**Daffodil Chromoplast DNA: Comparison with Chloroplast DNA, Physical Map, and Gene Localization**

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In daffodil, development of chloroplasts or chromoplasts is not accompanied by plastid DNA modification. This has been shown by comparing the fragment pattern of different restriction endonucleases, and by the lack of methylation of CCGG sequences. A physical map has been constructed for the plastome using the restriction endonucleases Sal I, Pst I, and Bgl I. The fragments containing the genes for the large subunit of ribulose bisphosphate carboxylase/oxygenase (rbcL), the alpha, beta, and epsilon subunits of the ATP synthase complex (atpA, atpB, atpE), cytochrome f (petA), and for the 51 kDa chlorophyll apoprotein of photosystem II (psbB) have been identified. The respective gene locations correspond to those on spinach chloroplast DNA.

**Introduction**

According to the endosymbiont hypothesis, chloroplasts phylogenetically represent the original plastid type [1]. In the process of evolution, other plastid types with different functions have evolved in connection with the development of organs and corresponding cell differentiation. During ontogeny, the various types of plastids within a plant all develop directly or indirectly from proplastids, and they are interconvertible [2]. Therefore one might suppose that all plastidal forms of a single plant contain either the same, or a reversibly modified, genetic material. Modification through methylation of ptDNA has been found in the amyloplasts of sycamore [3] and during gametogenesis in Chlamydomonas [4].

Preliminary investigations revealed no apparent differences between the DNA of daffodil chromoplasts (crDNA) and chloroplasts (clDNA) [5], although these plastid types differ greatly in pigment content, fine structure, and lipid as well as protein composition [6]. The protein complements of the two plastid types, in particular, are very different [7]. The plastome of the daffodil appears to become inactivated during development of chromoplasts from prochromoplasts [8]. The aim of the present work was to ascertain whether DNA modifications occur in parallel to the changes found in the structure and protein composition of nucleoids during chloro- and chromoplast differentiation [9]. Further, in continuation of earlier work on the daffodil plastome [5, 9–14] and as a basis for further comparative studies on the genetic material of chloro- and chromoplasts, a physical map of the plastome together with the localization of some genes is presented.

**Material and Methods**

Daffodils (Narcissus pseudonarcissus L. cv. ‘Golden Harvest’) were bought from the local market. Chromoplasts from the daffodil coronae were isolated as described by Thompson et al. [14]. For isolation of the chloroplasts, 120 g of green leaves were homogenized in 600 ml buffer A (0.33 M sorbitol, 50 mM Tris/HCl pH 8.0, 3 mM Na2EDTA, 1 mM 2-mercaptoethanol, 0.1% (w/v) BSA) in a blender (Bühler, FRG). After filtration through three layers of nylon tissue and low speed centrifugation (5 min, 130 x g), the chloroplasts were pelleted from the supernatant (60 s, 4000 x g), resuspended in buffer A, and repelleted (60 s, 4000 x g). The pellet was resuspended in buffer A and overlaid onto discontinuous Percoll density gradients (0, 36, 72% (v/v) Percoll, each in buffer B (0.3 M sucrose, 50 mM Tris/HCl pH 8.0, 3 mM Na2EDTA)). After centrifugation in a swinging bucket rotor (8 min, 4000 x g), the lower green band (36/72% Percoll) containing the intact chloroplasts was removed, and diluted 1:3 with buffer A. After a final centrifugation (2 min, 4000 x g), the chloroplasts were stored at −70 °C as pellets prior to use. From plastidal pellets the

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**Abbreviations:** BSA, Bovine Serum Albumine; clDNA, Chloroplast DNA; crDNA, Chromoplast DNA; ptDNA, Plastid DNA.

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ptDNA was extracted according to Kolodner and Tewari [15], and concentrated and stored as described by Herrmann [16].

Primary digestions of the DNA by means of restriction endonucleases were carried out under conditions recommended by the suppliers. Double digestions were carried out consecutively, adjusting the ionic conditions for the second enzyme. For construction of the physical map the crDNA and the restriction enzymes \( \text{Sal} I, \text{Pst} I, \) and \( \text{Bgl} I \) were used. The orientation of the fragments was determined as described by Herrmann et al. [17]. Several genes were localized on the physical map of the chloroplast DNA using genespecific heterologous cloned spinach cDNA fragments (cf. Table I). The following procedures were applied: Southern blot [18], nick translation [19], hybridization [20].

**Results and Discussion**

Recent investigations have shown that the ptDNA molecules within one plant need not be identical. Microheterogeneity (\( \text{Euglena gracilis} [21] \)), inversions of whole regions within the plastome [22], and even different DNA molecules (\( \text{Acetabularia acetabulum} \) [23]; brown algae [24]) have been reported as variants of a seemingly uniform plastome. This data, plus the finding that some methylation occurs in amyloplast DNA of sycamore \( (\text{Acer pseudoplatanus}) \) suspension culture cells [3], may be taken as an indication that, during the development of the various plastid types, the plastome may be modified in some way.

During tomato fruit ripening the expression of several genes declines, and, with the exception of the mRNA for the 32 kDa protein, the corresponding mRNAs are no longer detectable in the chromoplasts of ripe fruits [25]. In the daffodil the plastome appears to become totally inactivated during chromoplast development [8]. Iwatsuki et al. [26] and Thompson [5] have shown already that cr- and cDNA are probably identical. This finding is confirmed in the present paper for the plastome of the daffodil. The ptDNA from chromoplasts and chloroplasts yield identical fragment patterns on digestion with six different restriction endonucleases (Fig. 1). Furthermore, endonuclease digestion with \( \text{Hpa} II \), which only cuts the unmethylated sequence C/CGG, shows that there are no differences in the methylation of this sequence (Fig. 1f). The fragment pattern of crDNA after \( \text{Msp} I \) digestion – this enzyme cuts the sequence C/GGG as well as the methylated sequence C/CmGG – is identical to the pattern yielded by \( \text{Hpa} II \) digestion (Fig. 1g). This means that neither of the ptDNAs are methylated in the sequence CCGG, i.e., cr- and cDNA appear to be identical. The possibility that gene expression is regulated by methylation, as in eukaryote nuclear DNA, can thus be ruled out in the case of daffodil chromoplasts. The regulation of gene expression during chloroplast and

![Fig. 1. Comparison of chloroplast (lane A) and chromoplast (lane B) DNA from the daffodil after Pst I (a), Sal I (b), Bgl I (c), Sac I (d), Bam HI (e), Hpa II (f), Msp I (g; B1) and Hpa II (g; B2) endonuclease treatment.](image_url)

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primary clone/subclone</th>
<th>Fragments used</th>
<th>Probe size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>atpA</td>
<td>pWHsp 210</td>
<td>Taq I-1 + 2</td>
<td>700 and 600</td>
</tr>
<tr>
<td>atpB</td>
<td>pWHsp 403/E1</td>
<td>Sac I-1</td>
<td>1200</td>
</tr>
<tr>
<td>atpE</td>
<td>pWHsp 403/E3</td>
<td>Xba I-2</td>
<td>420</td>
</tr>
<tr>
<td>rbcL</td>
<td>pWHsp 403/E2</td>
<td>Pst I-1</td>
<td>1260</td>
</tr>
<tr>
<td>pcrA</td>
<td>pWHsp 407/E1</td>
<td>E-1 total</td>
<td>830</td>
</tr>
<tr>
<td>psbB</td>
<td>pWHsp 207/E3</td>
<td>E3 total</td>
<td>1450</td>
</tr>
</tbody>
</table>

Table I. Chloroplast DNA clones from spinach used as probes for heterologous hybridizations (for nomenclature cf. [33]).
chromoplast development therefore does not appear to be due to changes at the DNA level. Epigenetic factors such as structure, composition and protein complement of the nucleoids seem to play a predominant role in differential gene expression of plastids [9].

Earlier investigations have shown the daffodil plastome DNA to be circular with a length of 161 kbp. Furthermore, the 16S and 23S rRNA genes were shown to be localized in an inverted repeat sequence [12, 14]. A physical map was constructed using the restriction endonucleases Pst I, Sal I, and Bgl I (for fragment patterns, cf. Fig. 1a–c). The respective fragment sizes could be ascertained on calibrated gels from marker DNAs (Table II). By means of consecutive digestion with different enzymes, a physical map for the daffodil crDNA for the three enzymes applied could be constructed (Fig. 2). The locations of the Sal I fragments S3 and S7 were determined with the help of a fourth enzyme (Sac I). Circularity and overall size of the daffodil genome, as determined by Thompson et al. [14], could be confirmed by the physical map as presented here.

Comparative analyses of the organization of ptDNAs from a variety of higher plants revealed a very stable order of genes. The spinach gene order and arrangement appears to be the ancestral one. In some cases, however, the gene arrangement is changed by inversions (cf. [27]). In order to locate the genes for certain proteins in the daffodil plastome, the ptDNA was digested with Sal I, Sal I + Pst I, Bgl I, and Bgl I + Pst I, and the resulting fragments were separated in agarose gels. Fig. 3 shows ethidium bromide-fluorescence photographs of the gels, which were used for Southern transfer, together with their corresponding autoradiograms for each localized gene. The position of the genes is shown in

Table II. Restriction fragment sizes of chromoplast DNA from the daffodil.

<table>
<thead>
<tr>
<th>Sal I</th>
<th>Size (kbp)</th>
<th>Pst I</th>
<th>Size (kbp)</th>
<th>Bgl I</th>
<th>Size (kbp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>90.8</td>
<td>P1</td>
<td>45.9</td>
<td>B1</td>
<td>43.9</td>
</tr>
<tr>
<td>S2</td>
<td>25.6</td>
<td>P2</td>
<td>41.7</td>
<td>B2</td>
<td>33.0</td>
</tr>
<tr>
<td>S3</td>
<td>15.2</td>
<td>P3</td>
<td>29.7</td>
<td>B3</td>
<td>21.3</td>
</tr>
<tr>
<td>S4</td>
<td>13.9</td>
<td>P4</td>
<td>17.6</td>
<td>B4</td>
<td>16.9</td>
</tr>
<tr>
<td>S5</td>
<td>9.1</td>
<td>P5</td>
<td>12.1</td>
<td>B5</td>
<td>8.9</td>
</tr>
<tr>
<td>S6</td>
<td>7.2</td>
<td>P6</td>
<td>10.3</td>
<td>B6</td>
<td>7.5</td>
</tr>
<tr>
<td>S7</td>
<td>3.2</td>
<td>P7</td>
<td>5.0</td>
<td>B7 (2x)</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B8</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B9 (2x)</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Fig. 3. Localization of the genes for the alpha- (atpA; a), beta- (atpB; b), epsilon- (atpE; c) subunits of the ATP synthase complex, large subunit of the ribulose-bisphosphate carboxylase (rbcL; d), cytochrome f (petA; e), and 51 kDa chlorophyll apoprotein of the photosystem II (psbB; f). Daffodil chromoplast DNA digests (lane A: Sal I; B: Sal I + Psi I; C: Bgl I; D: Bgl I + Psi I) were separated in 0.6% agarose gels, filter immobilized and hybridized with cloned spinach chloroplast DNA fragments containing only structural parts of the genes (cf. Table I). 1) Ethidium bromide fluorescence photographs; 2) autoradiograms.

Acknowledgements

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