INCORPORATION OF THYMIDINE INTO CHLOROPLASTS OF SPIROGYRA

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One area of uncertainty in a consideration of the nuclear cytoplasmic interaction in green plant cells is the possible role of nucleic acids in the synthetic activities of chloroplasts. Three methods have been used to investigate the nucleic acid content of chloroplasts: (1) ultraviolet microspectrophotometry (Frey-Wyssling, Ruch, and Berger, 1955); (2) cytological staining methods (Metzner, 1952 ; Littau, 1958); (3) chemical analysis of isolated chloroplasts (Jagendorf and Wildman, 1954 ; Sisakjan and Odintsova, 1956 ; Chiba and Sugahara, 1957). Although serious objections have been raised against each method, small but significant amounts of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) have been reported to be present in chloroplasts by Metzner (1952); Frey-Wyssling, Ruch, and Berger (1955); Sisakjan and Odintsova (1956); and Chiba and Sugahara (1957). Other investigators have failed to show the presence of appreciable amounts of nucleic acids in chloroplasts (Jagendorf and Wildman, 1954; Littau, 1958).

With the ready availability of tritiated thymidine, numerous studies have been made recently on the incorporation of this nucleoside into DNA. Reports on animal tissue (Friedkin, Tilson, and Roberts, 1956) and on meristematic tissue of plants (Taylor, Woods, and Hughes, 1957) indicate that in low concentration C^{14}-labeled and H^3-labeled thymidine is utilized effectively in the biosynthesis of DNA without appreciable diversion of radioactivity into other compounds. We have used H^3-thymidine to study the possible synthesis of DNA in chloroplasts.

Method

Actively growing Spirogyra filaments were cultured in the light in half

strength Hoagland's solution. The filaments were incubated for various lengths of time in nutrient solution containing 10 μc of H^3-thymidine per ml. (Schwarz Laboratories, Inc.). This gave a final concentration of about 3×10^{-5}M thymidine. The label was contained in the pyrimidine portion of the molecule. After exposure, the filaments were fixed in formaldehyde-propionic acid-alcohol fixative, washed repeatedly with alcohol and water, and mounted on microscope slides with Haupt's adhesive. Kodak (AR-10) autoradiographic stripping film was applied to the slides, and after a two week exposure, the slides were developed. Post development staining was made with Harris's hematoxylin.

Results and Discussion

Although the alga was not grown in sterile culture, the following observations indicate that bacterial contamination did not significantly contribute to the absorption of thymidine. Spirogyra does not have the type of sheath in which bacteria would be trapped, and a study of the prepared material under oil failed to reveal bacterial contamination. When the algal filaments were imbedded in paraffin and sectioned prior to autoradiography, the autoradiograms showed that the radioactivity was not confined to the cell wall surface as would have been the case if bacterial contamination contained the radioactivity, but the radioactivity was present in the protoplasm of Spirogyra. Similar observations of whole cells in which the protoplast had separated from the cell wall during the fixation process showed the radioactivity only in the protoplasm and not in or on the walls.

The autoradiographs showed that under the conditions of the experiment the thymidine was incorporated into the cytoplasm rather than into the nuclei of Spirogyra cells. Further, it is evident that the radioactivity is concentrated in association with the chloroplasts (Fig. 1, Table 1). The average cell surface in contact with the film was approximately 0.016 square millimeters (0.32 mm x 0.05 mm) while that of the chloroplast was approximately 0.005 square millimeters or about one third of the surface. However, the chloroplast contained between 81 to 94 per cent of the total radioactivity

found in the cell (Table 1).

Table 1

Intracellular Distribution of Radioactivity in

Spirogyra Cells Treated with H^3-thymidine

Cell No.	Incubation time in H^3-thymidine	Number of silver grains reduced in emulsion over cell part indicated			% of total activity found in chloroplasts
		Chloroplasts	Nonplastid cytoplasm	Nuclei	
1	95 hrs.	760	44	8	94
2	95 hrs.	940	140	12	86
3	22 hrs.	392	38	0	91
4	22 hrs.	418	88	9	81

The nuclei do not become preferentially labeled even after 95 hours of exposure of the filaments to thymidine.

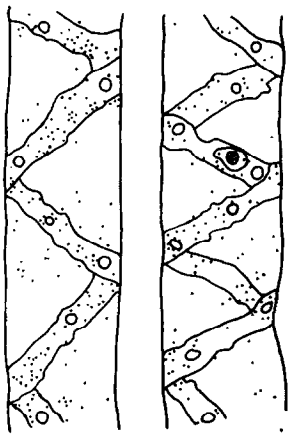

Fig. 1. Tracing of a photograph of parts of two Spirogyra cells treated with H^3-thymidine for 24 hours and then for 22 hours in unlabeled thymidine. Pyrenoids in the spiral chloroplasts are indicated as clear circles, the nucleolus is shaded with diagonal lines, and the radioactivity is indicated by small dots in the positions where silver grains appeared on the autoradiograph.

In order to test the nature of the thymidine uptake, large celled Spirogyra filaments were transferred to half strength Hoagland's solution after incubating in nutrient solution containing H^3-thymidine. The freely diffusible thymidine in the cells was determined by radioactive assay of the nutrient solution with a scintillation counter (Fig. 2). After 30 minutes the

filaments were transferred to nutrient solution containing unlabeled thymidine (3×10^{-5}M) and the exchangeable thymidine determined.

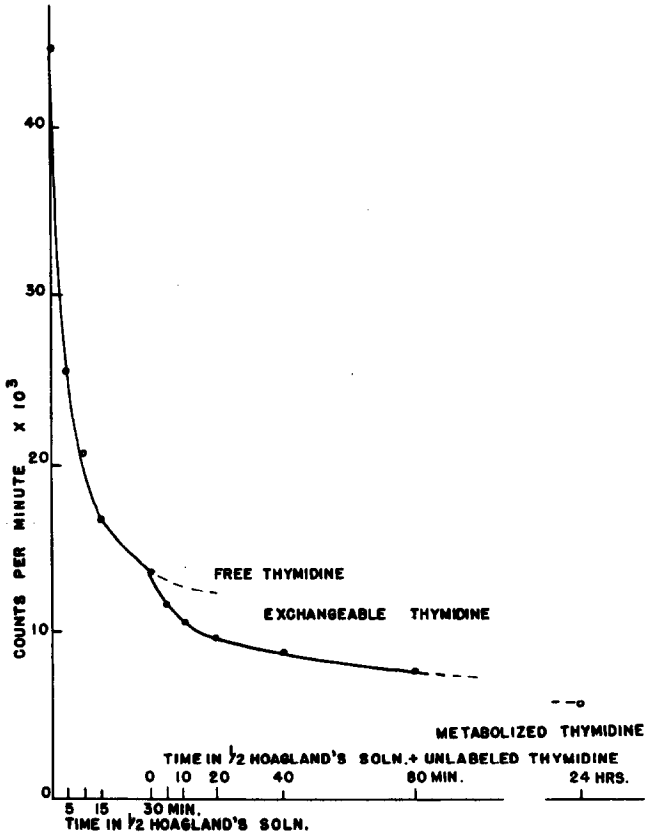

Fig. 2. Relative amounts of free thymidine, readily exchangeable thymidine, and nonexchangeable thymidine in Spirogyra treated with 10 µc H^3-thymidine per ml. for 48 hours.

Figure 2 shows that approximately half of the radioactivity that was incorporated into the filaments and not loosely held in the apparent free space was not exchangeable with unlabeled thymidine and presumably had been metabolized into nucleic acid while about half was held in a form that was not readily removed by the ions in the nutrient solution but was easily exchanged with unlabeled thymidine.

One interesting feature of Spirogyra is that it does not readily stain with the usual nuclear Feulgen stain for deoxyribose. Since this investiga-

tion was undertaken, Brachet (1959) has reported that short time exposure of the unicellular green alga Acetabularia to thymidine results in the incorporation of thymidine "into the cytoplasm (apparently in the chloroplasts)." In this alga also, no detectable amount of Feulgen positive material was found. Plaut and Sagen (1958) report that the nucleus of Amoeba proteus gives only a faintly positive Feulgen reaction and in this organism thymidine is incorporated into the cytoplasm.

The failure of the nuclei of Spirogyra to stain readily with the Feulgen test (under conditions in which nuclei of higher plant cells give a strong positive reaction) and the incorporation of thymidine into the chloroplasts rather than specifically into nuclei of Spirogyra could point to a peculiar nucleic acid metabolism in this alga.

Although the specificity of thymidine for DNA synthesis has been demonstrated in higher plants and in animals, one must use caution in regarding thymidine as an absolute specific DNA precursor when dealing with these more primitive organisms. Before one can conclude positively that DNA is specifically formed in the chloroplasts of Spirogyra further work must be carried out on the nature of the radioactive material that is accumulated in these chloroplasts when the cells are fed H^3-thymidine.

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