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INHIBITION OF REGENERATION OF *ACETABULARIA MEDITERRANEA* ENUCLEATED POSTERIOR STALK SEGMENTS BY ELECTRICAL ISOLATION*

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SUMMARY

In 1959, J. and Ch. Hämmerling have shown that posterior stalk segments (EPSS) of *Acetabularia mediterranea* are able to regenerate in the light after enucleation, if they have been previously kept for more than 5 days in the dark in the presence of the nucleus. This experiment was repeated but with a modification which isolated electrically the apical and basal ends of the EPSS segments during their illumination. The modification consisted in separating the culture medium (artificial sea water — ASW) into compartments in such a way that the apical end of the EPSS was dipping in one compartment, while its basal end was dipping in another compartment. In this case the regeneration was inhibited. When establishing an electrical connection between the compartments by addition of an agar—ASW bridge a normal regeneration was obtained, in conformity to the Hämmerling's result.

INTRODUCTION

The giant cell of Acetabularia is uniquely suited for cell regeneration experiments. Hämmerling [1] first observed its ability for regeneration. He usually divided the cell into 3 segments:
(*1*) the posterior stalk segment at the basis, without the nucleus; (2) the middle stalk segment; (*3*) the anterior stalk segment at the apex.
We label them EPSS, MSS and ASS, respectively.

*The results described in this paper were partially presented by one of us (B.N.) at the III. International Symposium on Acetabularia held at the University of Paris, France, July 11—12, 1974. Abstracts of this Symposium have been published in Protoplasma (1975).

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Abbreviations: ASS, anterior stalk segment; ASW, artificial sea water; EPSS, enucleated posterior stalk segment; MSS, middle stalk segment.

The ability for regeneration of such enucleated fragments was shown to depend on the position of the segment along the stalk. The EPSSs of the normal cells could not regenerate at all when kept in the light [2].

However, EPSSs were shown to regenerate in the light when they had been kept in the dark for at least 5 days before enucleation [2]. Although darkness stops photosynthesis, the activity of the nucleus for controlling developmental processes appears to be maintained in these circumstances. The nucleus is thought to release morphogenetical substances into the cytoplasm during the dark period. The EPSSs do not grow in darkness; their growth starts after they have been transferred in the light. Complementary, related observations were made recently by Sandakhiev et al. [3].

In 1959 J. and Ch. Hämmerling [4] experimented the regeneration of EPSSs (kept in the dark for several days before enucleation; 10—15 mm in length) in the earth Schreiber solution (in petri dishes; 21—22°; 2500 lux; 12 h light—12 h darkness programme). They found that 80 to 90 % of the EPSSs regenerated, and that the stalk outgrowth (several mm long) could develop into whorl or cap. They also maintained EPSSs for 2 to 3 days in darkness after enucleation, divided them into two parts of equal lengths, and found then that after transfer in the light, the apical differentiation into whorl occurred either at the original apical part, or at the original basal part of the EPSSs. The latter case implies a reversal of the original polarity of the EPSSs.

We repeated the experiment of J. and Ch. Hämmerling [4], but with a modification in which the surrounding medium of the EPSSs was artificially divided by separating walls. In our experimental arrangement, there was no electrical or mechanical connection through the surrounding medium between basal and apical ends of the EPSSs (Fig.2). This changed the result considerably, as reported here.

Previous experiments by Novak [5] had already proved that it was possible to inhibit regeneration in the light by holding the potential difference between both ends of the EPSS segment to zero (see also ref. 6).

THE EXPERIMENT

Algae (*Acetabularia mediterranea*) were kindly provided by Dr. Bonotto. They had been grown following the method described by Lateur [7]. The culture proceeded in a 12 h light and dark program and at a temperature of 21 ± 1°. The light intensity was 1500 lux (fluorescent light source "Phytor", L-F 40 W ACEC). Before the experiment, all cells were placed for at least 24 h in ASW of the following composition:
495 m*M* CaCl, 27.6 m*M* $MgSO_4 \cdot 7\,H_2O$, 24.9 m*M* $MgCl_2 \cdot 6\,H_2O$, 10.0 m*M* $CaCl_2 \cdot 2\,H_2O$, 9.7 m*M* KCl, 2.0 m*M* $NaHCO_3$; 10 m*M* TRIS/HCl was added to adjust the pH to 7.9.

We used a special cuvette (Fig.1) with thin walls a, a′, b, b′ to separate the ASW into three compartments A, B, C. 20 to 30 cells prepared as described

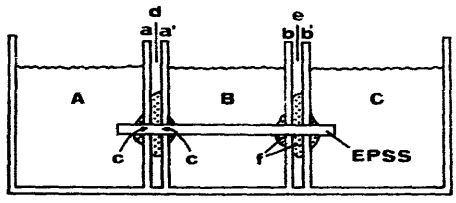

Fig.1. A longitudinal section through the experimental cuvette and through an EPSS segment in the cuvette. The spaces (d) and (e) are empty, allowing for the introduction of silicone grease (f), which isolates compartment A from compartment C electrically. Other explanations in the text.

above (in a developmental stage just before cap formation, or with very small caps; 50 mm long) were gently laid perpendicularly to the walls through appropriate grooves (c) in such a way that the apical end dipped in compartment A and the basal end in compartment B. They were very carefully dried in the wall-spaces (d,e). A silicone grease was applied around the cellular stalk, at both sides of the walls including the wall spaces d, e, to isolate electrically and mechanically the neighbouring compartments. The medium on both sides of the walls was ASW.

2 h after fixation into the cuvette the apical part of each cell including the cap region was cut off at a distance of 17—19 mm from rhizoid. A short time afterwards, the EPSSs were placed in darkness for 5 days. After that time, the rhizoids with nucleus were cut off in a position very near to the basal end. This manipulation occurred in a low-intensity green safelight. The EPSSs (15—17 mm long) were then kept in darkness for 1—2 days again.

Finally EPSSs in the cuvettes were transferred in the light (fluorescent light source Phytor, 1500Lux, 12 h light—12 h darkness; 21 ± 1°), and two types of series were made: (*a*) a control series, in which an agar—ASW bridge (2% agar in ASW) linked compartment A to compartment C allowing electrical

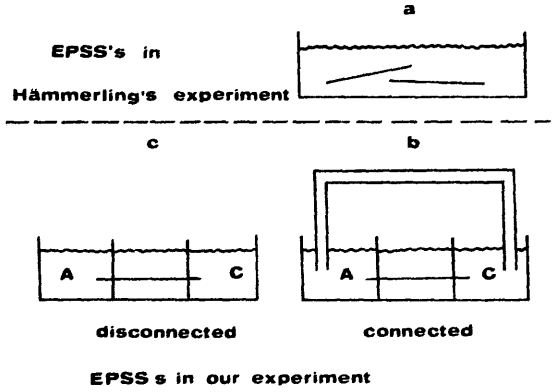

Fig.2. J. and Ch. Hämmerling's experiment compared to ours. The situation in b is analogous to that in a. In c, the connection between compartments A and C is lacking.

connection between them(= connected series; Fig.2b)
(*b*) an experimental series: the same as in (*a*), but without the agar—ASW bridge (= disconnected series; Fig.2c).

An observation of the state of both EPSS ends, originally apical or basal, was made every two days, the time being measured starting from the exposure of the EPSSs to the light.

RESULTS

The regeneration started 2 to 3 days after transfer of the EPSSs in the light. At that time a little dome 0.5 to 1 mm long, or growing tip, was seen at the regenerating end. When this dome was absent after 15 days in the light, the lack of regeneration ability of the segment was recorded.

The percentages of regeneration found after 15 days in each disconnected and in each connected (control) series are given in Table I; average values for all repetitions of the experiment are given in the Table II. Connecting the ends of the EPSSs by addition of an agar—ASW bridge between compartments A and C enhanced the total regeneration of the EPSSs drastically: from 15 % for disconnected series to 85 % for connected series.

TABLE I

EFFECT OF ELECTRICALLY DISCONNECTING PARTS A AND C OF THE EXPERIMENTAL CUVETTE ON THE REGENERATION OF EPSSs IN ACETABULARIA

(A) Control, connected series

Series No.	1	2	3	4
n	44	21	18	39
TR (%)	82	100	72	87
TR_a (%)	89	95	77	88
TR_b (%)	11	5	23	12

(B) Disconnected series

Series No.	1	2	3	4	5	6	7
n	80	84	95	33	60	19	30
TR (%)	12	16	11	18	20	11	16
TR_a (%)	100	93	73	100	83	50.0	100
TR_b (%)	0	7	27	0	17	50.0	0

n, number of EPSSs in each experimental series.
TR (%), percent of the EPSSs which were found regenerating at the apical and/or at the basal end after 15 days.
TR_a (%) and TR_b (%), regenerations which were seen at the apical (a) or basal (b) ends respectively after 15 days, in % of the total regeneration (TR (%) = 100).
The time was measured from the exposure of the EPSSs to light.

TABEL II

EFFECTS OF ELECTRICALLY DISCONNECTING PARTS A AND C OF THE EXPERIMENTAL CUVETTE ON THE REGENERATION OF EPSSs IN ACETABULARIA

Average values for all repetitions of the experiment.

EC	n	TR(%)	TR_a(%)	TR_b(%)	P_a(%)	P_b(%)	W_a(%)	W_b(%)	C_a(%)	C_b(%)
-	401	15	85	15	89	95	11	5	0	0
+	122	85	87	13	50	91	33	9	17	0

EC, electrical connection of the ends.
n, total number of EPSSs.
TR (%), percent of the EPSSs which did regenerate.
TR_a (%) and TR_b (%), regeneration which were seen at the apical (a), or at the basal (b) end respectively, in % of the total regeneration (TR (%) = 100).
P_a (%), tip, regeneration at the apical end
P_b (%), tip, regeneration at the basal end
W_a (%), whorl, regeneration at the apical end
W_b (%), whorl, regeneration at the basal end
C_a (%), cap, regeneration at the apical end
C_b (%), cap, regeneration at the basal end
} in % of the regeneration which occurred, either at the apical (a), or at the basal end (b) (TR_a (%), or TR_b (%) = 100)
The average values are calculated from all experimental series. The figures refer to observations made after 15 days' exposure of the EPSSs to light.

The majority of the EPSSs regenerated at the original apical end (85 % of the regenerating EPSSs for the disconnected series; 87 % for the connected series). This preference shows that the polarity has been essentially preserved in our experiments. This is possibly related to the length of the EPSSs and/or to the short duration of the starvation period after excision of the rhizoids. After 15 days, growing tips, caps and whorls were found in the control series, whereas practically tips only and some whorls were found in the disconnected series.

CONCLUSION

The occurrence of an 85 % regeneration of the EPSSs in the connected series, i.e. in the presence of the agar—ASW bridge, is in conformity with the result of the experiment of J. and Ch. Hämmerling [4]. On the contrary, when the agar—ASW bridge was omitted, and hence both ends of the EPSSs were dipped in the electrically disconnected parts A and C of the experimental cuvette the result was not Hämmerling's result, although all other conditions were exactly the same as in the presence of the bridge. There was no exception to this rule for all repetitions of the experiments. We are forced to admit that there must be a flow of a transcellular electrical current through the EPSS segments if they are to regenerate; regeneration ability and occurrence of a current flow within the cell segment are related in some way. This conclusion

is in agreement with the observations of Novak on the considerable inhibition of regeneration in the voltage clamp experiments, when the potential difference between compartments A and C was kept to zero.

In forthcoming papers we shall show that (1) the disconnection of the electrical contact between compartments A and C of the experimental cuvette by means of the isolating walls enables us to measure a transcellular current; (2) the differences of the particular ionic concentration in the bathing medium for a single compartment (e.g. ASW in compartment A and the modified ASW, with 1/10 of K^+ concentration instead of Na^+, in compartment C) create a transcellular current, which causes drastical changes in the regeneration distribution between the original apical and basal poles; in that this way the reversal of the original polarity was achieved (for details, see next papers).

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