PHOSPHATE INCORPORATION INTO THE SUBCELLULAR PARTICLES OF A BLEACHED EUGLENA[1]

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The production of energy for synthetic functions in the non-chlorophyll-containing Euglena gracilis var. bacillaris (streptomycin-bleached) is of considerable interest because these nonphotosynthetic organisms are able to grow and divide at a rate equal to that of normal chloroplast-containing cells if a carbon source is added to the medium (Wilson and Levedahl, 1964). This behavior raises the following questions: (1) What kind of intracellular organization in these nonphotosynthetic cells is responsible for the synthesis of macromolecules, and (2) what subcellular structures are involved in the production and utilization of different energy sources? As an initial approach to these problems, we have performed experiments to study (a) the distribution of subcellular particles from this organism according to sedimentation rate and banding density and (b) the sequential incorporation of labeled phosphate

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into these subcellular particles. Results presented here indicate that radioactive phosphate is rapidly incorporated into an acid in soluble form in subcellular fractions of Euglena, and that addition of divalent cations (magnesium) affects this incorporation.

Methods

Growth and labeling procedures. Cells of Euglena gracilis var. bacillaris (streptomycin-bleached) were grown on a modified medium (Cramer and Meyers, 1952; Padilla and James, 1960) at pH 6.8 at 25°C, with either 0.0368 M sodium succinate or 0.0368 M sodium acetate being used as the carbon source. Fifty-ml volumes of cell suspension were labeled for different time periods in physiological medium; 0.5 to 2.0 mc of inorganic ^{32}P (Reactor Division of the Oak Ridge National Laboratory) was used as a weak acid solution in HCl. The specific activity of the labeled material varied from 12 mc/ml to 8 mc/ml during the period of experimentation. At the end of each labeling interval, equal aliquots of cells were either diluted directly with 5 volumes of ice-cold phosphate buffer (0.1 M KH_2PO_4/Na_2HPO_4, pH 7.5) and centrifuged, or were made to 0.05 M with KCN to stop further incorporating activity. Cells were washed twice with 100 times their sedimented volume with fresh phosphate buffer and resuspended in Tris buffer pH 7.5 before fractionation.

Fractionation and counting procedures. Cells were disrupted in an ice-cold French Pressure Cell at 14,000 psig (lbs.-per-square-ince gauge). The homogenate was centrifuged at 500 rpm for 5 minutes in the No. 256 head of the Refrigerated International Centrifuge Model PR-2 to remove unbroken cells and larger cellular debris. The resulting supernatant was layered over a linear sucrose-density gradient and then sedimented in a B-XV zonal ultracentrifuge rotor in a Spinco Model L Centrifuge (Anderson, 1966). Sedimentation coefficients of zones in the gradient were determined by a computer program (Bishop, 1966). Collected fractions were diluted with buffer, divided, and then centrifuged for 14 hours in duplicate screw-cap tubes in the No. 30 Spinco rotor. One-tenth ml of each resuspended pellet was then adsorbed onto 12-mm discs of Whatman No. 1 filter paper and washed sequentially with trichloroacetic acid and ethanol-ether solutions (Bollum, 1959). The discs were counted in the Packard Tricarb (Model No. 574) Liquid Scintillation Spectrometer or in the Beckman Liquid Scintillation System (1650); in either case a mixture of toluene-dimethyl POPOP and PPO, as described in the Packard Tricarb operation manual, was used.

Results

Figure 1a shows an absorbance profile obtained in the absence of magnesium in the homogenizing medium and the gradient. The 30S and 50S ribosomal fractions are centered in fractions 8 and 10, respectively. The low, broad absorbance peak at the right in the profile represents membranous material that bands isopycnically in 40% to 45% sucrose. The densest portion of the cell

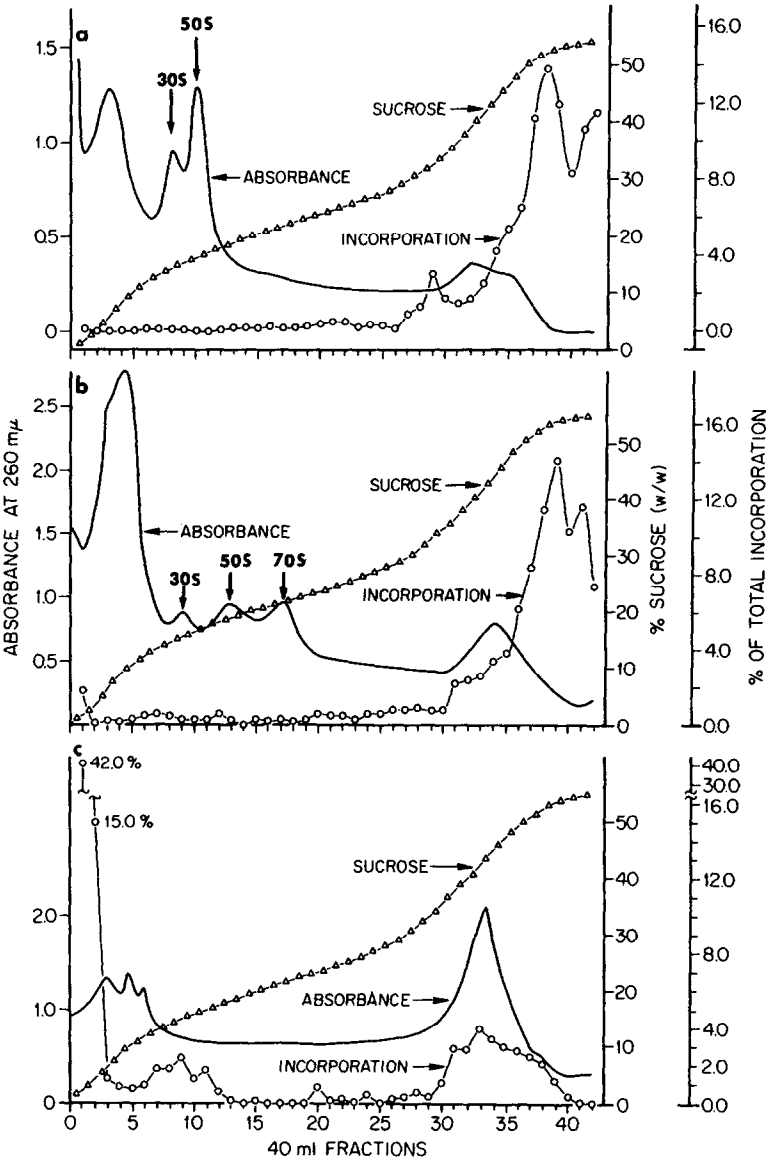

Fig. 1. Absorbance tracings of ^{32}P incorporation into subcellular particles of Euglena after pulse labeling for different periods of time. (a) Results of pulse labeling Euglena for 2 minutes in the absence of magnesium. As an example of the amount of ^{32}P that was fixed, the level of activity in fraction 38 was 12,500 detected counts per minute. (b) After 2 hours in ^{32}P with 10^{-4} magnesium present. (c) 2-hour pulse-labeling with ^{32}P with 10^{-2} M magnesium.

membrane fractions (fraction 38), contained most of the labeled phosphate.

Figure 1b shows another profile of subcellular particles pulse-labeled for 2 hours

but prepared and sedimented in a gradient containing 10^{-4} M magnesium. Here there were three ribosomal zones, 30S, 50S, and 70S, but most of the label was still in the membrane fractions. Figure 1c shows a profile of particles which were pulse-labeled for 2 hours and then prepared in 10^{-2} M magnesium. Here no discernable monomeric ribosomes appeared but there was more material in the membrane zone.

Figure 2 shows incorporation data derived from another series of experiments but plotted to show the level of radioactivity as a function of magnesium concentration. Figure 2a shows the distribution of acid-precipitable material in the starting zone as a function of both the duration of incubation and the magnesium concentration. The data show that more label was fixed when the cells are incubated and separated in (low) 10^{-4} M magnesium. The data also show that the largest amounts of label were found in this fraction after 5 to 30 minutes of incubation with ^{32}P. Figure 2b shows that more label was fixed

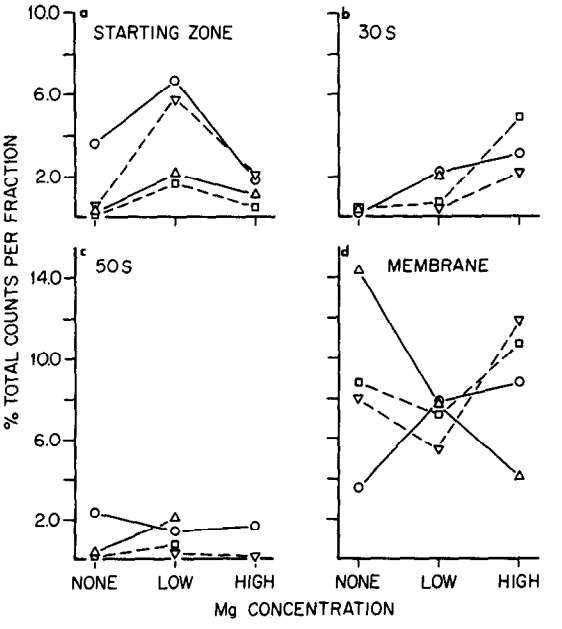

Fig. 2. The effect of magnesium ions on the percent total radioactivity (^{32}P) in various subcellular fractions. Symbols: □ — □ , 2-minute labeling periods; ▽ — ▽, 5 minutes; ○—○ , 30 minutes; and △— △ , 60-minute. The percent of total counts per fraction was obtained by comparing the amount in each subcellular fraction with the total incorporated into the total amount of material in the density gradient. High magnesium is 10^{-2}M; low is 10^{-4}M; none means no magnesium was added.

into the 30S ribosomal fraction at higher magnesium concentrations than at either 10^{-4} M or in the absence of magnesium. Figure 2c shows the effects of magnesium on ^{32}P fixation in the 50S ribosomal fraction. While low levels of activity were found in this subcellular fraction following either a 2- or 5-minute incorporation of P^{32}, somewhat more label was present after 30 minutes and is not affected appreciably by various magnesium concentrations. Figure 2d shows the effects of magnesium concentration on ^{32}P incorporation into the membrane fraction. During short time pulses, less label was fixed into fractions in the presence of 10^{-4} M magnesium than in the absence of magnesium or in higher concentrations of magnesium (10^{-2} M). In the absence of magnesium, more label was associated with the membrane fraction after 60 minutes of labeling, whereas the opposite was true for cell membranes obtained from cells grown in high magnesium (10^{-2} M).

Discussion and Conclusions

Results of this investigation indicate that: (1) The size distribution of particles obtained from Euglena resembles that of similar particles in bacterial cells (Schaechter, 1963; Schlessinger, 1963; Cline, 1966). (2) Divalent ions (magnesium) affect the integrity of the subcellular particles after cells are dissrupted. (3) Addition of magnesium affects the incorporation of labelled phosphate into subcellular particles in vivo. (4) The membrane fraction, which initially contained the most ^{32}P, loses label more rapidly with time and appears to be the most active site of synthesis. Other studies with intact Euglena cells have already shown that certain products are synthesized preferentially, depending upon which precursor molecule is available (Levedahl, 1966). While we have no information on the types of membranes in the isopycnic membrane fraction, unpublished results obtained with the aid of electron microscopy indicate that the smaller membrane fraction does not contain attached particles, whereas the larger membrane fraction (higher density) contains attached electron-dense particles. A similar distribution of isopycnically banded membrane-ribosome complexes has been reported in Escherichia coli (Cline, 1966). Additional information is now needed for Euglena to determine whether these attached particles are ribosomes in these cells and whether newly synthesized informational RNA is present.

Summary

The amount of acid-precipitable ^{32}P incorporated into subcellular fractions of Euglena depends upon both the duraction of labeling and the concentration of magnesium ion in the incubation and homogenizing medium. The site of earliest incorporation is a large-sized particulate fraction which bands isopycnically in sucrose-density gradients where membrane-ribosome complexes normally band. Label is moved or lost from this membrane fraction with time and with lowered magnesium concentrations, with label appearing simultaneously in slower-sedimenting fractions.

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