EVIDENCE FOR CYTOPLASMIC DNA IN
ROOT CELLS OF *NICOTIANA*

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ABSTRACT

Sterile root cultures from *Nicotiana tabacum* were grown with H^3-thymidine added to the medium for various intervals. Incorporation of the labeled nucleoside into nuclear DNA occurred in a fraction of the nuclei which increased with time. In addition, the cytoplasm of all cells incorporated enough tritium to be readily detected by autoradiography. The tritium was not removed by hydrolysis in 1 N HCl at 60°C for 10 minutes, but was removed by digestion in a DNase solution which also removed nuclear DNA. The amount of tritium in the cytoplasm increased during the first 2 hours, but did not appear to increase significantly during the following 5 hours. If the roots were transferred to unlabeled medium after 2 hours, the label was diluted faster than expected by growth without turnover of the labeled component. If FUdR was added to the unlabeled medium, the depletion occurred faster during the first 6 hours, but later appeared to level off so that at 10 hours these cultures did not differ from those incubated without FUdR. However, the addition of an excess of unlabeled carrier had no effect on the rate of depletion of the cytoplasmic label. Actinomycin D, which inhibited the incorporation of H^3-cytidine into RNA in the root tips, had no effect on the incorporation of H^3-thymidine into the cytoplasmic component. However, Mitomycin C or a high concentration of deoxyadenosine inhibited the incorporation of H^3-thymidine into the cytoplasmic component as well as into the nuclear DNA. It is concluded that H^3-thymidine is incorporated into a cytoplasmic fraction which has the characteristics of DNA, with a measurable rate of turnover. This fraction is synthesized regardless of whether or not the nucleus is synthesizing DNA. Although the function of cytoplasmic fraction is not yet known, it does not appear to be that of supplying precursors for the synthesis of the nuclear DNA.

INTRODUCTION

DNA[1] has usually been considered to be synthesized only in nuclei which are about to divide or in those which undergo chromosome replication without cell division and, therefore, attain polyteny or polyploidy. We thus assume that the amount of DNA per chromosome set remains constant throughout development and that DNA is metabolically stable. However, in developing embryos, extranuclear DNA-like substances have been reported a number of times (Hoff-Jørgensen and Zeuthen, 1952; Gregg and Løtrup, 1955; Finamore and Volkin, 1958).

In the present investigation, an autoradiographically detectable amount of H^3-thymidine was found to be incorporated into a cytoplasmic

[1] Abbreviations used: DNA(deoxyribose nucleic acid), RNA(ribose nucleic acid), DNase(deoxyribonuclease), RNase(ribonuclease), FUdR(5-fluorouracil deoxyriboside), BUdR(bromouracil deoxyriboside), and TCA(trichloroacetic acid).

fraction which has the characteristics of DNA. The turnover kinetics and the effect of inhibitors of DNA and RNA synthesis on this fraction were studied with the objective of learning more concerning its identity and possible function.

MATERIALS AND METHODS

Cultures of excised roots of *Nicotiana tabacum* were obtained from Dr. R. F. Dawson, Columbia University, New York. The roots were grown in 125-ml Erlenmeyer flasks in a sterile aqueous solution of inorganic salts, sucrose, vitamin B, and yeast extract (Dawson's modification of the medium devised by White, 1938). The cultures were kept in an incubator at 28–29°C. Under such conditions, the roots can be

way. Slides were prepared in 4 replicas by using short segments of ribbon made by cutting cross-sections of the root tips at a thickness of 4μ. One replica was stored for future use. The other three slides were dipped in xylene to remove the paraffin. They were then treated as follows:

1. Ether-alcohol (1:3) for 5 minutes at 60°C.

2. Alcohol series and 5 per cent TCA at 2–4°C for 5 minutes.

3. (a) One replica washed in cold water and stored in 70 per cent alcohol at 5°C until filmed.

(b) One replica hydrolyzed for 10 minutes in 1 N HCl at 60°C. This will remove almost all of the RNA-uracil while only 6 to 15 per cent of the DNA-thymine is lost (Taylor, 1958; Taylor and McMaster, 1954). The slide was then rinsed in water, stained
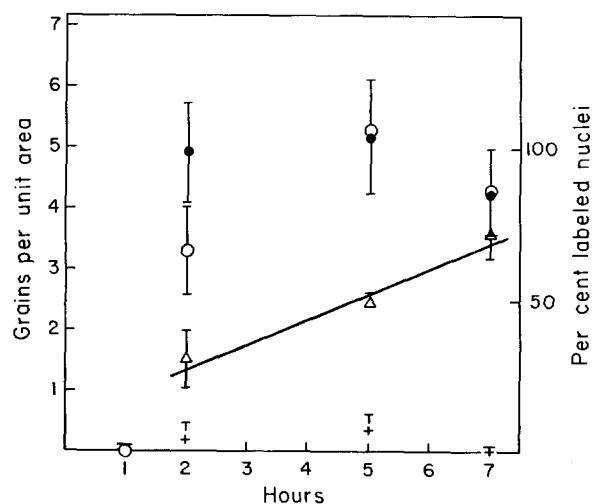

FIGURE 1 Incorporation of H^3-thymidine into the cytoplasm, as measured by number of grains per unit area, and the percentages of labeled nuclei, at different periods of incubation with H^3-thymidine. Replica slides were treated with 5 per cent TCA at 2–4°C (O), or with 1 N HCl at 60°C for 10 minutes (●); or with DNase (+). The per cent of labeled nuclei at each time interval is indicated (△) by the scale on the right.

grown at a logarithmic rate through an indefinite number of passages. The unit of transfer is a single root preferably with a side branch on it. Part of the stock solution of H^3-thymidine (sp. act. 5 c/mM) was diluted with unlabeled thymidine so that its specific activity was 500 mc/mM. This solution was always used at a concentration of 1 μc/ml. The cultures were incubated in a medium with labeled thymidine for varying periods of time. In one experiment, the roots were fixed in FAA (18 parts 70 per cent alcohol, one part glacial acetic acid, one part formalin). For other experiments, the roots were frozen in isopentane, cooled by liquid nitrogen, and dehydrated in absolute ethanol at about −50°C according to a modification (Woods and Pollister, 1955) of the method originally used by Simpson (1941). The roots were then allowed to warm up to room temperature and were fixed by heating in 75 per cent ethanol at 60°C for 1 hour. The material was then dehydrated, infiltrated, and embedded in the usual

by the Feulgen reaction, and then rinsed in water and stored in 70 per cent alcohol until filmed.

(c) One replica was digested in DNase (0.01 per cent solution in 0.005 M MgSO$_4$ containing 0.1 per cent gelatin at pH 6.6) at 37°C for 2 hours (Taylor and McMaster, 1954), followed by 3 minutes in 5 per cent TCA at 2–4°C, rinsed in water, and stored in 70 per cent alcohol until filmed. Stripping film (AR-10 Kodak) was applied to the slides according to the procedure outlined by Taylor and McMaster (1954). In all experiments, the film was exposed for 13 to 14 days and then developed according to the technique of Taylor (1960). After developing, the tissues which had not been subjected to the Feulgen reaction were stained with azure B bromide (0.025 per cent at pH 4.0 for 15 minutes).

A reticle with concentric rings was placed in the eyepiece of the microscope. The smallest circle of the reticle with a diameter of 5 μ enclosed an area of 19.64 μ^2 at 1250 magnification. This area was taken

as the unit area for grain counting. The background fog was estimated by the examination of 20 unit areas around the tissue on each slide.

In the experiment in which the cultures were treated with Actinomycin D (a gift from Merck, Sharp and Dohme Research Laboratory, West Point, Pennsylvania) and were labeled with H^3-cytidine, triplicate slides were made. Replicas a and b were treated the same way as replicas a and b described above. The third replica was digested in RNase (0.02 per cent solution in distilled water at pH 6–6.5) for 2 hours at 37°C (Kaufmann et al., 1951), followed by 3 minutes in 5 per cent TCA at 2–4°C, and then rinsed in water. The slides were stored in 70 per cent alcohol until filmed. The cells labeled with H^3-cytidine were exposed to the film for 13 days before development.

and Feulgen-stained, and the third replica was digested in DNase solution.

The number of grains per unit area was counted over the chromatin and cytoplasm of the cross-sections of the root tips. The numbers of labeled and unlabeled nuclei were also counted in each case on the Feulgen-stained slides. The grains over cytoplasm of the cells with labeled and unlabeled nuclei were counted separately to see if there was any difference in the incorporation of the tritium in the two cases. Twenty areas were counted for each treatment. At the 95 per cent confidence level, the difference between the number of grains per unit area over the cytoplasm of cells with labeled nuclei and those with unlabeled nuclei was

TABLE I

The Rate of Turnover of Cytoplasmic H^3-Thymidine Label and the Effect of FUdR or Carrier on This Turnover Rate

Time out of the labeled medium	Regular medium	Medium + carrier	Medium with 10^{-7} M FUdR
hrs.			
0	4.9 ± 0.84*	4.9 ± 0.84*	4.9 ± 0.84*
2	4.65 ± 0.88		
4	3.75 ± 0.53		
6	3.65 ± 0.59	3.25 ± 0.59	2.7 ± 0.47
8	2.2 ± 0.5		
10	1.55 ± 0.55	2.0 ± 0.57	1.7 ± 0.55
12	1.35 ± 0.53		

* The numbers represent mean number of grains per unit area ± 95 per cent confidence interval after the subtraction of the background fog.

H^3-thymidine was purchased from New England Nuclear Corp., Boston, RNase and DNase I from Worthington Biochemical Corp., Freehold, New Jersey, and BUDR from the California Corp. for Biochemical Research, Los Angeles. The Mitomycin C was a gift from Bristol Laboratories, Inc., Syracuse, New York.

RESULTS

To investigate the rate of incorporation of H^3-thymidine into the cytoplasm and the effect of acid hydrolysis and DNase digestion on the incorporated label, root cultures were incubated in medium containing H^3-thymidine (1 μc/ml). Two root tips were then fixed by freeze-substitution at each interval, 2 hours, 5 hours, and 7 hours. Triplicate slides were made of each root tip. One replica was treated with TCA at 2–4°C, another was hydrolyzed in 1 N HCl for 10 minutes at 60°C

not significantly different. The mean values are plotted in Fig. 1. At each time interval, there was no significant difference between the number of grains over the cytoplasm in the slides treated with cold TCA and those treated with 1 N HCl. On the other hand, DNase removed the tritium from both the labeled cytoplasm and the labeled nuclei (Figs. 1 to 3), which indicates that the tritium resides in DNA.

Labeled DNA was not detectable in the cytoplasm at the end of the 1st hour of incubation in the H^3-thymidine, but the amount which appeared after 2 hours did not appear to increase with time, even though the percentage of labeled interphase nuclei was increasing. In a similar experiment with FAA as a fixative, almost identical results were obtained.

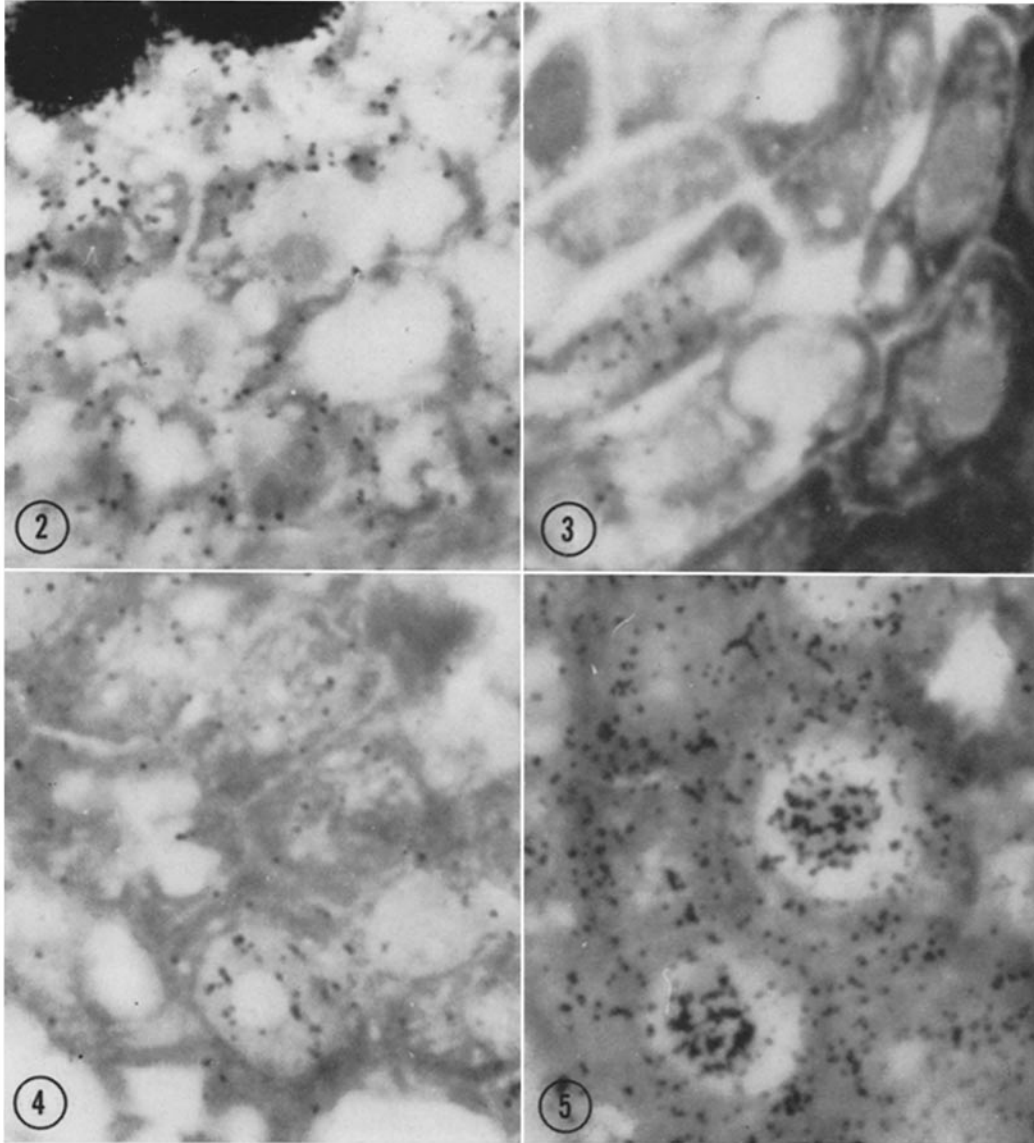That the H^3-thymidine in the medium was not degraded during incubation is indicated by the

FIGURE 2 The incorporation of H-thymidine into the cytoplasm and nuclei after 5 hours' incubation in a medium with H³-thymidine. This slide was treated with 5 per cent TCA at 2–4°C. Film exposed 14 days. × 2500.

FIGURE 3 Cells from another section of the root shown in Fig. 2. This slide was digested in DNase solution. Film exposed 14 days. × 2500.

FIGURE 4 Cells from a root tip that was incubated for 3 hours in medium that contains Actinomycin D at a concentration of 6.97 × 10^{-5} M and 2 per cent ethanol to dissolve this amount of the inhibitor. The roots were then placed in H³-cytidine for 2 hours. This slide was treated with 5 per cent TCA at 2–4°C for 5 minutes. Film exposed 13 days. × 2500.

FIGURE 5 Control roots for Actinomycin D experiment. Roots were incubated in medium containing 2 per cent ethanol for 3 hours and then H³-cytidine was added for 2 hours. This slide was treated with 5 per cent TCA at 2–4°C. Film exposed 13 days. × 2500.
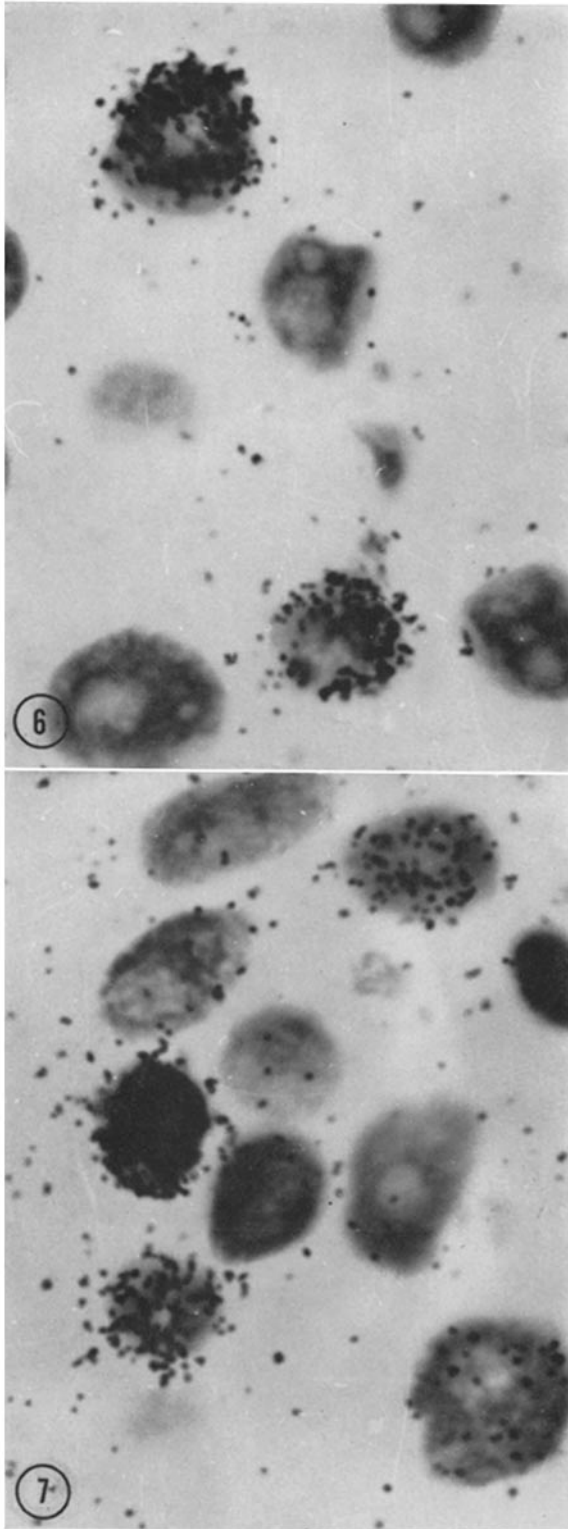
FIGURE 6 The incorporation of H^3-thymidine into the cytoplasm and the nucleus in root tips that were incubated for 3 hours in medium containing 6.97×10^{-5} M Actinomycin D and 2 per cent ethanol to dissolve this amount of the inhibitor. The roots were then placed for 2 hours in medium with H^3-thymidine. This slide was hydrolyzed in 1 N HCl at 60°C for 10 minutes and Feulgen-stained. Film exposed 14 days. × 2500.

FIGURE 7 Control roots for the above-mentioned experiment. The roots were incubated for 3 hours in medium with 2 per cent ethanol and then labeled with H^3-thymidine for 2 hours. This slide was hydrolyzed in 1 N HCl for 10 minutes and Feulgen-stained. Film exposed 14 days. × 2500.

chromatograms of samples of the medium before and after 5 hours of incubation. The distribution of radioactivity in the two chromatograms was quite similar.

To investigate the fate of the cytoplasmic component, another experiment was perfomed. The roots were labeled for 2 hours with H^3-thymidine, and then they were transferred either back to regular medium, or to a medium to which unlabeled thymidine $(2 \times 10^{-4} \, \text{M})$ was added, or to a medium containing FUdR $(10^{-7} \, \text{M})$. Two roots were fixed at each interval. The slides were hydrolyzed in 1 N HCl at 60°C for 10 minutes and stained with the Feulgen procedure. Autoradiographs were prepared and the grains per unit area over the cytoplasm of 20 cells were counted for each treatment.

medium with FUdR. However, within 10 hours the grain counts were similar in the two cases. Thus, it appears that at the beginning FUdR caused the cytoplasmic label to disappear faster. However, after 10 hours in FUdR-containing medium the difference was no longer evident.

Actinomycin D, an inhibitor for RNA synthesis, was used to test its effect on the incorporation of H^3-thymidine and H^3-cytidine into the cytoplasmic component. The cultures were incubated for 3 hours in medium containing Actinomycin D at a concentration of $6.97 \times 10^{-5} \, \text{M}$ (90 µg/ml). The medium also contained 2 per cent ethanol in order to dissolve this amount of the inhibitor (Bal and Gross, 1963). Part of the roots was then transferred to a medium with H^3-cytidine (sp. act. 360 mc/mM, and at a concentration of 1 µc/ml), and the

TABLE II
The Effect of Actinomycin D on the Incorporation of H^3-Cytidine

Extraction treatment		Grain numbers per unit area over:		
		Chromatin	Nucleolus	Cytoplasm
Cold TCA	Expt'l	1.9 ± 0.9*	0.05 ± 0.43*	0.3 ± 0.45*
	Control	6.1 ± 1.37	17.45 ± 1.94	5.7 ± 1.23
1 N HCl	Expt'l	0.95 ± 0.65	0.1 ± 0.31	0.25 ± 0.29
	Control	2.9 ± 1.72	1.0 ± 0.92	0.1 ± 0.25
RNase	Expt'l	1.2 ± 0.49	0.35 ± 0.55	0.25 ± 0.29
	Control	2.45 ± 1.86	0 ± 0.41	0.05 ± 0.22

* The numbers refer to mean number of grains per unit area ± 95 per cent confidence intervals.

Within 4 hours after removal from the H^3-thymidine, the grain count was down significantly (Table I). A theoretical point of 2.45, based on the dilution by growth after one cell generation (10 hours), was compared with the grain count obtained after 10 hours in unlabeled medium. At the 95 per cent level, there was a significant difference between the two values; i.e., the depletion of the cytoplasmic label is faster than would be expected if it were due only to cell division. In addition, the grain counts for roots transferred to regular medium after labeling and those grown in medium with carrier were quite similar. Therefore, the turnover of the cytoplasmic component did not appear to be affected by added carrier. After 6 hours there was a difference, significant at the 95 per cent level, between the grain counts for roots grown in regular medium and those grown in a

remainder was transferred to a medium with H^3-thymidine. Controls for each experiment were made by incubating two cultures in medium containing 2 per cent ethanol for 3 hours, and then labeling them with H^3-cytidine or H^3-thymidine. In all cases, the roots were incubated in labeled media for 2 hours and then fixed by freeze-substitution. The roots were sectioned at 4 µ and triplicate slides made. One replica was treated with cold TCA, another was hydrolyzed in 1 N HCl at 60°C for 10 minutes, and the third was digested wtih RNase. The labeled sections were hydrolyzed in 1 N HCl at 60°C for 10 minutes and stained by the Feulgen procedure. Autoradiographs were prepared, and grains were counted over the chromatin, nucleolus, and cytoplasm.

Actinomycin D inhibited the incorporation of H^3-cytidine into all regions of the cell (Figs. 4 to

TABLE III

The Effects of Actinomycin D and of 2 Per Cent Ethanol on the Incorporation of H³-Thymidine

	Cytoplasm	
	Mean grains/unit area ± 95 per cent confidence interval	Labeled nuclei
		per cent
Experimental (Actinomycin D)	1.9 ± 0.45	19.66 ± 3.92
Control (with 2 per cent ethanol)	2.35 ± 0.55	22.05 ± 4.7
Control (in regular medium)	4.9 ± 0.84	30.48 ± 9.6

TABLE IV

The Effect of Deoxyadenosine (5 × 10⁻³ M) on the Incorporation of H³-Thymidine

Time of exposure to H³-thymidine		Mean grains/unit area ±95 per cent confidence interval				DNase		Labeled nuclei
		Cold TCA		1 N HCL				
		Chromatin	Cytoplasm	Chromatin	Cytoplasm	Chromatin	Cytoplasm	
hrs.								*per cent*
2	Control	50 plus*	3.3 ±0.73	50 plus*	4.9 ±0.84	5.15 ±2.35	0.20 ±0.29	30.48 ±9.6
	Expt'l	9.8 ±1.86	0.25 ±0.25	10.95 ±4.31	0 ± 0.25	0.1 ±0.196	0 ± 0	14.8 ±5.88
5	Control	50 plus*	5.3 ±0.86	50 plus*	5.15 ±0.92	4.8 ±1.74	0.4 ±0.24	49.22 ±3.1
	Expt'l	17.4 ±4.12	0.55 ±0.47	18.75 ±5.49	0.85 ±0.55	0.75 ±1.0	0 ± 0.1	17.3 ±7.84
7	Control	50 plus*	4.3 ±0.69	50 plus*	4.25 ±0.73	5.9 ±1.96	−0.05 ±0.21	72.44 ±8.6
	Expt'l	19.7 ±5.68	0.35 ±0.25	18.1 ±2.94	0.75 ±0.58	0.45 ±0.43	0.1 ±0.16	26.1 ±4.9

* Grains too numerous and closely packed to count accurately.

5) but did not prevent the incorporation of H³-thymidine into either the nucleus or the cytoplasm (Tables II and III and Figs. 6 to 7). In control roots the H³-cytidine was incorporated into nucleoli faster than in any other part of the cell (Fig. 5). Since digestion with RNase or hydrolysis in 1 N HCl at 60°C for 10 minutes removed all tritium except a small fraction of that in the chromatin, the labeled component is assumed to be largely RNA, with some label in DNA. Although the Actinomycin D had no significant effect on the incorporation of H³-thymidine into the DNA of the nucleus or cytoplasm, the presence of the ethanol in the medium caused a decrease in cytoplasmic incorporation and perhaps some decrease in nuclear incorporation.

To test the effects of inhibitors of DNA synthesis on the incorporation of H³-thymidine into the cytoplasmic component, two different inhibitors were used, deoxyadenosine and Mitomycin C. The incorporation of H³-thymidine into the cytoplasm was greatly inhibited in the roots grown for 24 hours in a medium containing 5 × 10⁻³ M deoxyadenosine and then labeled for various periods

Effect of Mitomycin C on the Incorporation of H^3-Thymidine in the Nucleus and Cytoplasm in the Root Tips of Nicotiana tabacum

Cultures labeled in H^3-thymidine for 2 hours before fixation.

Time in Mitomycin C	Mean grains/unit area ± 95 per cent confidence interval		Labeled interphases
	Cytoplasm	Chromatin	
hrs.			per cent
0 (control)	4.9 ± 0.84	50 plus*	30.48 ± 9.6
3	1.15 ± 0.43	13.3 ± 3.33	15.27 ± 2.94
6	0.4 ± 0.35	8.0 ± 1.37	8.64 ± 2.35

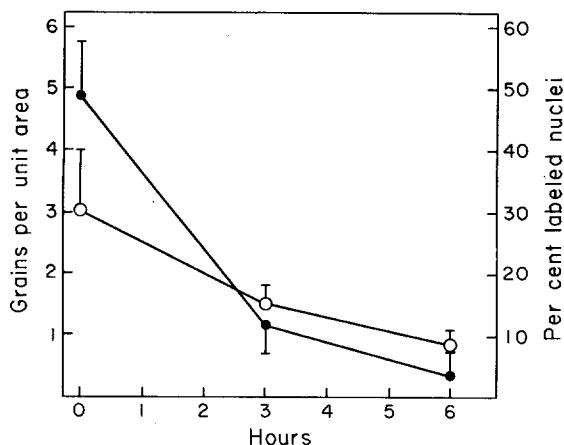* Grains too numerous and closely packed to count accurately.

FIGURE 8 Effect of different periods of incubation in medium containing Mitomycin C (0.005 per cent) on the incorporation of H^3-thymidine in the nucleus and cytoplasm. The roots were labeled with H^3-thymidine for 2 hours. (O) Frequency of labeled nuclei, (●) mean number of grains per unit area of the cytoplasm ± 95 per cent confidence interval.

of time with H^3-thymidine (Table IV). Percentages of labeled nuclei as well as the number of grains per unit area over the nuclei are significantly lower when compared with the controls.

Mitomycin C inhibited the incorporation of tritium into nuclear DNA and the cytoplasmic component when the roots were grown in a medium containing 1 μc/ml of H^3-thymidine. The grain counts per unit area over cytoplasm and nuclei are shown in Table V. The inhibitor greatly reduced the incorporation of H^3-thymidine into the nucleus and the cytoplasm within 6 hours (Fig. 8).

DISCUSSION

The tritium in the cytoplasm of cells after incubation in H^3-thymidine is neither in an acid-soluble fraction nor in RNA as was suggested by Brachet (1959), since it was not removed either by cold TCA or by hydrolysis for 10 minutes in 1 N HCl at 60°C (Taylor, 1958). Since the tritium was

removed by DNase, it is probably in a deoxyribonucleotide polymer. Two hours' incubation in the medium containing the labeled thymidine was required to bring about the accumulation of enough tritium in the polymer to be clearly demonstrated by autoradiography.

That the polymer had a rapid turnover is suggested by the fact that increasing the exposure time in the H^3-thymidine did not cause increase in the incorporation of the tritium into the cytoplasm. It is further indicated by the fact that within 4 hours after removal from H^3-thymidine the grain count went down significantly. After one generation time, the grain count was significantly less than would be expected if the decrease in the number of grains was due only to the growth and division of the cells.

If the H^3-thymidine in the cytoplasm is incorporated into a precursor for the synthesis of DNA in the nucleus, one would expect that in the presence of excess of unlabeled thymidine the nuclei might

not use the cytoplasmic precursor as fast as they would if the carrier were not present, However, this did not prove to be the case. This observation, along with the observation that the incorporation of labeled thymidine into the cytoplasm of cells whose nuclei were not synthesizing DNA occurred to the same extent as it did into the cytoplasm of cells whose nuclei were synthesizing DNA, suggests that the cytoplasmic polymer is not primarily a precursor for the synthesis of DNA in the nucleus. However, in the presence of FUdR, in an amount sufficient to inhibit the enzyme thymidylate synthetase, the cytoplasmic component was depleted faster than in cultures in which FUdR was not present. For example, in cells incubated for 6 hours in the medium containing FUdR, the amount of tritium in the cytoplasmic fraction was lower than in cells grown in regular medium. However, after 10 hours the difference had disappeared. These observations may be explained on the basis of a turnover of the cytoplasmic DNA. When thymidylate is in limited supply, it will tend to accumulate in the more stable polymer, the nuclear DNA in this case.

Actinomycin D inhibited the incorporation of H^3-cytidine into RNA in the root tips. However, it had no effect on the incorporation of H^3-thymidine. This suggests that the thymidine-containing component does not utilize a template mechanism similar to that for RNA synthesis (Hurwitz et al., 1962; Kahan et al., 1963) and/or that the template for its synthesis does not contain deoxyguanosine residues which are supposed to be the sites on DNA to which the inhibitor binds (Kersten, 1961).

Mitomycin C and deoxyadenosine at high concentrations inhibit or, at least, considerably slow down cytoplasmic as well as nuclear incorporation of H^3-thymidine. The inhibitory effect of deoxyadenosine on DNA synthesis in ascites tumor cells could be overcome by the addition of deoxyguanosine (Longer and Klenow, 1960). Deoxyadenosine triphosphate inhibits the reduction of guanylic acid to deoxyguanlyic acid (Reichard, 1960). The results thus suggest that the substance which incorporates thymidine into the cytoplasm of root tips of Nicotiana tabacum is a polymer that also contains purine bases. According to Iyer and Szybalski (1963), action of Mitomycin C is explained by the cross-linking of the two strands of the DNA double helix so that they cannot separate and replicate.

In conclusion, the results indicate that the poly-

mers in the cytoplasm into which H^3-thymidine is incorporated are double-stranded molecules of DNA having a measureable rate of turnover. They are synthesized regardless of whether or not the nucleus is in the DNA synthetic phase. Their nucleotides can be used for the synthesis of the nuclear DNA under certain condition, but their principal role is uncertain. Whether they are part of the genetic system and whether they are peculiar to certain tissues is yet to be investigated. It would also be interesting to know with which cellular organelles they are associated. Perhaps, like the thymidine-incorporating component in Amoeba proteus and cells of chick embryo, this one will be associated with particulate elements of the cell (Plaut and Sagan, 1958; Rabinovitch and Plaut, 1962; Nass and Nass, 1963).

Extranuclear DNA-like substances have been reported to exist in developing amphibian embryos (Hoff-Jørgensen and Zeuthen, 1952; Gregg and Løtrup, 1955; Finamore and Volkin, 1958). The amount of deoxynucleotides in this fraction is far in excess of that in nuclear DNA (Grant, 1958; Kuriki and Okazaki, 1959). Recently, synthesis of DNA has been reported to occur in enucleated eggs of Rana pipiens (Mezger-Freed, 1963). It was assumed that this synthesized material is a reservoir of precursors for the synthesis of nuclear DNA.

Chèvremont and Chèvremont, 1957, and Chèvremont et al., 1959 and 1960, using DNase II on animal cells in culture, were able to get a Feulgen reaction in modified mitochondria. When they used H^3-thymidine, the label appeared in the mitochondria and also in the cytoplasm. They argued that this DNA is normally used for the synthesis of DNA in the nucleus, and that DNase II blocks a step so that it accumulates in the cytoplasm in concentrations sufficient to give a detectable Feulgen reaction. The results of the present studies, however, suggest that the function of the cytoplasmic DNA in Nicotiana tabacum is not primarily that of a reservoir for synthesis of nuclear DNA.

The existence of DNA in chloroplasts has been reported a number of times, in algae as well as flowering plants (Chiba, 1951; Stocking and Gifford, 1959; Iwamura, 1962; Ris and Plaut, 1962; Baltus and Brachet, 1963). Recently Brachet and Quertier (1963) have reported a Feulgen-positive reaction in oocytes of Triturus when ethanol-HCl was used for hydrolysis instead of

aqueous HCl. Takats (1962; Takats and Smellie, 1963) concluded that the incorporation of tritium into a cytoplasmic component occurred by way of H³-thymidine rather than its degradation products in flower buds of *Lilium longiflorum* and root tips of *Vicia faba*. DNA with a rapid turnover has been reported to occur in growing tissues of higher plants (Sampson *et al.*, 1963). The base composition of this DNA appeared to differ from that of its high molecular weight counterpart (4 to 5 × 10⁶), but both fractions showed melting profiles typical of double-stranded helices. Nass and Nass (1963) have reported a fibrous component, identified by electron microscopy after heavy metal staining of sections, in mitochondria in cells of chick embryos. The fibers were destroyed by DNase.

All of these reports along with the results reported here begin to make a convincing case for the existence of DNA in various cytoplasmic organelles, but its functional role remains unclear except possibly in the chloroplast in which a genetic system has been identified.

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