SURFACE GLYCOPROTEINS OF THE MULTICELLULAR ALGA *VOLVOX CARTERI*

Developmental regulation, exclusive Con A binding and induced redistribution

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1. Introduction

The alternate developmental pathways exhibited by the green alga *Volvox carteri*, which are dependent on the action of a specific glycoprotein sex hormone, together with the simple polar cellular organization and the small number of cell types, render this alga particularly suitable for the study of the regulation of differentiation and development in multicellular eukaryotes. Moreover, the alga can grow and develop in minimal synthetic medium and genetic analysis of selected developmental mutants is possible [1–3].

Asexual and sexual development in *Volvox carteri* have been described previously [4–6]. The spherical organism, composed of only two cell types, has terminally differentiated somatic cells organized in a monocellular layer with their biflagellated pole facing the outside, and the reproductive cells located inside the spheroid. These cell types arise from asymmetric cell division which takes place at a specific stage of cell cleavage during embryogenesis. All cell divisions occur during embryogenesis and the final polar cellular organization of the matured spheroid is directly related to the pattern of cell cleavage. At the end of cell division the embryo exhibits an inside-out cell organization. The reproductive cells project outside the spheroid and the somatic cells are placed with their biflagellated pole towards the inside. Cellular organization of the mature spheroid is achieved by a rapid inversion of the embryo. This process was recently shown to involve the coordinate change in cell shape [7] and to be inhibited by cytochalasin B [8]. Somatic and reproductive cells can be separated and isolated following disruption of the intercellular matrix which is produced by the somatic cells.

The asexual reproductive cells, in both male and female strains, are responsive to the glycoprotein sex hormone [5,9–11] during a defined limited period in the asexual cycle. The commitment to sexual development is first expressed in a change in the pattern of cell cleavage. The asymmetric cell division which occurs at the 32-cell stage in asexual development is delayed to a later stage, and differs in female and male sexual embryogenesis.

The present study was aimed at the elucidation of developmentally controlled cell surface properties of this alga. Membrane mediated control of *Volvox* development was infered from the effect of Con A on specific developmental processes [8]. Con A concomitantly inhibited the induction of sexual development by the glycoprotein sex hormone and the process of embryo inversion. This suggested that the pattern of cell cleavage and the subsequent differentiation of reproductive cells was regulated by a transmembrane control involving surface receptor reorganization and submembraneous cytoskeletal assembly. To further examine this hypothesis, we have investigated cell surface composition and the effect of Con A binding on the induction of surface receptor redistribution.

2. Materials and methods

The species used in this study is *Volvox carteri f. nagariensis* female strain HK10, which was obtained from Dr R. C. Starr. Cultures were grown axenically in defined *Volvox* medium [12], at 25°C to 28°C with 16 h light and 8 h dark cycle. Stock cultures

were maintained on 1% agar in *Volvox* medium. The lectins employed were selected for their different carbohydrate binding specificities and were from peanut (PNA), wheat germ (WGA), wax bean (WBA), soybean (SBA), jack bean (Con A) and lentil (LCA).

With the exception of FITC-Con A, the various FITC-lectins were prepared by reacting fluorescein isothiocyanate (isomer I), Sigma, with the corresponding lectin (1 : 20, w:w, respectively) in 0.1 M bicarbonate buffer (pH 8.6) for 16 h, at 4°C. FITC-Con A was prepared at pH 7.8.

Binding of fluorescein conjugated lectins to *Volvox* asexual spheroids was detected by fluorescence microscopy following incubation with the respective lectin at 20 to 40 μg/ml for 15 min, at room temperature. To allow detection of lectin binding to gonidial cytoplasmic membrane, the spherical structure of the intact organism was mechanically disrupted by passage through a drawn capillary, thus releasing the gonidia. Radioactive labeling of outer surface proteins was achieved via iodination by the lactoperoxidase method [13]. ^{125}I-labeled surface proteins were extracted with NP40 (0.5%) and resolved by SDS–polyacrylamide (5.6%) slab gel electrophoresis [14]. The slab gels were dried on paper and autoradiographs made on Curix film, Agfa-Geraeret.

3. Results and discussion

Two approaches were taken to the study of cell surface composition. In the first, fluorescein conjugated lectins were employed as probes to examine the appearance and distribution of surface glycoconjugates. In the second approach surface proteins were studied following lactoperoxidase catalyzed iodination. These methods are directed towards extracellular components and thus allow the study of cytoplasmic

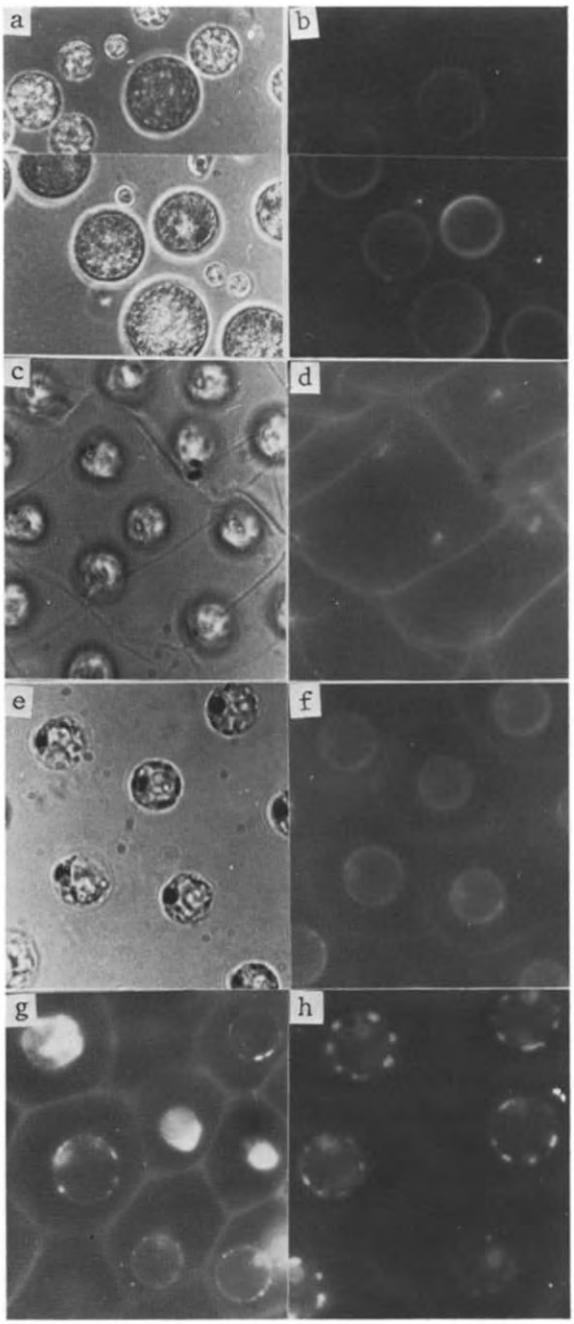

Fig.1. Fluorescent staining of *Volvox* cells labeled with FITC-Con A. Asexual female spheroids were mechanically disrupted to yield free gonidia and sheets of somatic cells within the intercellular matrix. a–f represent preparations incubated at pH 8.0, glycylglycine buffer; g and h represent preparations incubated at pH 6.5, Hepes buffer. a and b – phase contrast and fluorescent micrographs of the released gonidia; c and d – flagella and flagellar outlets (the two tiny adjacent circles) stained with FITC-Con A. e to h – somatic cells within the intercellular matrix. Fluorescent staining with FITC-Con A reveals the boundaries of the hexagonal somatic cell domains of the intercellular matrix, and the binding to the cytoplasmic membranes. Surface receptor redistribution is evident in g and h.

membrane, flagellar membrane and the intercellular glycoprotein matrix.

Of all the various fluorescein conjugated lectins tested in this study, only Con A, and to a lesser extent LcA, both of which are specific for mannose-like residues, were found to bind to *Volvox* surface glycoconjugates. Even after long incubation periods at high lectin concentration (up to 100 μg/ml), fluorescently labeled PNA, SBA, WGA and WBA did not stain *Volvox* spheroids to any extent. Binding of FITC-Con A to the bulk of the intercellular matrix resulted in a faint, diffused fluorescence which did not interfere with the detection of Con A binding to the cytoplasmic membrane of the somatic cells (fig.1e–h) to the flagellar membrane (fig.1c,d) or to the specific structures with the intercellular matrix. The latter, flagellar outlet (fig.1d), and the boundaries of hexagonal somatic cell domains (fig.1f,g), exhibited increased lectin binding capacity. The exclusive Con A binding specificity shared by the various surface components, may indicate a common and unique glycosylation pattern for all surface glycoconjugates.

The exclusive Con A binding to the cytoplasmic membrane did not change following enzymatic digestion of the intercellular matrix with a specific matrix digesting enzyme (Kurn, N., Colb, M. and Shapiro, L., in preparation). We could thus conclude that the intercellular matrix did not mask additional carbohydrate residues of surface glycoconjugates other than the specific Con A binding receptors. This was also supported by the exclusive Con A binding specificity of flagellar membrane.

Whereas Con A binding capacity to cytoplasmic membrane of somatic cells did not change with development, staining of gonidial cytoplasmic membrane by FITC-Con A increased with gonidial maturation. Binding of FITC-Con A to gonidial cell membrane resulted in a uniform ring-like staining. As shown in fig.1a,b young gonidia (smaller sized) show little or no fluorescence as compared to larger, more mature gonidia. This was also observed when synchronously grown cultures were exposed to FITC-Con A. It would thus appear that gonidial maturation is accompanied by increased density of Con A receptors.

The two cell types of the asexual spheroid differ considerably in their differentiation state. Contrary to the multipotent gonidia, which can become committed to either asexual or sexual development, the somatic cells are terminally differentiated. Cell specific surface proteins may have an integral role in the control of *Volvox* development and cell differentiation. Surface proteins of whole, intact, spheroids, as well as of pure somatic and gondial cell populations were analyzed following lactoperoxidase catalyzed iodination. ^{125}I-labeled proteins were detected by autoradiography following extraction with NP40 and resolution by electrophoresis on SDS–polyacrylamide slab gels (see legend to fig.2). The NP40 extraction resulted in a large, colorless residue which was radioactively labeled. This residue may contain components of the intercellular matrix. As shown in fig.2, this

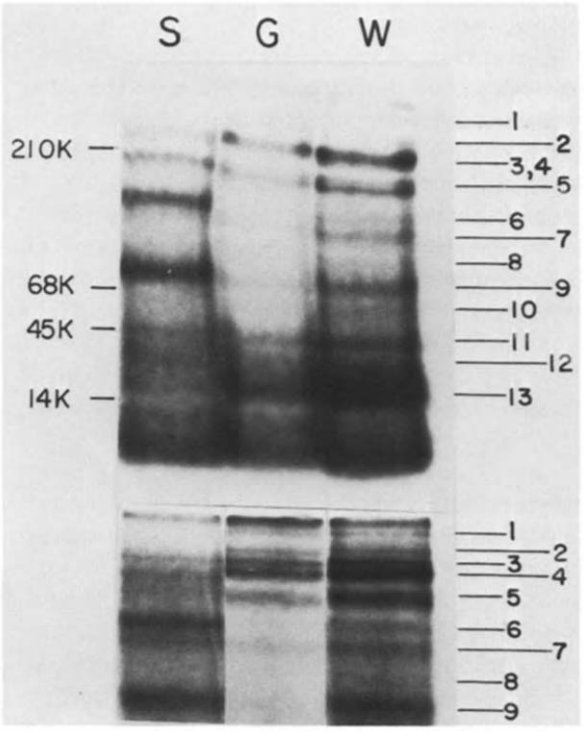

Fig.2. Autoradiographs of *Volvox carteri* surface protein, iodinated by the lactoperoxidase method [12]. Lanes S, G and W represent resolution of surface proteins from somatic and gonidial cell preparations and from intact spheroids. Pure cell populations were obtained following passage of the smaller somatic cells through Nitex nylon cloth, pore size 10 nm. a and b represent autoradiographs of ^{125}I-labeled surface proteins of two independent preparations. In b, only the upper part (higher molecular weight proteins) of the slab gel is shown, where improved resolution of band 3 and 4 is evident.

procedure revealed distinct profiles of somatic cell and gonidial surface proteins and allowed the identification of cell specific surface proteins. The gonidial cell surface lacks band 6, the major component of the somatic cell surface, and shows only traces of band 9, a prominent somatic cell surface component. Bands 7 and 13 appear to be specific to gonidia cell surface. All but band 11 appear to be glycoproteins, as these were found to be retained on Con A–sepharose affinity columns (not shown).

Iodinated NP40 extracts were applied to affinity columns of Con A–, LcA– and SBA–Sepharose. Whereas radioactively labeled surface proteins were found to be retained on the Con A and LcA bound matrices, none was retained on the SBA column. This finding is consistent with the exclusive Con A and LcA binding observed with intact cells.

Con A induced modulation of surface receptor distribution is shown in fig.1g,h. Receptor redistribution by Con A was evident only in cultures at low pH (6.5). Lectin binding to cells grown at pH 8.0, on the other hand, resulted in a uniform ring-like fluorescent staining. The pH sensitivity may imply the involvement of ion fluxes or intracellular ion concentration in the regulation of surface receptor mobility. Unlike the case in mammalian lymphocytes, where cap formation, but not the appearance of patches, was found to be energy dependent [15], the Con A induced patch formation in *Volvox* was inhibited by sodium azide (10^{-3} M) and 2,4-dinitrophenol (10^{-4} M).

Con A was previously shown to inhibit the induction of sexual development and the process of embryo inversion [8]. It is possible that the glycoprotein sex hormone binds to specific membrane receptors, thus activating intramembranous effectors, which in turn trigger a signal for the commitment to sexual development. An alteration in the distribution of membrane components caused by Con A binding could result in the uncoupling of the hormone–receptor complexes and their effectors, thus inhibiting the induction of sexual development. The demonstration of Con A induced surface receptor redistribution supports this hypothesis.

The characterization of cell specific surface glycoproteins can be now extended to relevant mutant strains in the hope of elucidating the role of the cell membrane in the regulation of somatic cell differentiation and development. Numerous mutant strains are now available that are defective in the maintenance of stable terminal somatic cell differentiation and in the process of commitment of the asexual gonidia to a specific developmental pathway. These will be used in future studies.

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