DIRECT COUNTS OF THE RIBOSOMES PER CELL IN THE ZOOSPORES OF BLASTOCLADIELLA EMERSONII

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Summary. The volume of the nuclear cap in a Blastocladiella emersonii zoospore was computed to be 4.8845×10^{-8} mm^3 using a median bisecting section selected from a set of serial longitudinal sections. Nuclear cap ribosomes measured 174 ± 29 Å by 202 ± 25 Å. The total number of such ribosomes, established from direct counts of ribosome population densities in thin sections, was estimated to be $8.23 \pm 1.47 \times 10^5$.

INTRODUCTION

Ongoing experimental attempts to understand the mechanism by which various organisms initiate protein synthesis are so numerous and well known as to require no documentation. The fungus Blastocladiella emersonii (1) is being employed in several laboratories (2, 3, 4) for similar developmental studies. Meaningful quantitative interpretations of results obtained with this and other organisms have sometimes been limited by the unavailability of direct estimates of a cell's total ribosome complement (as contrasted to indirect estimates of its "active" ribosomes); such direct estimates are provided here for the zoospores of B. emersonii.

METHODS

Zoospores of B. emersonii were produced from light grown plants and prepared for electron microscopy as previously described (5); thin sections were examined with Zeiss EM 9A and Phillips 300 electron microscopes.

Establishing the volume of a nuclear cap. From a continuous series of ten bilaterally symmetrical serial longitudinal sections through a zoospore, one was selected (Fig.1) as the spore bisector. Its identity as the bisector was based upon the magnitude of the nuclear cap and nuclear areas, and the presence (and disposition) or absence of the kinetosome and its adjoining rootlet (see 6 for three dimensional details), in the section selected and in several of its nearest neighbors.

The volume of the nuclear cap and nucleus, together, was measured by superimposing on the median profile (Fig. 1) equally spaced horizontal

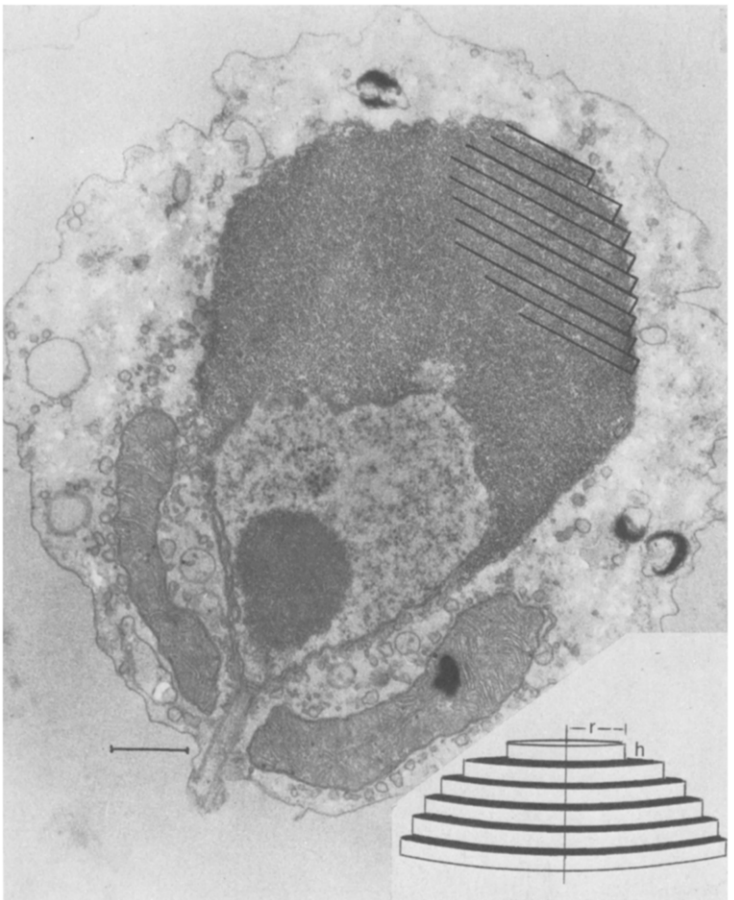

Fig. 1. Median section bisecting a zoospore and used to estimate the size of the nuclear cap. Dimensions calculated from this section were: areas of nuclear cap and nucleus, 2.0172×10^{-5} mm^2 and 0.5983×10^{-5} mm^2, respectively; volumes of nuclear cap and nucleus, 4.8845×10^{-8} mm^3 and 1.1008×10^{-8} mm^3, respectively. The bar represents 0.8072 μm.

lines, thus subdividing it totally into a vertical series of rectangles of radius $\underline{r}$ and height $\underline{h}$. These, in turn, corresponded to a series of shallow cylinders of volume $\pi \underline{r}^2\underline{h}$. From this, the volume of the nucleus was subtracted. Although nuclear profiles in these particular sections were somewhat distorted, experience suggests that the nucleus is generally symmetrical and roughly spheroidal; hence the nuclear area in Fig. 1 was measured with a mm grid, then assumed to represent a circle, its radius ($\underline{r}_1$) obtained, and the volume of the corresponding sphere deduced therefrom. The volume of the nuclear cap was then calculated:

$$\pi \underline{r}^2\underline{h} - 4/3\,\pi \underline{r}_1^3 = \text{nuclear cap volume}$$

From two usable series of serial sections, the one judged to be the most reliable (which included the section in Fig. 1) yielded a nuclear cap volume of 4.8845×10^{-8} mm^3.

Table 1.

Steps in a typical determination of the number of nuclear cap ribosomes in a zoospore of B. emersonii

1) Magnification of section to be scored for ribosomes ..... X 64600

2) Thickness of section..... 600 Å = 6×10^{-5} mm (= A)

3) Volume of nuclear cap..... 4.8845×10^{-8} mm^3 (= B)

4) Number of ribosomes counted with grid

..... $564/25$ cm^2 = 0.2256 ribosomes/mm^2 (= C)

5) Number of ribosomes/mm^2 at magnification X1

$C \cdot (64600)^2 = 9.4147 \times 10^8$ ribosomes/mm^2 (= D)

6) Number of ribosomes/nuclear cap at magnification X1

$.....(B \cdot D)/A = 7.664 \times 10^5$

7) Mean center-to-center spacing among ribosomes in population

$(D/A)^{-1/3} = 3.994 \times 10^{-5}$ mm = 399 Å

Establishing the ribosomal population density and total count in the nuclear cap. Sections (see Table 2 for thickness) through the nuclear caps of seven different zoospores were enlarged from ca 25000 to 236500 times. Areas thereon were overlaid with a transparent grid, and ribosome profiles were counted in square areas of 25 to 64 cm^2. The sizes of grids selected were large enough so that ribosome counts were minimally affected by variations in ribosome density due to clustering. From these counts, ribosome densities per unit volume, and therefrom total volume, of the nuclear cap were calculated.

RESULTS AND DISCUSSION

Table 1 illustrates the steps involved in a typical analysis. The final results obtained for the seven different zoospores are listed in Table 2. When magnified 236500 times (Fig. 2, before reduction to journal size), nuclear cap ribosomes appear to be slightly elongated, measuring 174 ± 29 Å by 202 ± 25 Å*. When treated as if it were a sphere (i.e., 188 ± 27 Å in diameter) to simplify calculations, the average ribosome occupies $3.4793 \times 10^{-15} \pm 0.0092 \times 10^{-15}$ mm^3. From their population

*) Throughout this paper, values preceded by "±" represent one standard deviation.

Table 2

Number of ribosomes in the nuclear caps of seven B. emersonii zoospores

Identifying (negative) number	Magnification on print	Ribosomes per nuclear cap*	Mean spacing (Å) (center-to-center) between ribosomes
11637	71250	6.387×10^5	425
11664	71250	6.888×10^5	414
11898	24774	9.797×10^5	368
12007	80750	7.531×10^5	402
12186	64600	7.664×10^5	399
C16-4 26/30	150000	9.452×10^5	373
C16-4 26/25	236500	9.960×10^5	366
Mean values**		$8.230 \pm 1.468 \times 10^5$	392 ± 24

*) Since all calculations had to be based upon the volume of one nuclear cap, this range of density values probably reflects, mainly, variations in the ribosome load of the nuclear caps in the different zoospores.

**) Although calculations were based upon an average section thickness of 600 A, its range was estimated as ca. 400 Å to 800 Å. The corresponding mean values for the number of ribosomes per nuclear cap at these extreme limits would have been $12.33 \pm 2.18 \times 10^{-5}$ and $6.17 \pm 1.10 \times 10^{-5}$, respectively.

densities (Table 2) and the size of the nuclear cap, it can be calculated that ribosomes occupy about 6% (outer limits, ca. 4% and 9%; see ft.nt. in Table 2) of the nuclear cap volume. Bearing in mind that all ribosomes caught at various depths in the section are in focus and therefore registered, it is evident (Fig. 2) that the apparent calculated spacing (Table 2) of the ribosome profiles is not inconsistent with this conclusion. In any case, even if ribosomes were uniformly packed as closely as possible to one another (without compression or distortion), and assuming them to be

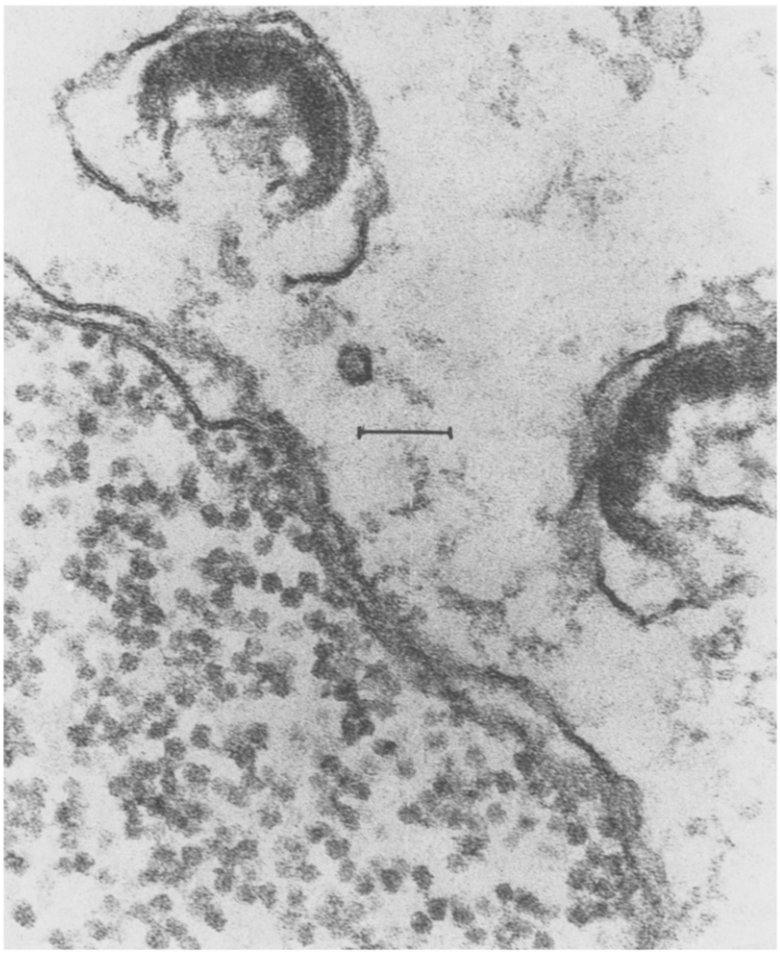

Fig. 2. Representative section through part of nuclear cap and two gamma particles; used at magnifications of 180000 and 236500 X for measurements of ribosome size. The bar represents 0.0844 μm.

spheres, they could not occupy more than about half the volume of the nuclear cap.

The present report points to the likelyhood that, with additional studies, accurate knowledge about the total ribosomes per cell of B. emersonii can be established. Its principle significance lies in the fact that, in other organisms, estimates of the number of ribosomes active in protein synthesis has generally been derived indirectly from calculations based upon a combination of measurements: total cell RNA, ribosomal

RNA as a per-cent of cell RNA, and polysomes as a per-cent of total ribosomes (7). Similarly, the number of ribosomes in a spore of B. emersonii was recently calculated (4) to be 1.17×10^6. However, not only is this approximation necessarily indirect, but its statistical variance is not known. Furthermore, the method permits only an estimation of "active" ribosomes. Knowing the total ribosomes per spore will help establish what proportion of the ribosome population becomes involved in protein synthesis*; it should also facilitate direct measurements -- as compared to indirect ones based on kinetic studies -- of the rates of protein synthesis, of ribosome attachments to mRNA, of initiation of protein synthesis, of translation, and of mobilization of monomeric ribosomes into active polyribosomes in B. emersonii.

Except for a small number of ribosome-like particles in its single mitochondrion, all of the extranuclear ribosomes in the zoospore of B. emersonii are neatly packaged together in its membrane bound nuclear cap. The fact that one can count the ribosomes in a zoospore before it is induced to encyst emphasizes yet another significant advantage that this organism offers for investigations of the mechanisms that trigger protein synthesis and the initiation of development.

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*) As a preliminary approximation, and overlooking some uncertainty about section thicknesses (ft. nt., Table 2), a comparison of our results in Table 2 with those of Leaver and Lovett (4) seems to suggest that most of the nuclear cap ribosomes may become active in protein synthesis.