or the resistance to trypsinization induced by histones in HeLa cells. Cytotoxic effects were not observed. It would be of interest to learn what structural features of the cell membranes are responsible for the difference between these cells and HeLa cells.

Desai & Foley showed that treating certain human cell lines with histones inhibited RNA synthesis [6]. The effect was influenced by histone concentration, source, and the molecular fraction of histone used. From their work it was clear that arginine-rich fractions were most inhibitory. The concentration of such fractions in our preparations was probably low enough to avoid cytotoxic effects without eliminating the cell surface effects shown in this report.

Stimulation of HeLa cell surface attachment by histones resembles the effect of cyclic AMP on the adherence of fibroblasts to plastic surfaces [7] and to some degree the induction of mutual restriction of movement and density-dependent inhibition of growth by cyclic AMP [8] or concanavalin A [9]. The relation, if any, between the mode of action of these agents is not yet known.

This work was supported by USPHS grant CA-10271 and the Mildred Werner League of Great Neck, New York. Excellent assistance of Jay Feingold is gratefully acknowledged.

References

1. Ryser, H J P & Hancock, R, Science 150 (1965) 501.
2. Latner, A L & Longstaff, E, Brit j cancer 25 (1971) 280.
3. Stoker, M G P & Rubin, H, Nature 215, (1967) 171.
4. Eagle, H, Science 130 (1959) 432.
5. Bassin, R H, Tuttle, N, & Fischinger, P J, Nature 229 (1971) 564.
6. Desai, L S & Foley, G E, Exptl cell res 66 (1971) 1.
7. Johnson, G S & Pastan, I, Nature new biol 236 (1972) 247.
8. Sheppard, J R, Proc natl acad sci US 68 (1971) 1316.
9. Burger, M M & Noonan, K D, Nature (London) 228 (1970) 512.
10. Bases, R & Mendez, F, Exptl cell res 69 (1971) 289.

Received May 29, 1972

On orientation of rhizoid outgrowth of *Ulva mutabilis* by applied electric fields

O. SAND, *Zoological Laboratory, University of Oslo, Oslo 3, Norway*

Summary

Both the wild-type and the mutant bubble of *Ulva mutabilis* showed anodal orientation of rhizoid outgrowth in applied electric fields, but the detailed orientation pattern was markedly different. It is concluded that the available data on outgrowth orientation of plant cells in applied electric fields do not weaken the theory of self-electrophoresis as a factor in cell development.

A possible mechanism of intracellular self-electrophoresis in plant cell differentiation, by patterning of charged cytoplasmatic particles, was long ago proposed by Went [12]. Strong evidence of a self-electrophoretic mechanism in differentiation has been given by Jaffe [4, 5, 6], who was able to show that the developing Fucales embryo drives a substantial electric current through itself.

Studies of orientation of plant cell polarity by applied electric fields have given ambiguous results, in that the elongation direction for different species can be cathodal or anodal (table 1). This investigation was undertaken to compare the orientation of cell growth in applied electric fields for different mutants within the same species. The experiments were performed on the wild type [8] and the mutant bubble [3] of the multicellular green alga *Ulva mutabilis* (Føyn).

Materials and Methods

Ulva mutabilis is a haplo-diplont, and the development from zoospores and zygotes is morphologically similar. The first cell division is unequal, leading to a cell which through elongation develops to the primary rhizoid, and a cell which gives rise to further division and development. If exposed to directed light, the rhizoid will point towards the dark. The experiments were performed on zygotes, and the culture medium was enriched sea water [9].

The experimental equipment for exposure of the cells to electric fields was in principal similar to that described by Bentrup [1]. The cells were exposed to diffuse white light of about 4 000 lux from underneath

cells to Petri dishes and greatly increased their resistance to detachment by trypsin and versene treatment, even when a dish contained 10^7 cells and extreme crowding and piling up was evident. Histones may be serving as positively charged interlayers between plastic surfaces and cells. Histones and polylysines adhere avidly to plastic surfaces and the changes described above could also be produced by pretreating empty Petri dishes with histone-containing medium for 1 h, removing unattached histones by rinsing, and then plating cells in the pretreated dishes. On the other hand, very small quantities of histones may be acting to specifically stimulate certain membrane sites which are exposed during cell attachment. From our results and those of Ryser [1] it is evident that cell membrane stimulation can be achieved with histone concentrations below 50 μg/ml.

Fig. 4 shows gel electropherograms of the

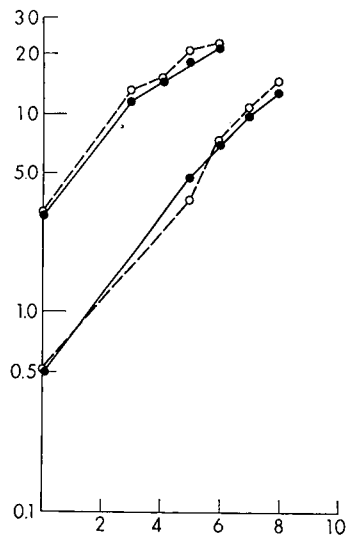

Fig. 3. Abscissa: days; *ordinate:* cells/dish $\times 10^{-6}$. ○, control; ●, histone treated.

Growth of HeLa cells in the presence of histones. The lower curve shows numbers of cells harvested from replicate dishes in the experiment of fig. 1 at times indicated. 2.5 ml of medium was added to dishes remaining on the 6th day. The upper curve is from a similar experiment. Medium was changed and suspended cells replaced on the 8th day in the second experiment.

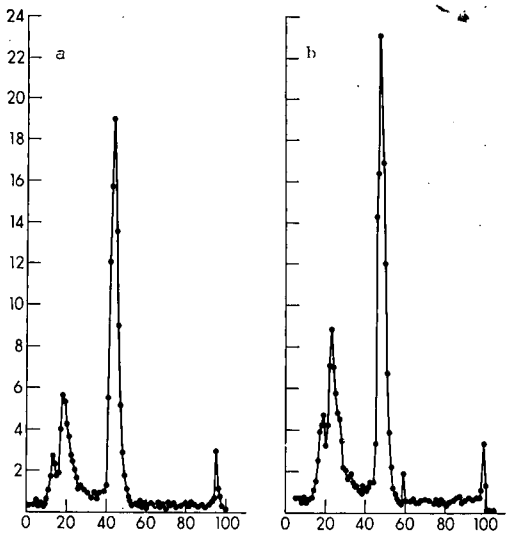

Fig. 4. Abscissa: fraction number; *ordinate:* ^{3}H cpm $\times 10^{-2}$.

Gel electropherograms of HeLa cell histones: (*a*) histones recovered from dish; (*b*) histones before absorption. ^{3}H-Lysine-labeled histones were extracted from the nuclei of HeLa cells as described [10], but were then dialysed against 0.15 N NaCl, 0.01 M Tris, pH 7.4 in the absence of sodium dodecyl sulfate (SDS) at 4°C and sterilized by filtration. Such preparations contained realtively less radiolabel in the first large peak than when dialysis included SDS at room temperature, suggesting some loss of F2a, F2b, and F3 arginine-rich histones may have occurred. Histones recovered from acid-extracted nuclei constituted about 50 % of the protein. Fifty per cent of the radiolabel was accounted for in the histone gel fractions in numerous experiments using ^{14}C-lysine labeled histones. Migration is to the anode at the left.

^{3}H-lysine labeled histones used in these experiments. About 25 % of the histone label could be absorbed to the surface of a Petri dish after 1 h of incubation in the absence of cells. Furthermore, the radiolabel was not easily removed by rinsing, but could be recovered for counting by trypsin treatment or by determining the radiolabel left on fragments of such dishes by placing the broken fragments directly into counting vials containing Bray solution (not shown).

In contrast to HeLa cells, a Moloney sarcoma virus transformed mouse cell line [5] failed to exhibit increased area of attachment

Table 1. *Outgrowth orientation by applied electric fields*

Organism	Cell	Outgrowth	Outgrowth direction	Reference
Griffitshia bornetiana	Thallus	Rhizoid	Anodal	[11]
Fucus inflatus	Egg	Rhizoid	Anodal	[7]
Fucus serratus[a]	Egg	Rhizoid	Cathodal	[1]
Equisetum limosum[b]	Spore	Rhizoid	Anodal	[1]
Equisetum variegatum[b]	Spore	Rhizoid	Anodal	[1]
Funaria hygrometrica	Spore	Chloronema	Cathodal	[1]
Vinca rosea	Pollen	Tube	Cathodal	[10]
Ulva mutabilis	Zygote	Rhizoid	Anodal	The present work

[a] Abnormal high (K^+) in the medium caused anodal orientation.
[b] At certain developmental stages the orientation was cathodal.

during the experiments, and the temperature of the medium was about 18°C.

The angles of the elongating primary rhizoids to the electric field were measured, and the following parameter was used as a measure of the polar alignment of a distribution [1]:

$$V = \frac{1}{N} \Sigma (n_\alpha \cdot \cos\alpha)\, 100\%$$

where N is the total number of plants and n_α is the number of plants in the angle category α.

Eight categories of 45° were used. The statistical treatment of this parameter has been discussed previously [1], and the data presented are given $\pm 2\sigma$. In this work V is chosen positive for orientation towards the anode.

Results

In both the wild-type and the mutant the rhizoid outgrowth was orientated towards the anode. To determine the time course of susceptibility of the cells to the electric field, the cells were exposed to constant field strengths for periods of 12 h. The results from an experiment of this type are shown in fig. 1. In regard to the wild-type, the anodal orientation was greatest when the cells were exposed at an age of about 36–48 h, while the corresponding age of the bubble mutant was about 12–24 h. Comparable experiments, performed with horizontally directed white light as the orientating vector, showed the time periods giving greatest orientation towards the dark to be coinciding with the periods giving most pronounced anodal orientation. The first cell division occurred at the same time for both cell types, and 50 % of the cells were divided at an age of about 78 h.

With cells of the same age experiments were performed using fields of various strengths, and fig. 2 shows the results from an experiment of this type. The cells were exposed for 12 h at their most sensitive phase. The anodal orientation grew with increasing strength for both cell types, but a given field strength gave less orientation for the bubble mutant than for the wild-type.

Cell populations from different crossings could give slightly different results, the variation being greatest within the mutant.

Discussion

In *Fucus* eggs several substances concentrate in the rhizoid end of the developing eggs [see 5]. A self-electrophoretic mechanism in this patterning process has heavy experimental support, and it is tempting to suggest that even an applied field acts through a direct electrophoretic mechanism.

It is reasonable to suppose the particles transported to a wall growth point to have the same electric charge in different species. If an applied electric field acts on the cell

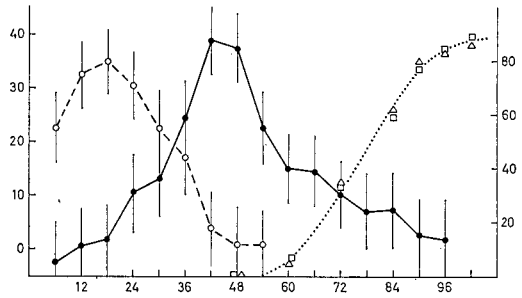

Fig. 1. Abscissa: age of cells (hours); *ordinate: (left)* V (%); *(right)* cells divided (%). ●, wild-type orientation at 0.9 V/cm; ○, bubble orientation at 2.2 V/cm; △, wild-type division; □, bubble division.

Anodal orientation of the wild-type and the mutant bubble, as a function of center time of the exposure periods, compared to the time course of the first cell division.

mainly by cytoplasmatic electrophoresis, the cell outgrowth thus ought to have the same orientation in all species. However, as the available data show (table 1), this is not so.

On the basis of this difference in orientation in different species, Bentrup [1, 2] concluded that the main effect of applied fields is not electrophoretic. He suggested the action site of the field to be the cell membrane, and he claimed that this precludes that self-electrophoresis can be of importance in development.

However, due to the high resistance of cell membranes, only less than 0.1 % of the voltage drop will occur across the cytoplasms.

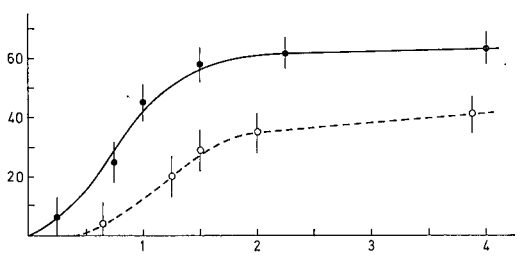

Fig. 2. Abscissa: electric field strength (V/cm); *ordinate:* V (%). ●, wild-type exposed 36–48 h; ○, bubble exposed 12–24 h.

Anodal orientation of the wild-type and the mutant bubble as a function of field strength.

when a cell is exposed to applied electric fields. A self-generated electric field has its origin in local changes of cell membrane permeabilities, and the potential drops across the cell membranes and the cytoplasm are of the same order of magnitude [6]. Measurements of self-generated cytoplasmatic fields in different systems have shown the gradients to be about 1 V/cm [see 6], whereas the cytoplasmatic components of the applied fields used in the orientation experiments have been far less than 0.1 V/cm. If the effect of applied fields is to orientate the self-generated field, by changing locally the cell membrane permeability, the direction of the net cytoplasmatic field will thus be in accordance with the self-generated field. Different cell membranes in biological systems show a wide diversity in reaction patterns when stimulated, and the difference in outgrowth orientation summarized in table 1 seems thus not to be unreasonable.

It may be concluded that the theory of self-electrophoresis as a factor in cell development is not weakened by the available data of the effect of applied electric fields on outgrowth orientation in plants.

I am greatly indebted to Dr A Løvlie for valuable criticism and assistance, both during the experimental work and the preparation of the manuscript.

References

1. Bentrup, F W, Z Pflanzenphysiol 59 (1968) 309.
2. — Ber deut botan Ges 7 (1968) 311.
3. Fjeld, A, Genet res Cambr 15 (1970) 309.
4. Jaffe, L F, Proc natl acad sci US 56 (1966) 1102.
5. — Advan morphogenesis 7 (1968) 295.
6. — Dev biol, suppl. 3 (1969) 83.
7. Lund, E J, Botan gaz 76 (1923) 288.
8. Løvlie, A, Compt rend trav lab Carlsberg 34 (1964) 77.
9. — Dev biol 20 (1969) 349.
10. Marsh, F & Beams, H W, J cell comp physiol 25 (1945) 195.
11. Schechter, V, J gen physiol 18 (1935) 1.
12. Went, F W, Jahrb wiss Bot 76 (1932) 528.

Received July 4, 1972