

# Incorporation of T<sub>4</sub> Phage DNA into a Specific DNA Fraction from the Higher Plant *Matthiola incana*

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DNA uptake, Bacteriophage T<sub>4</sub> DNA, Plant Seedlings, CsCl Gradients, Hybridization

Isolated T<sub>4</sub> phage DNA ( $\rho=1.694$  g/ml) is applied to seedlings of the crucifer *Matthiola incana* (DNA density  $\rho=1.698$  g/ml). The phage DNA can partly be reextracted from the plants in a specific DNA fraction, which is predominantly characterized by its unusual high density (high density complex=HDC;  $\rho=1.724$  g/ml).

DNA:DNA hybridization studies show that phage specific DNA sequences are preserved in the HDC. Results of BrdUrd labeling of the plant DNA before and during incubation with T<sub>4</sub> DNA suggest that the HDC is composed of T<sub>4</sub> DNA and a plant DNA component of high density. The analysis of ultrasonicated HDC confirms this suggestion.

The ability of plant cells to recognize and handle T<sub>4</sub> DNA specifically is discussed.

## Introduction

Attempts to change the genetic material of plant cells by application of exogenous DNA have involved the use of recipient organisms and donor DNA from a variety of sources. Some workers have attempted to establish whether or not proteins coded for by foreign DNA are synthesized in plant cells (Carlson<sup>1</sup>, Doy *et al.*<sup>2</sup>, Johnson *et al.*<sup>3</sup>). Other investigations have been concerned with the fate of exogenous DNA at the molecular level (Anker and Stroun<sup>4</sup>, Bendich and Filner<sup>5</sup>, Hemleben *et al.*<sup>6</sup>, Hess *et al.*<sup>7</sup>, Ledoux and Huart<sup>8</sup>, Rebel *et al.*<sup>9</sup>). These studies on the uptake and subsequent fate of donor DNA in recipient cells are necessary before various phenomena observed after application of foreign DNA can be interpreted.

Biochemical and biophysical investigations on the fate of exogenous DNA have postulated that the donor DNA is integrated into the recipient plant genome. For bacterial donor DNA such an integration has been reported (Ledoux and Huart<sup>8</sup>), although recently the interpretation of these results has been questioned (Kleinhofs *et al.*<sup>10</sup>). Homologous DNA applied to *Matthiola* seedlings is preserved in the recipient plants by covalent linkage to

the plant genome (Hemleben *et al.*<sup>6</sup>). The application of phage DNA to plants has been carried out by Sander<sup>11</sup>. However, the fate of phage DNA has not yet been investigated at the molecular level despite the fact that phage DNA is less complex than bacterial or plant DNA and therefore should serve as a suitable model system for DNA uptake and integration studies.

Several experiments suggest that the DNA from transducing bacteriophages may be expressed when phage particles are applied to plants (Carlson<sup>1</sup>, Doy *et al.*<sup>2</sup>, Johnson *et al.*<sup>3</sup>), although these results have also been disputed (Smith *et al.*<sup>12</sup>). Furthermore biological expression of the introduced bacterial genes was emphasized and less attention was paid to the molecular fate of the exogenous genetic material.

Recent investigations have shown that T<sub>4</sub> phage DNA ( $\rho=1.694$  g/ml) is taken up and can be recovered in macromolecular form from seedlings of the crucifer *Matthiola incana* (Rebel *et al.*<sup>9</sup>). The heterologous DNA is reextracted from the plants (plant DNA density  $\rho=1.698$  g/ml) as a DNA complex of high density (HDC;  $\rho=1.724$  g/ml). The following paper describes experiments which attempt to elucidate the incorporation of T<sub>4</sub> DNA into the plant cell genome.

## Material and Methods

### Strains

*E. coli* B<sub>1.5</sub> and T<sub>4</sub> phages were kindly supplied by Dr. A. Klein, Heidelberg, and seeds of *Matthiola incana* by Prof. W. Seyffert, Tübingen.

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**Abbreviations:** BrdUrd, 5-bromodeoxyuridine; HDC, high buoyant density complex between DNA from *Matthiola incana* and from T<sub>4</sub>; SDS, sodium dodecyl sulfate; SSC, standard saline citrate buffer.



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### Phage DNA preparation

*E. coli* B<sub>1.5</sub> was grown in 500 ml of TG medium after Kozinski and Szybalski<sup>13</sup> to  $5 \times 10^7$  cells/ml, inoculated with T<sub>4</sub> phages (m.o.i. = 5), and the progeny labeled with 5  $\mu$ Ci/ml <sup>32</sup>P<sub>i</sub> (Amersham-Buchler). After lysis the bacterial debris was discarded, the phage particles of the supernatant were pelleted by centrifugation, purified by banding in CsCl gradients in phage buffer (0.02 M Tris, 0.15 M NaCl, 0.001 M MgCl<sub>2</sub>,  $2 \times 10^{-5}$  g gelatin per ml, pH 7.5) and washed. The phage DNA was isolated by phenol extraction and alcohol precipitation. Before application to recipient plants donor DNA was redissolved in sterile distilled water and extensively dialyzed against water. The mean specific radioactivity was  $5 \times 10^7$  cpm/mg DNA.

### Application of DNA to seedlings

Seeds of *Matthiola incana* were surface sterilized with Orthozid (Merck), washed and grown aseptically in 200  $\mu$ l of distilled water in glass tubes (3 seeds per tube) for 6 days, then incubated with DNA or DNA precursors for 24 h, washed, and grown for another 48 h in water (for detail see legends to the figures). No bacterial or fungal contamination could be detected in the culture medium or in the homogenates of the seedlings after plating on nutrient broth agar. At the end of the growth period (9 days) the seedlings were incubated three times in DNase (20  $\mu$ g/ml) and three times in pronase (50  $\mu$ g/ml), each treatment for 20 min at 37 °C. The seedlings were gently homogenized in a glass homogenizer in 0.1 M Tris-HCl–0.005 M EDTA buffer pH 7.5 containing 1% SDS. Plant DNA was isolated and purified by phenol extraction with subsequent RNase (20  $\mu$ g/ml, 2 h, 37 °C) and pronase (100  $\mu$ g/ml, 2 h, 37 °C) treatment, reextraction of DNA by shaking with a mixture of chloroform and isoamylalcohol (9:1 v/v), and precipitation by ethanol at –28 °C (Hemleben *et al.*<sup>6</sup>).

### DNA fractionation in CsCl gradients

Plant DNA was dissolved in Tris-HCl-EDTA buffer pH 7.5, mixed with crystalline CsCl to the appropriate mean buoyant density and centrifuged in a Beckman preparative ultracentrifuge to equilibrium (see legends). Every tenth fraction was analyzed for buoyant density (Zeiss refractometer) and alternate fractions were taken for optical density measurement (260 nm) and determination of acid precipitable radioactivity (Hemleben *et al.*<sup>6</sup>). For DNA : DNA hybridization and ultrasonication

studies every second fraction, which had been used for optical density measurements, was saved.

### Ultrasonication

Pooled fractions from CsCl gradients were extensively dialyzed against 1:10 diluted Tris-HCl-EDTA buffer pH 7.5 and concentrated by 30% dextran T 70 (Pharmacia), then ultrasonicated for 2 min in an ice bath (50 W, Branson sonifier).

### DNA : DNA hybridization

a) Filter preparation: Unlabeled T<sub>4</sub> phage DNA was denatured (100 °C for 10 min in 0.01  $\times$  SSC) and quickly poured into icecold 20  $\times$  SSC to give a final concentration of 6  $\times$  SSC. Each filter (nitrocellulose membrane filters, 24 mm, 0.45  $\mu$ m, Sartorius) was soaked in 6  $\times$  SSC for 30 min, washed with 5 ml 6  $\times$  SSC, and 5 ml of DNA solution (20  $\mu$ g/ml) were passed through slowly. Each filter was washed with 50 ml 6  $\times$  SSC, dried in glass scintillation vials at ambient conditions for 2 h and at 80 °C under vacuum for 4 h. Immediately before incubation with labeled DNA the filters were preincubated with 1 ml preincubation medium for 8 h at 70 °C.

b) Hybridization assay: For detection of T<sub>4</sub> homologous sequences within a CsCl gradient fractions from the gradient were dialyzed separately against 2 l of 0.01  $\times$  SSC, changing buffer three times, and concentrated by 30% dextran T 70 (Pharmacia). The fractions were then sonicated for 2 min, denatured as described above and diluted to 3  $\times$  SSC. Filters loaded with 100  $\mu$ g of unlabeled DNA were incubated for 24 h at 70 °C with 0.55 ml fractions of the radioactive DNA probe. Filters were washed with excess 0.003 M Tris-HCl buffer pH 9.4, and another 30 ml of this buffer were passed through the filters from each side (Ludwig *et al.*<sup>14</sup>). For standardization of the hybridization assay filters loaded with 100  $\mu$ g of unlabeled DNA (T<sub>4</sub>, *Matthiola*, *E. coli*) were incubated with denatured <sup>32</sup>P-labeled T<sub>4</sub> DNA of different concentrations (0.01–100  $\mu$ g) under the same conditions as described above. After drying, radioactivity bound to filters was measured in 2 ml toluene-butyl-PBD in a liquid scintillation counter (Packard). All data were corrected for <sup>32</sup>P decay.

## Results

### Characterization of the donor and recipient DNA

<sup>32</sup>P-labeled donor DNA isolated from the bacteriophage T<sub>4</sub> ( $\rho$  = 1.694 g/ml) and DNA from the recipient plant *Matthiola* ( $\rho$  = 1.698 g/ml) were

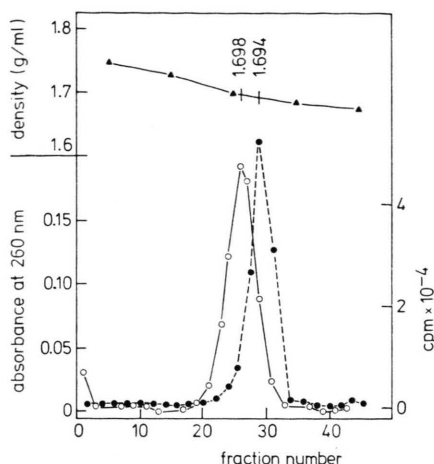


Fig. 1. Buoyant density profile of donor and recipient DNA. 86  $\mu$ g of DNA isolated from 9-day-old seedlings of *Matthiola incana* grown in distilled water is mixed *in vitro* with 40  $\mu$ g of <sup>32</sup>P-labeled T<sub>4</sub> DNA ( $2.5 \times 10^7$  cpm/mg) and fractionated on a CsCl gradient with a mean density of 1.70 g/ml. Centrifugation conditions: 45 h, 77000 $\times$ g, rotor SW 65, 20 °C. (○—○) Absorbance at 260 nm; (●—●) <sup>32</sup>P-radioactivity; (▲—▲) buoyant density.

mixed *in vitro* and characterized by fractionation on a CsCl gradient. Fig. 1 shows that both donor and recipient DNA band within a sharp peak demonstrating a high degree of purity and uniformity.

#### Uptake of T<sub>4</sub> DNA

The uptake of radioactively labeled exogenous T<sub>4</sub> DNA into seedlings of *Matthiola incana* was determined by measurement of radioactivity in the acid precipitable DNA fraction from plants treated with T<sub>4</sub> DNA. The seedlings, grown under standard conditions (see methods), were incubated for 24 h with <sup>32</sup>P-labeled T<sub>4</sub> DNA when 6 days old and grown in water for another 48 h. An extensive treatment with DNase before DNA isolation from the plants ensured that no radioactive exogenous DNA was superficially absorbed to the plants. The DNA from plants treated in this way contained radioactivity representing about 0.1% of the applied <sup>32</sup>P-labeled T<sub>4</sub> DNA: In a typical experiment 1  $\mu$ g of T<sub>4</sub> DNA with a specific radioactivity of about  $10^7$  cpm per mg of DNA was offered to one seedling, from which a DNA with a specific radioactivity of about  $3 \times 10^3$  cpm per mg of plant DNA was extractable. This regain of radioactivity is equivalent to about  $10^{-9}$  g of T<sub>4</sub> DNA per seedling or 0.05% of exogenous DNA material in a plant.

#### HDC and DNA synthesis

The DNA from plants treated with <sup>32</sup>P-labeled T<sub>4</sub> DNA as described above was fractionated on a CsCl gradient (Fig. 2). As shown previously (Rebel *et al.* <sup>9</sup>), this DNA exhibits two radioactively labeled

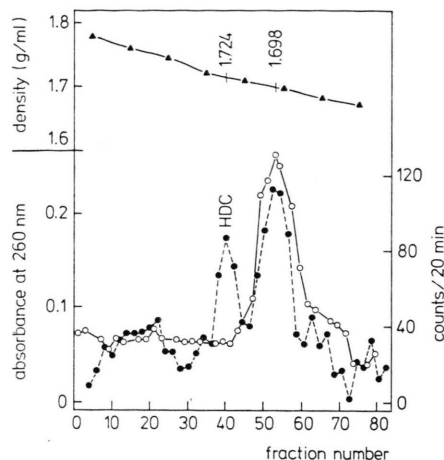


Fig. 2. Buoyant density profile of DNA from plants treated with <sup>32</sup>P-labeled T<sub>4</sub> DNA. 225  $\mu$ g of DNA isolated from 9-day-old seedlings of *Matthiola incana* are fractionated on a CsCl gradient with an mean density of 1.70 g/ml. The seedlings were incubated with <sup>32</sup>P-labeled T<sub>4</sub> DNA (20  $\mu$ g/ml,  $3 \times 10^7$  cpm/mg) for 24 h when 6 days old and grown for another 48 h in distilled water. Centrifugation conditions: 45 h, 77000 $\times$ g, rotor SW 40, 20 °C. (○—○) Absorbance at 260 nm; (●—●) <sup>32</sup>P-radioactivity; (▲—▲) buoyant density.

fractions. One of them (density about 1.698 g/ml) probably represents either native T<sub>4</sub> DNA or plant DNA labeled by reincorporation of degradation products from T<sub>4</sub> DNA. The second labeled fraction with a density of 1.724 g/ml cannot easily be classified as plant DNA or native T<sub>4</sub> DNA because of its high buoyant density. This fraction will be referred to as HDC (high density complex).

The role of DNA synthesis *de novo* in the formation of the HDC was tested by the following experiment. 6-day-old seedlings were incubated with unlabeled T<sub>4</sub> DNA and <sup>32</sup>P<sub>i</sub> simultaneously for 24 h and grown in water for another 48 h. The buoyant density profile of DNA isolated from plants treated in this way is shown in Fig. 3. No radioactivity is incorporated into a fraction with a density in the range of 1.724 g/ml, only the bulk of plant DNA ( $\rho = 1.698$  g/ml) is labeled. Therefore the HDC of the experiment in which labeled T<sub>4</sub> DNA is applied (see Fig. 2) cannot be produced by *de novo* DNA

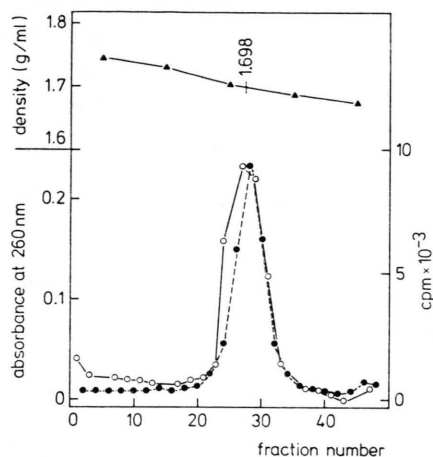


Fig. 3. Test for DNA *de novo* synthesis in the HDC. 100  $\mu$ g of DNA ( $8 \times 10^5$  cpm/mg) isolated from 9-day-old seedlings of *Matthiola incana* are fractionated on a CsCl gradient with a mean density of 1.70 g/ml. The seedlings were incubated with  $^{32}\text{P}_i$  (5  $\mu\text{Ci/ml}$ ) and unlabeled T<sub>4</sub> DNA (30  $\mu\text{g/ml}$ ) for 24 h when 6 days old and grown for another 48 h in distilled water. Centrifugation conditions: 45 h, 77000 $\times g$ , rotor SW 40, 20  $^\circ\text{C}$ . (○—○) Absorbance at 260 nm; (●—●)  $^{32}\text{P}$ -radioactivity; (▲—▲) buoyant density.

synthesis utilizing labeled degradation products of the donor DNA, but results from native T<sub>4</sub>DNA.

#### Evidence for T<sub>4</sub> DNA in the HDC by DNA : DNA hybridization

T<sub>4</sub> DNA sequences in the HDC should be detectable by membrane filter DNA : DNA hybridization. In experiments for the standardization of the hybridization assay filters loaded with 100  $\mu$ g of unlabeled T<sub>4</sub> DNA were checked for hybridization by incubation with various amounts of  $^{32}\text{P}$ -labeled T<sub>4</sub> DNA (Table I). The ratio of regain to input

Table I. Hybridization of unlabeled T<sub>4</sub> DNA on filters with increasing amounts of labeled T<sub>4</sub> DNA. Preincubated filters loaded with 100  $\mu$ g of unlabeled T<sub>4</sub> DNA were incubated with increasing amounts of  $^{32}\text{P}$ -labeled T<sub>4</sub> DNA for 24 h at 70  $^\circ\text{C}$  (mean from two experiments).

Input radio- activity [ $^{32}\text{P}$ ]T <sub>4</sub> DNA $\mu\text{g}$	cpm	cpm bound to filters			Hybridiza- tion % bound to filters
		with 100 $\mu\text{g}$ T <sub>4</sub> DNA	blank	cor- rected	
100	$10^6$	283 979	233	283 746	28
10	$10^5$	58 243	213	58 030	58
1	$10^4$	10 918	170	10 748	107
0.1	$10^3$	1 032	89	943	94
0.01	$10^2$	117	32	85	85

radioactivity was found to be constant only in the range between 0.01 and 1  $\mu\text{g}$  of  $^{32}\text{P}$ -labeled T<sub>4</sub> DNA. Maximum hybridization level (about 100% hybridization) was between 0.01 and 1  $\mu\text{g}$  as well. More than 1  $\mu\text{g}$  of offered DNA was not hybridized to the same percentage as smaller amounts, thus giving an upper limit for reasonable input DNA quantities. Control filters, loaded with 100  $\mu\text{g}$  of *Matthiola* DNA and *E. coli* DNA respectively, did not give significant hybridization levels with  $^{32}\text{P}$ -labeled T<sub>4</sub> DNA. In these control experiments maximum hybridization level was 1.3% over the complete range of 0.01 to 100  $\mu\text{g}$  of input DNA. Therefore the hybridization assay is sufficiently sensitive to detect exogenous T<sub>4</sub> DNA in the CsCl gradients (about 0.01 – 0.05  $\mu\text{g}$  of T<sub>4</sub> DNA per gradient).

In order to detect T<sub>4</sub> DNA sequences in T<sub>4</sub> DNA treated plants a CsCl gradient was loaded with 200  $\mu\text{g}$  of DNA isolated from plants which were given  $^{32}\text{P}$ -labeled T<sub>4</sub> DNA as described above. After centrifugation and analysis of the gradient for UV-absorption and radioactivity every second fraction was assayed for homology with T<sub>4</sub> DNA. In

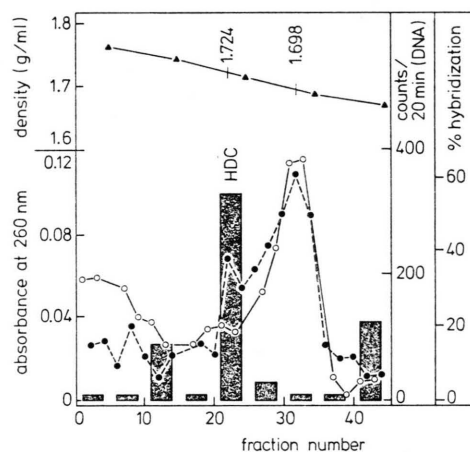


Fig. 4. Detection of T<sub>4</sub> DNA sequences in the HDC by DNA : DNA hybridization. 200  $\mu\text{g}$  of DNA ( $5 \times 10^3$  cpm/mg) isolated from 9-day-old seedlings of *Matthiola incana* are fractionated on a CsCl gradient with a mean density of 1.70 g/ml. The seedlings were incubated with  $^{32}\text{P}$ -labeled T<sub>4</sub> DNA (8  $\mu\text{g/ml}$ ,  $9 \times 10^7$  cpm/mg) for 24 h when 6 days old and grown for another 48 h in distilled water. Centrifugation conditions: 45 h, 77000 $\times g$ , rotor SW 40, 20  $^\circ\text{C}$ . Hybridization procedure: Filters loaded with 100  $\mu\text{g}$  of unlabeled T<sub>4</sub> DNA were hybridized to the DNA of two combined adjacent fractions from the CsCl gradient described above for 24 h at 70  $^\circ\text{C}$ . The amount of radioactive DNA hybridized is shown in percent of input radioactivity, calculated for every single fraction. (○—○) Absorbance at 260 nm; (●—●)  $^{32}\text{P}$ -radioactivity; (▲—▲) buoyant density; histogram, hybridization level.

Fig. 4 hybridization is shown with respect to input radioactivity. Only the HDC DNA reveals a significant homology with T<sub>4</sub> DNA, whereas the radioactive material from the main fraction of plant DNA does not hybridize with T<sub>4</sub> DNA on filters. This experiment was repeated twice with similar results.

#### A plant DNA component in the HDC

The T<sub>4</sub> DNA sequences present in the HDC must have been altered to give rise to the drastic density shift. One possible explanation for such a change in density is that a recipient plant DNA fraction of high buoyant density is involved in the formation of the HDC. This possibility may be tested by specific density labeling of plant DNA by BrdUrd before and during incubation with T<sub>4</sub> DNA. If the seedlings are grown in BrdUrd throughout the growth period, not only the bulk of plant DNA is density labeled but also the postulated plant DNA component of high density in the HDC should be labeled. Therefore the change of the HDC density to higher values after application of BrdUrd would indicate a plant DNA component in the HDC.

In the experiment BrdUrd (500 µg/ml) was applied to seedlings throughout the growth period in conjunction with a pulse of <sup>32</sup>P-labeled T<sub>4</sub> DNA for 24 h, when the seedlings were 6 days old. DNA isolated from plants which were treated in this way, exhibited a DNA density profile as shown in Fig. 5. The density of the main plant DNA fraction is shifted by 48 mg/ml to 1.746 g/ml. The density of the HDC rises as well, nearly by the same amount (45 mg/ml) to 1.769 g/ml. Therefore the T<sub>4</sub> DNA is probably associated with a plant DNA of higher density than the main band DNA.

#### High density plant DNA and T<sub>4</sub> DNA portions in the HDC

The suggestion that the HDC is composed of two different DNA species — T<sub>4</sub> DNA integrated into a plant DNA fraction — is further confirmed by analysis of ultrasonicated HDC DNA. HDC DNA from the appropriate region of a CsCl gradient from an experiment as described in Fig. 2 was ultrasonicated for 2 min and recentrifuged with unlabeled *Matthiola* DNA as marker. The radioactive labeled DNA fragments produced by this treatment exclusively band within a confined region of the CsCl gradient (Fig. 6). However, the density of these fragments is different from that of the original HDC

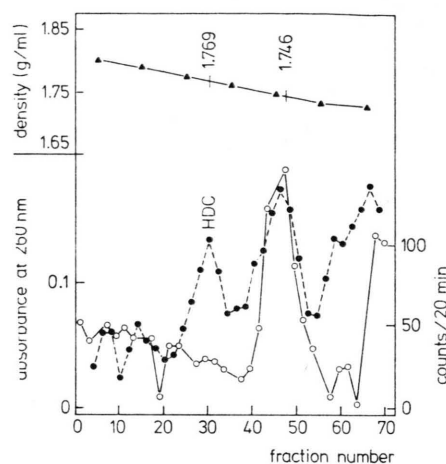


Fig. 5. Treatment of recipient plants with BrdUrd and <sup>32</sup>P-labeled T<sub>4</sub> DNA. 300 µg of DNA (2 × 10<sup>3</sup> cpm/mg) isolated from 9-day-old seedlings of *Matthiola incana* are fractionated on a CsCl gradient with a mean density of 1.74 g/ml. The seedlings were grown in a solution of BrdUrd (500 µg/ml) throughout the growth period and incubated with <sup>32</sup>P-labeled T<sub>4</sub> DNA (8 µg/ml, 5 × 10<sup>7</sup> cpm/mg) for 24 h when 6 days old and grown for another 48 h in BrdUrd solution. Centrifugation conditions: 45 h, 77000 × g, rotor SW 65, 20 °C. (○—○) Absorbance at 260 nm; (●—●) <sup>32</sup>P-radioactivity; (▲—▲) buoyant density.

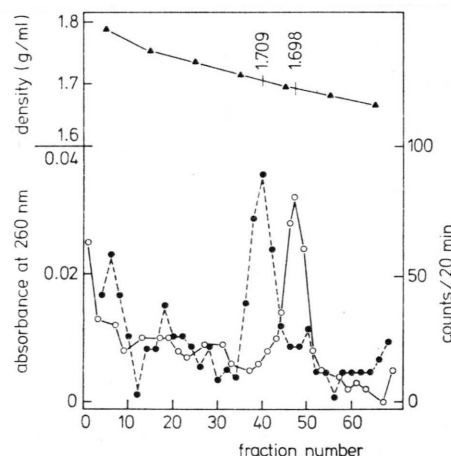


Fig. 6. Density analysis of ultrasonication products from the HDC. HDC DNA is ultrasonicated for two min and fractionated on a CsCl gradient with a mean density of 1.70 g/ml. 50 µg of unlabeled *Matthiola* DNA are included as marker DNA. The HDC DNA was recovered from a CsCl gradient loaded with *Matthiola* DNA as described in Fig. 2. Centrifugation conditions: 45 h, 77000 × g, rotor SW 65, 20 °C. (○—○) Absorbance at 260 nm; (●—●) <sup>32</sup>P-radioactivity; (▲—▲) buoyant density.

and of the native T<sub>4</sub> DNA. In this experiment the density is 1.709 g/ml, in two other experiments we found 1.713 and 1.714 g/ml, which gives a mean

density of  $1.712 \pm 0.002$  g/ml. This suggests that after ultrasonication the T<sub>4</sub> DNA still is recovered as a complex with plant DNA. The fact that the fragments band with a specific density indicates a rather constant ratio of exogenous to endogenous DNA after ultrasonication.

### Discussion

After uptake into seedlings of *Matthiola incana* exogenous T<sub>4</sub> DNA with normally nearly the same density than plant DNA is obviously modified in the recipient plant leading to a high density complex HDC (see Fig. 2). Several possible explanations for this phenomenon are discussed: Integration into the bulk of plant DNA is excluded, because this DNA and T<sub>4</sub> DNA nearly have the same density. Fractionation of the DNA from T<sub>4</sub> DNA treated plants on alkaline CsCl gradients results in an equal shift of density for plant DNA and the HDC of about 0.06 g/ml each (Rebel *et al.*<sup>9</sup>). The high density of the complex cannot be due to RNA association, because RNA is unstable in alkaline CsCl gradients. In addition, conformational changes of the phage DNA should lead to different shifts in neutral and alkaline assays. From these evidences, covalent linkage of double stranded T<sub>4</sub> DNA to a DNA fraction of high density is suggested.

Some DNA fractions in CsCl gradients from radioactively labeled plant DNA with higher density than the main DNA band are considered to be due to fast replicating satellite DNA or bacterial contamination (Kleinhofs *et al.*<sup>10</sup>). In our system this can be excluded by the experiment described in Fig. 3. Incubation of the seedlings with unlabeled phage DNA and <sup>32</sup>P<sub>i</sub> simultaneously does not produce a radioactive HDC. This fraction is only detectable, when labeled phage DNA is applied to the seedlings. Therefore this fraction cannot be newly formed by bacterial or plant DNA synthesis. The radioactive material from the exogenous DNA must have been incorporated into the HDC without being extensively degraded.

The DNA : DNA hybridization evidence for phage specific sequences in the HDC supports this interpretation. Within the density range of the HDC in a CsCl gradient ( $\rho = 1.724$  g/ml) there is a significant specific hybridization level with T<sub>4</sub> DNA (see Fig. 4) indicating the presence of T<sub>4</sub> DNA sequences, whereas the bulk of plant DNA ( $\rho = 1.698$  g/ml) does not show any homology to phage DNA. There-

fore the radioactivity in the main plant DNA fraction results from reutilization of some degraded T<sub>4</sub> DNA into plant specific sequences.

It is suggested that the T<sub>4</sub> DNA sequences are integrated into a DNA of high buoyant density. The evidence that this DNA is of plant origin is given by BrdUrd labeling of the recipient plants. The thymidine analogue alters not only the density of the main plant DNA, but also rises the density of the HDC (see Fig. 5). This indicates that a plant DNA component binds T<sub>4</sub> DNA sequences thus forming the HDC.

The results of the analysis of the HDC DNA by ultrasonication are compatible with the participation of a plant DNA in the formation of this fraction. After ultrasonication the radioactivity of the HDC is found in a new position in CsCl gradients (see Fig. 6) corresponding to a density of about 1.712 g/ml. This is quite different from the densities of the original T<sub>4</sub> donor DNA ( $\rho = 1.694$  g/ml) and the unsonicated HDC ( $\rho = 1.724$  g/ml). Since the hybridization results show the presence of T<sub>4</sub> DNA sequences in the HDC, the intermediate density of the ultrasonication products must be due to plant DNA which remains attached to T<sub>4</sub> DNA even after ultrasonication. This result implicates that the integrated T<sub>4</sub> DNA sequences are essentially smaller than the size of the ultrasonication products.

There are two possibilities for the mode of integration of T<sub>4</sub> DNA in the HDC. Random interspersal within the plant DNA should result in ultrasonication products with different amounts of T<sub>4</sub> DNA and plant DNA because of the double stranded covalent linkage (Rebel *et al.*<sup>9</sup>). After recentrifugation such fragments would band as a broad peak. However, the results show a sharp peak (see Fig. 4) with a comparatively constant buoyant density. This would only be compatible with random interspersal, if sites in the DNA are present which are preferentially sensitive to ultrasonication.

An alternative more probable explanation is that *in vivo* small segments of T<sub>4</sub> DNA are specifically attached to the ends of GC rich regions of plant DNA, which do exist in plant chromosomes (Timmis *et al.*<sup>15</sup>). After DNA extraction, which reduces the molecular weight of the DNA to about  $3 \times 10^6$ , a single T<sub>4</sub> DNA segment would be present at the end of a larger GC rich plant DNA fragment. Under these assumptions the size and molecular weight of the integrated DNA can be calculated:

The density of the ultrasonication products ( $\rho = 1.712$  g/ml) is composed of the T<sub>4</sub> DNA density 1.694 g/ml and the unknown density of a plant DNA component  $\rho_{pl}$ . During ultrasonication the molecular weight of the HDC (about  $3 \times 10^6$ ) is reduced to  $5 \times 10^5$ , equal to about 17% of the intact HDC molecule. Assuming that each molecule of the HDC has bound just one exogenous DNA fragment, the following two equations can be formulated:

$$a \times \rho_{fr} + (1 - a) \times \rho_{pl} = \rho_{HDC} [\text{g/ml}] \quad (1)$$

$$\rho_{fr} = y \times \rho_{T_4} + (1 - y) \times \rho_{pl} [\text{g/ml}] \quad (2)$$

where  $a$  represents the portion of one ultrasonication fragment in the HDC (0.17),  $y$  the portion of T<sub>4</sub> DNA in the ultrasonication fragments, and  $\rho_{T_4}$ ,  $\rho_{pl}$ ,  $\rho_{HDC}$ ,  $\rho_{fr}$  are the densities of T<sub>4</sub> DNA (1.694), plant component DNA, HDC DNA (1.724), and of the ultrasonication fragments (1.712) respectively.

The solution yields the two unknowns  $y = 0.42$  and  $\rho_{pl} = 1.725$  g/ml. Hence the radioactively labeled ultrasonication products consist of 42% T<sub>4</sub> DNA and 58% plant DNA of the density 1.725 g/ml. This corresponds to portions of 7% T<sub>4</sub> DNA

and 93% plant DNA in the intact HDC molecules, representing about 350 base pairs of exogenous DNA integrated. These calculations give a conception about the size of T<sub>4</sub> DNA integrated. These data also indicate that the amount of plant DNA involved in the formation of the HDC molecules is very small and therefore the plant DNA itself cannot be analyzed by conventional CsCl technique.

Experiments on *Matthiola* seedlings demonstrate that there is obviously no general mechanism for incorporation of exogenous DNA from different sources into plant DNA. Homologous donor DNA is integrated into the main fraction of *Matthiola* DNA (Hemleben *et al.*<sup>6</sup>), whereas T<sub>4</sub> DNA is preserved in the plant cells specifically bound to a plant DNA component of high buoyant density. Bacterial DNA from different species tested in this system is totally degraded and reutilized in the plant DNA synthesis (Gradmann-Rebel<sup>16</sup>). Therefore it is suggested that cells from *Matthiola incana* do discriminate DNA from different sources. Nucleases recognizing specific sequences may be involved here.

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