12.04 of *Schizosaccharomyces pombe* (aa143 and aa152, GenBank™ accession number Q14027) and the hypothetical protein *Mycobacterium thermoautotrophicum* (aa144 and aa152, GenBank™ accession number O26762), 5'-GAGA/AGGGGCGC-ATGATGIGGACG-3' (where *I* = inosine), in conjunction with the ZAPII 3'-end vector-specific primer T7 was used. The PCR reaction was carried out at an initial denaturation for 3 min at 95 °C, then 32 amplification cycles at 95 °C for 20 s, 67.5 °C for 45 s, 74 °C for 1.5 min, and a final extension step at 74 °C for 10 min at 74 °C. The reaction mixture was as described earlier (31). The fragment of 700-bp was used to isolate the cDNA from the library (32). The longest insert obtained was 1,106 nt (excluding the poly(A) tail). The clone was termed *SDERR* and was sequenced using an automated DNA sequencer (Li-Cor 4200).

**PCR Cloning of the Sponge CaM Kinase II—Calmodulin-dependent Protein Kinase II**—The cDNA, encoding the complete CaM kinase II, CcDPK_SUBDO, SDCCdPK was isolated using the same strategy. Here, the forward primer 5'-GCGA/AGGAGTC/CTCAAT/CTACGA/TGATGIGGACG-3' directed against one conserved region within the human CaM kinase II subunit δ-2 (33) (aa143, and aa152) and an annealing temperature of 50 °C were used. The 700-bp fragment was obtained and used for the isolation of the cDNA; the insert had a size of 1,714 nt.

**Sequence Comparisons**—The sequences were analyzed using computer programs BLAST (34) and FASTA (35). Multiple alignments were performed with CLUSTAL W version 1.6 (36). Phylogenetic trees were constructed on the basis of aa sequence alignments by neighbor-joining, as implemented in the “Neighbor” program from the PHYLIP package (37). The distance matrices were calculated using the Dayhoff peptidyl-glycine α-amidating monoxygenase matrix model as described (38). The degree of support for internal branches was further assessed by bootstrapping (37). The graphic presentations were prepared with GeneDoc (39).

**Northern Blotting**—RNA was extracted from liquid nitrogen-pulsed sponge tissue with TriZOL™ Reagent (Life Technologies, Inc.) as recommended by the manufacturer. 3 μg of total RNA was electrophoresed through a formaldehyde/agarose gel and blotted onto a Hybond N+ membrane following the manufacturer’s instructions (Amersham Pharmacia Biotech). Hybridization experiments were performed with the following probes: the −580-bp fragment of SDERR (nt50 to nt70) from *S. domuncula*, *S. domuncula* SDCCdPK (a segment of −530 bp was used; nt294 to nt330), and the *S. domuncula* β-tubulin SDBTUB (−800 bp). These probes were labeled with DIG-11-dUTP by the DIG DNA labeling kit. Hybridization was performed with the antisense DIG-labeled probes at 42 °C overnight using 50% formamide, containing 5× SSC, 2% blocking reagent, 7% (v/v) SDS, and 0.1% (w/v) N-lauroylsarcosine, following the instructions of the manufacturer (Roche Molecular Biochemicals). After washing DIG-labeled nucleic acid was detected with anti-DIG Fab fragments (conjugated to alkaline phosphatase) and visualized by the chemiluminescence technique using CDP-Star, the chemiluminescence substrate alkaline phosphatase, according to the instructions of the manufacturer.

To quantify the signals of the Northern blots, the chemiluminescence procedure was applied (40). The screen was scanned with the GS-525 Molecular Imager (Bio-Rad). The relative values for the expressions of the ERR_SUBDO and CDcDPK_SUBDO genes in *S. domuncula* cells were correlated with the intensities of the bands measured for the expression of the tubulin gene.

**RESULTS**

**Induction of Apoptosis in Primmorphs after Starvation and the Effect of Ethylene**—The primmorphs generally contain a small fraction of apoptotic cells (approximately 8 ± 3% of TUNEL-positive cells). If the primmorphs were cultured in SW lacking the supplement (Marine broth), the number of apoptotic cells increased and reached a value of 16 ± 4% after 24 h and 29 ± 5% after 48 h (Fig. 2). If primmorphs were kept under starvation (lack of Marine broth) but in the presence of 5 μM ethylene, the number of apoptotic cells did not change significantly; values of ~10 ± 4% were measured.

**Effect of Ethylene on the Intracellular Ca2+ Concentrations in Cells from *S. domuncula***—Dissociated cells from *S. domuncula* were incubated with 5 μM ethylene, and the [Ca2+]i level was determined. The assays were performed with Ca2+-containing incubation solution. If the cells were incubated for 23 min in the absence of ethylene no significant change of the [Ca2+]i was observed as checked by fluorescence (Fig. 3A, panels a–c). However, if the cells were treated with ethylene, an immediate shift of the fluorescence of most of the cells from blue to yellow/red is seen (Fig. 3B, panels a and b (5 min) and c (23 min)). The light microscopical aspects of the cells analyzed are given (Fig. 3, A, panel d and B, panel d).

A quantitative analysis of the changes of the [Ca2+]i, levels is summarized in Fig. 4. Cells not treated with ethylene did not show a change of the [Ca2+]i level; a ratio value (340/380 nm) of 1.73 ± 0.04 was measured. A significant shift of the ratio was measured if ethylene was added to the cells; it increased from 1.80 ± 0.04 (time zero) to 1.90 ± 0.02 (20 min after addition of ethylene) (Fig. 4).

**Cloning of the Ethylene-responsive Protein from *S. domuncula***—The complete cDNA, encoding the ethylene-responsive protein from *S. domuncula*, termed *SDERR*, is 1,106-nt long and has a potential open reading frame (ORF) from the putative AUG initiation codon at nt 39–41 to the stop codon at 957–959, that encodes a 306-aa long polypeptide (Fig. 5). The deduced aa sequence of the putative ethylene-responsive protein termed ERR_SUBDO has a putative size (Mw) of 32,704 and an isoelectric point (pI) of 5.87 (41). A bipartite nuclear targeting signature is present between aa63 and aa78 (Fig. 5). Northern blot analysis performed with the sponge *SDERR* clone as a probe yielded one prominent band of approximately 1.4 kilobases, confirming that a full-length cDNA was isolated (Fig. 6A).

A data bank search with the deduced aa sequence, ERR_SUBDO, revealed a high identity (similarity) to the plant sequence, the ethylene-inducible protein from *H. brasiliensis* (22) of 61% (82%), to the 31.4-kDa protein of *S. pombe* of 66% (81%), and to the protein MTH666 from *M. thermoautotrophicum* of 60% (76%).

**Cloning of the Sponge CaM Kinase II—**The sponge 1,714-nt long cDNA SDCCdPK encoding the CaM kinase II contains one potential open reading frame starting with the putative AUG initiation codon (nt 101–103) to the stop codon at nt 1,549–1,552. The deduced 483-aa-long polypeptide termed CDCCdPK_SUBDO (Fig. 7A) has a calculated Mw of 54,863 and pl of 7.07 (41). Northern blot analysis revealed a size of the transcripts of

**FIG. 2.** Induction of apoptosis in cells of primmorphs. Percentage of apoptotic cells in primmorphs after starvation by removing the Marine broth supplement from the SW medium. The cells were subjected to the TUNEL assay immediately after the beginning of the starvation period (time, 0 h), or after a period of 24 or 48 h. The primmorphs were incubated in the absence (closed bars) or presence (open bars) of 5 μM ethylene. Approximately 150 cells were counted in five independent experiments (mean ± S.D.).
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Fig. 3. Effect of ethylene on \([\text{Ca}^{2+}]\) in cells from \(S. \text{domuncula}\). A, incubation of cells in the absence of ethylene. Fluorescence images were recorded at time 0 (a), after 5 min (b), or 23 min (c). In panel d, the cells were inspected by light microscopy (Nomarsky interference contrast optics). B, treatment of cells with 5 \(\mu\text{m}\) ethylene. Initial fluorescence in the absence of the gas (a). Ethylene was added at 3 min, and the images were recorded at 5 min (b) and 23 min (c); d, light microscopic aspect. Magnifications, 50-fold.

Fig. 4. Change of \([\text{Ca}^{2+}]\) level. \(S. \text{domuncula}\) cells were incubated in the absence (●) or presence (△) of 5 \(\mu\text{m}\) ethylene. The ratios of the 360/380-nm images are shown. The arrow marks the time at which the gas was added to the sponge cells. The results are expressed as mean value ± S.E.; \(n = 98\).

2.0 kilobases (Fig. 6B). The deduced polypeptide comprises three autophosphorylation sites at Thr-283, Thr-302, and Ser-311, the autoinhibitory region (aa269 and aa299), which partially overlaps with the calmodulin-binding domain (aa288 and aa307) and the variable region, which spans in \(S. \text{domuncula}\) the region aa311 to aa370 (24). The \(~20\) aa at the COOH terminus, which are present in the \(\alpha\)-subunits of other CaM kinase II, are missing in the sponge sequence, whereas the Ser/Thr protein kinase active site (aa128 and aa141) and the ATP-binding signature (aa17 and aa25) are found as indicated in Fig. 7A.

Phylogenetic Analysis of Sponge CaM Kinase II—Until now no CaM kinase II enzymes have been described or cloned from evolutionary older phyla than from Porifera. The \(S. \text{domuncula}\) enzyme CaM kinase II with a \(M_r\) of 54,863 belongs, based on its size, to the \(\alpha\)-subunit of this kinase family (24). The closest similarity of the sponge protein was found to be the corresponding enzyme from \(Drosophila\) melanogaster, also an \(\alpha\)-subunit CaM kinase II (42) with an identity (similarity) of \(~52\%\) (\(~69\%\)). An unrooted phylogenetic tree was constructed (Fig. 7B) with the distantly related peripheral plasma membrane protein CASK from \(Rattus\) norvegicus. The tree revealed that the sponge CaM kinase II forms the basis for the related enzymes from protostomians, \(D.\) melanogaster, \(C.\) elegans and deuterostomian, chicken, Xenopus laevis and human.

Levels of Expression of Sponge Ethylene-responsive Protein and CaM Kinase II in Dependence upon Ethylene—In the absence of ethylene, the expression of the gene encoding the ethylene-responsive protein is low (Fig. 6A). However, already after an incubation of 1 day in the presence of ethylene the expression of \(SDERR\) increased significantly (1.8-fold) and reached a maximum after 3 days (6.6-fold). A similar pattern is seen for the expression of CaM kinase II. The steady state level of this enzyme is low in the absence of ethylene and increased drastically after 3 (5.5-fold) to 5 days (6.1-fold) (Fig. 6B). In parallel blots, the level of expression of \(\beta\)-tubulin RNA from ethylene-treated primmorphs was determined; no significant differences were seen, confirming that the same amount of RNA was applied (Fig. 6C).

DISCUSSION

The Porifera lived before the Cambrian Explosion, a time at which presumably a lower oxygen content in the atmosphere existed than at present (40). Consequently, during that period a less dense ozone screen protected living organisms against the ultraviolet (UV) fluxes (44) and precluded the development of terrestrial life (45). Therefore, it can be assumed that the animals living in the marine environment were exposed to higher levels of alkenes, due to UV-mediated photochemical degradation of dissolved organic carbon than today. Based on this consideration it was not too surprising that the hypothesis that sponges react to alkenes with an increase of their metabolism could experimentally at least partially be supported.

In a first set of experiments it was shown, in accordance with earlier observations (20), that sponge cells react to starvation with an increased rate of apoptosis. Treatment of cells in seawater supplemented with low concentrations of ethylene significantly reduces the number of apoptotic cells in the primmorphs. This result hints at a beneficial anabolic effect is caused by this volatile product. Ethylene occurs in ripening plants in high concentrations that are not toxic for humans (46). However, in Metazoa no report has been presented that ethylene can be used as energy source or for any other physiological and metabolically favorable process. Therefore, the following experiments were performed to substantiate the assumption that ethylene stimulates cell metabolism in sponges.

Intracellular \(\text{Ca}^{2+}\) is of pivotal importance for many biological processes, e.g. as stabilizer of intracellular structures or as second messenger in signal transduction pathways (47). Therefore, the determination of \([\text{Ca}^{2+}]\), in response to a given extracellular stimulus is a useful measure for cell response. Here we report that immediately after enrichment of the medium with ethylene the cells respond with a significant rise of \([\text{Ca}^{2+}]\). This finding might indicate that this alkene causes an immediate effect on cell metabolism. Because no data are available in Metazoa about whether ethylene binds to a membrane receptor that might be coupled to a G-protein, it is too early to speculate about a possible effect of ethylene on a signal transduction pathway that releases \(\text{Ca}^{2+}\) from intracellular stores.

In plants, ethylene very likely interacts with a putative...
From parallel, blots from ethylene-treated primmorphs were hybridized with the following proteins: the ethylene-inducible protein from *H. brasiliensis* (ER1_HEVBR, accession number Q39963; Ref. 22), the 31.4-kDa protein of *S. pombe* (YEM4_SCHPO, accession number O14027; Ref. 58), and the protein MTH666 from *S. pombe* following proteins: the ethylene-inducible protein from *H. brasiliensis* and CaM kinase II from *S. domuncula*.

The alignment was performed using CLUSTAL W program. Residues of aa, identical among all sequences, are shown in shaded type; those present in at least three sequences are shaded. The locations of the bipartite nuclear targeting signature (——) are indicated.

**FIG. 5.** Deduced ethylene-responsive protein from *S. domuncula*. The deduced sponge sequence, ERR_SUBDO, was aligned with the following proteins: the ethylene-inducible protein from *H. brasiliensis* (ER1_HEVBR, accession number Q39963; Ref. 22), the 31.4-kDa protein of *S. pombe* (YEM4_SCHPO, accession number O14027; Ref. 58), and the protein MTH666 from *M. thermoautotrophicum* (Y666_METTH, accession number O26762; Ref. 59). The alignment was performed using CLUSTAL W program. Residues of aa, identical among all sequences, are shown in shaded type; those present in at least three sequences are shaded. The locations of the bipartite nuclear targeting signature (——) are indicated.

**FIG. 6.** Effect of ethylene on the expression of ethylene-responsive protein and CaM kinase II from *S. domuncula*. A, primmorphs were incubated for 0 day (control; lane a), 1 day (lane b), 3 days (lane c) or 5 days (lane d) in the absence (ethylene: −) or the presence (ethylene: +) of 5 μM ethylene. Then Northern blot analyses were performed to estimate the level of expression of the genes using the probes for the ethylene-responsive protein, SDERR, or the CaM kinase II, SDCCdPK. RNA was extracted, and 3 μg of total RNA was size separated; after blot transfer hybridization was performed either with the SDERR probe (A, ERR) or the SDCCdPK probe (B, CaM II). In parallel, blots from ethylene-treated primmorphs were hybridized with a probe from *S. domuncula*, encoding β-tubulin (C). The intensities of the transcripts for SDERR and SDCCdPK are correlated with the expression of β-tubulin (parallel samples of the expression of SDERR (A; ethylene-treated) or of SDCCdPK (B; ethylene-treated) are shown.

ETR1 receptor, which displays similarity to both histidine kinase and response regulator domains of the bacterial two-component sensing system (48). Downstream within the signal transduction cascade, the CTR1 kinase has been identified (49). This enzyme belongs to the family of Ser/Thr protein kinases (50). CTR1 is differentially regulated by ethylene and air, a process which ultimately results in changed expression of various genes (51). Among those is the HEVER gene, which is induced in *H. brasiliensis* in response to ethylene (22). The deduced aa sequence of the sponge-related gene, SDSERR, shares high similarity to the plant molecule (>80% similar aa with the same physico-chemical properties). The expression of the sponge ethylene-responsive gene is strongly up-regulated 1 day after ethylene exposure. It is the first ethylene-responsive gene that has been described in Metazoa, which may be due to the fact that either this gene is missing in higher metazoans or it has not yet been searched for.

The modulatory effect of ethylene on gene expression in sponge cells is not restricted to SDSERR alone. It is shown that ethylene also causes an increased expression of CaM kinase II in *S. domuncula* cells. The deduced aa sequence of the enzyme shares the characteristic signatures for this family of kinases (24). The sponge CaM kinase II may be preliminarily classified to the α-subunits because of its size; it comprises 483 aa residues in comparison with the 478-aa-long α-subunits of higher metazoans, whereas the other subunits are of longer size (24). In higher metazoans, the α-subunits of CaM kinases II are especially abundant in brain (52). It remains to be tested whether, besides an induction of this gene, the resulting protein is activated by phosphorylation, as it is known to be for CaM kinases II from higher Metazoa (53). The results presented here identify an ethylene-mediated Ca^{2+}-triggering signal cascade in which the sponge CaM kinase II might be involved. In mammalian cells it has been established that Ca^{2+} mediates also the CaM kinase II cascade and thereby prevents apoptosis (54). CaM kinases II are known to be involved in signal transduction reactions, mediating signal transmission or metabolic activity, and also in gene regulation (55). Here we show that the expression of CaM kinase II parallels the expression of the ethylene-responsive protein; future studies must show whether the expression of the latter gene is connected with a phosphorylation process mediated by CaM kinase II.

It should be stressed at this point that the CaM kinase II from *S. domuncula* is the phylogenetically oldest kinase of this family. Hence this enzyme represents a further autapomorphic character and a novel signal transduction molecule of Metazoa, such as the receptor tyrosine kinases (56) or integrin (26), which have been described previously from sponges.
This study establishes that sponge cells react to ethylene with an activation of cell metabolism including gene activation. It will be interesting to perform studies of specific biological effects, such as the determination of ethylene on the development of sponge cells in primmorphs, or on the growth of bacteria in situ, which usually live in symbiosis with sponges (57).

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