The extracellular matrix (ECM) of *Volvox* is modified during development or in response to external stimuli, like the sex-inducing pheromone. It has recently been demonstrated that a number of genes triggered by the sex-inducing pheromone are also inducible by wounding. By differential screening of a cDNA library, a novel gene was identified that is transcribed in response to the pheromone. Its gene product was characterized as an ECM glycoprotein with a striking feature: it exhibits a hydroxyproline content of 68% and therefore is an extreme member of the family of hydroxyproline-rich glycoproteins (HRGPs). HRGPs are known as constituents of higher plant ECMs and seem to function as structural barriers in defense responses. The *Volvox* HRGP is also found to be inducible by wounding. This indicates that the wound response scenarios of higher plants and multicellular green algae may be evolutionary related.

The evolution of a complex extracellular matrix (ECM) from a simple cell wall was one of the prerequisites to promote the transition from unicellularity to multicellularity. The volvocine algae provide the unique opportunity for exploring the pathways that led from a simple cell wall to a complex ECM that stabilizes the shape of an organism and mediates many developmental responses of cells to internal as well as external stimuli. The volvocine algae range in complexity from unicellular *Chlamydomonas* to multicellular organisms, with differentiated cells and complete division of labor, in the genus *Volvox*. The asexually growing organism of *Volvox carteri* is composed of only two cell types: 2000–4000 biflagellate *Chlamydomonas*-like somatic cells are arranged in a monolayer at the surface of a hollow sphere (1, 2) and 16 much larger reproductive cells (“gonidia”) lie just below the somatic cell sheet. *Volvox* cells are surrounded and held together by a glycoprotein-rich ECM (reviewed in Refs. 3 and 4). Cell walls and ECMs of the volvocine algae are assembled entirely from glycoproteins (5) and a high content of hydroxyproline has been detected. Hydroxyproline-rich glycoproteins (HRGPs) represent a constituent of higher plant ECMs, and much work has been done to analyze the structures of these proteins (6–11). However, there are few examples in the literature where multiple ECM proteins have been examined in molecular detail from a single species or from closely related species. This approach has been initiated with volvocine algae to allow a more integrated approach to elucidate the structure, assembly, and function of ECM proteins.

A remarkably rapid remodelling of the ECM is observed under the influence of the sex-inducing pheromone (a glycoprotein) that triggers initiation of the sexual life cycle of *Volvox carteri* (12–14). In particular, synthesis of some members of the pherophorin family of ECM proteins (15–17) is strongly induced by the pheromone. Pherophorins are ECM glycoproteins that contain a C-terminal domain with homology to the sex-inducing pheromone.

By differential screening of a cDNA library, additional genes were recently identified that are transcribed under the control of the sex-inducing pheromone (18). Unexpectedly, genes were found, in addition to those encoding the pherophorins, that encode extracellular chitinases and proteinases. In higher plants, similar protein families are known to play an important role in defense against fungi. Indeed, it could be demonstrated that the same set of genes triggered by the sex-inducing pheromone is also inducible by wounding of *Volvox* spheroids.

Pheromone-induced changes in the composition of the ECM have been characterized in detail within the cellular zone of the ECM (12, 13, 15, 16, 19) and to a lesser extent within the deep zone (DZ) (17), which contains all ECM components internal to the cellular zone (for nomenclature see Ref. 3). The DZ appears as a relatively amorphous component that fills the deepest regions of the spheroid and that may constitute more than 90% of the total volume of the organism. In this paper, we characterize a HRGP exhibiting an extreme composition that is expressed in response to the sex-inducing pheromone and to wounding and that is part of the DZ compartment of the ECM.

**Experimental Procedures**

*Culture Conditions*—The female *V. carteri* f. *nagariensis* strains HK10 (wild type) and 153-48 (mitA−) were obtained from R. C. Starr (Culture Collection of Algae, University of Texas, Austin, TX) or from D. L. Kirk (Washington University, St. Louis, MO). Synchronous cultures were grown in *Volvox* medium (20) at 28 °C in a 8 h dark/16 h light (10000 lux) cycle (21). Strain 153-48 was grown in the presence of 1 mM NH₄Cl. The sex-inducing pheromone was used as described (22).

* Differential Screening of a cDNA Library*—Total RNA was isolated from *V. carteri* spheroids (HK10) at various times after the addition of the sex-inducing pheromone. RNA samples isolated 3, 6, and 12 h after the application of the pheromone were pooled, and a cDNA library was prepared from the corresponding poly(A)+ mRNA preparation (18) by using the *λ*ZAP cDNA synthesis kit (Stratagene, La Jolla, CA). Replica filters were probed with 32P-labeled cDNA prepared from polyadeny-
lated RNA extracted from sexually induced or asexually growing V. carteri spheroids. Hybridization was performed according to standard procedures (23). The cDNA fragment of dz-hrgp yielded only a 32P signal from sexually induced spheroids, not from asexually growing organisms.

Cloning of the dz-hrgp Gene—The dz-hrgp cDNA fragment obtained by differential screening of the cDNA library was used as a probe to screen a V. carteri genomic library (19) in λEMBL 3 (24).Cloning of the dz-hrgp gene followed standard techniques (23).

PCR Amplification of dz-hrgp cDNA Fragments—RNA from sexually induced (2.5 h) V. carteri spheroids (HK10) was used to construct a cDNA library covalently linked to magnetic beads according to the instructions of the manufacturer of the beads (Deutsche Dynal, Hamburg, Germany), and cDNA fragments of dz-hrgp were amplified by PCR. Alternatively, cDNA fragments were amplified by reverse transcription PCR as described (25). RACE-PCR technique was performed as described (26).

DNA Sequencing—Genomic and cDNA clones of dz-hrgp were mapped with standard restriction enzymes, and restriction fragments were subcloned. To create targeted breakpoints for DNA sequencing, these subclones were digested unidirectionally with exonuclease III (27). This was done from both sides of the subclones. Products were transformed into ultracompetent Escherichia coli cells (Epicurian Coli, Stratagene). The precise length of a given insert was determined by gel electrophoresis. Reverse transcription from both strands was achieved by oligo(dT) hybridizing with (152P)ATP. Sequencing of GC-rich stretches was improved by the addition of Me 2SO 0.5-mm hypodermic needle. Reverse transcription-PCR from 20

**Northern Blot Analysis—**Total RNA (10 μg) from V. carteri strain HK10 was separated on a 1.0% denaturing gel (23), vacuum-blotted, UV-cross-linked onto Hybond-N membrane (Amersham Pharmacia Biotech), and hybridized with a 0.6-kb 32P-labeled cDNA fragment of dz-hrgp. This cDNA fragment came from the 3′-untranslated region of dz-hrgp to prevent cross-reactions with other polyproline-encoding genes.

**Reverse Transcription-PCR—**Volvox spheroids were incubated with or without the presence of the sex-inducing pheromone or were wounded by forcing a concentrated Volvox suspension through a 0.5-mm hypodermic needle. Reverse transcription-PCR from 20 Volvox spheroids was performed as described (25). The antisense oligonucleotide primer 5′-GTGTTTCCCACCAAGTGCGA and the sense oligonucleotide primer 5′-GAGCCATGTGGAAAGTCG were used for PCR amplification of a 117-base pair dz-hrgp cDNA fragment. Products of PCR amplification were cloned and sequenced.

**Construction of Chimeric Genes—**The fusion regions of the chimeric dz-hrgp promoter-dz-hrgp gene or the chimeric dz-hrgp-arylsulfatase gene were generated by the recombinant PCR technique (33). The final construction was performed by standard techniques (23).

**Stable Transformation of Volvox—**Transformation of Volvox was as described (34) but using a BioListic PDS-1000/He particle gun (Bio-Rad) (35) at 1500 psi, 153-48 (nA) with Volvox-coated microprojectiles.

**Genomic PCR—**Genomic PCR was used to confirm stable transformation of Volvox. 50 spheroids were selected under a stereomicroscope and transferred into 10 ml of sterile lysis buffer (0.1 M NaOH, 2.0 M NaCl, 0.5% SDS). After 5 min at 95 °C 200 μl of 50 mM Tris/HCl, pH 7.5, were added immediately. 2 μl of the resulting lysate was used for PCR (in a total volume of 100 μl). PCR was performed by standard protocols. Products of PCR amplification were cloned into the Smal I site of pUC18 and sequenced.

**Preparation of Anti-DZ-HRGP Antiserum—**The peptide PRRSV-VALVETC (amino acids 26–37 of DZ-HRGP) with an artificial cysteine at the C-terminal end was synthesized by using Fmoc (9-fluorenylethoxycarbonyl) amino acid derivatives. The peptide was purified on a reversed phase C18 HPLC column (Nucleosil 100–7, 7 μm; Macherey-Nagel, Düren, Germany). The predicted molecular mass and the sequence of the peptide were confirmed by electrospray mass spectrometry and by Edman degradation. The synthetic peptide was covalently linked to a maleimide-activated carrier protein (keyhole limpet hemocyanin) via the SH group of the artificial cysteine and used to raise antibodies in rabbits. Antibodies were purified by protein G-Sepharose column chromatography (Amersham Pharmacia Biotech). Further purification of anti DZ-HRGP antiserum was on an affinity column (SulfoLink Coupling Gel, Pierce) with covalently linked DZ-HRGP peptide. The column was produced and handled as described (36).

**Amino Acid Analysis and Mass Spectrometry—**Amino acid analysis was performed as described by Cohen and Strydom (39). Molecular masses of fractions of interest were determined by electrospray mass spectrometry using an Applied Biosystems, Foster City, CA).

**Radioactive labeling of DZ-HRGP with [14C]Bicarbonate—**Pulse labeling with [14C]bicarbonate was performed in vivo as described (38).

**Amino Acid Analysis and Mass Spectrometry—**Amino acid analysis was performed as described by Cohen and Strydom (39). Molecular masses of fractions of interest were determined by electrospray mass spectrometry using a SSQ 7000 mass spectrometer (Finnigan).

**RESULTS**

**Differential Screening—**Total RNA was isolated from V. carteri spheroids (female HK10) harvested at various times after the addition of the sex-inducing pheromone. RNA samples isolated 3, 6, and 12 h after the application of the pheromone were pooled, and a cDNA library (αZAP) was constructed from the corresponding poly(A)+ mRNA preparation. Repeated differential screenings of the cDNA library with cDNA derived from asexual versus sexually induced organisms resulted in the detection of novel clones in addition to the already known members of the pherophorin family (15–17) and to the chitinase and the cysteine protease described recently (18). One of these novel clones was named dz-hrgp for reasons explained below. The kinetics of dz-hrgp mRNA accumulation in response to the sex-inducing pheromone was analyzed by Northern blotting. As shown in Fig. 1, hybridizing RNA started to accumulate only about 30 min after pheromone treatment and reached its maximum 2 h later. No significant signals were observed in asexually growing organisms. The mRNA detected by the dz-hrgp cDNA fragment is ~2.0 kb in length.

**DNA Sequencing and Deduced Amino Acid Sequence—**To extend the cDNA sequence information obtained from the originally isolated 0.6-kb cDNA, the RACE-PCR (26) was used to obtain the missing 5′ stretches. But the RACE-PCR yielded only a 0.25-kb fragment because a stretch of unusually high G+C-content caused premature termination of reverse transcription. To circumvent this problem, the dz-hrgp cDNA fragment was used to clone the corresponding genomic DNA. Sequencing of this unusual stretch of genomic DNA also produced particular problems and was only possible by the application of special techniques like creation of targetable breakpoints for DNA sequencing by exonucleolytic III digestion, label synthesis (for details, see “Experimental Procedures”). The strategy applied to collect the complete nucleotide sequence of dz-hrgp cDNA is shown in Fig. 2. The sequence was submitted to the GenBankTM/EBI Data Bank with accession number AJ242540. The dz-hrgp gene contains a single intron within its coding region. The deduced amino acid sequence for the DZ-HRGP is shown in

**Localization of DZ-HRGP—**Whole Volvox spheroids were separated into defined fractions to allow localization of DZ-HRGP. A DZ extract was prepared as described below. The remaining material (intact cells and cell-bound ECM) was extracted with 2 M NaCl (2 h) and then disrupted ultrasonically (Sonifier B15, Branson, Danbury, CT). Soluble and insoluble components were separated by ultracentrifugation (100,000 × g, 30 min). All fractions were lyophilized, deglycosylated by anhydrous HF (37), and analyzed by a Western blot using the polyclonal DZ-HRGP antibody.

**Purification of DZ-HRGP from Volvox—**Sexually induced Volvox spheroids from three 20 l cultures were harvested at the stage of embryogenesis by filtration on a 100-μm mesh nylon screen. The spheroids were broken up by forcing them through a 0.5-mm hypodermic needle. The disrupted spheroids were centrifuged at 25,000 × g for 30 min. The supernatant (DZ extract) was brought to 50 mM Tris/HCl, pH 9.0, 10 mM NaCl and applied to a QAE-Sephadex A-25 anion exchange column (Amersham Pharmacia Biotech) equilibrated with the same buffer. Elution was performed with 250, 500 and 800 mM NaCl. Fractions containing DZ-HRGP antigen were dialyzed against 4 mM NH4HCO3 and lyophilized. The dried material was deglycosylated by anhydrous HF (37), applied to SDS-PAGE, and blotted onto polyvinylidene difluoride membrane. The membrane was stained with Coomassie Blue R-250 (Serva, Heidelberg, Germany) in 50% methanol, destained with 50% methanol/10% acetic acid, and washed with water. The band attributed to the DZ-HRGP antigen was cut out and sequenced using an automated gas-phase peptide sequence (Applied Biosystems, Foster City, CA).

**radioactive labeling of DZ-HRGP with [14C]bicarbonate—**Pulse labeling with [14C]bicarbonate was performed in vivo as described (38).

**Amino Acid Analysis and Mass Spectrometry—**Amino acid analysis was performed as described by Cohen and Strydom (39). Molecular masses of fractions of interest were determined by electrospray mass spectrometry using a SSQ 7000 mass spectrometer (Finnigan).
Fig. 1. Northern blot analysis of dz-hrgp mRNA. The accumulation of dz-hrgp mRNA in vegetative or sexually induced organisms (by treatment with 10^{-12} M sex-inducing pheromone at 0 hr) was determined. Equal amounts of total RNA isolated after different periods of incubation from vegetative or sexually induced Volvox organisms were hybridized with a 0.6-kb cDNA fragment of dz-hrgp (see cDNA clone in Fig. 2).

Fig. 2. Strategy applied to collect the complete nucleotide sequence of dz-hrgp cDNA and the deduced amino acid sequence of DZ-HRGP. a, completion of cDNA was achieved by 5'-RACE-PCR, by reverse transcription-PCR, and by sequence analysis of a genomic clone. The position of an intron is indicated by an arrowhead. The sequence was submitted to the GenBank™/EBI Data Bank with accession number AJ242540. b, deduced amino acid sequence of DZ-HRGP. All prolines are shown in white letters on a black background. An arrow marks the signal peptidase cleavage site.

Fig. 2b. This amino acid sequence exhibits striking features. The open reading frame encodes a polypeptide 409 amino acid residues in length, including a typical signal sequence. Hydroxy-proline constitutes 68% of the amino acid residues of the mature polypeptide. Several stretches of up to 14 (hydroxy)-proline residues and numerous repeats of Ser-(Pro)_4 as well as (Ser/Arg)-(Pro)_4 elements are special features of this gene product. These data (and the data presented below) indicate a close relation of this novel Volvox protein to a well-known protein family, namely, to the HRGPs of higher plants (6–11). The reason for giving DZ-HRGP its name was this relationship and its localization within the DZ of the Volvox ECM (see below).

Because of the extended proline stretches of DZ-HRGP there are only short amino acid sequences near both the N and C termini of the polypeptide, which could serve as a suitable antigen in DZ-HRGP antibody production to prevent cross-reactions with other proline-rich ECM glycoproteins. Therefore, a sequence derived from the N-terminal end of DZ-HRGP was used to synthesize the peptide PRRSPVVIVALVETC. An artificial cysteine at the C terminus of this peptide simplified coupling to a carrier protein. Peptide specific polyclonal antibodies were raised in rabbit.

Identification and Homologous Overexpression of DZ-HRGP—The deduced DZ-HRGP amino acid sequence includes a typical signal peptide indicating an extracellular localization of DZ-HRGP. The peptide-specific antibody was used to search for DZ-HRGP in different extracts prepared from sexually induced Volvox spheroids. However, neither complete lysates nor ECM fractions produced any signal in immunodetection experiments. The putative extracellular localization of DZ-HRGP suggests extensive glycosylation of hydroxyproline residues, and this in turn could prevent immunodetection by our peptide-specific antibody. Therefore, the components of Volvox extracts were deglycosylated by treatment with anhydrous HF. Indeed, after deglycosylation, positive signals at ~150 kDa could be obtained in extracts from complete Volvox spheroids as well as in an ECM extract representing the DZ of Volvox ECM. The material of the DZ is selectively released by mild mechanical stress as may be exerted by forcing Volvox spheroids through a hypodermic needle.

Because Western blots yielded only a weak DZ-HRGP signal, overexpression of DZ-HRGP in Volvox was thought to get sufficient amounts of DZ-HRGP for structural studies. Random integration by illegitimate recombination events is the preferred mode of DNA integration into the Volvox genome, and transformants often integrate multiple copies of the plasmids used for transformation (34). Therefore, transgenic Volvox were generated that express additional copies of the dz-hrgp gene under the control of its own promoter. Stable transformants were produced as described previously (25, 34, 35, 40). The transgenic Volvox strain did not show any visible change in phenotype, but the expression rate of DZ-HRGP was clearly higher than in wild-type algae. Again, the polyclonal DZ-HRGP antibody leads to a positive immunosignal at ~150 kDa after deglycosylation of extracts from sexually induced transformants. ~50% of DZ-HRGP is liberated just by forcing the Volvox spheroids through a hypodermic needle (DZ extract); all of the remaining DZ-HRGP is extracted in the presence of 2 M NaCl.

A molecular mass of 39.6 kDa is calculated for the mature polypeptide chain of DZ-HRGP (Fig. 2b), but this is much less than the apparent molecular mass of deglycosylated DZ-HRGP (~150 kDa) shown on Western blot gels (Fig. 3b). The extreme (hydroxy-)proline content of DZ-HRGP explains the difference between the observed and calculated molecular masses, because stretches of poly-(hydroxy)-proline have a reduced ability to bind SDS (41).

Purification of DZ-HRGP—To prove the identity of the immunoreactive material and the DZ-HRGP, the components of the DZ extract of the ECM were fractionated by ion exchange chromatography (QAE-Sephadex). Because proteins from the DZ are hardly stained with standard procedures on SDS-PAGE gels, the Volvox spheroids were grown in the presence of [14C]bicarbonate prior to preparation of the DZ extract, and the SDS-PAGE gels were analyzed by fluorography. As demonstrated by analytical SDS-PAGE, the QAE-Sephadex chromatography separated two main protein species of the deep zone extract completely from each other. These protein species exhibit apparent molecular masses of ~300 and ~240 kDa and elute at 500 and at 800 mM NaCl, respectively (Fig. 3a). The material of both fractions was then deglycosylated by treatment with anhydrous HF and fractionated by 6% SDS-PAGE.
After blotting, an immunoreactive polypeptide with an apparent molecular mass of ~150 kDa could be detected in the 500 mM NaCl fraction (Fig. 3b). To confirm that it is indeed the ~300-kDa protein (glycosylated) that produces the ~150-kDa immunosignal (deglycosylated), the ~300-kDa protein was further purified by excision from a SDS-polyacrylamide gel. After elution the ~300-kDa protein was deglycosylated and subjected to SDS-PAGE. Again, the ~150-kDa immunosignal was detectable in a Western blot (data not shown). The immunoreactive polypeptide was subjected to automated Edman degradation, resulting in the N-terminal sequence Ala-Hyp-Ala-Arg-Lys-Hyp-Hyp-Hyp-Arg-Arg-Ser-Hyp, matching the N-terminal sequence deduced for mature DZ-HRGP. Remarkably, even the very first proline residue of the polypeptide turned out to be posttranslationally modified to hydroxyproline. Amino acid analyses of DZ-HRGP resulted in molar ratios of the predominant amino acids hydroxyproline, arginine, and serine of 10:1:1.4. On the basis of the cDNA sequence the ratios of (hydroxy)proline, arginine, and serine were calculated as 10.2:1:1.6. All the other amino acids within DZ-HRGP could not be quantified exactly in amino acid analyses because of the small amounts detected. As shown in Fig. 4, all of the prolines of DZ-HRGP are found to be modified to hydroxyproline.

Carbohydrate Composition of DZ-HRGP—The carbohydrate composition of DZ-HRGP was determined by radio gas chromatography. DZ-HRGP purified from Volvox spheroids grown in the presence of [14C]bicarbonate was hydrolyzed, and the resulting monosaccharides were analyzed as the alditol acetates. DZ-HRGP contains the neutral sugars arabinose and galactose in a 2:1 ratio (Fig. 5).

The Promoter of dz-hrgp Gene Mediates Pheromone-dependent Transcription—To examine the properties of the dz-hrgp promoter, the 5′-nontranslated region (~3 kb) of dz-hrgp was placed in front of a reporter gene, the arylsulfatase gene from Volvox (25, 42) (Fig. 6a). In wild-type Volvox, arylsulfatase is only expressed under sulfate starvation; no activity is detectable in organisms grown in sulfate-containing medium (42).

After transformation of Volvox with the chimeric dz-hrgp/aryl sulfatase gene, the reverse transcription-PCR technique was used to verify the existence of hybrid mRNA in transformants (see “Experimental Procedures”). Volvox transformants containing the arylsulfatase gene under the control of the dz-hrgp promoter were incubated with or without the sex-inducing pheromone in the presence of the chromogenic enzyme substrate 4-nitrophenyl sulfate. Arylsulfatase activity was determined photometrically by measuring the absorbance of the liberated 4-nitrophenol. After treatment with the sex-inducing pheromone, only transformants exhibited enzyme activity (Fig. 6b). Thus, the promoter fragment from the dz-hrgp gene is sufficient to mediate transcription in response to the sex-inducing pheromone.

Transcription of dz-hrgp Gene Is Cell Type-specific—In the Northern blotting experiments described above whole Volvox spheroids were used for RNA preparation. To investigate whether transcription of the dz-hrgp gene is cell type-specific, both cell types of (sexually induced) Volvox spheroids, somatic and reproductive, were separated from each other by size frac-
tionation. RNA from both cell types was extracted and reverse transcribed, and a dz-hrgp cDNA fragment was amplified by PCR. Transcription of the dz-hrgp gene was detectable mainly in somatic cells, as shown in Fig. 7a.

Wounding Induces Transcription of dz-hrgp Gene—As demonstrated recently (18), all genes known so far to be under the control of the sex-inducing pheromone are also triggered by wounding. This observation led us to investigate whether transcription of dz-hrgp gene is also responsive to wounding. Volvox spheroids were slit by mild mechanical stress simply by forcing them through a hypodermic needle. 2.5 h later total RNA from 20 spheroids was extracted and reverse transcribed, and a dz-hrgp cDNA fragment amplified by PCR. As shown in Fig. 7b, transcription of dz-hrgp gene is not detectable in asexually grown Volvox colonies. In contrast, the sex-inducing pheromone as well as wounding induces transcription of this gene (Fig. 7b). Wounding even appears to stimulate a higher rate of transcription as compared with the induction by the sex-inducing pheromone.

DISCUSSION

This paper describes the structure and properties of a novel component of the Volvox ECM with the striking feature of being composed of 68% hydroxyproline. Some of these hydroxyprolines are arranged in Ser-(Pro)₃ and Ser-(Pro)₄ elements that are typical of higher plant extensins (48, 49). Among the modifications identified in Volvox are O-glycosylations with oligoarabinosides, attachment of saccharides containing phosphodiester bridges between arabinose residues, and in a single case, the additional attachment of a highly sulfated arabinomannan (19). As analyzed in more detail for the ECM protein SSG 185, the HR module is also involved in covalent cross-linking of the monomeric units (19). If DZ-HRPG serves a similar function, this rod-shaped molecule could be involved in the creation of the fibrous networks observed in the deep zone compartment (3). The fact that overexpression of DZ-HRPG did not create an aberrant ECM morphology would indicate the participation of a second ECM molecule in the cross-linking reaction. In this case, only the concomitant overexpression of both ECM partners interacting in a stoichiometric relation would be expected to cause a visible phenotype.

The natural resistance of higher plants to diseases involves an array of inducible defense responses, including synthesis of extracellular hydrolytic enzymes such as proteases and chiti-
nases and the accumulation of HRGPs within the ECM. The latter glycoproteins are hypothesized to function in defense as structural barriers (50) or as specific microbial agglutinins (51, 52) against pathogen attack. Surprisingly, the simple multicellular green alga Volvox responds to wounding in much the same way as observed in higher plants. As was demonstrated recently (18), Volvox responds to wounding with the synthesis of a chitinase as well as a protease that is combined with chitin-binding modules. With the additional demonstration of a typical HRGP that is produced in response to wounding, it now appears that much of the response scenario found in higher plants already exists in multicellular green algae. Even more surprising is the fact that these algal pathways are also triggered by the sex-inducing pheromone. However, Kirk and Kirk (53) were able to demonstrate that synthesis of the sex-inducing pheromone can be triggered in somatic cells by a short heat shock applied to asexually growing organisms. This response induces the production of dormant zygotes that survive unfavorable conditions like drought. Although wounding is unable to induce pheromone production, similar biochemical responses are observed after wounding and pheromone application (18). As induction of sexuality and subsequent production of zygotes obviously is part of the strategy of the organism to escape from environmental stress, it appears to make sense that apparently obviously is part of the strategy of the organism to escape from environmental stress, it appears to make sense that apparently

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