EVIDENCE FOR MEMBRANE-MEDIATED CONTROL OF DIFFERENTIATION DURING EMBRYOGENESIS OF *VOLVOX CARTERI*

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1. Introduction

In the preceding paper [1] a model was proposed which offers an explanation to many aspects of embryogenesis in *Volvox*. In particular, the model explains pattern formation during embryogenesis, i.e., it correctly predicts the very regular positioning of the reproductive cells (gonidia) within the *Volvox* spheroids. The assumption was made that membrane proteins mediating cell–cell contacts are involved in this control. In order to support this assumption experimentally, we treated synchronously developing gonidia with several substances known to modify cell surfaces. It is demonstrated here that these substances strongly disturb the developmental program if applied during the early stages of embryogenesis. Treatment at other times does not affect the developmental program.

2. Materials and methods

2.1. *Growth of* Volvox carteri

Volvox carteri f. *nagariensis*, strain HK 10, used in this investigation was a gift from Professor L. Jaenicke, Cologne. The organism was grown in *Volvox* medium designed [2] according to the procedures in [3]. Illumination of 12 000 lux intensity on a 16 h light/8 h dark cycle and a temperature of 27–29°C during the light period (25°C during dark) resulted in a synchronously growing culture.

2.2. *Preparation of isolated gonidia*

A clonal culture of strain HK 10 was obtained by inoculating one mature spheroid from a synchronously growing culture into a test tube with 10 ml *Volvox* medium, adjusted to pH 8.0. After 4 days, a 500 ml Erlenmeyer flask containing 200 ml *Volvox* medium was inoculated with one test-tube culture and grown with aeration under the above conditions. Two days later, exactly at the time of release of daughter spheroids from the parent colonies, the spheroids were concentrated by filtration through a 40 μm filter (wire netting, Haver und Boecker, 4740 Oelde, FRG) and resuspended in 1–2 ml *Volvox* medium. Gonidia and somatic cells were dissociated by breaking down the spheroid matrix by protease treatment [4,5]. Of several proteases tested, subtilisin was the most efficient. *Volvox* colonies were treated with 250 μg/ml subtilisin for 30 min at 30°C. The gonidia were trapped on a 10 μm screen cloth and washed several times with *Volvox* medium until all the somatic cells had passed through the filter [5]. The isolated gonidia were then resuspended in 1.5 ml *Volvox* medium. Test tubes containing 5 ml *Volvox* medium were inoculated with 100–200 μl of the gonidia suspension and incubated under illumination (12 000 lux). Under our conditions, gonidia began synchronous divisions after a further 12 h incubation. Division of gonidia was completed within 8–10 h. In the time scale of fig.1, zero time denotes the moment when the *Volvox* spheroids have been dissociated. Gonidia were treated by substances (proteases, glycosidases, lectins, borate $Na_2B_4O_7$) simply by adding these chemicals to the suspension at defined times. After 60 min incubation the applied substance was removed by filtration through a 10 μm screen cloth and washing with *Volvox* medium. The trapped gonidia were

resuspended in 5 ml *Volvox* medium and incubated further until mature *Volvox* spheroids had developed.

Photographs were taken by a stereomicroscope, M7S, Wild, Heerbrugg. Magnification 25 X.

3. Results and discussion

Volvox colonies from a synchronously growing culture were dissociated with subtilisin exactly at the time of release of daughter spheroids (zero time in fig.1). The gonidia were isolated by filtration and resuspended in *Volvox* medium. After 12 h continuation of their enlargement (maturation) the gonidia began division, cleavages occurring approximately every 50–60 min. Synchronized development in the isolated gonidia was not as uniform as in intact spheroids, but usually >65% of the gonidia were found to be in the same stage of division. The isolated gonidia develop into *Volvox* spheroids on the same time scale as gonidia developing inside the parental individual; however, these *Volvox* individuals contain a reduced number of gonidia (10–16). In the experiment shown in fig.1, the isolated gonidia were treated with subtilisin (40 μg/ml) for 60 min at different times of their developmental program. The resulting *Volvox* colonies were then analysed for their number of gonidia. In addition, the relative spatial positions of the gonidia in the mature spheroid were checked and denoted as regular if they matched the normal patterns (fig.2A) or as irregular, if not. As shown in fig.1 gonidia which were treated with subtilisin during any time of their maturation period develop completely normally to *Volvox* spheroids. The only effect observed was a slight reduction of the number of gonidia. In sharp contrast, if subtilisin was applied after the initiation of cell division, the developmental program is strongly disturbed. Although viable *Volvox* colonies developed, the number of gonidia was reduced and the arrangement of gonidia found to be completely irregular. In many cases, the gonidia were scattered around the whole sphere without any recognizable symmetry or in other cases were concentrated in clusters of three or four. Typical examples of the resulting *Volvox* spheroids are shown in fig.2B. In some aberrant colonies, gonidia were even located in the anterior region of the spheroid which never bears gonidia in normally developed individuals. If grown

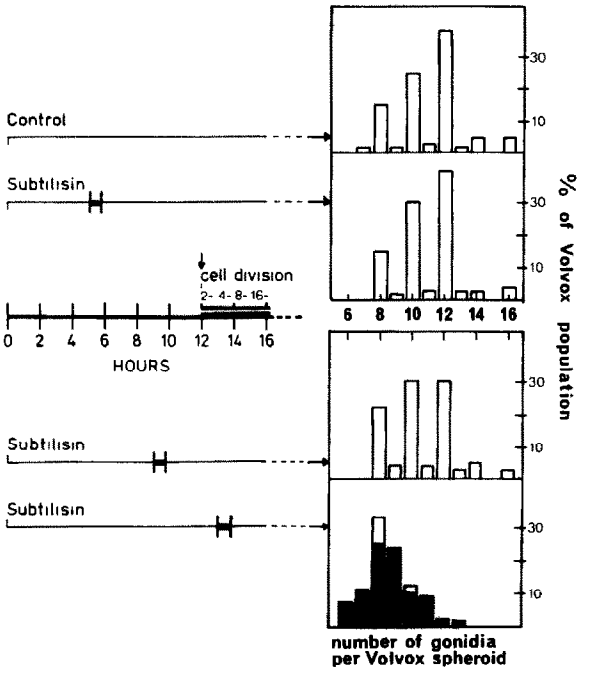

Fig.1. Number and positioning of gonidia in *Volvox* spheroids produced from isolated gonidia treated with subtilisin. Isolated gonidia were treated with 40 μg/ml subtilisin for 60 min at different stages of their developmental program (see time scales on the left). 100–200 individuals of the resulting *Volvox* population were analysed with respect to number and positioning of their gonidia. White bars indicate regular, black bars irregular spatial positioning of the gonidia. Subtilisin treatment did not affect the viability of the developing gonidia as compared to the control.

for one more generation, the gonidia of these irregular colonies produced organisms with normal numbers and positioning of reproductive cells. Essentially identical results were obtained with some other proteases, e.g., with trypsin (5 μg/ml) and thermolysin (40 μg/ml).

Surface glycoproteins are possible candidates for mediation of cell–cell contacts [6]. Therefore, several substances which are known to modify glycoproteins were tested for their ability to disturb the developmental program. The lectin Con A (10 μg/ml) as well as a mixture of 4 glycosidases (containing α-, β-glucosidase, β-galactosidase and β-glucuronidase, 10 μg/ml each), were found to affect the developmental regulation. Treatment of the gonidia with these agents was

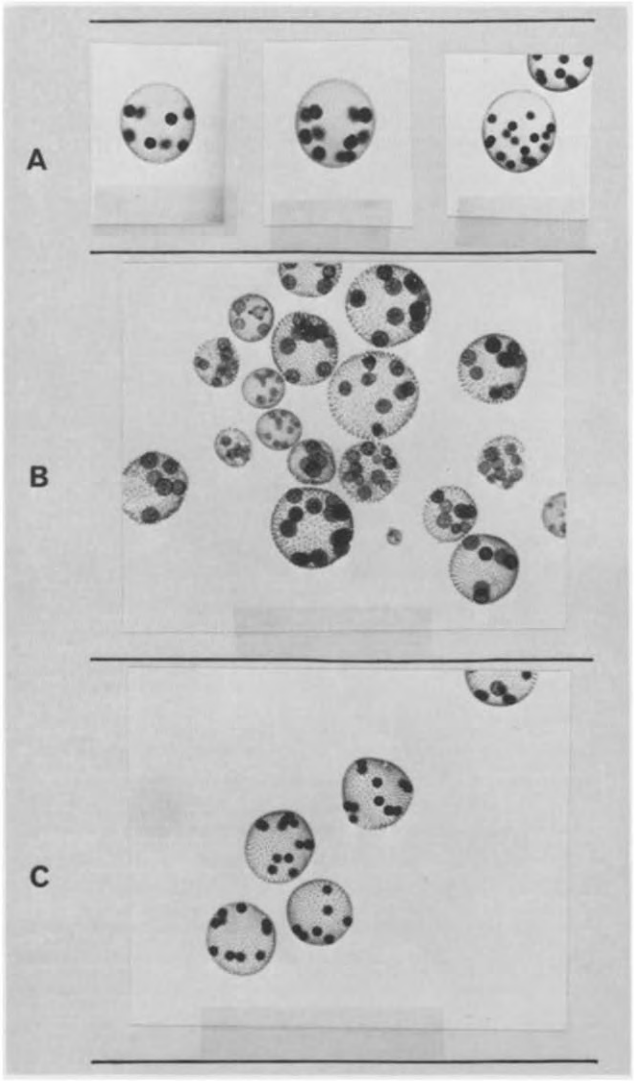

Fig.2. *Volvox* spheroids produced from isolated gonidia treated with subtilisin (B) or borate (C) for 60 min immediately after the initiation of cell division. 40 μg/ml subtilisin were applied. The concentration of $Na_2B_4O_7$ was 20 mM. (A) *Volvox* spheroids developed from untreated gonidia, containing 8, 12 or 16 gonidia in a regular spatial positioning.

carried out exactly as described for the subtilisin procedure and the result was also qualitatively similar; the number and positioning of the gonidia within the developing embryos were disturbed only when the lectin (or the glycosidases) were applied during the defined limited period of the first cleavage stages.

Particularly drastic disturbance of the control of differentiation was achieved by treating embryos during the early cleavage stages with borate (10–20 mM $Na_2B_4O_7$, adjusted to pH 8.5–9.0). Some examples of the resulting *Volvox* individuals are shown in fig.2C. Possibly, the sugar-complexing property of borate causes the disturbance of the developmental control.

In summary, the group-specific reagents (proteases, Con A, glycosidases) applied in this study to alter the cell surface molecules provide evidence for a membrane-mediated (glycoprotein-mediated) control of differentiation in *Volvox carteri* embryogenesis.

During the preparation of this manuscript, a paper appeared [7] mentioning inhibiting effects of Con A on the process of sex induction and inversion, indicating that these developmental processes are also membrane-mediated.

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