Apparent Identity of Chromoplast and Chloroplast DNA in the Daffodil, *Narcissus pseudonarcissus*

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A comparison of the EcoRI restriction endonuclease digest fragments from daffodil chromoplast and chloroplast DNAs on agarose gels has yielded identical results. The sum of the fragments for each DNA gives a minimum molecular weight of 98.6×10^6.

Introduction

Although chromoplasts and chloroplasts are morphologically and functionally very different, it has been shown in certain plants and under certain conditions, that they are interconvertible [see 1–5]. One could then expect such plastids to contain, at least qualitatively, the same plastome.

It appears questionable, however, whether this applies to all plastid types. For example, Frey-Wyssling *et al.* [6] postulated that chromoplasts represent an irreversible end stage of plastid development. This concept, although not universally applicable, probably describes the situation correctly in some cases, particularly in gerontoplasts [7; see, however, 8]. Further, one could envisage that if the plastome of a given plastid does not represent a single homogenous species, but rather may be, to a limited extent, heterogenous, as suggested by Bedbrook and Bogorad for maize chloroplast DNA [9], then such minor plastome variants in the chloroplast may become selected for and even prevail in the morphologically different chromoplasts.

A general comparison of contour length and buoyant density has shown that chloroplast and chromoplast DNA in *Tropaeolum majus* could be identical [10]. However, a more detailed comparison must be made before this assumption can be verified. Such comparisons between chloroplast DNAs from different plants have been carried out using restriction enzymes [11–15], as well as by determining the amount of hybridisation between one chloroplast DNA and other [16]. Variations in sequence have thus been found between chloroplast DNAs from different plants. For example, Lamppa and Bendich [16] found that daffodil and pea chloroplast DNAs exhibit only 27% sequence homology.

In this communication, a restriction enzyme analysis of chromoplast DNA, isolated from the coronae of the daffodil flower, and chloroplast DNA from daffodil leaves will be discussed.

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Experimental

Chromoplast isolation

This was carried out, with modifications, after Liedvogel *et al.* [17]. 40 g coronae were used for the isolation. After low speed centrifugation of the filtered homogenate, the supernatant was overlayered onto a cushion of 40% sucrose, 5 mM $MgCl_2$, 0.067 M phosphate buffer (pH 7.5). This was centrifuged for 30 min in a swinging bucket rotor at $16\,500 \times g$. The band at the interphase was then removed, and 100 µg/ml DNase I (Boehringer, Mannheim) and $MgCl_2$ (final concentration 10 mM) were added. After a 40 min incubation at 4 °C, 30 µg/ml phosphodiesterase (Boehringer, Mannheim) was added, and the incubation continued for a further 20 min at 4 °C. 3 volumes 0.47 M sucrose, 0.067 M phosphate buffer, 10 mM EDTA, pH 7.5, were then added, and the mixture was spun down at $16\,500 \times g$ for 20 min in an 8×38 ml fixed angle rotor. The pellet was washed once again in the EDTA buffer and centrifuged as above, yielding the final chromoplast pellet.

Chloroplast isolation

The chloroplasts were isolated from 60 g leaves after Liedvogel *et al.* [17]. However, the 1500 g crude pellet was resuspended in 10 ml isolation medium containing DNase I (150 µg/ml) and 10 mM $MgCl_2$. After a 1 h incubation at 4 °C, 30 ml 0.6 M sucrose, 0.1 M phosphate buffer, 10 mM EDTA were added, and the mixture was spun down at $1500 \times g$ for 10 min. The pellet was resuspended in the latter buffer and the above centrifugation repeated, yielding the final chloroplast pellet.

Isolation of plastid DNA

Chromoplast and chloroplast pellets were lysed and the DNA isolated according to Kolodner and Tewari [18]. Separation of plastidal DNA from mitochondrial DNA was achieved on CsCl density gradients (see [10]).

Restriction enzyme digestion

After ethanol precipitation, 5 µg plastidal DNA were resuspended in 100 µl 10 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 100 mM NaCl, 10 mM β-mercaptoethanol, and 3 µl EcoRI endonuclease (Boehringer, Mannheim) were added. Incubation at 37 °C for 3 h was ended by adding EDTA to a final concentration of 10 mM, and the fragments were stored at −26 °C, prior to being separated on 0.8% agarose gels.

Results and Discussion

Fig. 1 demonstrates the purity of the plastid DNA fractions, which are free from nuclear DNA contamination, but contain slight mitochondrial DNA contamination. The latter was, however, easily removed after fractionation of the CsCl gradients. Fig. 2 reveals that the chromoplast and chloroplast DNA from *Narcissus pseudonarcissus* are identical in their EcoRI endonuclease fragment patterns. The sum of the fragments gives in both cases a minimal molecular weight of 98.65×10^6 (27 fragments determined), which is slightly higher, but comparable with the size of 92×10^6 calculated by Falk *et al.* [19], on the basis of contour length measurements.

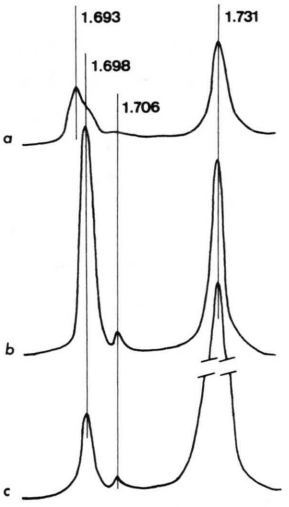

Fig. 1. Buoyant density profiles of DNA centrifuged in CsCl gradients, using *Micrococcus lysodeikticus* DNA of density 1.731 g/cm³ as a marker. a) Preparative gradient of total petal DNA (after chromoplast isolation, but excluding the 40% sucrose cushion from the 16 500 × *g* centrifugation step, and no DNase I/phosphodiesterase treatment). Nuclear DNA has a density of 1.693 g/cm³, chromoplast DNA 1.698 g/cm³ and mitochondrial DNA of 1.706 g/cm³. b) Preparative gradient of DNA from "chromoplast" pellet. c) Preparative gradient of DNA from "chloroplast" pellet showing chloroplast DNA at a density of 1.698 g/cm³.

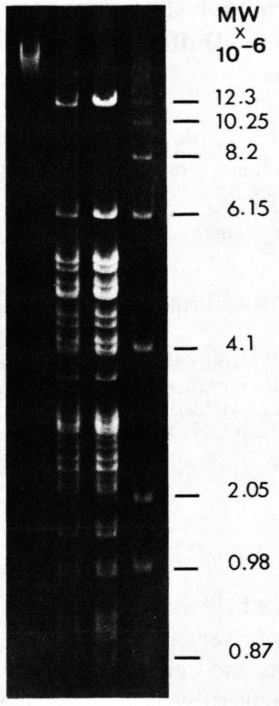

Fig. 2. Restriction enzyme analysis of plastid DNAs after EcoRI endonuclease treatment. 50 µl of the DNA mixtures (ca. 2.5 µg) were used per lane. From left to right, undigested chromoplast DNA, digest of chloroplast DNA, digest of chromoplast DNA and finally marker DNA including a mixture of EcoRI digested λdv 21/8 DNA and a Hae III digest of λdv 1 DNA. 0.8% agarose gel in 40 mM Tris, 5 mM Na acetate, 1 mM EDTA pH 7.8, plus 1 µg/ml ethidium bromide. The running buffer was as for the gel, but containing 0.6 µg/ml ethidium bromide. The 30 cm gels were run for 6−8 h at 200 V (50 mA) and the bands visualised and photographed under long wave ultra violet light (366 nm).

On completion of this work we have since been informed by Prof. R. G. Herrman (Univ. of Düsseldorf), that identical results, to be published in detail later, have been independently attained in his group. One can therefore assume the plastome of both plastid types to be identical. However, the

protein patterns, as analysed on SDS-polyacrylamide gels are indeed very different in isolated chromoplasts from those obtained from isolated chloroplasts within the same plant species (results not shown). If the plastome is active in chromoplasts, which may be presumed (at least for early developmental stages, where plastidal ribosomes are visible under the electron microscope), then one could expect other proteins to be produced in chromoplasts than in chloroplasts, suggesting a differential transcription of this plastome. This result may be of some interest as so far only a small portion of the plastome appears to be transcribed in chloroplasts (see [20]). Investigations are now being continued in our laboratory regarding the expression of the plastome genes in developing chromoplasts.

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