SYNTHESIS OF CHLOROPLAST RNA AT THE SITE OF CHLOROPLAST DNA

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There is increasing evidence that chloroplast RNA is synthesized within the chloroplast, coded by chloroplast DNA. Studies by Kirk (1964) on broad bean, Schweiger and Berger (1964) on Acetabularia, and Shah and Lyman (1966) on Euglena have shown that RNA synthesis occurs in isolated chloroplasts and that it is inhibited to a greater or lesser degree by DNase and actinomycin D. Kirk gives good evidence that the results he observed were not due to the presence of contaminating nuclear DNA, and in the case of Acetabularia, the plants had been enucleated 24 hours prior to the isolation of the chloroplasts. Other studies performed in vivo on enucleated plants of Acetabularia also indicate that chloroplasts contain an RNA-polymerase dependent on chloroplast DNA (Shephard, 1965; Janowski, 1965). In the present study it is shown by electron microscopic autoradiography that newly synthesized chloroplast RNA first appears over the regions of the chloroplast DNA. After labeling for 30 minutes with uridine-H^3, the labeled RNA in the chloroplast is concentrated near the chloroplast DNA, whereas after 2 hours incorporation, the labeled RNA is distributed uniformly throughout the chloroplast.

MATERIALS AND METHODS

Cells of the flagellate, Ochromonas danica, were grown in the dark and then exposed to the light as described previously (Gibbs, 1962). After 24 hours in the light, at which time the chloroplast of each cell is growing and differentiating rapidly, the cells were concentrated and re-suspended in fresh medium containing 50 μc/ml uridine-5,6-H^3 and gently shaken in the light for 30 minutes or 2 hours. The incorporation was terminated by flooding the cells with an excess of fixative (1% osmium tetroxide in acetate-veronal buffer). The fixed cells were dehydrated in ethanol and embedded in methacrylate. Uniform silver sections were coated with recently manufactured Ilford L-4 emulsion by the loop method of Caro and van Tubergen (1962) and exposed for 5 1/2 months. Control cells were fixed in 1.6% glutaraldehyde and embedded in glycol methacrylate by the method of Leduc et al (1963). Digestion of thin sections with ribonuclease (1 mg/ml) for 30 minutes at 38° C reduced the number of grains to background level.

RESULTS

The chloroplast of Ochromonas danica proved ideal for this study, since the chloroplast DNA has a restricted and easily identified location. In sections of higher plant chloroplasts (Kislev et al, 1965; Gunning, 1965) and some algal chloroplasts (Bisalputra et al, 1967), one sees scattered DNA areas which appear to be randomly distributed throughout the chloroplast matrix. It is not yet known whether these areas represent separate nucleoids or whether they are interconnected. In cross sections through the chloroplast of Ochromonas, however, one characteristically sees two DNA areas, one at each end of the chloroplast, located just inside the two or three outermost lamellar bands which loop around the end of the chloroplast. These DNA areas are easily identified in low-powered

autoradiographs by their very low electron density which contrasts with the moderate density of the remainder of the chloroplast matrix. At high magnification, 40 A fibrils are visible within these areas. Proof that these areas do indeed contain DNA has been obtained by electron microscopic autoradiography using thymidine-H^3 as precursor (Gibbs, 1967). Since every cross section through the chloroplast of Ochromonas displays a DNA area at each extremity, the chloroplast DNA in three dimensions has the shape of a cord, or ring, which encircles the periphery of the plate-like chloroplast a short distance in from its rim.

If the RNA of the chloroplast is indeed synthesized at this peripheral ring of DNA, a time sequence of electron microscopic autoradiographs after different periods of labeling should reveal an initial concentration of label at the ends of each chloroplast section, followed by a dispersion of this label throughout the chloroplast. The present results are based on labeling times of 30 minutes and 2 hours. Shorter pulses were not feasible since even a 30 minute treatment gives an average of only 5 grains per cell section after almost 6 months exposure of the photographic emulsion.

The distribution of labeled RNA within the chloroplast was determined by measuring the distance of each chloroplast grain from the center of the nearest DNA area in every chloroplast cut transversely. This was done by superimposing on the micrographs a transparent grid of concentric circles, which marked off the distance from the DNA in 1/4 μ intervals. Because of the shape of the chloroplast sections, the successive areas so delimited are roughly equal. The results obtained are summarized in Fig. 1. The solid black rectangles represent the actual number of grains counted at each distance from the nearest DNA area, up to a maximum of 2.5 μ. However, in chloroplast sections less than 5 μ long, it is obviously not possible to observe grains at distances up to 2.5 μ from the DNA. Thus the decrease in grain count with distance is partly due to the fact that not all the sections of chloroplasts analysed were 5 μ long. This can easily be

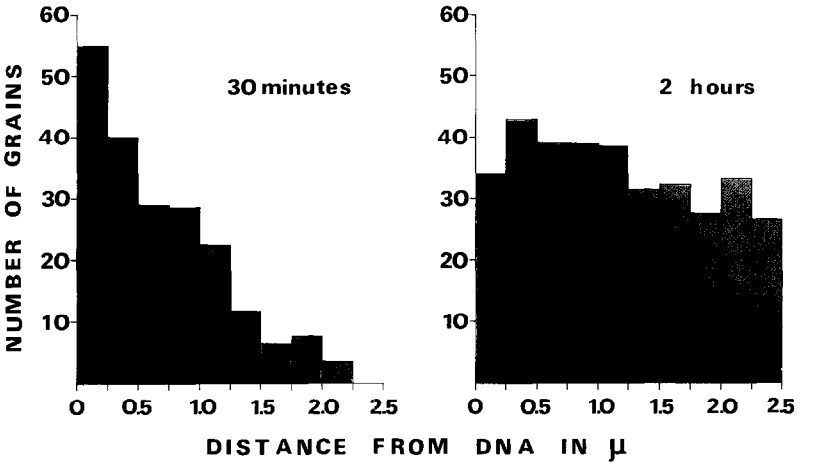

Fig. 1. Distance of the chloroplast grains from the chloroplast DNA after labeling with uridine-5,6-H^3 for 30 minutes and for 2 hours. The solid black rectangles are the actual numbers of grains observed, and the shaded rectangles represent a correction for the variation in length of the chloroplast sections as explained in the text.

corrected for, and the shaded rectangles represent the additional number of grains which would have been observed at each distance if all the chloroplasts sectioned had been 5 μ long. Looking at the normalized data in Fig. 1, one can see that the labeled RNA appears to be almost randomly distributed throughout the chloroplast after 2 hours isotope incorporation, whereas after 30 minutes labeling, there is a highly significant ($P<0.001$) concentration of labeled RNA near the DNA, with 28% of the grains actually over the DNA. It is concluded that most, if not all, chloroplast RNA is synthesized at the site of the chloroplast DNA, using this DNA as template.

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