

Buds of hydra also elongated despite total inhibition of mitotic activity by radiation<sup>9</sup>. Hydranth initiation increased in *Podocoryne* colonies subjected to starvation<sup>10</sup>, although mitotic activity would be expected to decrease under these conditions. Localized cellular proliferation is probably not a common morphogenetic mechanism in the hydrozoans.

No elongation was detected in 7 stolons during a 4 h interval between tip ablation and fixation, although the presence of dividing cells was subsequently confirmed. Tipless stolons left undisturbed for 5 h or more usually regenerated motile tip regions and resumed elongation. Isolated sections of stolons sometimes regenerated motile tips at both ends and extended in both directions.

The observations described above suggest that active motility of the stolon tip in *Proboscoidactyla* does not depend upon cellular proliferation and serves a necessary function in stolon elongation. This is not to say that physical stretching is the only way stolons increase in length. Some mitotic activity does occur in the proximal portions of non-irradiated stolons. It has also been suggested that viable cells migrate to growing regions of

hydroid colonies through the gastrovascular cavity<sup>2,5</sup>, although this seems improbable in isolated stolon tips.

Whether cellular proliferation and migration actually figure significantly in the normal elongation of *Proboscoidactyla* stolons cannot be resolved on the basis of the evidence given. However, the stolon tip is clearly identified as a motile organ whose locomotory activity is an essential concomitant of stolon elongation.

**Résumé.** Les portions distales des stolons du *Proboscoidactyla flavicirrata* sont des organes moteurs dont les activités jouent un rôle essentiel dans l'élongation du stolon.

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Victoria, (British Columbia), 9 October 1972.

<sup>9</sup> S. G. CLARKSON and L. WOLPERT, *Nature*, Lond. 214, 780 (1967).

<sup>10</sup> M. BRAVERMANN, *Biol. Bull. mar. biol. Lab., Woods Hole*, 139, 404 (1970).

### Micronuclear Mitosis in the Life Cycle of a Plurimicronucleate Strain of *Euplotes crassus*<sup>1</sup>

In *Euplotes crassus*, a ciliate marine species, it has been found<sup>2</sup> that starvation may bring about a physiological reorganization of pellicular structures as well as a mitotic division of the single micronucleus, not accompanied by cytokinesis. By successive reorganization processes, some micronuclei may accumulate in a common cytoplasm. However, only 1 micronucleus divides during the vegetative reproduction which ensues after reorganization, while the supernumerary ones are sorted out in daughter cells without resorption. In this way unimicronucleate cells are again formed as asexual reproduction goes on.

A search has been made to find out whether any one of the nuclei present in the cytoplasm can enter mitosis in the life cycle of the ciliate, or if the nuclear products of the same nucleus always take part in the division process. It must be noted that all nuclei, no matter how many, are derived through mitosis from the same mother-nucleus and that all of them should be in G<sub>2</sub> phase as DNA duplication stage begins at late telophase in *Euplotes*<sup>2,3</sup>.

The routine techniques used are reported in detail elsewhere<sup>2</sup>. Only those strictly necessary will be described here. As material, strain 17 of *E. crassus*, Pisa collection, has been used extensively. Lines of this strain consists of uni- and plurimicronucleate cells.

**Results.** First of all, it was ascertained by spectrophotometric analysis that the amount of Feulgen positive material of different micronuclei in resting stage inside a cell, and of different cells, averaged around the same arbitrary units (Figure 1), confirming that all nuclei are

in G<sub>2</sub> phase. This analysis was carried out using the apparatus described by BENEDETTI and VIOLA MAGNI<sup>4</sup>.

Cells of a plurimicronucleate line were allowed to divide 2 or 3 times in culture fluid with 3H-thymidine

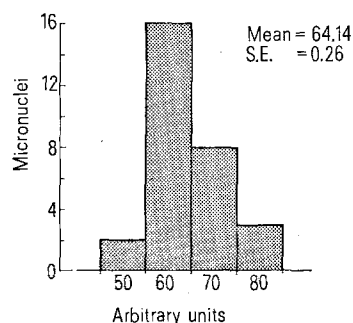


Fig. 1. Content of micronuclear Feulgen positive material expressed in arbitrary units (abscissa); in ordinate, number of micronuclei. The data were derived from 14 cells.

<sup>1</sup> Supported by a grant from Consiglio Nazionale delle Ricerche (C.N.R.).

<sup>2</sup> P. LUPORINI and P. BRACCHI, *Monitore zool. ital.*, in press (1973).

<sup>3</sup> D. M. PRESCOTT, R. F. KIMBALL and R. F. CARRIER, *J. cell Biol.* 13, 175 (1962).

<sup>4</sup> P. A. BENEDETTI and M. P. VIOLA-MAGNI, *J. scient. Instrum.* 43, 141 (1966).

Life cycle phases	No. specimens examined	Unimicronucleate specimens	Plurimicronucleate specimens	Specimens with 1 labelled micronuclei	Specimens with 2 labelled micronuclei
Asexual reproduction	577	406	171	577	—
Conjugation	215	155	60	215 <sup>a</sup>	—
Reorganization	176	59	75	134	42
1st Post-reorganization fission	438	223	191	414	24

Micronuclear behaviour in the plurimicronucleate strain 17 in different life cycle phases. Strain 17 includes uni- and plurimicronucleate cells. Other explanations in the text. <sup>a</sup> In conjugation, the labelled micronucleus is that undergoing the first pregamic fission.

(20 $\mu$ Ci/ml) added and then washed, fixed and prepared for autoradiography. The results reported in the Table, first row, indicate that only one micronucleus, among the many present, is labelled (Figure 2), thus suggesting that it is always one and the products of the same micronucleus which divide during vegetative reproduction.

Cells of plurimicronucleate line with one micronucleus labelled during binary fissions were repeatedly washed

out and then mixed with cells of complementary mating type having only one micronucleus. Pairs were fixed after 3–4 h from the onset of conjugation when the micronucleus is normally engaged in the mitotic pregamic division. The active micronucleus of the plurimicronucleate partner is always and uniquely the labelled one (Figure 3; Table, second row) which is to be identified with the micronucleus active also during asexual reproduction.

Cells of a plurimicronucleate line with one, 3H-thymidine labelled micronucleus were sent through reorganization by starvation. As soon as reorganizing cells, recognizable by their comma-shaped form, appeared in the culture, they were fixed and prepared for autoradiography. As one must recall, during this process one micronucleus undergoes mitosis in a variable percentage of reorganizing cells. It was found that 29.5% of the cells examined showed 2 labelled micronuclei (Figure 4); the remaining cells had only one labelled micronucleus (Table, third row). Moreover, in a parallel experiment with animals of the same line, it was ascertained that 32% of reorganizing cells had undergone micronuclear mitosis. As the percentage values of the 2 experiments are close enough, one may conclude that the 2 labelled nuclei found in reorganizing cells derive from the active micronucleus labelled during binary fissions.

Reorganizing cells of a plurimicronucleate line were singly isolated, at the end of the process, in fresh culture fluid with 3H-thymidine added. They were allowed to divide once and then both products of the division were fixed and prepared for autoradiography. All the cells examined showed either 1 or 2 labelled micronuclei. An experiment, simultaneously carried out on cells of the same line, indicated that, among 190 specimens, 57% had undergone micronuclear mitosis during reorganization. The data reported in the Table, 4th row, are therefore interpreted in this way: in about 125 cells (57% of 432/2) micronuclear mitosis occurred during reorganization, but 2 micronuclei divided at the first post-reorganization fission in only 12 of these cells. This would account for

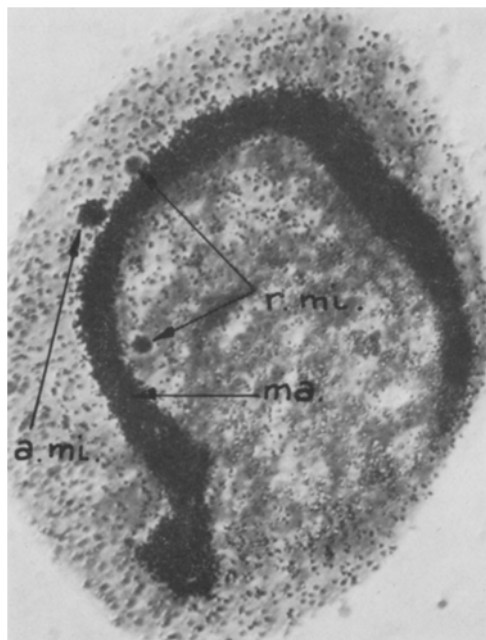


Fig. 2. Cell which passed through fissions in  $^3\text{H}$ -thymidine medium. Macronucleus (ma.) heavily labeled; only 1 micronucleus (a. mi.) labelled out of the 3 present; r. mi. = unlabelled micronuclei.

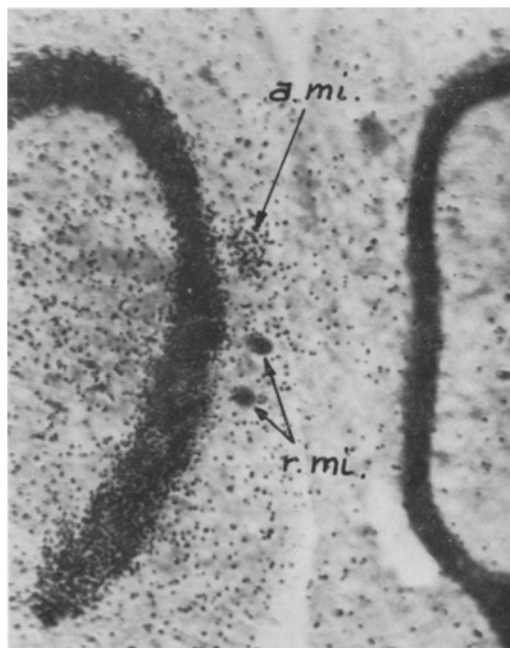


Fig. 3. Conjugating pair; the plurimicronucleate partner shows a labelled micronucleus (a.mi.) engaged in the first pregamic mitosis and 2 resting, unlabelled micronuclei (r. mi.).

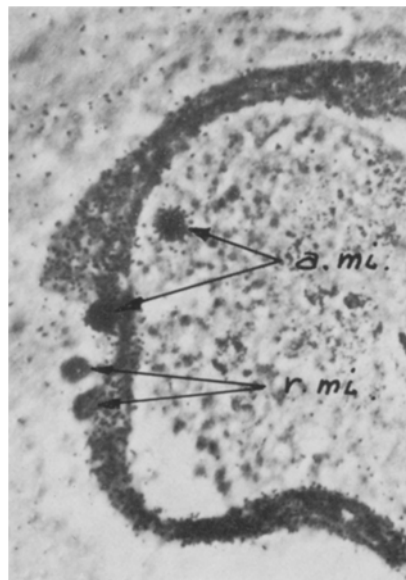


Fig. 4. Reorganizing specimen with 2 labelled micronuclei (a. mi.) and 2 unlabelled ones (r. mi.).

the 24 cells found with 2 labelled micro nuclei otherwise unexplicable, unless one assumes a misdistribution of the 2 mitotic products, which was never observed. In all other cells, only 1 micronucleus divides, although it is impossible to track down which one in the case of plurimicronucleate cells.

**Conclusion.** The data reported above point to a micronuclear genetic continuity in plurimicronucleate cells of *E. crassus*. In successive cell generations, the nucleus which divides is always a product of the preceding mitosis. An exception might be found in the first post-reorganization fission as, for example, if one assumes that in those plurimicronucleate cells which did not undergo micronuclear mitosis while reorganizing, the dividing nucleus was not a product of the last mitosis preceding reorganization. Such genetic continuity might be accounted for by the existence of unique information available per cell carried by the micronucleus itself and duplicating with it. Although nothing is known at present about the nature of such information, it is worthwhile to mention one of the hypothesis put forward by MURTI and PRESCOTT<sup>5</sup> to explain the presence of RNA in the micronucleus of *Tetrahymena*: 'micronuclear DNA is synthesized in situ but functions within the micronucleus rather than migrating out. Such a situation might imply a role of micronuclear RNA in the replication of micronuclear DNA, etc. .

A direct macronuclear involvement in the information transmission appears less valid. Indeed, in that case there would be an accumulation of information in the cytoplasm which in turn should cause a random choice of the target

micronuclei. Any one, or more than one, micronucleus would then be expected to divide.

The hypothesis of unique micronuclear information explains all the data with the exception of what happens at the first post-reorganization fission when in 90% of the cells with 2 mitotic products only one, instead of two, divides. But we might assume that micronuclear information could be regulated and controlled at this stage, as it is during sexual reproduction when nuclear differentiation of pregamic and metagamic divisions is associated with specific cytoplasmic regions<sup>6,7</sup>.

An experimental approach to the hypothesis set forth above is under examination by inducing mitosis of supernumerary nuclei while blocking the normal, active one.

**Résumé.** Dans les différents phases du cycle reproductif d'un stock plurimicronucléaire d'*Euplotes crassus* c'est toujours le même micronoyau qui est sujet à la division mitotique. Nous avons expliqué cette continuité génétique micronucléaire par l'existence d'une unique information cellulaire résidant dans le micronoyau et qui se doublerait avec le micronoyau même.

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<sup>5</sup> K. G. MURTI and D. M. PRESCOTT, *J. cell Biol.* **47**, 460 (1970).

<sup>6</sup> D. L. NANNEY, *Biol. Bull. mar. biol. Lab., Woods Hole* **105**, 132 (1953).

<sup>7</sup> T. M. SONNEBORN, *Caryologia, Suppl.* **6**, 307 (1954).

## Lumière et morphogénèse du thalle de l'*Acrochaetium* sp. (Rhodophytes)

Si la réaction des plantes supérieures à la lumière est maintenue bien connue, il n'en est pas de même chez les algues<sup>1</sup>. Pour une même algue, les cultures sont souvent maintenues dans des conditions d'éclairement très variables (en durée et en qualité) d'un expérimentateur à l'autre, ce qui rend difficile la comparaison des différents résultats.

Nous avons pu démontrer que pour une souche d'*Acrochaetium* sp. (Rhodophycée), la croissance est stimulée par des éclaircements longs (de 18 h par 24 h). Un éclairciment continu en lumière blanche (24 h/24 h) est encore plus favorable à la croissance. Par contre un éclairciment de 23 h sur 24 h est au contraire beaucoup moins efficace et même inhibiteur<sup>2</sup>. Lorsque les radiations monochromatiques remplacent en partie l'éclairciment blanc, elles sont d'autant plus efficaces qu'elles coïncident avec les zones d'absorption des systèmes photosynthétiques, ce qui suggère un rôle prédominant de la photosynthèse par rapport au photopériodisme proprement dit.

Nous avons effectué le spectre d'absorption in vivo de l'*Acrochaetium* sp.; l'absorption est élevée à partir du violet jusqu'au jaune; elle diminue rapidement aux longueurs d'onde supérieure sauf dans le rouge clair. Ce spectre d'absorption est voisin de celui réalisé par FORK<sup>3</sup> sur *Porphyra perforata*; or chez cette algue rouge, le même auteur montre que l'activité photosynthétique mesurée en éclaircements monochromatiques est essentiellement limitée aux radiations de longueurs d'onde comprise entre 500 et 660 nm. Or un effet spécifique stimulateur des radiations bleues sur la croissance en longueur des filaments a été mis en évidence. L'effet est inexplicable par le seul mécanisme de la photosynthèse. L'allongement des filaments étant uniquement dû, chez cette Rhodophycée aux divi-

sions cellulaires, il faut donc noter que les radiations bleues de 476 nm stimulent la division cellulaire<sup>4</sup>.

Compte tenu de ces données nous avons étudié l'influence de l'intensité d'éclairement sur la morphogénèse du thalle ramifié de l'*Acrochaetium*. Les souches sont entretenues sur le milieu de FRIES<sup>5</sup>. Après purification par passages successifs sur des milieux contenant divers antibiotiques, les cultures sont maintenues sur milieu de FRIES additionné de tellurite de potassium à 10 mg/l; cet inhibiteur empêche tout développement bactérien, mais ne modifie pratiquement pas le développement de l'algue<sup>4</sup>. Les cultures sont placées en éclairciment continu blanc fluorescent « Blanc super » Mazda, à une température de  $16^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

1. L'éclairciment optimal, pour la croissance en longueur des filaments, est de  $3500 \text{ ergs cm}^{-2}\text{s}^{-1}$ . Les faibles comme les fortes énergies sont inhibitrices ( $150 \text{ ergs cm}^{-2}\text{s}^{-1}$  et  $9000 \text{ ergs cm}^{-2}\text{s}^{-1}$ ).

2. La longueur (50–60  $\mu\text{m}$ ) atteinte par chaque cellule est indépendante de l'éclairciment. Le résultat oppose donc une fois de plus les algues aux plantes supérieures soumises au phénomène de l'étiollement<sup>6</sup>.

<sup>1</sup> M. J. DRING, 4th Marine Biology Symposium (Ed. D. J. CRISP, Cambridge University Press 1971), p. 375.

<sup>2</sup> J. P. LARPENT and R. JACQUES, *Plant Sci. Lett.*, **1**, 339 (1973).

<sup>3</sup> D. C. FORK, in: *The Physiological Aspects of Photosynthesis* (Ed. O. V. S. HEATH; Stanford University Press, Stanford 1969), p. 213.

<sup>4</sup> M. LARPENT-GOURGAUD, J. P. LARPENT et R. JACQUES, *C. r. Acad. Sci.*, Paris **274**, 2988 (1972).

<sup>5</sup> L. FRIES, *Physiologia plant.* **16**, 695 (1963).

<sup>6</sup> J. P. LARPENT, *C. r. Acad. Sci.*, Paris **267**, 1953 (1968).