Theor Appl Genet (1988) 75:282–285

THEORETICAL AND APPLIED GENETICS

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Homologies to chloroplast DNA in the nuclear DNA of a number of Chenopod species

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Received June 16, 1987; Accepted August 19, 1987
Communicated by R. Hagemann

Summary. Sequences homologous to chloroplast (ct)DNA have been found in nuclear DNA in five species of the *Chenopodiaceae,* extending the earlier observations of 'promiscuous' DNA in *Spinacia oleracea* (Timmis and Scott 1983). Using the 7.7 kbp spinach ctDNA Pst I fragment as a hybridization probe, several separately located homologies to ctDNA were resolved in the nuclear DNA of *Beta vulgaris, Chenopodium quinoa,* and *Enchylaena tomentosa.* In *Chenopodium album* and *Atriplex cinerea* the major region of homology was to a nuclear Eco RI fragment (6 kbp) indistinguishable from that in ctDNA. These homologies may therefore involve larger tracts of ctDNA because the same restriction sites are apparently retained in the nucleus. This suggests that in these latter two species there is a contrasting, more homogeneous arrangement of ctDNA transpositions in the nucleus.

Key words: Chloroplast DNA – Nuclear DNA – DNA homologies – Chenopods

Introduction

The occurrence of DNA sequences common to more than one genetic compartment, so called 'promiscuous' DNA, has been described in a range of organisms (for review, see Timmis and Scott 1984). We have previously shown, using Southern blot analysis of purified nuclear DNA, that virtually the entire chloroplast (ct) genome of *Spinacea oleracea* is represented in nuclear (n)DNA, with the nucleus containing the equivalent of four to five copies of ctDNA per haploid genome (Timmis and Scott 1983; Scott and Timmis 1984). The nucleotide sequence of several of these nuclear DNA tracts homologous to ctDNA will be reported separately (Cheung and Scott, unpubl. results). It was of interest to see if the phenomenon of 'promiscuous' ctDNA sequences could be observed in other Chenopod species and, if so, whether the same nuclear organization of the transposed DNA was involved. In this study we have used the spinach chloroplast 7.7 kbp Pst I fragment as a probe to total DNA preparations of five other Chenopod species. All of the species investigated showed homology between chloroplast and nuclear DNA to varying degrees.

Materials and methods

Fresh leaves of *Beta vulgaris* and *Chenopodium quinoa* were obtained from glasshouse grown plants and those of *C. album, Enchylaena tomentosa* and *Atriplex cinerea* from the Adelaide Botanic Gardens.

Total DNA was prepared from leaves as described by Scott and Possingham (1980), except that 0.05 to 0.1 M β-mercaptoethanol was added to the initial extraction medium to retard oxidation of polyphenols. Samples were digested with restriction enzymes under the conditions recommended by the manufacturer (Boehringer), with 5U Eco R1/μg of DNA for 8 h and 3U Hpa II/μg of DNA for 5 h, and the fragments resolved on 0.8% agarose gels. Southern blots of the gels were probed with the ctDNA Pst I 7.7 kbp fragment (Palmer and Thompson 1981), labelled with ^{32}P ($> 1 \times 10^8$ cpm/μg), and hybridized to Southern transfers at a concentration of 3×10^6 cpm/ml as previously described (Scott and Timmis 1984).

The spinach ctDNA probe was used in all experiments reported here rather than isolating individual probes for each species examined. This simplification was possible because of the extensive conservation of ctDNA sequences between all higher plants studied so far (Dyer 1984).

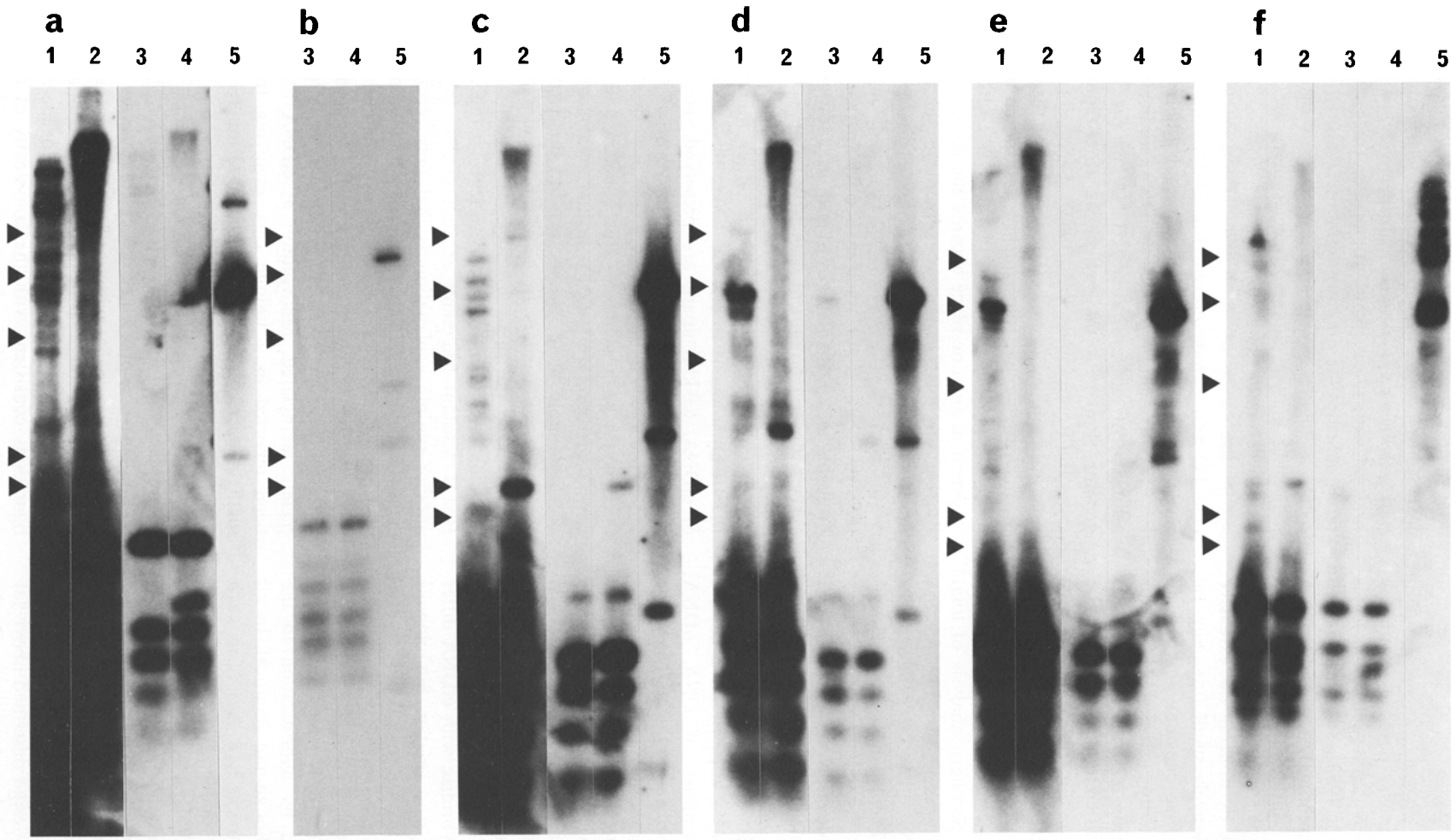

Fig. 1. Hybridization of the 7.7 kbp spinach ctDNA Pst I fragment with total leaf DNA from (**a**) *Beta vulgaris,* (**c**) *Chenopodium quinoa,* (**d**) *C. album,* (**e**) *Atriplex cinerea,* (**f**) *Enchylaena tomentosum* and (**b**) purified spinach chloroplast DNA following restriction endonuclease digestion, gel electrophoresis and Southern transfer of the DNA. In all cases tracks 1 and 2 contained 5 μg of DNA and *tracks 3, 4* and *5* 500 ng of DNA except for Fig. 1 b when *tracks 3, 4* and *5* contained 100 ng of DNA. The DNA in *tracks 1* and *3* was digested with both Hpa II and Eco RI, in *tracks 2* and *4* with Hpa II alone, and in *track 5* with Eco RI alone. The markers in each figure ( ) represent MW standards of 9.6, 6.6, 4.3, 2.3 and 2.0 kbp, respectively

Results and discussion

In our earlier experiments it was possible to prepare spinach nuclei that contained barely detectable levels of ctDNA (Timmis and Scott 1983), and this enabled the identification of different ctDNA and nDNA restriction fragments on Southern blots. We also showed that it was possible to separate a large proportion of nDNA from ctDNA by utilizing the extensive cleavage of ctDNA into relatively small fragments by Hpa II (a methylation sensitive enzyme). In contrast, the nDNA remains essentially undigested by this enzyme, and the experiments reported here (Fig. 1) take advantage of this differential digestion. The difference in sensitivity to Hpa II is due to the extensive methylation of cytosine residues in nuclear DNA of higher plants (Gruenbaum et al. 1981) and the lack of base modification in ctDNA. (Smillie and Scott 1969).

For example, following Southern analysis of total leaf DNA of *Beta vulgaris* digested with Eco R1 and hybridized with the 7.7 kbp probe, large fragments (> 2 kbp) characteristic of Eco RI digestion of *B. vulgaris* ctDNA were seen (Fig. 1 a, track 5). These patterns identify the bona-fide ctDNA fragments which are most clearly resolved in the appropriately loaded and exposed tracks 3, 4 and 5. Following digestion with Hpa II (track 4) or Hpa II digestion and Eco RI (track 3), small fragments (< 2 kbp) of ctDNA were generated. With higher loadings of DNA, nDNA resistant to Hpa II and hybridizing to the ctDNA probe (track 2) appeared as a continuum of unresolved bands of high MW and undigested DNA fragments. After digestion with Eco R1 as well as Hpa II (track 1) this DNA previously undigested by Hpa II was resolved into a large number of discrete Eco RI bands, the majority of which were greater than 4 kbp. In this case (*B. vulgaris*) the majority of the relatively large number of nDNA fragments that hybridize with ctDNA differ in size from the bona fide, and smaller, homologous ctDNA fragments (compare tracks 1 and 3).

The size and number of fragments generated by digestion of purified spinach ctDNA (Fig. 1 b) with Hpa II plus Eco RI (track 3), Hpa II (track 4), and Eco RI (track 5) and identified by hybridization to the 7.7 kbp probe is similar, though not identical, in all species studied (compare tracks 3, 4 and 5 in each series). Thus, fragments of total leaf DNA > 2–3 kbp generated by digestion with Hpa II plus Eco RI and hybridizing with the probe are most likely to be nuclear in origin. In all instances there is a heavy overexposure of hybridized ctDNA fragments, (tracks 1 and 2) due to the large numbers of ctDNA copies per cell compared with nDNA (Scott and Possingham 1980).

The same experimental design described above for *B. vulgaris* applies to the comparable gel tracks of Fig. 1 c, d, e and f, where total leaf DNA preparations from *Chenopodium quinoa, C. album, Atriplex cinerea* and *Enchylaena tomentosum* respectively, were hybridized with the 7.7 kbp Pst I probe. Track 1 of each series indicates the presence of methylated nDNA fragments of higher MW than the fragments of ctDNA, indicating that in all these species there are nuclear sequences homologous with ctDNA.

In the cases of *B. vulgaris* (Fig. 1 a), *C. quinoa* (Fig. 1 c) and *E. tomentosum* (Fig. 1 f), the homologies revealed in each track 1 are to several different sized Eco RI fragments of nuclear DNA, indicating an arrangement of transposed ctDNA in the nuclear genome similar to that which we first reported in spinach (Timmis and Scott 1983). There are several sites of homology to this ctDNA fragment throughout the nuclear genome. The sequences transposed may not include very large pieces of the ctDNA 7.7 kbp Pst 1 fragment in any one place because the internal Eco RI sites of the ctDNA are not present in the nuclear homologies (track 1).

In contrast, *C. album* (Fig. 1 d) and *A. cinerea* (Fig. 1 e) show fewer nuclear Eco RI fragments hybridizing to ctDNA (track 1), and in addition each species shows a prominent high MW (approx 6.0 kbp) fragment which is the same MW as that in the corresponding ctDNA. This is a larger fragment of nDNA homologous to ctDNA, where the Eco RI sites of ctDNA, 6.0 kbp apart, are preserved. This single nuclear fragment is responsible for most of the observed homology in these two species.

It could be argued that this effect is due to the partial digestion of ctDNA in the total leaf DNA preparations in these two species, but this is unlikely because there appears to be complete digestion by Hpa II alone and there are no signs of partial Hpa II digestion of DNA in these experiments. If these larger Hpa II resistant fragments are not nuclear in origin, another posible explanation is that they represent a small population of ctDNA molecules that are methylated. If this was the case, we would expect a minority of additional bands to appear on Hpa II digestion alone unless a subgroup of chloroplast genomes were completely methylated. Methylation of ctDNA has only been reported in a unique case involving transient methylation associated with uniparental inheritance in *Chlamydomonas* (Sano et al. 1984).

Another possible explanation of these non-chloroplast homologies to ctDNA revealed by this double digestion procedure is that they may be due to sequence homologies with mtDNA in the total leaf DNA preparations. The majority of homologies detected, however, are organized in heterogeneous configurations consistent with the complexity of nuclear rather than mitochondrial genomes, and the copy

numbers in individual bands are probably lower than would be expected for mtDNA homologies (Timmis and Scott 1983). Further, the tract of mtDNA which we found to show homology to ctDNA in spinach (Timmis and Scott 1983) was also distinguishable from nuclear DNA by differential Hpa II digestion.

This study shows that the phenomenon of DNA sequence homology between ctDNA and nDNA is widespread in the *Chenopodiaceae,* and that the number and types of homology vary even within one family. This latter observation suggests the interesting possibility that ctDNA incorporation into the nucleus is a frequent evolutionary event and perhaps an ongoing process.

Acknowledgements. The authors wish to acknowledge the support of the CSIRO/University of Adelaide collaboration research fund (MAA), and the gift of plants from the Adelaide Botanic Gardens.

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