

# A Novel Glycosphingolipid That May Participate in Embryo Inversion in *Volvox carteri*

Stephan Wenzl and Manfred Sumper

Lehrstuhl Biochemie I

Universität Regensburg, Universitätsstr. 31

8400 Regensburg

Federal Republic of Germany

## Summary

**Mouse monoclonal antibody 4-I-244 detects a developmentally regulated antigen in embryos of *Volvox carteri* and inhibits specifically the morphogenetic process of inversion (the process by which the embryo turns inside out). Antigen 4-I-244 was chemically characterized as a complex phytosphingolipid containing the neutral sugars xylose, galactose, and glucose as well as inositol and phosphate.**

## Introduction

The curling of cellular sheets in a spatially programmed manner is an ubiquitous process during embryogenesis of multicellular organisms. This process generates species-specific form. At the end of embryogenesis of the multicellular alga *Volvox*, a morphogenetic process of relative simplicity takes place which turns the embryo inside out ("inversion"). This inversion has successfully been used for a detailed geometric analysis. It has been demonstrated that the observed changes of cell shapes together with the locations of cell-cell connections are sufficient to account for the shape of the embryo at all stages of inversion (J. L. Kelland, [1964]. Ph. D. thesis, Princeton University, Princeton, NJ: Kelland, 1977; Pickett-Heaps, 1970; Viamontes and Kirk, 1977; Viamontes et al., 1979; Green et al., 1981).

The asexual organism of *Volvox carteri* consists of only two cell types: somatic and reproductive. Two thousand to four thousand somatic cells are located as a single layer on the surface of a hollow sphere (spheroid); 16 reproductive cells (gonidia) are positioned within the spheroid in the posterior region. The life cycle of *V. carteri* has been repeatedly described in full detail (Starr, 1969, 1970).

Eleven or twelve rapid synchronous cleavage divisions of a gonidium generate all the cells of an adult spheroid. The resulting embryonic cells are arranged in a hollow sphere, but their orientation with respect to the surface is the reverse of that found in the adult: the flagellar ends of the somatic cells are directed toward the center of the sphere and gonidia protrude from the surface. During inversion, the embryo turns completely inside out through a cross-shaped slit (the "phialopore") and thereby establishes the adult configuration. Inversion is initiated when cells bordering the phialopore undergo a transition in shape and begin to resemble the classic "bottle cells" seen in a gastrulating amphibian embryo (Holtfreter, 1943, 1944). The sequence of changes of cell shape pro-

gresses in a wave-like fashion toward the pole opposite the phialopore.

Not surprisingly, the cell-shape changes accompanying inversion are mediated by the cytoskeleton (Viamontes et al., 1979). However, the control mechanism by which these cell-shape changes are initiated and precisely propagated are unknown.

In order to define extracellular molecules that might be involved in this mechanism, we prepared monoclonal antibodies against crude envelope fractions of inverting embryos and screened the resulting monoclonal antibodies for their capability to inhibit inversion *in vivo*. Antibody 4-I-244 showed exactly this inhibitory action and in this paper we describe the characterization of the corresponding antigen.

## Results

Monoclonal antibodies were raised against a crude membrane preparation from isolated *Volvox carteri* embryos at the stage of inversion. The resultant monoclonal antibodies were tested for their ability to interfere *in vivo* with the morphogenetic process of inversion. A single hybridoma line (4-I-244) produced an antibody exhibiting this inhibitory action (see Figure 1). If added to a population of synchronously developing embryos at least 3 hr prior to the initiation of inversion, antibody 4-I-244 blocks nearly all embryos of the population from entering inversion. Thus the reproductive cells remain protruding from the embryonic surface (Figure 1, B1). This inhibition is specific in that further development of the reproductive cells proceeds normally. After maturation, the cells enter a new cycle of cell divisions and develop into daughter colonies which are now located outside the mother spheroid (Figure 1, B2). Antibody 4-I-244 does not interfere with any other developmental process if added during periods of the life cycle other than that preceding inversion. Antibody 4-I-244 was characterized as an IgM antibody. Therefore antibody 4-I-244 was cleaved to a mixture of monovalent and divalent antibodies by a recently introduced technique (Matthew and Reichardt, 1982). After cleavage, antibody 4-I-244 fully retained its inhibitory action on the inversion process.

## Indirect Immunofluorescence Microscopy at Various Embryonic Stages

Embryos fixed with formaldehyde at different stages of development were mixed and stained with monoclonal antibody 4-I-244 and fluorescein-conjugated sheep anti-mouse IgM. Early embryos (2- to 32-cell stage) did not bind antibody 4-I-244 appreciably, whereas all cell surfaces of late embryos (all stages beyond the 64-cell stage) were stained intensely (Figure 2). This finding suggests that antigen 4-I-244 is synthesized in a developmentally regulated fashion.

Another nine hybridoma lines obtained from the same

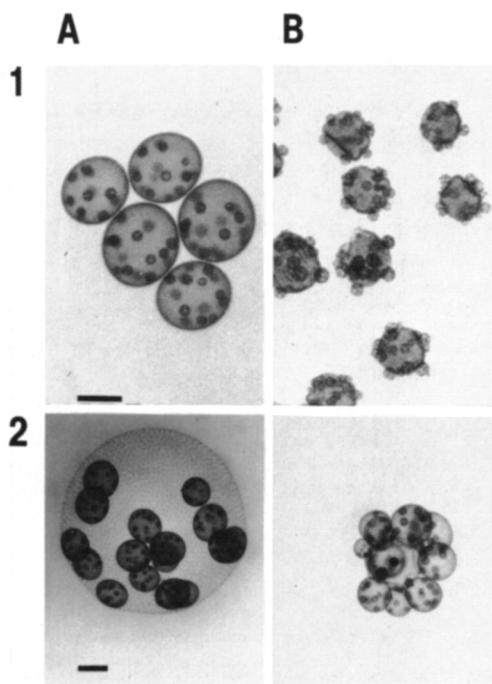


Figure 1. Monoclonal Antibody 4-I-244 Inhibits the Morphogenetic Process of Inversion

(A) Control culture of isolated embryos.

(B) Antibody 4-I-244 (50  $\mu\text{g/ml}$ ) added to isolated embryos 3 hr prior to inversion.

Photographs were taken 10 hr (1) and 50 hr (2) after inversion. A total of 52 monoclonal antibodies derived from the same fusion experiment were tested; only antibody 4-I-244 inhibited inversion. Bars = 100  $\mu\text{m}$ .

fusion experiment produced antibodies which stained the cell surface of embryos as intensely as did antibody 4-I-244. However, none of these nine monoclonal antibodies interfered with the process of inversion.

#### Isolation of Antigen

In a preliminary experiment, a lysate from inverting embryos was run on 6% and 15% SDS-polyacrylamide gels followed by blotting on a nitrocellulose filter. Antibody 4-I-244 recognized a single component migrating within the low molecular weight range of the gels. There was absolutely no cross-reaction with any of the high molecular weight (glyco-) proteins, such as I-SG (Wenzl and Sumper, 1982), of *Volvox carteri* embryos. The antigenic component was completely resistant to proteolytic digestion. Furthermore, the antigen could be extracted into organic solvent mixtures. Complete extraction was achieved with chloroform:methanol:water (10:10:3). Thus the antigen behaves like a lipid with strong amphiphilic character. For further characterization of the antigen, an ultrasonic lysate from  $^{14}\text{C}$ - $\text{NaHCO}_3$ -labeled inverting embryos was dried and extracted with chloroform:methanol (3:2), followed by chloroform:methanol:water (10:10:3). The material of the latter extract was further separated by silica gel thin-layer chromatography, followed by blotting on a nitrocellulose filter and immunostaining with antibody 4-I-244

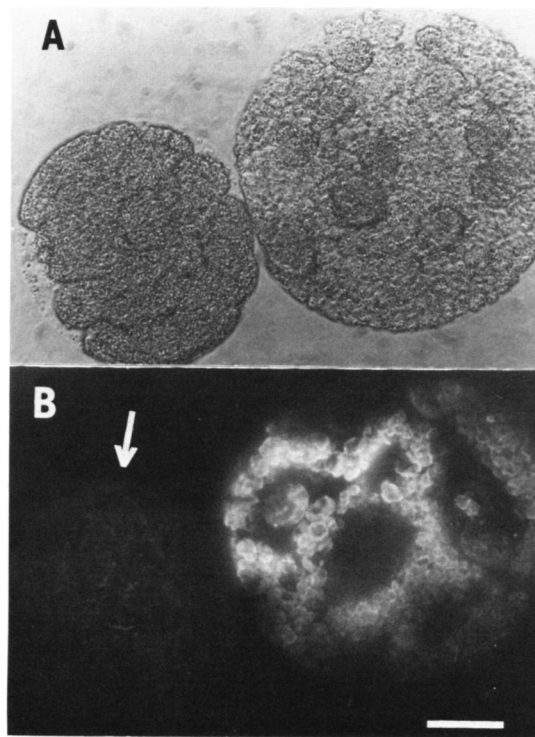


Figure 2. Expression of Antigen 4-I-244 by *Volvox carteri* Embryos at Different Cleavage Stages Studied by Indirect Immunofluorescence

A mixture of early embryos (8- and 16-cell stage) and late embryos (>240-cell stage) was labeled with antibody (see Experimental Procedures). (A) Corresponding phase-contrast micrographs of fluorescence image (B) which shows an early (arrow) embryonic and a late embryonic stage. Bar = 25  $\mu\text{m}$ .

(Figures 3A and 3B). Again, antibody 4-I-244 recognized only a single component. After elution from the thin-layer chromatogram, this component turned out to be homogeneous as judged by two-dimensional thin-layer chromatography (Figure 3C).

#### Chemical Characterization of Antigen

To obtain a first insight into the chemical structure of the antigen, homogeneous radioactive antigen (see Experimental Procedures) was subjected to acid hydrolysis (4-N-trifluoroacetic acid, 4 hr at 100°C). The water soluble products were analyzed for the presence of neutral sugars by gas chromatography of the corresponding alditol acetates. As documented in Figure 4A, radioactive xylose, galactose, and glucose were detected in a ratio of about 2:1:1. No radioactive amino sugars could be found by radio gas chromatographic analysis, even after strong acid hydrolysis (6-N-HCl, 6–12 hr at 105°C) and subsequent conversion to N-acetylhexosaminitol acetates (Michalski et al., 1984).

Radioactive material extractable into organic solvent from an acid hydrolysate was methylated and analyzed for the presence of fatty acids by radio gas chromatography. A single radioactive substance could be found (Figure 4C) which on both polar (SP 2330) and nonpolar stationary phases (SE 30) cochromatographed with hexaeicosanoic



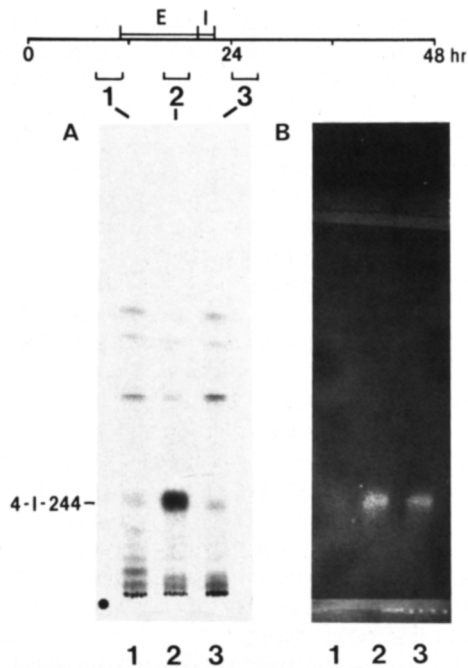


Figure 5. Expression of Antigen 4-I-244 in Asexual Embryos as a Function of Developmental Stage Analyzed by Thin-Layer Chromatography and Immunostaining

(A) Isolated uncleaved reproductive cells (1), preinversion embryos (2), and postinversion embryos (3) were pulse-labeled with  $^{14}\text{C-NaHCO}_3$  (3 hr) and the chloroform:methanol:water (10:10:3) extract (40,000 cpm each) analyzed by thin-layer chromatography (solvent 1) and autoradiography (see Experimental Procedures).

(B) The same chromatogram was blotted on a nitrocellulose filter and immunostained with antibody 4-I-244.

The drawing on top of the chromatograms shows a time scale of the life cycle of asexually growing *V. carteri*. The periods of pulse labeling are marked.

chromatography for the presence of antigen 4-I-244. Autoradiography reveals no detectable synthesis in uncleaved reproductive cells (Figure 5, lane 1). A high rate of synthesis of antigen was observed 3–4 hr immediately preceding the initiation of inversion (Figure 5, lane 2). Shortly after termination of inversion, the rate of synthesis of antigen 4-I-244 drops markedly (lane 3) whereas other lipid components continue to be synthesized at essentially unchanged rates. The same thin-layer chromatogram was blotted onto a nitrocellulose filter. Antibody 4-I-244 detected antigen only at late embryonic stages and at stages following inversion (Figure 5B). Thus antigen 4-I-244 remains stable for at least 4 hr after synthesis.

A unique feature of sexual development in the male strain (69-1b) that leads to sperm-cell-containing *Volvox carteri* spheroids is the occurrence of an additional morphogenetic process: in the first inversion process the embryo turns inside out exactly as during asexual development. Afterward (20 hr later) each reproductive cell (now called an androgonidium) undergoes a series of cleavages to form a packet of sperm cells. This bowl-shaped sperm cell mass again performs a morphogenetic process (second inversion) to form a sperm bundle which is

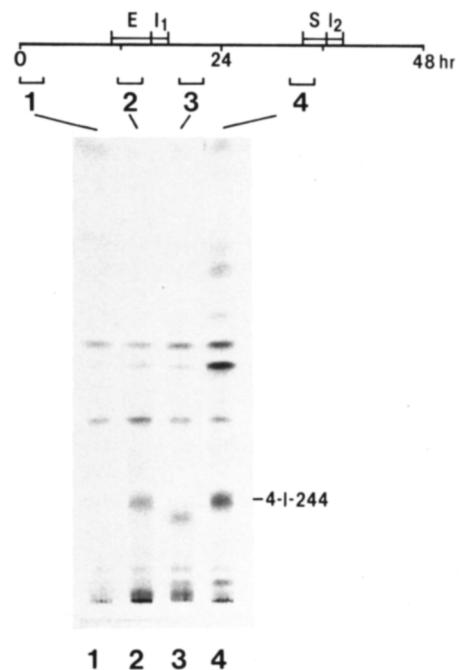


Figure 6. Expression of Antigen 4-I-244 in Sexual Male Embryos (strain 69-1b) as a Function of Developmental Stage Analyzed by Pulse Labeling and Thin-Layer Chromatography

Isolated uncleaved reproductive cells (1), preinversion embryos (2), postinversion embryos (3), and isolated preinversion sperm packets (4) were pulse-labeled with  $^{14}\text{C-NaHCO}_3$  (3 hr) and the glycolipid fraction (40,000 cpm each) applied to silica gel thin layers (solvent 1). Autoradiography was overnight. The drawing on top of the chromatograms shows a time scale of the life cycle of sexually growing *V. carteri* (69-1b). The periods of pulse labeling are marked.

convex on its anterior side (Birchem and Kochert, 1979). A functional role for antigen 4-I-244 in the inversion process would become even more likely if its synthesis were reinitiated within sperm packets at the time of this second inversion.  $^{14}\text{C-NaHCO}_3$  pulse-labeling experiments over the whole life cycle of sexually induced male embryos clearly show that antigen 4-I-244 is indeed synthesized during both the first and second preinversion period (Figure 6, lanes 2 and 4). Furthermore, antigen 4-I-244 is produced within the isolated sperm packets during the second inversion process (Figure 6, lane 4).

#### Localization of Antigen Synthesis within a *Volvox carteri* Spheroid

All experiments described so far were done with isolated gonidia or embryos at various developmental stages with the assumption that only these reproductive units are synthesizing antigen 4-I-244. The experiment described in Figure 5 gave essentially the same result when performed with intact *Volvox carteri* spheroids containing embryos at corresponding developmental stages.

To exclude antigen synthesis by somatic cells of the mother colony, *Volvox carteri* spheroids containing preinversion embryos were pulse-labeled with  $^{14}\text{C-NaHCO}_3$  and dissociated mechanically into somatic cell sheets and free embryos (see Experimental Procedures). The so-

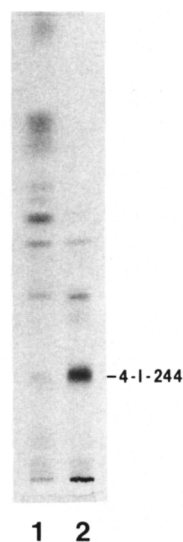


Figure 7. Localization of Newly Synthesized Antigen 4-I-244 within a *Volvox carteri* Spheroid

Synchronously developing *Volvox carteri* spheroids containing preinversion embryos were pulse-labeled with  $^{14}\text{C}$ - $\text{NaHCO}_3$  (3 hr) and then dissociated into somatic cells and embryos (see Experimental Procedures). A chloroform:methanol:water (10:10:3) extract (40,000 cpm each) of both cell fractions was analyzed by thin-layer chromatography (solvent 1) and autoradiography. (1) somatic cell extract. (2) embryonic cell extract.

matic cell sheets of the mother spheroid were separated from the embryos by differential sieving. The glycolipids of both cellular fractions were then extracted and analyzed by thin-layer chromatography (Figure 7). Nearly all of the labeled antigen was associated with the embryo fraction, indicating that antigen 4-I-244 is mainly synthesized within preinversion embryos. Thus antigen 4-I-244 is produced in both a spatially and temporarily controlled fashion.

## Discussion

Antigen 4-I-244 turned out to be a complex sphingolipid containing phosphate and inositol and the neutral sugars xylose, galactose, and glucose. In a previous work, in which the main lipid components of *Volvox carteri* were characterized (Moseley and Thompson, 1980), this type of lipid remained undetected, indicating that this lipid class represents only a minor constituent of *Volvox carteri* membranes. To estimate what percentage of total lipid antigen 4-I-244 is, we determined the ratio of  $\text{C}_{16}$  plus  $\text{C}_{18}$  fatty acids (the main constituents of *Volvox carteri* lipids) and  $\text{C}_{26}$  fatty acid (the constituent of antigen 4-I-244) by gas chromatographic analysis after hydrolysis of total lipid extracts. From this analysis, antigen 4-I-244 represents less than 0.2% of total *Volvox carteri* lipids.

The *Volvox carteri* sphingolipid superficially resembles the complex phosphorus-containing glycolipids that had been isolated and characterized by Carter and associates (1958, 1969) from plant material. These compounds were called phyto-glycolipids. The remarkable similarity in gen-

eral structure between plant and animal cerebroside suggests similar functions for these sphingoglycolipids in plant and animal cell membranes. Growing evidence supports the idea that these lipids are extremely important in a number of specialized functions. For instance, the ganglioside GQ1b enhances greatly neurite outgrowth and cell proliferation in neuroblastoma cell lines (Tsuji et al., 1983). Gangliosides detected by a monoclonal antibody were shown to be developmentally regulated in chicken retina and brain (Grunwald et al., 1985), and a phosphorylated ganglioside was reported to stimulate species-specific adhesion of chicken hepatocytes (Roseman, 1985).

Monoclonal antibody 4-I-244 specifically inhibits the morphogenetic process of inversion during *Volvox carteri* embryogenesis. However, this specific inhibitory action does not unequivocally prove a functional role of this glycolipid during inversion. We cannot exclude the possibility that the inhibitory action is due to an unspecific steric hindrance or due to crosslinking caused by antibody binding to the cell surface that might interfere with cell-shape changes that occur during inversion. Several observations, however, argue against an unspecific effect:

- Antigen 4-I-244 is a developmentally regulated glycolipid, i.e., it is only synthesized during a rather limited period of the *Volvox carteri* life cycle and this period coincides with the preinversion stage.
- Synthesis of antigen 4-I-244 is locally restricted, taking place within the preinversion embryo.
- The different developmental program of sexually induced males includes a second inversion event, taking place within the sperm cell packets. Again, coinciding with the latter event, synthesis of antigen 4-I-244 is initiated within sperm packets at a high rate.
- Antibody 4-I-244 does not inhibit any other developmental event of the *Volvox carteri* life cycle. Even in the permanent presence of this monoclonal antibody, *Volvox carteri* develops completely normally except for the inversion process. If antibody is added immediately after the inversion event, development again continues completely undisturbed.
- After cleavage into small fragments, IgM-antibody 4-I-244 retains its inhibitory action on the inversion process.

The last cell division during embryogenesis was shown to have finished between 50 and 100 min before inversion. During this time a cell-shape change from pear- to spindle-shaped occurs within the embryo. To elicit full inhibition of inversion, antibody 4-I-244 must not be added later than about 120 min before inversion. This observation suggests that antibody 4-I-244 interferes with an early event of the inversion process.

The immunofluorescence data demonstrate that antigen 4-I-244 is accessible and therefore associated with the external surface of the embryonic cells. Previously, we characterized an extracellular sulfated glycoprotein (I-SG) which is likely to have a function during inversion (Wenzl and Sumpster, 1982). These observations indicate that, besides the cytoskeleton, a number of extracellular mole-

cules seem to be involved, suggesting a mechanism of transmembrane coordination of cytoskeletal components.

## Experimental Procedures

### Culture Conditions

*Volvox carteri* f. *nagariensis*, strains HK 10 (female) and 69-1b (male) were obtained from the Culture Collection of Algae at the University of Texas at Austin (R. C. Starr). Synchronous cultures were grown in *Volvox* medium (Provasoli and Pintner, 1959) at 28°C in an 8 hr dark/16 hr light (10,000 lux) cycle (Starr and Jaenicke, 1974).

### Isolation of *Volvox carteri* Embryos

The following procedure refers to cleaving embryos. Slightly different experimental conditions are required for the isolation of uncleaved embryos (gonidia) which are given in brackets. Spheroids at the appropriate developmental stage were disrupted by forcing them through a 0.5 mm (0.4 mm) hypodermic needle. The suspension was filtered through a 90 µm mesh nylon cloth through which only free embryos and single somatic cells pass. Embryos were collected on a 40 µm (10 µm) mesh nylon cloth, which retains only embryos. Subsequently, the embryo suspension was allowed to settle several times and the supernatant, which might contain some somatic cell sheets, was discarded each time.

### Preparation of the Immunogen

Inverted embryos of about 8000 *Volvox carteri* spheroids were isolated and disintegrated by ultrasonic treatment (30 sec total). The lysate (0.5 ml) was layered over a 3 ml cushion of 20% sucrose in *Volvox carteri* medium. Centrifugation was at 100,000 g for 3 hr. The green pellet was dissolved in 300 µl in phosphate-buffered saline containing 1% SDS and heated at 95°C for 2 min. One hundred and fifty microliters were mixed with the same volume of the appropriate adjuvant for one immunization.

### Preparation of Monoclonal Antibodies

BALB/c mice were immunized by intraperitoneal injections of a crude membrane fraction from *Volvox carteri* embryos at the developmental stage of inversion with Freund's complete adjuvant. Six weeks later an intraperitoneal boost of the same immunogen with Freund's incomplete adjuvant was given followed by fusion with NSO/U myeloma cells (Clark, Wright, and Milstein, unpublished results) four days later. Hybridomas interfering with the morphogenetic event of inversion were detected in an *in vivo* assay. About 100 isolated *Volvox carteri* embryos in 0.5 ml *Volvox carteri* medium were incubated with 100 µl concentrated hybridoma culture supernatants 4 hr before the onset of inversion: 0.45 ml culture fluid aliquots were precipitated by the addition of 0.55 ml of saturated ammonium sulfate, followed by centrifugation and resuspension of the pellet in 0.1 ml *Volvox carteri* medium (concentration factor about 5), and dialysed against the same medium overnight. Ten hours after inversion all samples were checked for abnormal inversion by inspection under a stereo microscope. Positive hybridomas were cloned under microscopic observation by deposition of small droplets into microtiter plates. To droplets containing only one cell, medium with macrophages was added (Ochiai et al., 1982). Determination of antibody class was done via Ouchterlony analysis. Antibody 4-I-244 (IgM) was precipitated from hybridoma culture supernatants by 55% saturation of ammonium-sulfate, dialysed against 0.14 M phosphate buffer (pH 8.0) (Ochiai et al., 1982), and purified by protein-A-sepharose column chromatography (Pharmacia, Uppsala). Antibody was eluted with 0.1 M acetic acid containing 0.15 M NaCl.

### Isolation of <sup>14</sup>C-Labeled Antigen 4-I-244

For chemical characterization via radio gas chromatography, isolated embryos (4 hr before the onset of inversion) of about 8000 *Volvox carteri* spheroids were suspended in 2 ml Na-glycerophosphate-free *Volvox carteri* medium. After addition of 400 µCi (15MBq) <sup>14</sup>C-NaHCO<sub>3</sub> the suspension was shaken under illumination (10000 lux) in a water bath at 27°C for 3 hr. <sup>14</sup>C-labeled embryos were disintegrated by ultrasonic treatment (30 sec total). The lysate was dried and extracted four times with 0.8 ml chloroform:methanol (3:2). Antigen was then extracted twice with 0.4 ml chloroform:methanol:water (10:10:3). The latter extracts were pooled, dried, and the material was dissolved in 50

µl propanol:water 65:35 and applied to silica gel thin layers (Kieselgel 60; Merck, Darmstadt). Chromatography was performed with solvent 1: chloroform:methanol:4 N ammonium hydroxide (9:7:2 v/v) with 0.2 M ammonium acetate (Kaul and Lester, 1975). After autoradiography antigen 4-I-244 was localized either by immunoblotting or by comparison with authentic antigen run on the same chromatogram. The antigen containing area was scraped off and the antigen eluted with propanol:water 65:35 and rechromatographed on a silica gel thin layer in propanol:water 65:35. After autoradiography antigen 4-I-244 was isolated as described for the first chromatography. Antigen 4-I-244 was homogeneous as checked by two-dimensional thin-layer chromatography on silica gel plates.

### Chemical Characteristics

Radio gas chromatography was performed with a Packard 423 gas chromatograph. The outlet end of the column was fitted with a 1:10 stream splitter: the radioactivity of 0.9 of the sample was measured as <sup>14</sup>CO<sub>2</sub> in a Packard model 894 gas proportional counter. One tenth of the sample served for mass detection (FID) of internal standards.

### Carbohydrate Analysis

Neutral and amino sugars were analyzed by GLC as their alditol acetates (Laine et al., 1972; Spiro, 1972) on a 1 m x 2 mm glass column (3% SP-2340 on 100/120 Supelcoport, Supelco) with a linear temperature program from 180°C to 265°C (4°/min). Samples were hydrolyzed in 4-N-trifluoroacetic acid for various times at 100°C.

### Fatty Acid Analysis

<sup>14</sup>C-labeled lipid was hydrolyzed in conc. HCl:CH<sub>3</sub>OH:H<sub>2</sub>O (3:29:4, v/v) at 80°C for 18 hr (Carter and Hirschberg, 1968). Free fatty acids and their methylesters were extracted into petroleum ether and after the addition of appropriate unlabeled reference acids (20 µg each) the extract was dried and treated with 50 µl Methyl-8 (Sigma) at 60°C for 15 min to ensure complete methylation. GLC was performed either on a 2 m x 2 mm 10% SP 2330 (Supelco) glass column (temperature program 180°C to 240°C with 3°C/min) or on a 2 m x 2 mm 3% SE-30 (Supelco) glass column (temperature program 210°C to 290°C with 4°C/min).

### Inositol Determination

Inositol was estimated gas chromatographically after strong acid hydrolysis according to Wells et al. (1985) as modified by Smith and Lester (1974). After peracetylation inositol was identified on a 3% SP-2340 (Supelco) 1 m x 2 mm glass column as described for sugar analysis.

### Periodate-Permanganate Oxidation of Intact Antigen

Long chain bases were characterized by identification of the fatty acids formed after a permanganate-periodate oxidation (Von Rudloff, 1956) of intact lipids by the modified procedure of Youngs and Subbaram (1964). To samples of <sup>14</sup>C-labeled antigen and standard phytylphosphoglycine (100 µg) 150 µl tert-butanol, 40 µl of H<sub>2</sub>O and 10 µl of K<sub>2</sub>CO<sub>3</sub> (8 mg/ml) were added. To this solution was added 50 µl of the oxidizing mixture: 0.21 g NaIO<sub>4</sub>, 0.25 ml of 0.1 M KMnO<sub>4</sub> and H<sub>2</sub>O to 10 ml. After 1 hr at 65°C another 20 µl of the oxidizing mixture was added and incubation continued for another 1.5 hr. The reaction was stopped by the addition of 3 µl ethylene glycol (1 hr, 60°C). The solution was evaporated to approximately 50 µl and 1 µl conc. HCl was added. Fatty acids were extracted with petroleum ether, methylated, and analyzed by GLC. (2 m x 2 mm 3% SE-30 glass column, temperature program 160°C to 260°C with 2°C/min).

### Analysis of the Expression of Antigen 4-I-244 during Development

The embryos of about 1500 *Volvox carteri* spheroids at the appropriate developmental stage were isolated as described and suspended in 0.5 ml Na-glycerophosphate-free *Volvox* medium. "Pulse"-labeling (3 hr) with 100 µCi (4 MBq) <sup>14</sup>C-NaHCO<sub>3</sub> and extraction was performed as described. Aliquots of the chloroform:methanol:water (10:10:3) extract corresponding to 40,000 cpm were subjected to thin-layer chromatography on silica gel in chloroform:methanol:4-N-ammoniumhydroxide with 0.2 M ammonium acetate (solvent 1). Labeled lipids were visualized by autoradiography (overnight). Antigen 4-I-244 was identified by comparison with authentic material or by immunological detection after blotting onto nitrocellulose filters.

### Immunodetection of Blotted Lipids

<sup>14</sup>C-labeled lipids were transferred to nitrocellulose filters (BA 85; Schleicher & Schuell, Dassel) as described by Towbin et al. (1984).

Filters were incubated in ammonium-sulfate-precipitated hybridoma culture fluid of hybridoma 4-I-244 and subsequently in 0.02 mg/ml FITC-labeled anti-mouse IgM from sheep (Sigma, Munich). The blots were inspected under monochromatic light of 490 nm produced by an interference filter (Schott, Mainz) through a 530 nm cut-off filter (Schott, Mainz). Photographs were taken using Ilford HP 5 film.

#### Fluorescence Microscopy

Isolated *Volvox carteri* embryos from various developmental stages were mixed and fixed in 2% paraformaldehyde for 15 min at 20°C. After washing with Tris-saline (pH 8.0) and 50 mM glycine (pH 8.0) the embryos were labeled with 0.01 mg/ml of monoclonal antibody 4-I-244 in the presence of 1 mg/ml horse IgG for 45 min at 20°C. After washing, 0.01 mg/ml FITC conjugated anti-mouse IgM from sheep (Sigma) in 5% horse serum was applied for 60 min. Labeled embryos were washed and viewed using a Leitz fluorescence microscope. Photographs were taken using Ilford HP 5 film.

#### Acknowledgments

Our work was supported by the Deutsche Forschungsgemeinschaft (SFB 43). We wish to thank Dr. Gerisch Martinsried, who helped us to establish monoclonal antibody techniques. We appreciate the excellent technical assistance of Brigitte Seidel and Ulrike Stöeckl. We are grateful to Manuela Biederer for cultivating hybridomas.

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Received March 3, 1986; revised April 23, 1986.

#### References

- Birchem, R., and Kochert, G. (1979). Development of sperm cells of *Volvox carteri* f. *weismannia* (Chlorophyceae). *Phycologia* 18, 409–419.
- Carter, H. E., and Hirschberg, C. B. (1968). Phytosphingosines and branched sphingosines in kidney. *Biochemistry* 7, 2296–2300.
- Carter, H. E., Celmer, W. D., Galanos, D. S., Gigg, R. H., Lands, W. E. M., Law, J. H., Mueller, K. L., Nakayama, T., Tomizawa, H. H., and Weber, E. (1958). Biochemistry of sphingolipids. X. Phytoglycolipide, a complex phytosphingosine-containing lipid from plant seeds. *J. Amer. Oil Chem. Soc.* 35, 335–343.
- Carter, H. E., Strobach, D. R., and Hawthorne, J. N. (1969). Biochemistry of the sphingolipids. XVIII. Complete structure of tetrasaccharide phytoglycolipid. *Biochemistry* 8, 383–388.
- Green, K. J., Viamontes, G. I., and Kirk, D. L. (1981). Mechanism of formation, ultrastructure, and function of the cytoplasmic bridge system during morphogenesis in *Volvox*. *J. Cell Biol.* 91, 756–769.
- Grunwald, G. B., Fredman, P., Magnani, J. L., Trisler, D., Ginsburg, V., and Nirenberg, M. (1985). Monoclonal antibody 18B8 detects gangliosides associated with neuronal differentiation and synapse formation. *Proc. Natl. Acad. Sci. USA* 82, 4008–4012.
- Holtfreter, J. (1943). A study of the mechanics of gastrulation. Part I. *J. Exp. Zool.* 94, 261–318.
- Holtfreter, J. (1944). A study of the mechanics of gastrulation. Part II. *J. Exp. Zool.* 95, 171–212.
- Kates, M. (1972). Techniques of lipidology: isolation, analysis and identification of lipids. In *Laboratory Techniques in Biochemistry and Molecular Biology*, T. S. Work and E. Work, eds. (New York: North-Holland), pp. 279–579.
- Kaul, K., and Lester, R. L. (1975). Characterization of inositol-containing phosphosphingolipids from tobacco leaves. *Plant Physiol.* 55, 120–129.
- Kelland, J. L. (1977). Inversion in *volvox* (Chlorophyceae). *J. Phycol.* 13, 373–378.
- Laine, R. A., Esselman, W. J., and Sweeley, C. C. (1972). Gas-liquid chromatography of carbohydrates. *Meth. Enzymol.* 28, 159–167.
- Matthew, W. D., and Reichardt, L. F. (1982). Development and application of an efficient procedure for converting mouse IgM into small, active fragments. *J. Immunol. Meth.* 50, 239–253.

- Michalski, J., Peter-Katalinic, J., Egge, H., Paze-Parente, J., Montreuil, J., and Strecker, G. (1984). Behavior of the 2-acetamido-2-deoxy-D-glucopyranosyl residue during sequential hydrozoinolysis, N-reactylation, reduction, and methylation of glycoasparagines. *Carbohydr. Res.* 134, 177–189.
- Moseley, K. R., and Thompson Jr., G. A. (1980). Lipid composition and metabolism of *Volvox carteri*. *Plant Physiol.* 65, 260–265.
- Ochiai, H., Schwarz, H., Merkl, R., Wagle, G., and Gerisch, G. (1982). Stage-specific antigens reacting with monoclonal antibodies against contact site A, a cell-surface glycoprotein of *Dictyostelium discoideum*. *Cell Differ.* 11, 1–13.
- Pickett-Heaps, J. D. (1970). Some ultrastructural features of *Volvox*, with particular reference to the phenomenon of inversion. *Planta* 90, 174–190.
- Provasoli, L., and Pintner, I. J. (1959). Artificial media for freshwater algae: problems and suggestions. In *The Ecology of Algae*, Special Publication No. 2, Pymatuning Laboratory of Field Biology, C. A. Tyrone and R. T. Hartman, eds. (Pittsburgh: University of Pittsburgh), pp. 84–96.
- Roseman, S. (1985). Studies on specific intercellular adhesion. *J. Biochem.* 97, 709–718.
- Smith, S. W., and Lester, R. L. (1974). Inositol-phosphoryl ceramide, a novel substance and the chief member of a major group of yeast sphingolipids containing a single inositol phosphate. *J. Biol. Chem.* 249, 3395–3405.
- Spiro, R. G. (1972). Study of the carbohydrate of glycoproteins. *Meth. Enzymol.* 28, 3–43.
- Starr, R. C. (1969). Structure, reproduction, and differentiation in *Volvox carteri* f. *nagariensis* Iyengar, strains Hk 9 & 10. *Arch. Protistenk.* 111, 204–222.
- Starr, R. C. (1970). Control of differentiation in *Volvox*. *Dev. Biol. Suppl.* 4, 59–100.
- Starr, R. C., and Jaenicke, L. (1974). Purification and characterization of the hormone initiating sexual morphogenesis in *Volvox carteri* f. *nagariensis* Iyengar. *Proc. Natl. Acad. Sci. USA* 71, 1050–1054.
- Towbin, H., Schoenenberger, C., Ball, R., Braun, D. G., and Rosenfelder, G. (1984). Glycosphingolipid-blotting: an immunological detection procedure after separation by thin layer chromatography. *J. Immunol. Meth.* 72, 471–479.
- Tsuji, S., Arita, M., and Nagai, Y. (1983). GQ1b, a bioactive ganglioside that exhibits novel nerve growth factor (NGF)-like activities in the two neuroblastoma cell lines. *J. Biochem.* 94, 303–306.
- Viamontes, G. I., and Kirk, D. L. (1977). Cell shape changes and the mechanism of inversion in *Volvox*. *J. Cell Biol.* 75, 719–730.
- Viamontes, G. I., Fochtmann, L. J., and Kirk, D. L. (1979). Morphogenesis in *Volvox*: analysis of critical variables. *Cell* 17, 537–550.
- Von Rudloff, E. (1956). Periodate-permanganate oxidations. V. Oxidation of lipids in media containing organic solvents. *Can. J. Chem.* 34, 1413–1418.
- Wells, W. W., Pittman, T. A., and Wells, H. J. (1965). Quantitative analysis of myoinositol in rat tissue by gas-liquid chromatography. *Anal. Biochem.* 10, 450–458.
- Wenzl, S., and Sumper, M. (1982). The occurrence of different sulphated cell surface glycoproteins correlates with defined developmental events in *Volvox*. *FEBS Lett.* 143, 311–315.
- Youngs, C. G., and Subbaram, M. R. (1964). Determination of the glyceride structure of fats: gas-liquid chromatography of oxidized glycerides. *J. Amer. Oil Chem. Soc.* 41, 218–221.