Theor Appl Genet (1988) 75:244–251

Clone bank and physical and genetic map of potato chloroplast DNA

S. Heinhorst[1]*, G. C. Gannon[1]*, E. Galun[2], L. Kenschaft[1] and A. Weissbach[1]**

[1] Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA
[2] The Weizmann Institute of Science, Rehovot 76100, Israel

Received July 29, 1987; Accepted August 19, 1987
Communicated by R. Hagemann

Summary. Clone banks of *Pvu*II, *Bam*HI and *Xho*I fragments were generated of the *Solanum tuberosum* cv Katahdin plastome. These clone banks, in conjunction with molecular hybridization to tobacco ctDNA probes, were used to construct a physical map of potato ctDNA. The potato plastome was found to be a circular molecule of 155–156 Kbp containing two inverted repeat regions of 23–27 Kbp. The arrangement of restriction sites is very similar to that of other Solanaceae plastomes. Heterologous hybridization to known ctDNA encoded gene probes from tobacco allowed us to establish a genetic map of the potato chloroplast genome. The arrangement of these genes on the potato plastome resembles that on most higher plant ctDNAs.

Key words: *Solanum tuberosum* – Potato plastome – Chloroplast DNA – DNA restriction profile – Restriction endonucleases

Introduction

In a span of less than one decade a wealth of information has accumulated regarding the physical structure of angiosperms' chloroplast DNA (ctDNA) (Palmer 1985; Palmer *et al.* 1987). From the study of over 200 species, the angiosperms' chloroplast genome (plastome) emerged as having a rather conserved structure: a circular molecule of double-stranded DNA measuring between 120 and 160 kilobase pairs (Kbp). Moreover, with the notable exception of some legumes, all angiosperm plastomes analyzed had virtually the same general organization: an inverted repeat region containing the ribosomal RNA genes (as well as several other genes) and two single copy regions.

Nicotiana was among the first angiosperm genera in which a physical map of the ctDNA was reported (Fluhr and Edelman 1981a, b). *Nicotiana tabacum* is also the first for which a complete ctDNA base sequence was reported (Shinozaki et al. 1986a, b). The physical maps of plastomes from only a few other genera of the extensive and economically important Solanaceae family have been published (e.g. *Petunia,* Bovenberg et al. 1984; *Lycopersicon,* Phillips 1985). Restriction profiles of ctDNA from potato (*Solanum tuberosum*) and its relatives were analyzed by Hosaka et al. (1984), Buckner and Hyde (1985), and Hosaka (1986), but these studies did not lead to the construction of physical maps.

While information on ctDNA restriction profiles can provide phylogenetic and evolutionary indications (e.g. Palmer and Zamir 1982; Hosaka *et al.* 1984; Perl-Treves and Galun 1985; Perl-Treves et al. 1985; Green et al. 1986), the availability of a physical map allows for a much more detailed analysis of rearrangements and deletions of homologous plastome sequences among closely related as well as unrelated species (Palmer et al. 1987).

The genus *Solanum* contains about 160 tuberous species and probably over 1,000 other species (Hawkes 1978). The most important among these species is obviously the potato (*Solanum tuberosum*), which is regarded as a major food crop ranking highest with respect to production increase in developing countries (Anonymous 1984).

Protoplast manipulation methods are available to transfer plastomes from a donor species to a recipient

* Present address: Department of Chemistry, University of Southern Mississippi, Southern Station, Box 5043, Hattisburg, Ms 39406, USA
** To whom correspondence should be addressed

species (Galun and Aviv 1986). This opens an interesting field of investigating plastome/nuclear compatibilities in the genus *Solanum*. Such studies as well as the interspecific transfers of plastomes in *Solanum*, require detailed knowledge of the plastome of this genus as well as the availability of a ctDNA clone bank. With these latter requirements in mind we have established a clone bank and show the construction of the physical map of the potato plastome and the location of some known chloroplast genes.

Materials and methods

Biological material

Greenhouse and field grown potato (*Solanum tuberosum* cv Katahdin) plants were obtained from Drs. Catherine Carter and Melvin Henninger, Rutgers University. We also utilized plants that were grown from seeds obtained from the International Potato Introduction Station, Sturgeon Bay, Wisconsin.

Escherichia coli RRI was the recipient for plasmids using pMK2004 (Kahn *et al.* 1979) or pJSC73 (Cordingly *et al.* 1983) as a vector, whereas pUC19 (Norrander et al. 1983) derived recombinant DNA was propagated in strain RRIΔM15 (Ruether 1982). The transformed bacteria were grown in M9 medium supplemented with 80 μg/ml of Ampicillin.

Isolation of potato ctDNA

Potato plants were kept in the dark 24 h prior to harvest. All steps were performed on ice unless otherwise indicated.

Young leaves (approximately 30 g) were washed in ice cold water and freed of their midribs. The leaf tissue was cut into small pieces which were homogenized in 200 ml of isolation buffer [50 mM N-2-hydroxymethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 8, 300 mM sorbitol, 1 mM dithiothreitol (DTT)] in a Waring blender with 4 high speed pulses of 4 s each. The homogenate was filtered successively through nylon nets of 100 μm, 50 μm, and 20 μm pore size and then centrifuged at 400×g for 3 min. After pelleting the chloroplasts from the supernatant by centrifugation for 10 min at 3,400×g, the dark green pellet was gently resuspended with a paint brush in 50 ml of isolation buffer and pelleted once more at 3,400×g. The chloroplasts were resuspended in 8 ml of isolation buffer and layered onto a density step gradient of Percoll (Pharmacia) in isolation buffer (7 ml per step of 20%, 40% and 60% Percoll). The gradients were centrifuged for 10 min at 10,000×g. The green layers at the 20%/40% interface, which presumably consists of broken chloroplasts, and of the 40%/60% interface, which contains intact organelles, were pooled. After a 1 : 1 dilution with isolation buffer the plastids were pelleted as described above, resuspended in isolation buffer, and centrifuged once more to rid the organelles of Percoll. This procedure removes mitochondria which under those conditions do not enter the gradient.

The green pellet was resuspended in 10 ml of breaking buffer (100 mM Tris-HCl, pH 8, 50 mM disodium ethylenediamine tetraacetate (EDTA), 100 mM NaCl, 10 mM β-mercaptoethanol). Proteinase K (Merck, Darmstadt) and sodium dodecyl sulfate (SDS) were added to 50 μg/ml and 0.5% final concentration, respectively, and the sample was incubated at 37 °C for 3 h. The sample was extracted twice with phenol/chloroform (1 : 1) equilibrated in TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA). Residual phenol in the aqueous phase was removed by two extractions with diethylether. Nucleic acids were precipitated with 0.54 volumes of cold (–20 °C) isopropanol and collected by centrifugation at 10,000×g for 10 min. The precipitate was dissolved in 200 μl of TE buffer and incubated for 1 h at 37 °C with 35 μg/ml of ribonuclease A (Boehringer, Mannheim). The sample was extracted twice with phenol/chloroform as outlined above, made 0.2 M in Na-acetate, pH 5.5 and precipitated with two volumes of cold ethanol. The final preparation of ctDNA was resuspended in 100 μl of 10 mM Tris-HCl, pH 8. A typical yield was 50–80 μg of DNA.

Cloning

Potato ctDNA was digested with either *Bam*HI, *Xho*I or *Pvu*II (New England Biolabs as prescribed by the manufacturer). After digestion the mixture was extracted with phenol/chloroform and the aqueous phase precipitated with ethanol. Vector DNA was digested with the appropriate restriction enzyme and subsequently treated with calf intestine alkaline phosphatase (Boehringer, Mannheim) as described in Maniatis et al. (1982). Plasmid pUC19 served as vector for insertion into the *Bam*HI site, pMK2004 for cloning into the *Xho*I site, and pJSC73 for cloning into the *Pvu*II site. Ligations were carried out in 5 μl volumes at an insert to vector ratio of 5 : 1 (w : w) in a total DNA concentration of 1 μg/μl. The incubation contained one unit of T4 DNA ligase (New England Biolabs) and was carried out overnight at 16 °C in ligation buffer (Maniatis et al. 1982) containing 1 mM hexamine cobalt chloride (Rusche and Howard-Flanders 1985). In order to obtain clones of the five large *Pvu*II fragments of ctDNA, a restriction digest of the ctDNA was first size fractionated on a Prepgel apparatus (Bethesda Research Labs). Those fractions containing fragments of the desired length were pooled, concentrated on an Elutip-D column (Schleicher and Schuell), and ethanol-precipitated prior to ligation.

Competent *E. coli* cells were either prepared by the method of Dagbert and Ehrlich (1979) or were purchased from Bethesda Research Labs and transformed with the DNA. Recombinants were selected on plates containing the appropriate antibiotic and/or color reagents (IPTG/X-Gal), and screened on a small scale for ctDNA inserts by the miniprep procedure outlined by Palmer (1986). Positives were grown on a preparative scale, their plasmid DNA purified on CsCl gradients and further characterized by single and double restriction digests with *Bam*HI, *Xho*I and *Pvu*II. All clones are available upon request.

Molecular hybridization

Restriction digests of either total ctDNA or of individual clones were subjected to agarose gel electrophoresis and blotted onto nylon membranes (Gene Screen Plus from New England Nuclear). In order to minimize loss of DNA from the support during subsequent hybridization, the membranes were UV-irradiated as described previously (Cannon et al. 1985). Hybridization was carried out at 65 °C in 0.5 M Na^+ in the presence of 10% dextran sulfate. The conditions for blotting and hybridization were those recommended by the manufacturer.

Probe DNA was labeled with (α-^{32}P) dCTP by random primer extension (Summers 1975).

Results

Restriction endonuclease analysis of potato ctDNA

Potato ctDNA was digested with the enzymes *Bam*HI, *Pvu*II and *Xho*I, and the resulting fragments are shown

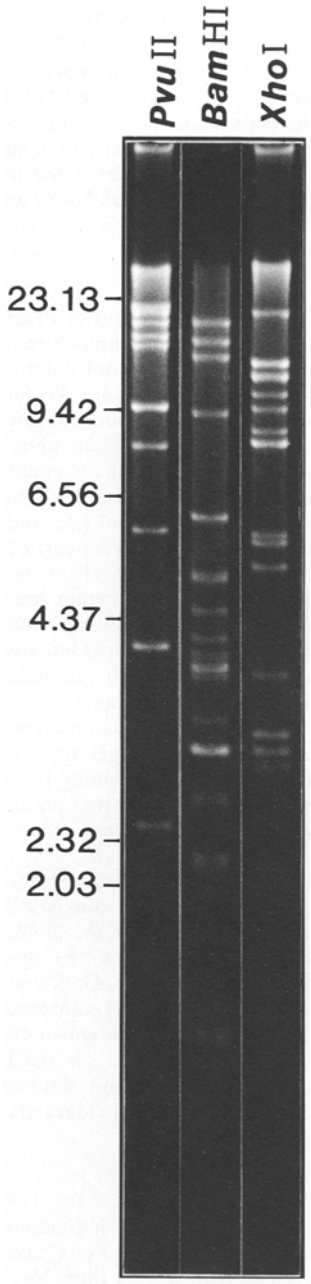

Fig. 1. Restriction fragment patterns of the *S. tuberosum* cv Katahdin chloroplast genome. Electrophoresis was performed in 0.8% agarose gels. The numbers on the *left* indicate the sizes (Kbp) of marker fragments

in Fig. 1. The restriction patterns obtained corroborate those previously reported for *Solanum tuberosum* ssp. tuberosum by Hosaka et al. (1984) and Buckner and Hyde (1985).

To complete the list of restriction fragments produced by each enzyme, we also included small *Bam*HI and *Xho*I fragments that had not been previously reported by the above authors. Since some of these small fragments cannot be readily resolved by gel electrophoresis of a total ctDNA digest, their numbers and sizes were inferred from secondary digests of the cloned *Pvu*II fragments that were separated on 1,5% agarose gels. Table 1 summarizes the potato ctDNA restriction fragments for all three enzymes. The sum of the fragments in the *Pvu*II and the *Xho*I digest vary by only 0.9 Kbp (155.6 and 156.5 Kbp, respectively). The sum of all *Bam*HI fragments detected, however, is somewhat smaller (151.7 Kbp), possibly due to the accumulation of errors when sizing the large number of fragments produced by this enzyme.

Generation of a potato ctDNA clone bank

We chose to use a *Pvu*II digest of potato ctDNA to generate a clone bank, since the number of different fragments generated by this enzyme is low (Table 1). Furthermore, with the exception of the very large pieces, the fragments were well separated on a gel, facilitating the identification of cloned inserts. Our initial approach of "shotgun" cloning of a total ctDNA digest yielded predominantly clones containing the smaller fragments. To obtain plasmids with large inserts, it became necessary to fractionate the *Pvu*II digested DNA by size prior to ligation. We were unable to clone *Pvu*II fragment P6b.

The recombinant plasmids were digested with *Pvu*II and their size determined by gel electrophoresis. In order to determine which *Bam*HI and *Xho*I subfragments were contained in each *Pvu*II fragment, *Bam*HI/*Pvu*II and *Xho*I/*Pvu*II double digestions were performed. Table 2 lists the sizes of these subfragments. The sum of the subfragment sizes differs from that calculated for the respective *Pvu*II insert by less than 5%.

To verify that the cloned *Pvu*II fragments were indeed derived from ctDNA, the recombinant plasmids were hybridized to Southern blots of potato ctDNA digested with either *Bam*HI, *Pvu*II or *Xho*I. Table 3 gives a summary of the hybridization data. It should be noted that we have generated other, partially complete clone banks of *Bam*HI and *Xho*I fragments to aid in further fine-mapping of chloroplast encoded genes.

Physical mapping of the potato ctDNA

The data obtained from molecular hybridization of cloned *Pvu*II fragments to total ctDNA restriction digests (Table 3) shows a pattern of overlapping fragments. To extend these studies, clones of *Bam*HI and *Xho*I fragments were cross-hybridized to single and double-digested cloned *Pvu*II fragments. This technique allows one to determine the extent of overlap between any two clones, to determine internal and border subfragments, and to assign positions of subfragments on a

Table 1. Numbers and sized in Kbp of *S. tuberosum* cv Katahdin ctDNA restriction fragments generated by *Pvu*II, *Xho*I and *Bam*HI. The numbers in brackets on the right of each column indicate the stoichiometry those fragments located in the inverted repeats

Fragment nos.	*Pvu*II	Fragment nos.	*Xho*I	Fragment nos.	*Bam*HI
1	a 19.9[a]	1	16.9	1	14.4[a]
	b 20.9[a]	2	12.2 (2X)	2	13.9
2	19.7[a]	3	a 11.2	3	12.3[a]
3	17.8[a]		b 11.2	4	9.2[a]
4	15.2[a]	4	10.8	5	6.1 (2X)
5	14.2[a]	5	9.7	6	5.1
6	a 10.0	6	8.8	7	5.0[a]
	b 10.0[a]	7	a 8.3[a]	8	4.4[a]
7	8.4[a]		b 8.3	9	4.0
8	6.1[a]	8	5.8[a]	10	3.9[a]
9	4.2[a] (2X)	9	5.7	11	a 3.8
10	2.5[a] (2X)	10	5.3		b 3.8[a]
		11	3.8		c 3.8
		12	3.2[a] (2X)	12	3.7[a]
		13	3.0[a] (2X)	13	3.2[a]
		14	2.9	14	a 3.0 (2X)
		15	2.2		b 3.0[a] (2X)
		16	2.1		c 3.0[a] (2X)
		17	1.5	15	2.5
		18	1.4	16	2.4
		19	0.82[a]	17	2.15
		20	0.75 (2X)	18	2.1[a]
		21	0.7	19	2.05
		22	0.5	20	1.92
		23	0.3	21	1.35
				22	1.33
				23	1.25 (2X)
				24	1.22
				25	1.20[a] (2X)
				26	1.17 (2X)
				27	1.10[a] (2X)
				28	a 1.07
					b 1.07
				29	0.4 (2X)
				30	0.36 (2X)
				31	0.3 (2X)
Sum	155.6		156.5		151.7

[a] Clones of these fragments are available

linear map. A circular map of potato ctDNA was drawn based on these results (Fig. 2). An exception, however, are *Pvu*II fragments P1b and P3, the digestion of which with *Bam*HI resulted in a great number of subfragments (Table 2). Most of these had no internal *Xho*I site, so that an unambiguous alignment of these fragments on a linear map of P1b and P3 was not possible. In order to establish a linear map of these two *Pvu*II fragments, various cloned *Pst*I fragments of *Nicotiana tabacum* ctDNA (Fluhr et al. 1983) covering that area were hybridized to double digests of these fragments. Since ctDNA fragments between the Solanaceae (Fluhr and Edelman 1981a), as well as between even more distantly related plants (Green et al. 1986) are essentially colinear, subfragments of the *Pvu*II fragments P1b and P3 of the potato chloroplast genome can be aligned based on their homology patterns to tobacco ctDNA probes. Tobacco ctDNA *Pst*I fragment PS5 hybridizes to *Bam*HI subfragments B7, B14, B27, B25, B31 and B17 of potato ctDNA fragment P1b. The *Bam*HI subfragment homologous to both PS5 and PS7 is B17 (data not shown). Since the order of subfragments B14a to B25 was known from experiments with cloned *Xho*I fragment X13, the linear map depicted in Fig. 2 was deduced from the data obtained from heterologous hybridization. The further positioning of subfragments B23 and B29 was accomplished by defining fragment B23 as the homologous fragment common to both tobacco ctDNA *Pst*I probes PS7 and PS3b.

Table 2. Sizes of subfragments generated by digestion of *S. tuberosum* cv Katahdin ctDNA *Pvu*II fragments with *Bam*HI or *Xho*I. The numbers in brackets indicate subfragment stoichiometry

*Pvu*II fragment (Kbp)		2nd endonuclease	Subfragment sizes (Kbp)
P1a	(19.9)	*Bam*HI	14.4, 3.6, 2.1
		*Xho*I	16.2, 2.3, 1.5
P1b	(20.9)	*Bam*HI	4.8, 2.9 (2X), 2.16, 1.93, 1.4, 1.23, 1.18, 1.11, 0.42, 0.31
		*Xho*I	10.5, 3.7, 2.95, 2.0, 0.82, 0.79, 0.48
P2	(19.7)	*Bam*HI	4.0, 3.65, 3.22, 2.26, 2.07, 1.94, 1.08 (2X), 0.34
		*Xho*I	8.4, 7.2, 2.11, 1.32, 0.77
P3	(17.8)	*Bam*HI	3.25, 2.9 (2X), 2.16, 1.4, 1.23, 1.18, 1.11, 0.42, 0.31
		*Xho*I	10.5, 4.3, 2.95
P4	(15.2)	*Bam*HI	6.7, 3.8, 2.6, 1.5
		*Xho*I	10.7, 4.2, 0.66
P5	(14.2)	*Bam*HI	6.3, 3.9, 2.6, 1.3
		*Xho*I	9.4, 4.75
P6a[a]	(10.0)	*Bam*HI	7.4, 2.5
		*Xho*I	5.0, 4.9
P6b	(10.0)	*Bam*HI	10.0
		*Xho*I	5.0, 3.2, 1.8
P7	(8.4)	*Bam*HI	3.9, 2.4, 1.6
		*Xho*I	4.05, 3.9
P8	(6.1)	*Bam*HI	2.1, 1.35, 1.2, 0.77, 0.3
		*Xho*I	4.8, 0.68, 0.26
P9	(4.2)	*Bam*HI	3.4, 0.74
		*Xho*I	2.7, 0.7, 0.6
P10	(2.5)	*Bam*HI	1.65, 0.58, 0.44
		*Xho*I	2.0, 0.44

[a] The subfragments of *Pvu*II fragment P6a were inferred from the map since no clone of this fragment was obtained

Table 3. Molecular hybridization of cloned *Pvu*II fragments to *S. tuberosum* cv Katahdin ctDNA digested with *Bam*HI or *Xho*I. Designation of the very small homologous *Bam*HI and *Xho*I fragments were inferred from size determinations of subfragments as indicated in Table 2

PvuII fragment probe	Hybridization to ctDNA fragments	
	*Bam*HI	*Xho*I
P1a	B1, B11	X1, X14, X17
P1b	B6, B7, B11, B14, B17, B23, B25, B27, B29, B31	X2, X6, X11, X13, X14, X16, X19, X22
P2	B4, B8, B9, B12, B13, B19, B20, B28	X3, X7, X15, X18
P3	B6, B7, B14, B17, B23, B25, B27, B29, B31	X2, X6, X11, X13
P4	B4, B6, B11, B15	X3, X6, X21
P5	B2, B5, B10, B21	X4, X5, X8
P6a[a]	B2, B5	X4, X5, X8
P6b	B3	X7, X9, X10
P7	B3, B8, B16	X3, X9
P8	B3, B11, B18, B22, B24	X1, X7, X23
P9	B5, B26	X4, X8, X12, X20
P10	B14, B26, B30	X2, X12

[a] The hybridization data for P6a are supported by data using cloned fragment X8 as a probe

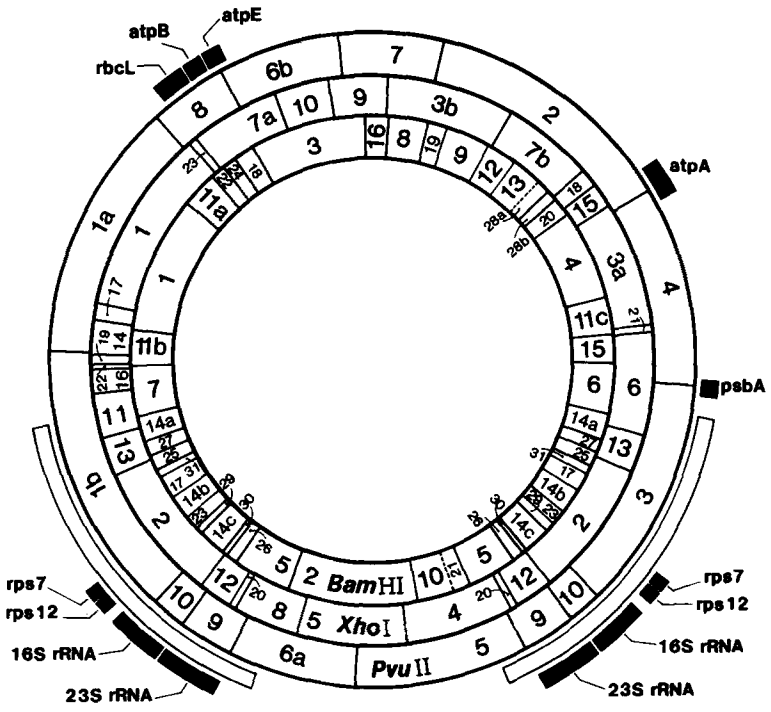

Fig. 2. Circular restriction map of *S. tuberosum* cv Katahdin ctDNA. The *open bars* indicate the approximate lengths of the inverted repeat regions. *Closed bars* denote plastome encoded genes that were mapped by heterologous hybridization. The approximate sizes of the genes were inferred from our hybridization data and by analogy to other known genetic maps of plastomes. *Dashed lines* indicate that the order of *Bam*HI fragments B10 and B21, as well as that of B13 and B28a is ambiguous

Heterologous hybridization to tobacco ctDNA *Pst*I fragments PS6, PS4 and PS3a (Fluhr et al. 1983) was used to establish a linear map of the potato *Pvu*II fragment P2. Since the potato *Bam*HI fragment B9, but not B19, has an internal *Pst*I site (data not shown), the alignment of B9 and B19 depicted in Fig. 2 is assumed to be correct, though data from heterologous hybridization does not allow for this conclusion, possibly due to too small an overlap of tobacco *Pvu*II fragment PS4 and potato *Bam*HI fragment B9. The positions of potato *Bam*HI fragments B13 and B28a relative to each other could not be resolved due to the lack of internal *Xho*I or *Pst*I recognition sites within these fragments. Likewise, the positions of *Bam*HI fragments B10 and B21 (in the small single copy region) relative to each other is ambiguous, since the tobacco probes covering that area are too large to determine if fragment B10 or B21 borders on fragment B5.

Gene mapping

To map known ctDNA encoded genes on the circular chloroplast genome, Southern blots of either total potato ctDNA restriction digests or of single and double digested cloned fragments were hybridized to tobacco ctDNA probes known to be specific for these genes. A synopsis of this data is displayed in Fig. 2.

In order to determine the location of the *psb*A gene on the potato plastome, two different probes were used. Plasmid pSP 247-5 (Fluhr et al. 1983) showed homology to *Bam*HI fragment B6 and to *Xho*I fragment X6 in a ctDNA restriction digest. The 3.2 Kbp EcoRI insert of pNT 32 (Cohen et al. 1984), which spans the coding region of the tobacco *psb*A gene, was hybridized to double restriction digests of cloned potato *Pvu*II fragments P3 and P4. Only the *Bam*HI and *Xho*I border fragments common to both *Pvu*II inserts gave a positive signal, thus establishing the location of this gene on the border of *Pvu*II fragments P3 and P4, on *Bam*HI fragment B6, and *Xho*I fragment X6.

The gene for the alpha subunit of the chloroplast ATPase (*atp*A) was located on *Bam*HI fragment B4, and on the border of *Pvu*II fragments P2 and P4 and *Xho*I fragments X3 and X15, respectively. The probe used for heterologous hybridization was plasmid pB1–9, which contains a 9.3 Kbp *Bam*HI insert that spans the *atp*A gene of *N. tabacum* (Fluhr et al. 1983).

The 2.2 Kbp *Bam*HI insert of plasmid pB1–20 (Fluhr et al. 1983) covers the 5′ ends of the genes for the large subunit of ribulose bisphosphate carboxylase (*rbc*L) and of the beta subunit of ATPase (*atp*B) of *N. tabacum.* This probe hybridized to potato *Xho*I fragment X7, *Pvu*II fragment P3, and *Bam*HI fragment B18. Further probing of the cloned potato *Pvu*II fragment P8 for the *rbc*L gene using the tobacco ctDNA insert of plasmid pEI-17 (Fromm, Edelman, Galun, unpublished) revealed that this gene extends to *Bam*HI fragments B24 and B22 and to *Xho*I fragment X23. Heterologous hybridization with the insert of plasmid pUBSX-1 derived from tobacco ctDNA (Avni and

Edelman, unpublished) revealed the site of the epsilon subunit of the ATPase gene (*atp*E) on the border of *Bam*HI fragments B18 and B3, *Pvu*II fragments P8 and P5b, and on *Xho*I fragment X7a.

The potato plastome region spanning the two 3′ exons of *rsp*12 was analyzed by the respective probes from the analogous region of tobacco (Fromm et al. 1986 and Fromm, Edelman and Galun, unpublished). Exon II was probed with the 0.9 Kbp (*Eco*RI) tobacco ctDNA insert of plasmid p2BE-2, and both exons (II and III) were probed with the 1.25 Kbp (*Bam*HI) insert of the tobacco ctDNA derived plasmid pBSB-9. This latter probe also spans the 5′ end of the gene for ribosomal protein S7 (*rps*7). Both probes hybridized to the same 1.25 Kbp *Bam*HI fragment B23 of the potato plastome that has been mapped within *Xho*I fragment X2 and *Pvu*II fragments P3 and P1b within the inverted repeat region.

The 16S rRNA gene was found to span *Bam*HI fragments B29, B14c and B30 in the inverted repeat, and was located on *Pvu*II fragments P3, P1b and P10 and *Xho*I fragment X2. A 3.3 Kbp *Bam*HI fragment of plasmid pXP23 (Koller et al. 1987) which carries the tobacco chloroplast 16S rRNA gene was used for heterologous hybridization.

In order to locate the gene for the 23S rRNA gene on the potato plastome, tobacco ctDNA fragment B5 was used as a probe. Homology was detected to potato *Bam*HI fragments B5 and B26, *Xho*I fragments X12 and X20, and *Pvu*II fragment 9 in the inverted repeat.

Discussion

A physical and genetic map of the *Solanum tuberosum* plastome was established with the help of a clone bank and molecular hybridization to tobacco ctDNA probes. The potato plastome of approximately 155–156 Kbp in size is slightly smaller than that of other Solanaceae (156–160 Kbp) (Fluhr and Edelman 1981b; Palmer and Zamir 1982; Zhu et al. 1982; Phillips 1985). Its structural features, namely the presence of two large inverted repeat regions of 23–27 Kbp separated by two single copy regions of approximately 19 Kbp and 109–113 Kbp, respectively, are common to most angiosperm plastomes, with the exception of some legumes (Palmer 1986).

Our clone bank of the potato ctDNA encompassed 150 of the total 155 Kbp of the plastome. Clones of all *Pvu*II fragments with the exception of P6a (10 Kbp) were obtained. Of the missing fragment P6a, 5 Kbp were covered by *Xho*I fragment X8. In addition, other cloned *Xho*I and *Bam*HI fragments from regions throughout the chloroplast genome should be efficient tools for detailed studies of genes and controlling sequences of the potato plastome. Our results indicate that the potato plastome is colinear and rather similar to the tobacco plastome (Fluhr et al. 1983; Hildebrand et al. 1985). Since the exact base sequence as well as the location of numerous genes and open reading frames have been established for the latter plastome (Shinozaki et al. 1986a, b), the location and isolation of further potato plastome encoded genes should be a relatively simple task.

Changes in chloroplast function have been implicated in potato cultivars and related *Solanum* species which were found to be cold tolerant or heat resistant (Hetherington et al. 1983; Smillie et al. 1983), but such changes have not been traced to either the plastome or the nuclear genome. The availability of a physical map and a clone bank of potato ctDNA in conjunction with chloroplast transfers from alien species to potato may clarify the role of the plastome in controlling these as well as other traits which are of great relevance to potato breeding. Organelle transfer by the donor-recipient protoplast fusion (see Galun and Aviv 1986) is applicable to potato. *Solanum* species serving as organelle donors (e.g. *S. commersonii, S. brevidence, S. demissum, S. chacoense, S. bertaulti, S. eutuberosum*) have recently been established (Perl, Aviv and Galun, unpublished).

Cybrids containing the nuclear genome of potato and chloroplasts from an alien species with specific, maternally inherited features of reactivity to environmental stress should become amenable to molecular investigation. It may then be possible to correlate alterations in base sequences of specific genes or controlling domains of ctDNA to an altered reaction to the environment. In general, acquaintance with the structure of the potato plastome and the availability of a ctDNA gene bank in *S. tuberosum* provide the tools for a thorough investigation of nuclear genome/plastome compatibilities in cybrids with *S. tuberosum* nuclei and chloroplasts of alien *Solanum* species.

Acknowledgements. We are grateful to Hillel Fromm, Department of Plant Genetics, The Weizmann Institute of Science, Rehovot, for providing cloned tobacco ctDNA fragments. We would also like to thank Drs. Catherine Carter and Melvin Henninger, Rutgers University, for supplying us with potato plants.

References

Anonymous (1984) Potatoes for the developing world. International Potato Center (CIP), Lima, Peru, p 148

Bovenberg WA, Howe CJ, Cool AJ, Nijkamp HJJ (1984) Physical mapping of genes for chloroplast DNA encoded subunit polypeptides of the ATPsynthase complex from *Petunia hybrida*. Curr Genet 8:283–290

Buckner B, Hyde BB (1985) Chloroplast DNA variation between the common cultivated potato (*Solanum tuberosum*

ssp. *tuberosum*) and several South American relatives. Theor Appl Genet 71:527–531

Cannon GC, Heinhorst S, Weissbach A (1985) Quantitative molecular hybridization on nylon membranes. Anal Biochem 149:229–237

Cohen BN, Coleman TA, Schmitt JJ, Weissbach H (1984) In vitro expression and characterization of the translation start site of the *psb*A gene product (Q_B protein) from higher plants. Nucleic Acids Res 12:6221–6230

Cordingly JS, Taylor DW, Dunne DW, Butterworth AE (1983) Clone banks of cDNA from the parasite *Schistosoma mansoni:* isolation of clones containing a potentially immunodiagnostic antigen gene. Gene 26:25–39

Dagbert M, Ehrlich SD (1979) Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene 6:23–28

Fluhr R, Edelman M (1981a) Conservation of sequence arrangement among the Solanaceae and between *Nicotiana* and *Spinacia.* Nucleic Acids Res 9:6841–6853

Fluhr R, Edelman M (1981b) Physical mapping of *Nicotiana tabacum* chloroplast DNA. Mol Gen Genet 181:484–490

Fluhr R, Fromm H, Edelman M (1983) Clone bank of *Nicotiana tabacum* chloroplast DNA: mapping of the alpha, beta and epsilon subunits of the ATPase coupling factor, the large subunit of ribulosebisphosphate carboxylase, and the 32-KDal membrane protein. Gene 25:271–280

Fromm H, Edelman M, Koller B, Goloubinoff P, Galun E (1986) The enigma of the gene coding for ribosomal protein S12 in the chloroplasts of *Nicotiana.* Nuclei Acids Res 14:883–898

Galun E, Aviv D (1986) Organelle transfer. Methods Enzymol 118:595–611

Green RM, Vardi A, Galun E (1986) The plastome of *Citrus.* Physical map, variation among *Citrus* cultivars and species and comparison with related genera. Theor Appl Genet 72:170–177

Hawkes JG (1978) Biosystematics of the potato. In: Harris PM (ed) The potato crop. Chapman and Hall, London, pp 15–69

Hetherington SE, Smillie RM, Malagamba P, Huaman Z (1983) Heat tolerance and cold tolerance of cultivated potatoes measured by chlorophyll-fluorescence method. Planta 159:119–124

Hildebrand M, Jurgenson JE, Ramage RT, Bourgue DP (1985) Derivation of a physical map of chloroplast DNA from *Nicotiana tabacum* by two-dimensional gel and computer-aided restriction analysis. Plasmid 14:64–79

Hosaka K (1986) Who is the mother of the potato? – restriction endonuclease analysis of chloroplast DNA of cultivated potato. Theor Appl Genet 72:606–618

Hosaka K, Ogihara Y, Matsubayashi M, Tsunewaki K (1984) Phylogenetic relationships between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jpn J Genet 59:349–369

Kahn M, Kolter R, Thomas C, Figurski D, Meyer R, Remaut E, Helinski DR (1979) Plasmid cloning vehicles derived from plasmids ColEI, F, R6K. In: Wu R (ed) Methods in enzymology, vol 68. pp 268–280

Koller B, Fromm H, Galun E, Edelman M (1987) Evidence for in vivo trans-splicing of pre-mRNAs in tobacco chloroplasts. Cell 48:111–119

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. Cold Spring Harbor Laboratory

Norrander J, Kempe T, Messing J (1983) Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene 26:101–106

Palmer JD (1985) Comparative organization of chloroplast genomes. Annu Rev Genet 19:325–354

Palmer JD (1986) Isolation and structural analysis of chloroplast DNA. In: Weissbach A, Weissbach H (eds) Methods in enzymology, vol 118. pp 167–185

Palmer JD, Osorio B, Aldrich J, Thompson WF (1987) Chloroplast DNA evolution among legumes: loss of a large inverted repeat occurred prior to other sequence rearrangements. Curr Genet 11:275–286

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationship in *Lycopersicon.* Proc Natl Acad Sci USA 79:5006–5010

Perl-Treves R, Galun E (1985) The *Cucumis* plastome: physical map, intragenic variation and phylogenetic relationships. Theor Appl Genet 71:417–429

Perl-Treves R, Zamir D, Navot N, Galun E (1985) Phylogeny of *Cucumis* based on isozyme variability and its comparison with plastome phylogeny. Theor Appl Genet 71: 430–436

Phillips A (1985) Restriction map and clone bank of tomato plastid DNA. Curr Genet 10: 147–152

Ruether U (1982) pUR250 allows rapid chemical sequencing of both DNA strands of its inserts. Nucleic Acids Res 10: 5765–5772

Rusche JR, Howard-Flanders P (1985) Hexamine cobalt chloride promotes intermolecular ligation of blunt end DNA fragments by T4 DNA ligase. Nucleic Acids Res 13: 1997–2008

Shinozaki K, Ohme T, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chungwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986a) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5:2043–2049

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayasha T, Zaita N, Chungwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kata A, Tohdoh N, Shimada H, Sugiura M (1986b) The complete nucleotide sequence of the tobacco chloroplast genome. Plant Mol Biol Rep 4:111–175

Smillie RM, Hetherington SE, Ochoa C, Malagamba P (1983) Tolerance of wild potato species from different altitudes to cold and heat. Planta 159:112–118

Summers J (1975) Physical map of Polyoma viral DNA fragments produced by cleavage with a restriction enzyme from *Haemophilus aegypticus:* endonuclease R. HaeIII. J Virol 15:946–953

Zhu YX, Duvall EJ, Lovett PS, Kung SD (1982) *Nicotiana* chloroplast genome. V. Construction, mapping and expression of clone library of *N. otophora* chloroplast DNA. Mol Gen Genet 187:61–66