This calculation indicated that the proportion of fibrinogen to fibrin incorporated into the complex was between 0.6 and 0.8 mole of fibrinogen per mole of fibrin.

The question whether fibrin-stabilizing factor might act to form "hybrid polymers" of fibrinogen and fibrin has been raised (*11*). Our results provide an affirmative answer. However, the speculation (*11*) that cross-linking action of fibrin-stabilizing factor is responsible for formation of the cold-insoluble globulin that we specifically characterized as "cryoprofibrin" proved invalid. It has been shown (*1*) that cryoprofibrin separates into component fibrinogen and fibrin when dissolved in saline. Nonseparability of the stable complex that is formed with aid of fibrin-stabilizing factor reaffirms our identification of cryoprofibrin as a labile complex.

Our previous work (*1*) has shown the significance of formation of the labile complex in endotoxin-treated rabbits. Intravascular deposition of fibrin may begin when the labile complex is elevated to threshold concentrations. It is not as yet known whether the stable complex also occurs. Formation of the stable complex is dependent on fibrin-stabilizing factor which may exist in an inactive form (*9*) in blood. Our results now show that fibrin spent in formation of the stable complex would remain soluble until the complex is acted upon by thrombin.

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Replication of Chloroplast DNA of Tobacco

Abstract. *An experimental method has been designed for determining the relative rates of replication of the chloroplast and nuclear DNA's of* Nicotiana tabacum. *By this method chloroplast DNA in week-old seedlings is being replicated several times faster than nuclear DNA.*

The presence of DNA in chloroplasts and mitochondria is now well established (*1*). In the organisms so far examined, it has been possible to show that these "satellite" DNA's have a base composition different from those of the corresponding nuclear DNA's, and therefore they can be separated from them in CsCl gradients (*2*). The evidence for cytoplasmic inheritance of chloroplasts and mitochondria, the work on chloroplast mutations in *Euglena* (*3*), and the absence of chloroplast satellite DNA in aplastidic mutants of *Euglena* (*4*) suggest strongly that the DNA of these organelles does carry genetic information which is vital to the existence and development of the organelles. In addition, there is some evidence for DNA-directed RNA synthesis in chloroplasts (*5*) and mitochondria (*6*) and for protein synthesis in chloroplasts (*7*).

It is generally believed (*8*) that the number of chloroplasts per cell in a higher plant increases as the cell matures, up to a relatively late stage of development. If chloroplast DNA is a stable carrier of essential genetic information, it should replicate when the chloroplasts or proplastids divide. It therefore should be replicating faster than the nuclear DNA. If young seedlings in which the primary leaves are still developing are pulse-labeled with P^{32} for a long enough time so that pools of nucleotide will have a chance to equilibrate, but a short enough time so that the nuclei will have gone through, on the average, less than one division, the relative specific activities of the chloroplast and nuclear DNA's at the end of this period should be equal to their relative rates of synthesis.

There are two satellite DNA's in *Nicotiana tabacum* (*9*). One of these, which forms bands at 1.706 g/cm^3 in CsCl as compared to 1.696 g/cm^3 for the nuclear DNA, is associated with the chloroplasts. The other satellite, which forms bands at 1.709 to 1.712 g/cm^3, has not been localized in the cell, but may be mitochondrial. The existence of chloroplast DNA in tobacco has been confirmed in essence by Shipp *et al.* (*10*), although their figures for its buoyant densities in CsCl do not agree with ours, nor with Marmur's value for nuclear DNA (*11*).

Since the nuclear and chloroplast DNA's of tobacco form bands close together, their densities being only 0.010 g/cm^3 apart, the two DNA's cannot be resolved in a preparative CsCl gradient unless very small amounts of DNA (~1 μg) are used, although it is possible to obtain a fraction enriched in satellite DNA by careful selection of fractions. Therefore, it is difficult to determine the absolute specific activities of the two DNA's; for example, when 1 μg of DNA is used there is not enough material in either peak to determine the optical density, and thence the weight of DNA in the test samples. It is, however, possible to determine the relative specific activities of the two DNA's by the following three-step experiment.

1) Seeds of *Nicotiana tabacum* var. Maryland were sterilized by soaking in saturated bromine water for 10 to 15 minutes (*12*); the seeds were then placed in petri plates on sterile glucose-agar (1 percent glucose, 0.4 percent agar) in the dark. After 6 to 7 days, the seedlings were 1.5 to 2 cm long and had two small, yellow, unfolded primary leaves. One millicurie of sterile, carrier-free P^{32}, in the form of the neutralized inorganic phosphate, was added to each plate of 100 to 200 seedlings. Half the plates were placed under light (1650 lu/m^2) for the 12- to 14-hour labeling period, and the other half were kept in the dark. At the end of the labeling period, the seedlings were washed off, and DNA was extracted from them by Ray and

Hanawalt's (*13*) modification of Marmur's method (*11*). One preparation was also made from seedlings labeled in the light from the 4th to the 6th day (Table 1, E).

The P^{32}-labeled seedling DNA, containing 2 to 3 percent of chloroplast DNA, was given a preliminary enrichment in a preparative CsCl gradient. The fractions on the high-density edge of the resulting single peak were combined and reduced in volume.

2) This enriched fraction was centrifuged to equilibrium in a CsCl gradient (Spinco model E analytical ultracentrifuge) to determine the relative proportions, on a weight basis, of the chloroplast and nuclear DNA's. The densities were checked by including in the sample 0.5 to 1.0 μg of DNA, from *Micrococcus lysodeikticus*, of buoyant density 1.731 g/cm^3 (*2*) as a marker. This DNA was not radioactive, so it did not contribute anything to step 3. After equilibrium had been attained (20 hours at 44,770 rev/min), ultraviolet photographs were taken at various intervals after exposure. The right hand column of Fig. 1 shows tracings (Analytrol densitometer) of the photographs of the bands. The proportions of chloroplast and nuclear DNA on an optical-density basis, and therefore a weight basis, were determined by integrating the areas under the curves of the tracings of several exposures; both a planimeter and simple square-counting were used. The results of five or six determinations agreed within 5 percent. There is no sign of the second satellite, probably because it is present only in very small amounts (*14*).

3) The sample was removed from the ultracentrifuge and mixed with a portion of purified H^3-nuclear DNA. This DNA had been isolated from seedlings grown for 2 weeks in glucose-agar containing H^3-thymidine, and it had been purified in a preparative CsCl gradient. Tris-ethylenediaminetetraacetate (EDTA), *p*H 8.6, and solid CsCl were added to give a standard volume of 7.72 cm^3 and a density of 1.70 g/cm^3. The solution was centrifuged in the Spinco SW39 rotor for 48 to 60 hours at 35,000 rev/min. Fractions (three drops each) were collected directly into brand-new scintillation counter vials, Bray's solution was added, and the H^3 and P^{32} activities were counted simultaneously (Fig. 1, left). About half the radioactivity (P^{32}, count/min) comes from the chloroplast DNA, although the chloroplast DNA is much less than half the total DNA on a weight basis. There is enough chloroplast DNA to cause a shift of the P^{32} peak away from the H^3 peak.

The radioactivity due to two species of DNA was resolved by the following calculations and a model experiment (Fig. 2). On the low-density side of the P^{32} peak, in the hatched region, all the P^{32} measured should be due to P^{32}-nuclear DNA only. Therefore, the ratio of P^{32} to H^3 for those fractions should be constant. With this ratio and the values of H^3 for all the other points on the curve, it should be possible to calculate for each fraction the P^{32} counts due to nuclear DNA, since the ratio of P^{32} (nuclear) to H^3 (nuclear) is equal to a constant, K. Therefore, for any point, P^{32} (nuclear) is equal to $K \times H^3$ (nuclear). When the calculated amount of nuclear P^{32}-DNA (count/min) is subtracted from the total P^{32} the difference is due to P^{32}-DNA of the satellite. If these values are plotted separately, the curves of Fig. 2*c* result. If the components of the satellite curve are simple Gaussian curves, with careful extrapolation, the satellite curve can be resolved into two components (Fig. 2*d*).

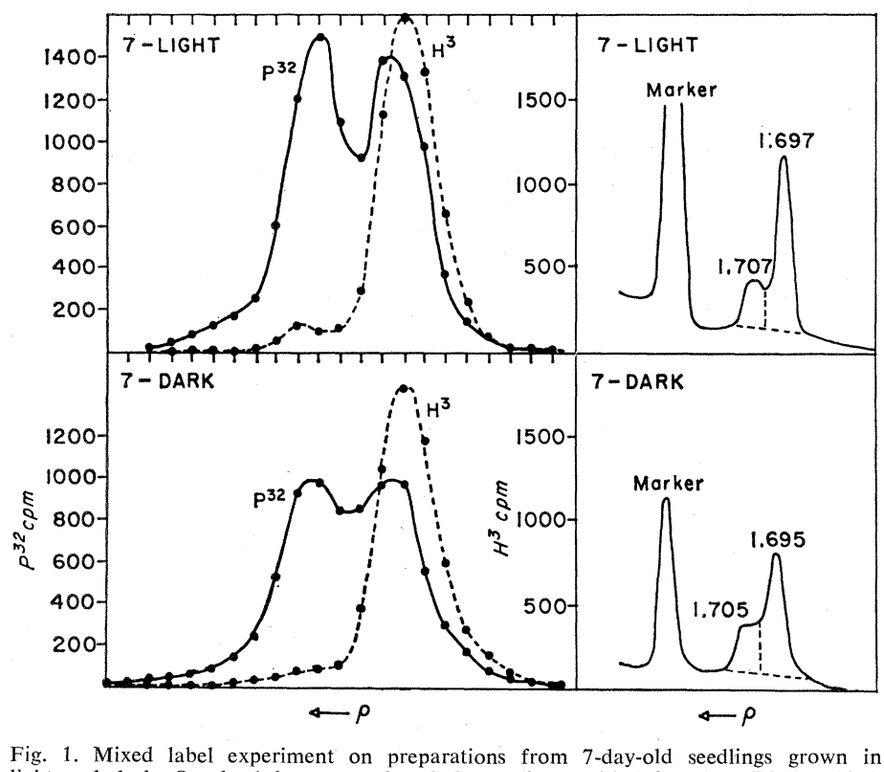

Fig. 1. Mixed label experiment on preparations from 7-day-old seedlings grown in light and dark. On the left, preparative CsCl gradients with H^3-nuclear DNA added. On the right, analytical CsCl gradients of the same sample before the addition of H^3-nuclear DNA.

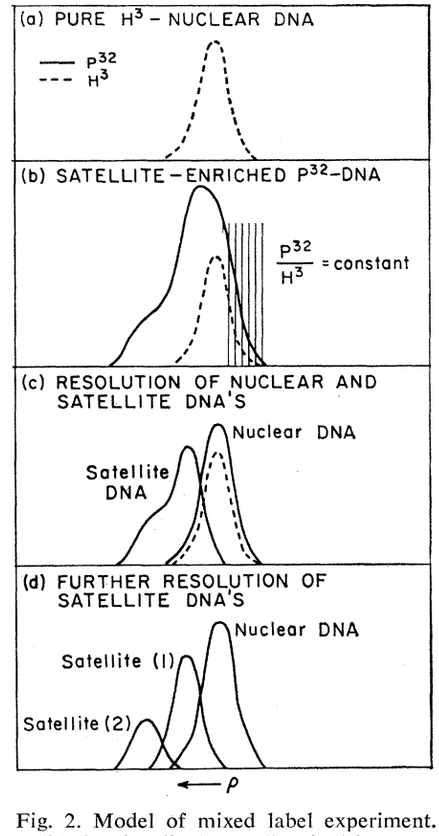

Fig. 2. Model of mixed label experiment. Solid line is P^{32}; dotted line is H^3.

Since it is not possible to calculate the absolute specific activities of the nuclear and satellite DNA's it is necessary to define the specific activities of a given DNA arbitrarily as the ratio of the percentage of the total P^{32} (count/min) to its weight (percent) determined from its optical density.

The relative specific activities of the satellite (chl) and nuclear (nuc) DNA's are then calculated from the equation:

$$\frac{\text{Specific activity chl DNA}}{\text{Specific activity nuc DNA}} = \frac{\%\ P^{32}\ (\text{chl})}{\%\ \text{by wt (chl)}} \times \frac{\%\ \text{by wt (nuc)}}{\%\ P^{32}\ (\text{nuc})}$$

Figure 3 shows the resolution of the nuclear and chloroplast DNA's. There is a trace of what may be the second satellite and one side of the satellite curve is not Gaussian. This was due to a very small amount of bacterial contamination. There were only about 10^5 bacteria per preparation (homogenate assayed before DNA extraction). This amount of bacterial contamination is too low, by several orders of magnitude, to contribute enough DNA to be detected in an analytical gradient. However, when carrier-free P^{32} is used, it can be calculated (*15*) that as little as 10^{-7} μg of fully labeled DNA could contribute noticeably to the "satellite" peak. All the bacteria from these preparations formed identical button-shaped colonies on tryptone agar and were identified as a species of *Bacillus* (*16*). A P^{32}-labeled DNA preparation was made from *Bacillus* sp., and it had a buoyant density (ρ) of 1.701 g/cm^3. It was used to calibrate the gradient along with H^3-DNA from *Escherichia coli* (ρ = 1.710 g/cm^3) and nuclear H^3-DNA (ρ = 1.696 g/cm^3) from tobacco plants. The contribution of *Bacillus* sp. was more clearly noticeable in preparations C and D (Table 1) which gave two "satellite" peaks and on which the model of Fig. 2 was based. In this case, after the gradient was calibrated, it was found that the middle peak was at the same density as *Bacillus* DNA. However, it was still possible to determine the relative specific activities of the chloroplast and nuclear DNA's by subtracting the *Bacillus* contribution from the total radioactivity (count/min). Preparations A and B were also corrected for the presence of *Bacillus* DNA.

With the possible exception of preparation E, the specific activity of the chloroplast DNA is higher than that of the nuclear DNA (Table 1), an indication that the chloroplast DNA is being replicated faster than the nuclear DNA. The low ratio of specific activities found in preparation E is consistent with this conclusion, because the specific activities of the two DNA's would approach each other after the DNA chains had each undergone a number of divisions; that is, the longer the labeling period, the lower the ratios expected. Apparently there is a difference in rates of replication be-

Table 1. Relative specific activities of chloroplast and nuclear DNA labeled with P^{32} and incubated in the light (1650 lu/m^2) or in the dark. A and B seedlings were 7 days old, C and D seedlings were 6½ days old, and E seedlings were 6 days old. The percentage of P^{32} in each fraction was judged from the number of counts per minute, and the weight was calculated from optical density. The column marked "Ratio" indicates the ratio of the specific activity of chloroplast DNA to that of nuclear DNA.

Expt.	Light	Labeling period (hr)	Chloroplast DNA		Nuclear DNA		Ratio
			P^{32} (%)	Wt. (%)	P^{32} (%)	Wt. (%)	
A	+	12	57	21	43	79	5.0
B	−	12	60	27	40	73	4.1
C	+	13	42	17	58	83	3.5
D	−	14	35	17	65	83	2.6
E	+	48	13	9.5	87	90.5	1.4

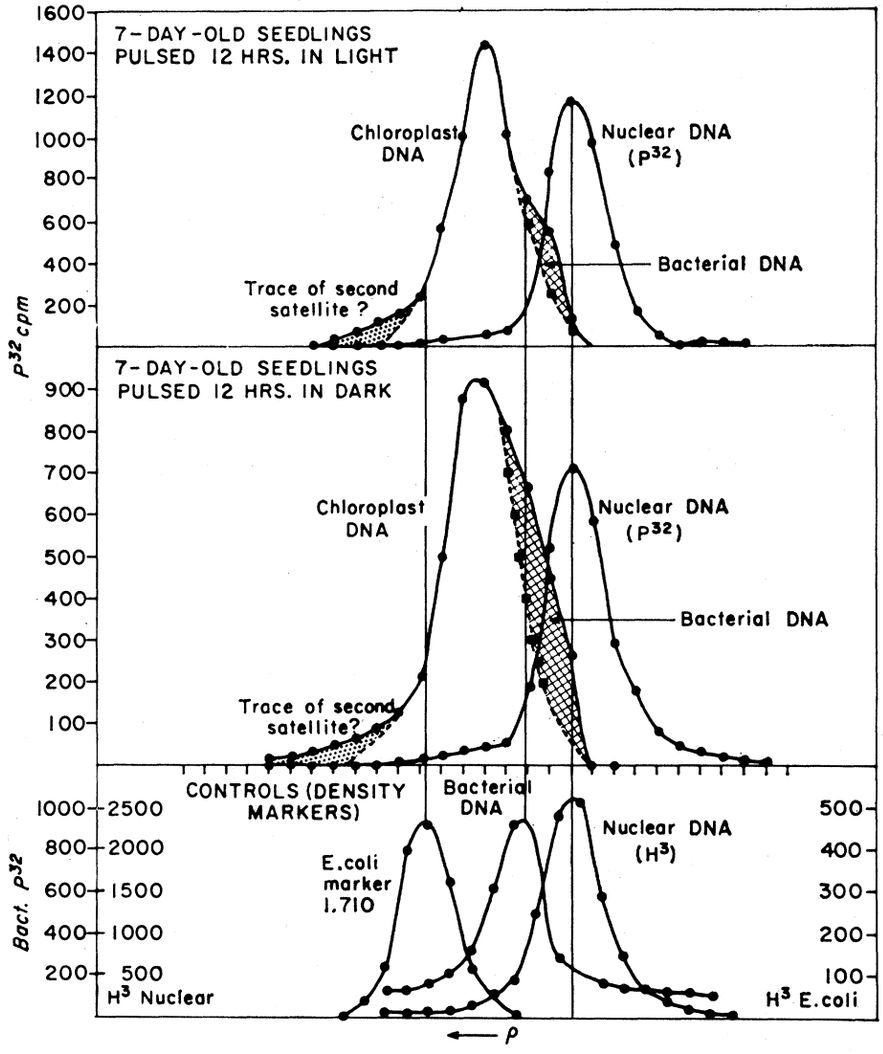

Fig. 3. (Top) Preparation A (7-day-old, light) resolved into nuclear and satellite peaks. (Middle) Preparation B (7-day-old, dark) resolved. (Bottom) Composite of calibration gradients of DNA's of known density. "Bacterial DNA" is DNA of *Bacillus* sp. with a density of 1.701 g/cm^3.

tween seedlings labeled in the light and in the dark, but the differences are not large enough to justify a definite conclusion.

There are two possible explanations for our results. The chloroplast DNA may simply be replicating faster than the nuclear DNA. This would be consistent with the hypothesis that the chloroplasts themselves are replicating, but does not prove it. On the other hand, chloroplast DNA may be turning over. From autoradiographic studies on tobacco root tips, Mourad has suggested (*18*) that there is turnover of cytoplasmic DNA, but she did not localize this DNA in a specific organelle. There have also been reports of the isolation of a rapidly labeling DNA fraction from a number of plant tissues (*19*), but unfortunately no precautions against, or assays for, bacterial contamination were reported in either paper.

Somewhat similar results have been reported by Shipp *et al.* (*10*). Using a different tobacco system, they detached small secondary leaves from the plant and labeled them with P^{32} in nutrient solution. They found almost all of the P^{32} label in a peak which formed bands in CsCl at the same density as unlabeled chloroplast DNA. They found approximately the same numbers of bacteria in their preparations as we did in ours, but they did not culture the bacteria to see if the buoyant density of the resulting DNA was the same as that of the chloroplast DNA and the majority of the P^{32} label. Considering the difficulties involved in eliminating the bacterial problem even when the seeds are sterilized with bromine water, it seems highly unlikely that leaves detached from the plant and labeled for periods up to 2 weeks would be any less likely to contain highly labeled bacterial DNA. However, the proportion of label in the chloroplast peak is decreased after 2 weeks of labeling, which would not be the case if bacterial DNA were being labeled. The ratios of the specific activities of chloroplast and nuclear DNA reported by Shipp *et al.* are much higher than those we found. In one experiment where the leaves were labeled for 6 days, approximately 80 percent of the label is in the chloroplast DNA, but it cannot be detected optically. If chloroplast DNA accounts for 2 percent of the total DNA by weight, by our calculations the ratio of specific activities for their experiment is 196. In an experiment where immature leaves were labeled for 12 hours, the ratio was 930. These differences may be a result of the differences between our two systems. The conclusion drawn from the experiments with both systems is essentially the same, namely, that chloroplast DNA in immature tissues is replicating faster than the nuclear DNA.

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Murine Leukemia Viruses: Antigenic Studies by Quantitative Complement Fixation

Abstract. *The murine leukemia viruses of Rauscher and Friend, derived from plasma of infected Balb/c mice, was purified. Their antigenic relationship was studied by quantitative complement-fixation reactions with the virion antigen and homologous antiserums. The complement-fixation curves observed in cross-reactions indicated close antigenic similarity between these two leukemia viruses. Highly purified viral preparations contained detectable amounts of host antigens.*

The interrelations of certain murine leukemia viruses have been studied by a number of investigators. On the basis of viral ultrastructure or pathology of induced leukemias these agents appear to represent a family of related viruses. Some of these agents seem to be distinct in certain immunological respects. Thus, Old *et al.* (*1*) demonstrated by cytotoxic tests that leukemias induced by Friend (*2*), Moloney (*3*), and Rauscher (*4*) (FMR) viruses are related, whereas Gross virus (*5*) leukemias showed no evidence of antigenic similarity. Moloney (*6*) demonstrated cross-neutralization between Friend and Moloney viruses but found no immunological relationship with Gross virus. On the other hand, results of Gross (*7*) seem to indicate neutralization of Moloney and Gross viruses by rabbit antiserum to Gross virus. Furthermore, immunological relations between the FMR and Gross leukemia agents were suggested by the demonstration of a common complement-fixing antigen (*8*).

We chose a highly sensitive, quantitative complement-fixation test (*9*), previously applied to the differentiation within type I polioviruses (*10*) to compare the antigenic character of Rauscher and Friend leukemia viruses purified by density gradient centrifugation. The antigens were derived from the plasma of Balb/c mice 3 to 4 weeks after intraperitoneal infection with Rauscher (*11*) or Friend virus. Mice were inoculated with extracts of spleens from infected Balb/c mice; control animals