ELECTRON MICROSCOPY OF THE MACRONUCLEUS OF *EUPLOTES EURYSTOMUS*

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INTRODUCTION

It has been demonstrated by Gall (1), Prescott and Kimball (2), and Kimball and Prescott (3) that the macronucleus of *Euplotes* is excellent material for studying relations between DNA, RNA, and protein synthesis during nuclear replication. Replication bands normally originate at the two tips of the long, rod-shaped macronucleus during the early hours of interphase and progress as two waves of DNA synthesis until they meet in the center. In the light microscope one sees that two marked changes in the cytological organization of DNA precede DNA replication. The reorganization or replication bands consist of two zones. At the forward edge of the forward zone DNA shifts from a granular state to an evenly dispersed state. In the rear zone, where DNA and histone syntheses occur, DNA is much less concentrated. In the remaining portion of the rear zone, chromatin aggregates are reconstituted from the duplicated material.

Fauré-Fremiet, Rouiller, and Gauchery (4), and Roth (5) have reported observations on the macronucleus and the replication band in *Euplotes* with the electron microscope. The present study confirms some of the earlier observations and also includes descriptions of several macronuclear structures not previously reported. Particular attention is devoted to the ultrastructure of the

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FIGURE 1

Electron micrograph of a small part of an *Euplotes* macronucleus stained with 1 per cent lanthanum acetate and 2 per cent uranyl acetate. The small, very dense particles are due to the lanthanum acetate which seems to deposit selectively on the chromatin bodies (*C*) and not on the nuclear bodies of lower density (*N*). *M*, mitochondrion; *NM*, nuclear membrane. *H*, helices. × 24,000.

FIGURE 2

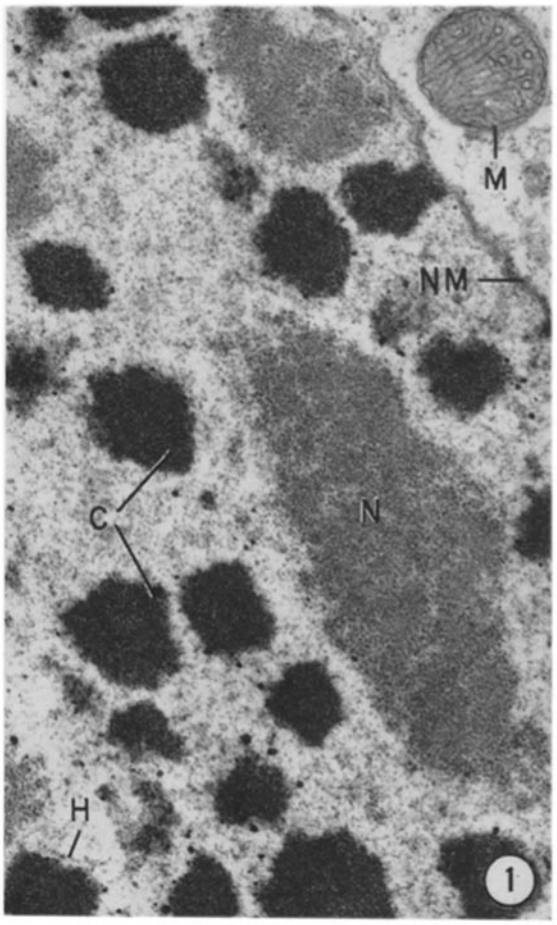
Electron micrograph of a macronucleus treated in the same manner as the one shown in Fig. 1. Rows of particles possibly representing helices are indicated (*H*). *C*, chromatin body; *N*, nuclear body of lower density. × 54,000

FIGURE 3

Macronucleus stained with 0.1 M ferric chloride. The replication band moves down the macronucleus in the direction indicated by the arrow. The chromatin bodies (*C*) at the bottom of the figure have already begun to break up and form the network of twisted microfibrils (*F*). *NM*, nuclear membrane. *SZ*, zone of DNA synthesis. × 11,000.

FIGURE 4

Macronucleus stained with 0.1 M ferric chloride. The zone of DNA synthesis (*SZ*) is immediately followed by a region where the minute microfibrils appear to be aggregating into small chromatin bodies (*C*). *N*, nuclear body of lower density. *F*, network of twisted microfibrils. × 54,000.


M
NM
C
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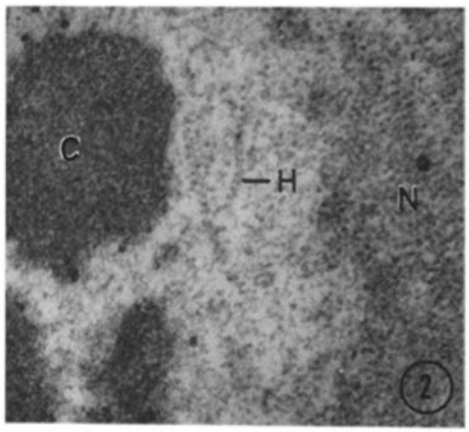
1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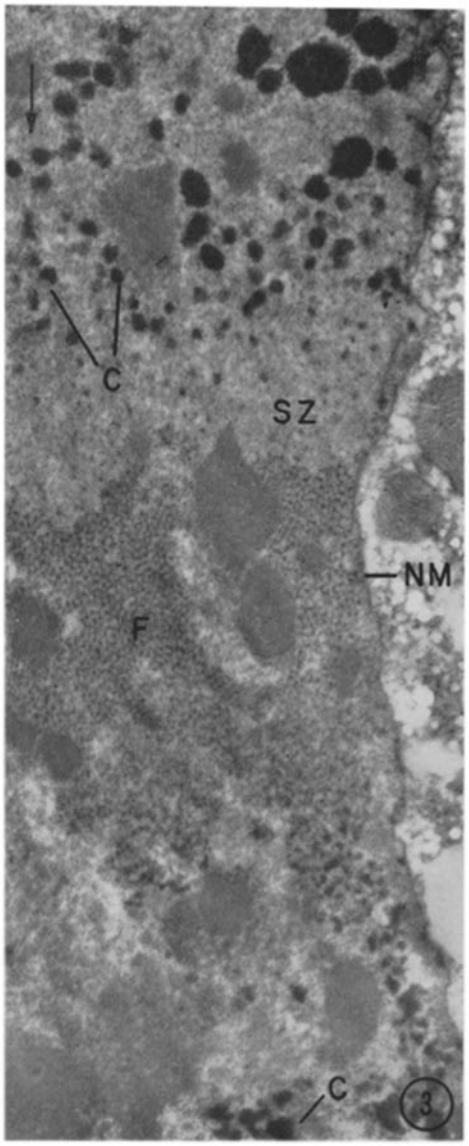
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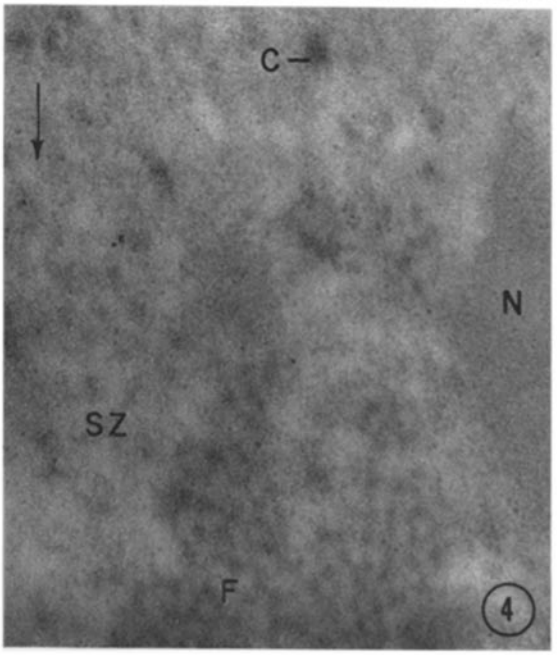
3


C
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4

replication band because it was recently discovered to be the place of DNA synthesis.

MATERIALS AND METHODS

E. eurystomus was grown on bacteria and *Tetrahymena pyriformis* HSM in dilute lettuce infusion at 20°C. A large number of euplotes was fixed for 1 hour at 0 to 5°C in Palade's (6) fixative, pH 7.4. The cells were placed in 30 per cent and 50 per cent acetone at 0 to 5°C for 10 minutes each. One-fourth of the euplotes was stained for 15 minutes in 0.1 M ferric chloride in 50 per cent acetone at 0 to 5°C. One-fourth was stained in 1 per cent lanthanum acetate in 50 per cent acetone, pH 7.0, at 0 to 5°C for 15 minutes, while the remaining cells were placed in 70 per cent acetone at 0 to 5°C. The euplotes were then further dehydrated in increasing concentrations of acetone at room temperature. One-half of the unstained cells was placed in 1 per cent potassium permanganate in 100 per cent acetone for 15 minutes, rinsed in 100 per cent acetone, and placed for a few minutes in acetone and methylacrylate at a concentration of 2 drops per 20 ml. All of the organisms were embedded in Epon according to the method of Luft (7). Sections were cut with glass or diamond knives on a Porter-Blum microtome and observed with an RCA 3 electron microscope. Some of the sections were stained with 2 per cent aqueous uranyl acetate for 4 hours by immersing the grids bearing the sections into the solution.

OBSERVATIONS

The macronuclei of *Euplotes* fixed in osmium tetroxide and embedded in Epon are difficult to study with the electron microscope because of a lack of contrast. Of the commonly used electron stains lanthanum acetate followed by uranyl acetate (double stain) (Figs. 1 and 2) and ferric chloride (Figs. 3 and 4) were the best for increasing the contrast. Two types of bodies were observed in the macronucleus. The more numerous and smaller bodies are believed to be chromatin bodies (Figs. 1 and 2, *C*) because they correspond in size, frequency, and location to the Feulgen-positive bodies of light microscopy. The larger, less dense bodies (Figs. 1 and 2, *N*) might correspond to nucleoli but designation as such is not warranted without knowledge of their chemical composition. Attempts to identify these bodies in the light microscope have given inconclusive results. In squashes of whole euplotes, pyronine stains all nuclear inclusions heavily, and methyl green-pyronine staining has also failed to differentiate between the two types of bodies. Fauré-Fremiet *et al.* (4) and Roth (5) have also distinguished these two bodies. The lanthanum staining (pH 7.0) results in the precipitation of many small dark granules on or near the chromatin bodies, and few or none on the nucleolar-like bodies, nucleoplasm, cytoplasm, or mitochondria (Fig. 1).

Even at fairly high magnifications it is not possible to resolve fine details of structure in either of the nuclear bodies. Both have a granular appearance. The chromatin aggregates apparently are composed of small, tightly packed granules and of fibrils 50 to 150 A diameter, some of which project out into the nucleoplasm. Perhaps the granules represent cross-sections through the fibrils. However, the nucleolar-type bodies consist of loosely packed granules without any fibrsou component (Fig. 1). The nucleoplasm also contains occasional rows of particles that could be interpreted as sectioned helices (Figs. 1 and 2, *H*). These have no visible attachment to chromatin aggregates.

The replication band (Figs. 3 and 4) moves down the macronucleus in the direction indicated by the arrow. The chromatin bodies just ahead of the band (Fig. 3) break up into small particles that continue to disperse until they give way to an organized network of twisted microfibrils (Figs. 3 and 4, *F*) having a diameter of about 110 A. At the transition from forward zone to rear zone of the band, the 110 A fibrils abruptly disappear and the beginning of the rear zone is composed of a mass of finer and less clearly resolvable fibrils. This zone is very short and is immediately followed by a region in which the fibrils appear to be reaggregating into small chromatin bodies (Figs. 3 and 4) that continue to increase in size until they reach the standard size of 0.5 to 0.7 microns.

The less dense bodies are somewhat reduced in size and number in the forward zone of the band and in the zone of DNA synthesis in the rear zone. The nuclear membrane (*NM*, Fig. 3) remains intact while the replication band sweeps along the macronucleus.

DISCUSSION

The objective of this study was a characterization of the changes in fine structure of the *Euplotes* macronucleus at the replication band, and the observations on this region are by far the most interesting results obtained. Prescott and Kimball (2) reported that RNA synthesis is continuous in all regions of the macronucleus in which chromatin

bodies are observable. As the band advances the aggregates break up and RNA synthesis apparently ceases completely as the original material of the aggregates becomes organized into the 110 A fibrils of the band's forward zone. Perhaps these fibrils are not a new formation, but rather originate through disaggregation and straightening out of the fibrils seen in the chromatin aggregates. Evidently these fibrils are metabolically inert in this region since neither DNA, RNA, nor protein syntheses are detectable here (2). Thus, the border between the forward zone and the rear zone is a point of sharp change in *both fine structure* and *function*. The 110 A fibrils apparently subdivide into much finer fibrils and, according to the work of Prescott and Kimball (2), this is the point, and the only point, at which DNA (and probably histone synthesis) takes place. Immediately behind this very short synthesis region the old and new DNA is aggregated directly into chromatin bodies, and only then is RNA synthesis resumed (2). The aggregation of nucleoprotein into chromatin bodies takes place over a short region in which very small aggregates at the rear edge of the synthesis region gradually increase in size as they are left behind by the moving band. This reformation of aggregates coincides with a gradual increase in Feulgen and methyl green staining of DNA (2).

The macronucleus of *Euplotes* offers a unique opportunity to correlate changes in fine structure of nuclear organization—and particularly the arrangement of DNA in chromatin—with shifts in function from RNA synthesis to DNA synthesis and back to RNA synthesis. However, the intriguing problem of the fine structure of the short DNA synthesis zone remains to be answered since structures in this region were not resolved by the methods used here.

Staining studies and interference microscope measurements have shown that the DNA synthesis zone has a sharply decreased density, and the electron microscope observations indicate the basis for this. In the electron micrographs the density of the synthesis zone is very likely sufficient to account for the low average density of the zone as measured by interference microscopy methods.

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