VARIABLE PERIODICITY IN THE RHIZOPLAST OF *NAEGLERIA* FLAGELLATES

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INTRODUCTION

Flagellar rootlets, rhizoplasts, and parabasal filaments are periodically banded structures which extend into the cytoplasm from the basal bodies and kinetosomes of flagella and cilia (Pitelka, 1969). In all but a few cases the banding pattern has been assumed to be constant for the rhizoplast of a particular organism. This note reports an electron microscope study of the banding pattern of the rhizoplast of the amebo-flagellate *Naegleria gruberi*, and presents evidence suggesting that while the organelle is in the cell the period is variable. Isolation of the rhizoplast greatly diminishes this variability.

MATERIALS AND METHODS

Amebae of the NB-1 strain of *N. gruberi* were grown and transformed into flagellates by means of the methods of Fulton and Dingle (1967), except that the buffer used was 0.025 M Tricine-HCl at pH 6.8. All flagellates were transformed at 25°C and harvested 120 min after initial suspension.

In order to examine rhizoplasts *in situ*, cells were fixed in 3% glutaraldehyde in pH 7.4 buffer (0.014 M Veronal acetate containing 0.012 M NaCl and 0.001 M $CaCl_2$) for 30 min at 22°–23°C, then postfixed 60 min on ice in 1% osmium tetroxide in the same buffer. Fixed cells were dehydrated through a graded series of ethanols and embedded in Araldite 502 (Coulter, 1967). Silver and gold sections were stained with 1%

aqueous uranyl acetate for 20 min and with lead citrate for 0.5–1 min (Reynolds, 1963).

Isolated rhizoplasts were prepared as follows. Flagellates were collected by centrifugation and brought to a concentration of greater than 10^7 cells per milliliter in cold Tricine-HCl containing 0.0025 M $MgSO_4$. The suspension was transferred to a chilled 7 ml capacity Dounce homogenizer (Kontes Glass Co., Vineland, N.J.) and given 80 strokes of the pestle, lysing the cells and releasing the rhizoplasts. Differential centrifugation removed the majority of the large and small contaminants (whole cells, nuclei, membrane fragments, ribosomes, etc.), leaving partially purified rhizoplasts in the suspension. A 1 min spin at rheostat setting 5 of an International Equipment Co. Model CL centrifuge was followed by a spin of the resulting supernatant for 2 min at rheostat setting 7. This second pellet was resuspended in a small volume of buffer with $MgSO_4$ and spun again at rheostat setting 5 for 1 min, after which the supernatant contained mostly rhizoplasts. Isolated organelles were prepared for observation by placing a few drops of the suspension on a parlodion-carbon-coated grid and by carrying out negative staining with uranyl acetate (Huxley, 1963).

Observations were made with RCA-EMU-3H and Zeiss EM-9 microscopes. All measurements were made on photographic prints at an approximate magnification of 70,000. Sectioned rhizoplast periodicities were measured using a 7 × comparator eyepiece. Isolated rhizoplast periodicities were computed by measuring the distance spanned by a number of bands (at least 100) within an organelle. Calibrations were performed using a carbon grating replica having 54,800 lines per inch (Ernest F. Fullman, Inc., Schenectady, N.Y.).

RESULTS AND DISCUSSION

The rhizoplast of *N. gruberi* flagellates exhibits variable periodicity in sectioned material. This period is composed of alternating electron-opaque (dark) and electron-lucent (light) bands with no visible subperiods in either band (Fig. 1). The two organelles shown in Fig. 1 were prepared identically: one has a narrow (13.3 nm) period, the other, a wide (19.7 nm) period. Such variation was observed only between separate organelles, never within a single organelle. For the 12 rhizoplasts measured in Fig. 2, the dark component exhibits a period of 13.6 nm, with a range of 9.4–19.5 nm and a standard deviation about the mean of ±2.4. The light component has a period of 5.0 nm with

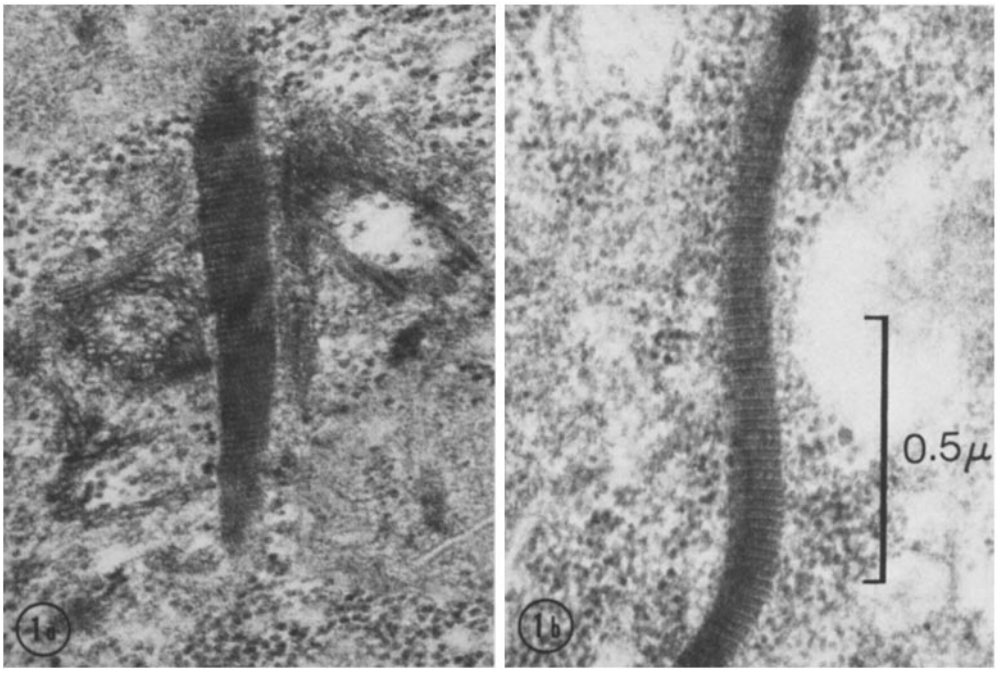

FIGURE 1 Variable periodicity of *Naegleria* rhizoplasts. The two organelles shown were fixed in Veronal acetate-buffered osmium tetroxide and are taken from cells treated identically in all respects. The rhizoplast in Fig. 1 *a* exhibits a total period (dark band plus light band) of 13.3 nm, that in Fig. 1 *b* has a total period of 19.7 nm. × 70,000.

a range of 3.8–6.9 nm and a standard deviation of ±0.9. The total period was 18.5 nm with a range of 13.8–25.2 nm and a standard deviation of ±2.9. The rhizoplasts having the widest dark bands do not necessarily have the widest light bands, and vice versa (Fig. 2). The data in this figure represent only a fraction of the measurements performed, those obtained with Veronal acetate-buffered glutaraldehyde fixation and Veronal acetate-buffered osmium tetroxide postfixation. Other fixation procedures (Veronal acetate-buffered osmium tetroxide, Tricine-buffered glutaraldehyde and postfixation with Tricine-buffered osmium tetroxide, and Tricine-buffered osmium tetroxide fixation) all gave similar results indicative of period variation.

The light and dark bands of isolated rhizoplasts cannot be easily measured due to the degree of staining (Fig. 3), and thus only the total period was measured. Measurements of 108 isolated rhizoplasts yield a mean period of 21.7 nm with a range of 20.0–23.6 nm and a standard deviation about the mean of ±0.2. Fig. 3 shows three isolated organelles with identical periods (22.0 nm)

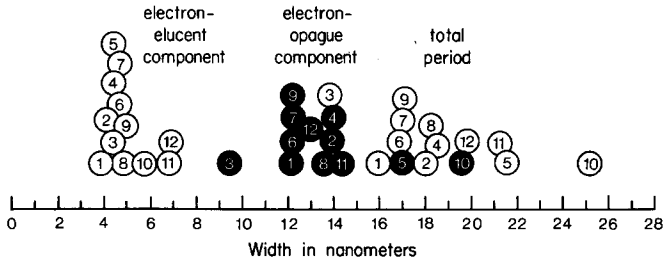

FIGURE 2 Distribution of widths of electron-lucent components, electron-opaque components, and total periods of 12 individual rhizoplasts fixed as in Fig. 1. The light bands were numbered in order of increasing width. Note that the widths of the dark bands do not correspond in order to those of the light bands.

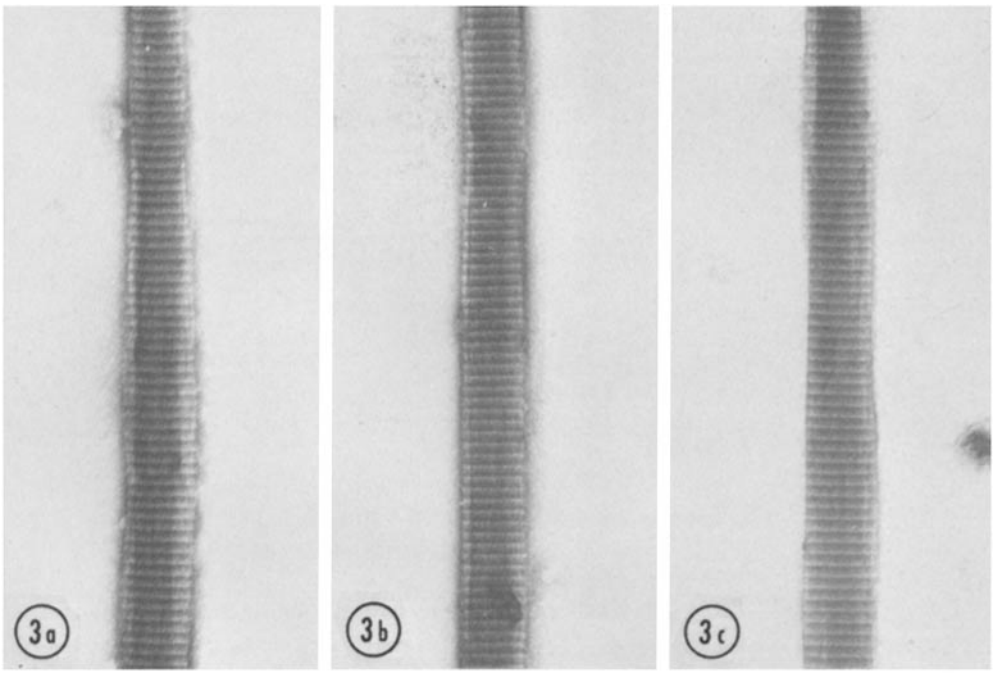

FIGURE 3 Constant periodicity of isolated, negatively-stained *Naegleria* rhizoplasts. The three isolated organelles in Figs. 3 *a*, 3 *b*, and 3 *c* exhibit identical total periods of 22.0 nm. × 70,000.

Comparison of the results of sectioned and isolated material using Bartlett's computation for the homogeneity of variance (Snedecor, 1956) gives a Chi-square value indicating that the constant periodicity of the isolated rhizoplasts and the variability of period of the sectioned organelles are different at the 5% confidence level. The difference in periodicity is shown dramatically in Fig. 4 which presents the total periods of 108 isolated and 78 fixed, sectioned rhizoplasts in frequency histograms.

The data thus confirm the preliminary observations of variable periodicity in *Naegleria* rhizoplasts (Dingle and Fulton, 1966) which conflicted with the report that the rhizoplast of *Naegleria* had a constant 18.0 nm period (Schuster, 1963). Numerous reports of measurements of periodically banded rootlets of cilia and flagella appear in the literature. In many of these, only a single value for the periodicity is given, e.g., 75 nm for the rootlets of *Anodonta cataracta* gill cilia (Gibbons, 1961), 14 nm for flagellar rootlets of the zoospore of *Oedogonium cardiacum* (Hoffman and Manton, 1962), and 26 nm for the kinetodesmal fibers of *Tetrahymena pyriformis* (Allen, 1967). In other reports, a range is given for the periodicity: 30–60 nm for the parabasal fibers of *Trichonympha* sp. (Pitelka and Schooley, 1958), 25–40 nm for fibers associated with the centriolar apparatus of the complex flagellates *Trichonympha* sp. and *Pseudotrichonympha* sp. (Grimstone and Gibbons, 1966), and 34–50 nm for the parabasal fibers of *Tritrichomonas gallinae* Rivolta (Mattern, Honigberg, and Daniel, 1967). One report briefly mentioned that the rhizoplast periodicity varies in sectioned *Tetramitus rostratus*, an amebo-flagellate closely related to *Naegleria* (Outka and Kluss, 1967). However, the authors give only average values for 10 measurements (12.0 nm period comprised of 8.0 nm wide bands and 4.0 nm narrow bands) and provide no further data. In none of the above cases in which variable periodicity was indicated did the authors comment on the nature or possible significance of such variations in periodicity.

Little information concerning the periodicities of isolated rootlets has appeared, perhaps due to the lack of isolated material. Hoffman and Manton (1962) described the rootlet of isolated flagellar apparatuses of the *O. cardiacum* zoospore as having a period of 14 nm. Pitelka (1965) and Hufnagel (1969) reported periods of 30–35 nm for isolated kinetodesmal fibers of *Paramecium multimicronucleatum* and *P. aurelia*, respectively. In the latter two cases, the maximum variation observed (15%) is no greater than that measured in our preparations of isolated rhizoplasts and does not compare at all with the 110% variation in period measured for *Naegleria* rhizoplasts in sectioned material. Thus, the large period variability measured *in situ* is greatly diminished when the rhizoplasts are isolated.

The possibility that the variations observed in *Naegleria* rhizoplast periodicity could be due to artifact is considered elsewhere (Simpson, 1970). Compression artifacts that arise during sectioning may play a role in creating period variability, but only a minor one for the following reasons. All sections are treated identically—collected on 10%

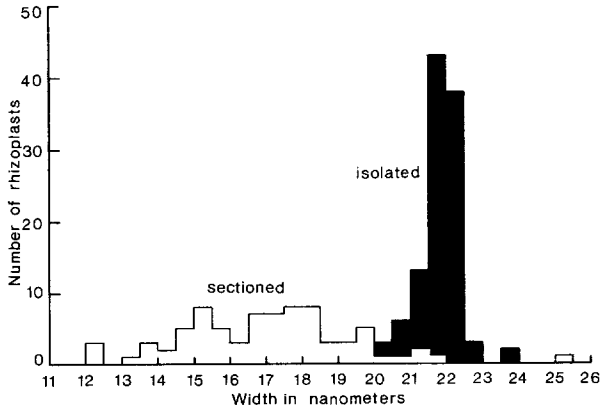

FIGURE 4 Frequency histograms showing variability of the total period of fixed, sectioned rhizoplasts and constancy of the total period of isolated rhizoplasts. The mean period for 108 isolated rhizoplasts was 21.7 nm, and that for 78 sectioned rhizoplasts, from four different fixation protocols (see text), was 17.1 nm.

acetone and stretched in chloroform vapors for 15 sec—before being picked up on grids. Secondly, single sections often contain more than one cell with a measurable rhizoplast in the same plane and orientation. Such rhizoplasts, which are presumably subject to identical compression forces, nevertheless exhibit variable periodicities. Another kind of sectioning artifact which has been described by Huxley (1957) is that due to the depth of a section taken from a regular geometric pattern (in that case, the hexagonal array of thick-thin filaments of striated muscle). That this type of artifact is unlikely in our measurements is indicated by the following consideration of the data presented in Fig. 4. The average number of bands measured for the 78 sectioned organelles was 62 per rhizoplast. In order to include 62 bands in a section through a rhizoplast of average dimensions (200 nm diameter, with a total period repeat of 20 nm), the angle of sectioning can be no greater than 10° from the long axis of the organelle. Calculations made on models of rhizoplasts (assumed to be cylindrical) indicate that the maximum variation in period possible at this angle is 3%, an amount far below the 110% maximum variation observed experimentally. Finally, since wide variations in periodicity were observed among rhizoplasts fixed, dehydrated, and embedded by identical procedures (Fig. 2), artifacts of the type described by Page and Huxley (1963) for the lengths of striated muscle filaments are eliminated as a possible cause of the observed variations. The differences in rhizoplast periodicities are thus considered to be due to factors other than preparation artifacts.

Although the function of the organelle is not known, the variable periodicity observed in sectioned intracellular rhizoplasts presumably reflects true differences in their functional states, since the cells are undoubtedly in different states of activity at the instant of fixation. Since the periodicities vary greatly *in situ*, but only slightly in isolated rhizoplasts, it is suggested that the observed variations are related to some function (either contractile or elastic) of the organelle, and that the long-period, isolated rhizoplasts are uncontracted or relaxed. Whether the variations are active or passive is unknown.

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