The Cytochemistry of hypotrichous-ciliates Onychodromus and Euplotes

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SUMMARY: Synthesis of specific macromolecules can be conveniently divided into two classes, « cyclic », that is those that appear only at certain times in each life cycle and « continual » those that are most persistent. The periodic synthesis of DNA; which has been known since the early days of cycle work is a matter of great importance. It provides two markers, the beginning and end of the synthesis period (S), to chart the progress of a cell through the cycle. In the majority of hypotrichous ciliates during the S period a replication band appears in the macronucleus and gradually passes through it. It has been established that DNA is synthesized and all types of macronuclear proteins become doubled during the passage of the replication band. Using the appearance of this band as a convenient starting point, a study of the localization of other macromolecules like proteins, lipids, carbohydrates has been made and a few oxidative and hydrolytic enzymes have been analysed at different stages in the life cycle of two hypotrichous ciliates, Euplotes and Onychodromus. Cytochemical investigations indicate that there is a periodic increase and decrease in the cell constituents as well as in their localization, during the different stages of cell cycle. The results are summarized and their significance discussed.

La cytochimie des ciliés hypotriches Onychodromus et Euplotes

RéSUMÉ : La synthèse des macromolécules spécifiques peut être commodément divisées en deux classes :
1. Cycliques, c'est-à-dire, celles qui apparaissent à certains stades seulement du cycle de vie ; et
2. « Continuelles » : celles qui persistent le plus longtemps.

La synthèse périodique de l'ADN, déjà observée depuis longtemps, est une affaire de grande importance. Elle prévoit deux étapes, le commencement et la fin du stade de synthèse (S), pour marquer le développement d'un organelle à travers le cycle. Dans la majorité des ciliés hypotriches, on observe dans le macronucleus une chaîne de réplication, qui passe, petit à petit, à travers le macronucleus tout entier.

La synthèse de l'ADN est confirmée, comme aussi la duplication de toutes espèces de protéines macronucléaires, pendant le passage de la chaîne de réplication. Se servant de la présence de cette chaîne comme point commode de départ, l'étude de la localisation d'autres macromolécules, telles que des protéines, lipides, carbohydrates, s'est poursuivie ; quelques enzymes oxydatives et hydroly-
tiques ont été explorées à des stades différents du cycle vital des deux ciliés hypotrichieux, - Euplotes et Onychodromus. Les recherches cytochimiques indiquent des augmentations et des diminutions périodiques dans le contenu ainsi que dans leur localisation pendant les stades différents du cycle vital. Les résultats sont résumés et leur signification est discutée.

**INTRODUCTION.**

In the majority of hypotrichous ciliates a replication band passes through the macronucleus, serving as a marker for denoting the period of nucleic acid synthesis. This marker has been made use of in the present study to find out whether besides nucleic acids, other products like carbohydrates, proteins and lipids are also concentrated in the cytoplasm at this period. Two hypotrichous ciliates, Onychodromus and Euplotes, in which a prominent replication band is present, were chosen and cytochemical investigations made.

**MATERIAL AND METHOD.**

The fresh water ciliate Onychodromus was cultured in an infusion of hay and garden soil fortified with horlicks and supplemented with Chalkley's and a solution of eleven essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, valine). The cultures thrived best at a temperature range of 23° C to 26° C.

Euplotes was cultured in a medium of hay infusion fortified with horlicks at room temperature.

For the cytochemical assay whole mounts of the organisms were used. The air dried animals were fixed in Carnoy to demonstrate proteins by Mercury Bromophenol blue (8), Carbohydrates and Glycogen by the PAS reaction (6). The organisms fixed in calcium-formal were tested for unsaturated lipids by Sudan Black B (2).

**Observations.**

Three stages have been distinguished in the present study for the cytochemical investigations, based on the appearance, and movement of the replication band through the macronucleus.

1. Presynthetic period characterized by the absence of the replication band in the macronucleus. (Fig. 1).

2. A period of synthesis-appearance of the replication band in the macronucleus. (Fig. 2).

3. Fission period - in which cytotomy is occurring. (Fig. 3).

The cytochemistry of these two ciliates have been studied at all these three stages to find out whether there is a difference in the localization of the chemical substances mentioned with reference to the replication band.

The results of the cytochemical investigations are summarized in Table I.
The life cycle of an animal is characterized by a well-coordinated synthesis of various macromolecules leading to an increase in cell mass and replication of genetic material — DNA and ultimately a sharing of macromolecules as a result of division (7). G₁ and G₂ represent periods to which it is not yet possible to ascribe any biochemical events that are specific parts of cycle progress or continuity, and the positive basis for the existence of these two intervals is not known. RNA and protein synthesis and general mass

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**Table I**

Cytochemistry of two hypotrichous ciliates

<table>
<thead>
<tr>
<th>Region</th>
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<th>Euplotes</th>
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<tbody>
<tr>
<td></td>
<td>Prior to replication</td>
<td>During replication</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>+</td>
<td>±</td>
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<tr>
<td>Macronucleus</td>
<td>-I</td>
<td>±</td>
</tr>
<tr>
<td>Basal granules</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Peristomial groove</td>
<td>1 I</td>
<td>±</td>
</tr>
<tr>
<td>Replication band:</td>
<td>Zone of rep.</td>
<td>1</td>
</tr>
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<td>Zone of syn.</td>
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- Negative.  
- Weak.  
+ Positive.  
++ Strong.  
+++ Intense.

rep. = Replication.  
syn. = Synthesis.

**DISCUSSION.**

The life cycle of an animal is characterized by a well-coordinated synthesis of various macromolecules leading to an increase in cell mass and replication of genetic material — DNA and ultimately a sharing of macromolecules as a result of division (7). G₁ and G₂ represent periods to which it is not yet possible to ascribe any biochemical events that are specific parts of cycle progress or continuity, and the positive basis for the existence of these two intervals is not known. RNA and protein synthesis and general mass
increase of the cell occur during $G_1$ and $G_2$ (and S), but the role of these activities in advancing the cell within the cycle is unknown. However, studies on the cell cycle have shown that doubling of DNA occurs during the S period and by far the major interest has been on DNA synthesis specially in ciliates. In the majority of hypotrichous ciliates, prior to fission a replication band appears in the macronucleus and passes through it, and has been established that the genetic material DNA is synthesized in the macronucleus at this period (5). In this investigation an evaluation of the time relationship between the synthesis period of the macromolecule DNA and localization of other macromolecules like proteins, carbohydrates and lipids has been examined. The replication band serves as a marker for the period of DNA synthesis and the presence of the above mentioned substances has been studied by standard cytochemical tests.

Fig. 1. — Euplotes in the presynthetic stage stained with mercury-bromophenol blue.
Fig. 2. — The synthetic stage of Euplotes showing replication band in the macronucleus with zone of replication and zone of synthesis.
Fig. 3. — Onychodromus during fission stained with mercury bromophenol blue. Protein concentration in the region of the cleavage furrow.
Fig. 4. — Presynthetic stage of Onychodromus, stained with mercury bromophenol blue. Cytoplasm, macro-nucleus and basal granules show high concentration of proteins.
Fig. 5. — Onychodromus during the synthetic stage. Stained with BPB. Replication band in the macronucleus indicated by the arrow.
Fig. 6. — Euplotes - different stages of the replication band. Stained with BPB. A. - beginning of the replication band in the macronucleus. B. - An advanced stage of the replication band. C. - Meeting of the replication bands in the centre of the macronucleus.
Fig. 7. — Onychodromus in the presynthetic stage. Stained with Sudan Black B. Macronucleus negative while the region round the macronucleus is positive.
Fig. 8. — Onychodromus during cytotomy. Stained with Sudan Black B for lipids.
Fig. 9. — Euplotes during the presynthetic stage stained with Sudan Black B. Basal granules of the Meridional row of cilia show positive reaction. Macronucleus shows negative reactions.
Fig. 10. — Onychodromus during presynthetic stage stained with PAS. Negative reaction in the region of the macronucleus. Basal granules show positive reaction.

R.B. Replication Band; C.F. Cleavage furrow; B.G. Basal granules; M.A. Macronucleus.
An analysis of the results of the cytochemistry of the three different periods show that, protein concentration is maximum in the macronucleus during the presynthetic stage, decreases during replication, and again increases during cytotomy being concentrated in the region of the cleavage furrow. The cytoplasm of Euplotes and Onychodromus show a positive reaction for the mercury bromophenol blue test in the presynthetic stage, the reaction is intense during cytotomy, but there is only a weak reaction during the synthetic period. Protein concentration in the basal granules follows the same pattern as that of the cytoplasm. (Figures : 1, 2, 3, 4, 5 and 6).

During the presynthetic period the zone of replication shows negative reaction while there is strong activity in the zone of synthesis (Fig. 2).

Carbohydrate distribution in the cytoplasm and basal granules during the presynthetic period is strong, weak in the synthetic stage and moderate during cytotomy. The macronucleus shows negative reaction during all the three stages. In Stentor a weak reaction has been observed by Tartar during cytotomy. According to his observation the carbohydrate reserves which are present in the cell are prediced between the daughter cells at fission. This division enables the anterior as well as the posterior daughter cell to begin life with a share of the original carbohydrate material. Approximately half the granular mass migrates forwards (8). This accounts for the weak reaction during cytotomy in our present cytochemical investigation.

Very little quantities of lipids are present in the cytoplasm during the pre-synthetic period. The macronucleus shows negative reaction while the region surrounding the macronucleus exhibits strong reaction, as lipids form one of the chemical constituents of the membrane system in biological systems. The basal granules show positive reaction in Onychodromus but in Euplotes the basal granules show very strong reaction. The arrangement of the meridional row of cilia can be seen clearly because of the basal granules. The peristomial region shows strong reaction, this is due to the basal granules of the cilia which line the peristomial groove.

The cytochemistry of the different cell organelles of Onychodromus and Euplotes are concurrent with those of Spirostomum and Blepharisma (9).

The staining reactions in the different stages of cell cycle show that the localization of the substances like carbohydrate, proteins and lipids occur after nucleic acid synthesis, and that during the different stages of the cycle, these substances have different concentrations. While nucleic acids are being synthesized other substances are present in small quantities.

During cytotomy the cleavage furrow shows strong reaction with bromophenol blue indicating that proteins are necessary for fission to occur. Once fission is over, in the succeeding cycle during the G1 period lipids and carbohydrates reach their normal concentration in the cytoplasm, whereas protein increase is both in the macronucleus and cytoplasm.

Electron-microscopic and autoradiographic studies would provide proof for the synthesis of these substances in relation to DNA synthesis.
As enzymes play an important role in cell metabolism, investigations on a few hydrolytic and oxidative enzymes are in progress to evaluate their significance in the metabolism of the macromolecules studied.

ACKNOWLEDGEMENT

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REFERENCES


