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Fate of T_4 Phage DNA in Seedlings of *Matthiola incana*

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After uptake of native T_4 phage DNA in seedlings of *Matthiola incana* this DNA is integrated as a double-stranded fraction with higher density than the unaltered phage DNA and the bulk of plant DNA.

It is known in bacteria, that under certain circumstances genetic information in the form of isolated DNA molecules can be taken up, incorporated and translated. For higher organisms such as plants the uptake of donor DNA is proven, and its integration into the plant genome has been traced [1–3]. According to the results of Ledoux *et al.* the exogenous DNA of bacteria with a higher density than the plant DNA is bound by covalent linkages. This results in a hybrid DNA fraction of intermediate density.

The present paper forms a discription and discussion of the behaviour of phage donor DNA of the same density as the recipient DNA in plant cells. The recipient organism was the annual crucifer *Matthiola incana* with a DNA density of 1.698 g/cm³, chosen (with an eye to future investigations) because of its well defined genetic system of flower pigmentation. The donor DNA used was T_4 phage DNA, which using [^{32}P] orthophosphate can be specifically highly labeled; its density of 1.694 g/cm³ lies within the density values of the recipient DNA.

Maximum uptake of [^{32}P]-labeled T_4 DNA occurred in 6 days old *Matthiola* seedlings. The plants were treated for 24 hours under sterile conditions with [^{32}P]-labeled T_4 DNA; the seedlings were then thoroughly rinsed in water and grown for a further 48 hours. The isolated DNA from seedlings thus treated was characterized on neutral and alkaline CsCl gradients.

Fig. 1 a shows the distribution on a neutral CsCl gradient. A maximum in radioactivity occurs at an unexpectedly high density of 1.724 g/cm³, in an area where the profile of optical density exhibits no related ultraviolet absorption. The shoulder in the radioactivity curve above the main peak for *Matthiola* DNA can be the result of either the incorporation of T_4 DNA fragments into the plant DNA or of unaltered phage DNA.

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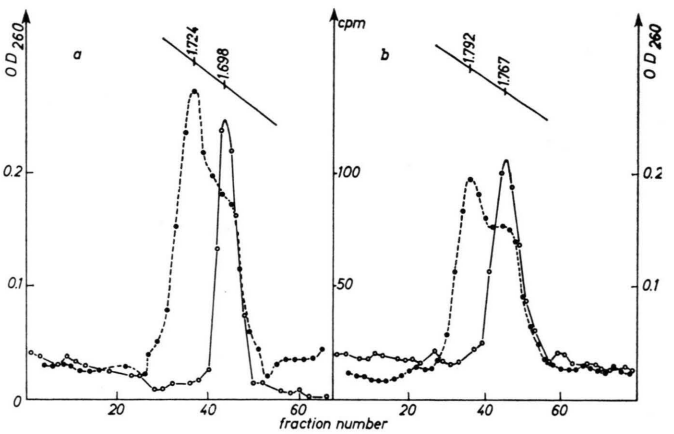

Fig. 1. CsCl gradient centrifugation of DNA from 9 days old seedlings of *Matthiola*. 6 Days old seedlings were incubated with [^{32}P]-labeled T_4 DNA ($1.7 \cdot 10^5$ cpm/plant) for 24 hours, thoroughly washed and grown for a further 48 hours in sterile water. After treatment with DNAase and pronase the whole plants were homogenized. DNA was isolated by the phenol method followed by RNAase treatment and separated on CsCl gradients (conditions of centrifugation: 50 000 rpm, 24 hours, 20 °C, rotor SW 65, Spinco). About 60–70 fractions of three drops each were collected; to every second fraction carrier RNA was added, the radioactive material precipitated with trichloracetic acid and counted on membrane filters. The optical density of the samples was measured in 1 ml buffer at 260 nm. The data of density were received by refractometer measurements and calculated from *Matthiola* DNA ($\varrho = 1.698$ g/cm³) as reference. a. Centrifugation in 2.5 ml neutral CsCl solution with a final density of 1.7 g/cm³. b. Centrifugation in 3.0 ml alkaline CsCl solution with a final density of 1.79 g/cm³ (pH 12.5). ○–○ Optical density at 260 nm; ●--● [^{32}P] radioactivity.

The distribution of the DNA from T_4 DNA treated seedlings on an alkaline CsCl gradient gives the same distribution of radioactivity and optical density with a uniform density shift of about 68 mg/cm³ (Fig. 1 b). It thus follows, that all components of this DNA preparation must have been double-stranded. The higher density fraction which recurs after incubation of the seedlings with T_4 DNA must therefore also be double-stranded DNA.

On the question of its origin it remains to clarify whether this DNA fraction results from native phage DNA or from the specific incorporation of T_4 DNA fragments into a more densely banded plant DNA fraction. In order to resolve this point [^{32}P]-labeled T_4 DNA was degraded with DNAase to oligonucleotide size. 6 Days old *Matthiola* seedlings were incubated for 24 hours with this preparation and then grown for a further 48 hours. The DNA isolated from these seedlings exhibits, after centrifugation in a neutral CsCl gradient, a similar radioactivity distribution pattern to that obtained for *Matthiola* seedlings grown under the same conditions after treatment with [^{32}P] orthophosphate (Figs. 2 a and 2 b). The bulk of the radioactivity now coincides with the *Matthiola* DNA at a density of 1.698 g/cm^3. The weak [^{32}P] radioactivity shoulder, which at a density of 1.714 g/cm^3 indicates a second DNA fraction, probably represents a plant satellite DNA [4]. The questionable DNA fraction with the high density of 1.724 g/cm^3 (Fig. 1 a) does not appear here. It occurs solely after incubation of plants with native phage DNA.

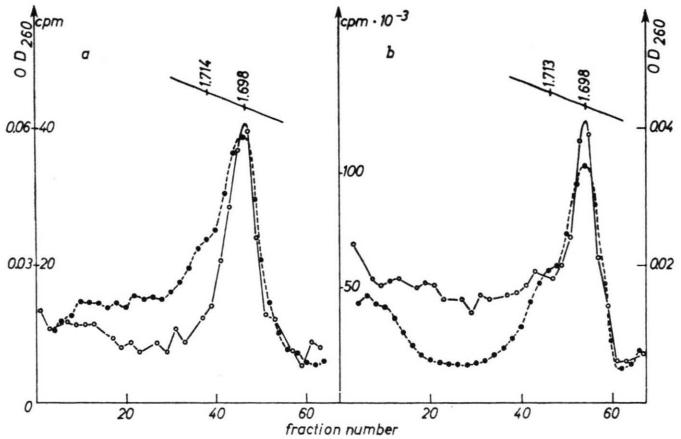

Fig. 2. CsCl gradient centrifugation of DNA from 9 days old seedlings of *Matthiola*. a. 6 Days old seedlings were incubated with [^{32}P]-labeled T_4 DNA ($4.35 \cdot 10^4$ cpm/plant) degraded by DNAase treatment. After 24 hours the seedlings were thoroughly washed and grown in sterile water for a further 48 hours. b. 7 Days old seedlings were incubated with [^{32}P] orthophosphate (5 μCi/plant) for 48 hours. Isolation of the DNA from the whole plants was done as described in the legend to Fig. 1. Neutral CsCl gradient centrifugation was carried out under the following conditions: 32 500 rpm, 45 hours, 20 °C, rotor SW 65, Spinco. Measurements of optical density, radioactivity and density were done as described for Fig. 1. ○—○ Optical density at 260 nm; ●--● [^{32}P] radioactivity.

Alteration in the density of phage DNA through change in conformation after uptake in the plants can be excluded, because ultrasonication of this densely banded fraction does not influence its position on the CsCl gradient.

Future investigation must elucidate how this new DNA fraction can form after uptake of T_4 phage DNA. The formation of hybrid DNA from plant and phage DNA, analogous to the results of Ledoux, postulates a recipient DNA fraction of very high density, which after association of the T_4 phage DNA would shift the latter to an intermediate density of 1.724 g/cm^3. Some preliminary results indicate the replication of the phage DNA fraction within the plant cells.

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