# A large deletion in the plastid DNA of the holoparasitic flowering plant *Cuscuta reflexa* concerning two ribosomal proteins (*rpl2*, *rpl23*), one transfer RNA (*trnI*) and an ORF 2280 homologue

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Abstract. We have determined the nucleotide sequence of a 5.3-kb region of the plastid DNA (ptDNA) from the heterotrophic holoparasitic plant Cuscuta reflexa. The cloned area contains genes for the D1-protein (32-kDa protein; *psbA*), tRNA<sup>His</sup> (*trnH*), ORF 740 (homologous to ORF 2280 from Nicotiana tabacum), ORF 77 (homologous to ORF 70), tRNA<sup>Leu</sup> (trnL) and a hypothetical ORF 55 which has no homology to any known gene among higher plants. This 5.3-kb area is colinear with a 12.4-kb region of tobacco ptDNA and has therefore undergone several deletions totalling 7.1 kb. Most of the missing nucleotides belong to one large deletion in the ptDNA of C. reflexa of approximately 6.5 kb. This deletion involves two ribosomal protein genes, rpl2 and rpl23, as well as the transfer RNA for Isoleucin (trnI) and a region encoding 1540 amino-acid residues of an ORF 2280 homologue, as compared to tobacco chloroplast DNA. This is remarkable since the remaining genes, especially the psbA gene, are highly conserved in C. re*flexa*. Furthermore, we found that the expression of the psbA gene is in the same range as in the autotrophic Ipomoea purpurea which belongs to the same family as *Cuscuta* (Convolvulaceae). Here we hypothesize a total loss of *rpl2* and *rpl23* in the entire genome of *C. reflexa*. The phylogenetic position of, and the evolutionary change of ptDNA from, Cuscuta are discussed.

Key words: Cuscuta – Parasitic plant – Chloroplast genome – Plastid evolution – psbA – Gene expression

# Introduction

The genus *Cuscuta* exclusively consists of holoparasitic flowering plants wich have lost their ability to grow photoautotrophically. In this genus, different degrees of adaptation to parasitic life can be observed. We have demonstrated this at the cytological as well as the biochemical level. While *Cuscuta europaea* is totally achlorophyllous, *Cuscuta reflexa* contains small amounts of chlorophyll, but in both species no Rubisco activity is detectable in crude extracts (Machado and Zetsche 1990; Haberhausen et al. 1992). At the cytological level, the plastids of *C. reflexa* show only a small number of thylakoid membranes, while in *C. europaea* no thylakoid membranes can be observed (Machado and Zetsche 1990).

We now have to elucidate whether this different adaptation to parasitic life could also be seen at the molecular level, and especially in the plastid DNA (ptDNA) of *Cuscuta*. We believe that *Cuscuta* is an excellent tool to answer questions regarding the stability and the evolution of ptDNA.

The chloroplast genome has been extensively examined among photoautotrophic angiosperms (for reviews see: Whitfeld and Bottomley 1983; Palmer 1985; Sugiura 1992) and the complete sequence is known from two higher land plants, tobacco and rice (Shinozaki et al. 1986; Hiratsuka et al. 1989). Comparison of these sequences reveals a great similarity in both the gene arrangement and the coding capacity of chloroplast DNA. Therefore, the ptDNA from higher plants has been described as a very stable genetic system during land plant evolution with a relatively low mutation rate as compared to that of the nucleus (Wolfe et al. 1987; Zurawski and Clegg 1987; Palmer 1990).

Chloroplasts of higher plants encode for about 30 tRNAs which are probably sufficient to recognize all 64 codons (for review see Sugiura 1992). As in the case of the ribosomal protein genes, chloroplasts code for one-third of the 60 different protein components found in chloroplast ribosomes (Sugiura 1992). In tobacco, several ribosomal protein genes are clustered near the junction between the inverted repeat (IR) and the large single copy region (LSC). The genes rpl23 and rpl2 are duplicated in *Nicotiana tabacum* by being part of both segments of the IR (Shinozaki et al. 1986).

Little is known about the arrangement and development of ptDNA in parasitic plants during the course of

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evolution, and where presumably no selective constraint effects are any longer operative. There are only three non-photosynthetic species reported so far. The root-parasitic Epifagus virginiana (dePamphilis and Palmer 1990) lacks most of the photosynthetic and putative chlororespiratory (ndh) genes. This results in a chloroplast genome of only 71 kb in size. A similar situation has been found in a related member of the Orobanchaceae, Conopholis americana (Wimpee et al. 1991, 1992). In Conopholis, some regions in the ptDNA are highly conserved, whereas other regions are either absent or highly divergent (Wimpee et al. 1991). In a previous paper (Haberhausen et al. 1992), we have shown that in the stem parasite C. reflexa most of the photosynthesis-related genes are present. The nucleotide sequences of the *rbcL* and *atpB* genes are highly conserved as compared to those of tobacco but the regulatory sequences (promoter, termination sites) show Cuscuta-specific deletions. In the present study, we describe an area of ptDNA from C. reflexa which shows more differences to the corresponding region in tobacco and where a large deletion has taken place. Two ribosomal protein genes, rpl2 and rpl23, are affected by this deletion. For this reason, the whole translation apparatus might be non-functional in C. reflexa.

# Materials and methods

Nucleic acid isolation. C. reflexa Roxb. was grown in a greenhouse on Coleus sp. host plants. Total cellular DNA was isolated from C. *reflexa* and *N. tabacum* as described (Haberhausen et al. 1992). For the isolation of total RNA from *C. reflexa* and *Ipomoea purpurea* (L.), small stem pieces of both plants were ground in liquid nitrogen and RNA was isolated according to the LiCl method (Ausubel et al. 1988).

Molecular cloning of the psbA gene and its flanking regions. Total cellular DNA from C. reflexa was digested with HindIII and the psbA gene was located on a 6.1-kb fragment by heterologous hybridization (psbA gene probe kindly provided by P. Westhoff and R. G. Herrmann). This fragment was cloned into pUC18 using standard methods (Maniatis et al. 1982).

Sequence analysis. Parts of the 6.1-kb clone were subcloned into pUC18 and sequenced using a T7 sequencing system (Pharmacia). The sequence of both strands was determined and all restriction sites were confirmed. Computer analysis was performed using DNASIS (HIBIOsystems) and 'Kröger-menu' (Kröger and Kröger-Block 1984). The sequences reported in this paper have been deposited in the EMBL Data Library under the accession number X67512.

Southern analysis. To check the deletion in C. reflexa ptDNA, two gene probes from N. tabacum containing parts of ORF2280 and the rpl2 and rpl23 genes were hybridized to N. tabacum and C. reflexa total cellular DNA (1  $\mu$ g/lane) using the ECL direct labelling and detection system (Amersham). Gene probes were kindly provided by M. Sugiura.

Northern analysis. Total cellular RNA isolated from the stems of C. reflexa and I. purpurea was electrophoresed on 1.5% formaldehydagarose gels (15  $\mu$ g/lane) and transferred onto nitrocellulose as described by Davies et al. (1986). Filters were hybridized to direct-labelled probes (ECL, Amersham).

..ORF509-A

1	GCTTGGGCTTAAAATTATTGGAAGAATTCTTTACGTCGGAAGAGCAAATTATTTTTTTAACTTTCCCAAACGCTTCGTTGAATTTGCGAGGAGTGTCGAAAGGGCGGATTTGGTATTTTG LeuGlyLeuLysLeuLeuGluGluPhePheThrSerGluGluGlnIleIlePheLeuThrPheProAsnAlaSerLeuAsnLeuArgGlyValSerLysGlyArgIleTrpTyrPheAsp
121	ATATAGTITATATCAATGAGCAGGCCAATTITTAATGATTCAGTCITCAATCITCAATATTCITAAAATCAAATTITGAATAAATTCITAAGGITGICTITITCCACTATAATCIGAAAT IlevaltyrileasnGluginalaasnPhe-c
241	CTTGATTCAAAATGATAAATGAAAATTTACCCGAGGCTAAAGCCGTGTGCAATGAAAAACTAAAGCAATATAAATTTTTTTT
361	
481	psbA GTCCTTGATGATTAAATAAACCAAGAATTTACCATGACTGCAATTTTAGAGAGAG
601	ATTGGATGGTTTGGCGTTTTGATGATCCCTACTTTATTGACCGCAACTTCTGTATTATTATTGCCTTCATTGCTGCTCCAGTAGATATAGATGGTATTCGGGAACCTGTTTCTGGA lleGlyTrpPheGlyValLeuMetIleProThrLeuLeuThrAlaThrSerValPheIleIleAlaPheIleAlaAlaProProValAspIleAspGlyIleArgGluProValSerGly
721	TCTCTGCTTTACGGAAACAATATTGTTTCGGGTGCCATTATTCCGACTTCTGCAGCTATAGGTTTACACTTTTACCCAATATGGGAAGCGGCGTCTGTTGATGAATGGTTATACAACGGT SerLeuLeuTyrGlyAsnAsnIleValSerGlyAlaIleIleProThrSerAlaAlaIleGlyLeuHisPheTyrProIleTrpGluAlaAlaSerValAspGluTrpLeuTyrAsnGly
841	GGTCCTTATGAACTAATTGTTCTACACTTTTTACTTGGTGTAGCTTGTACATGGGTCGTGAGTGGGAACTGAGTTTCCGTTTGGGTATGCGCCCTTGGATTGCTGTTGCGTACTCAGCT GlyProTyrGluLeuIleValLeuHisPheLeuLeuGlyValAlaCysTyrMetGlyArgGluTrpGluLeuSerPheArgLeuGlyMetArgProTrpIleAlaValAlaTyrSerAla
961	CCTGTTGCAGCTGCTACCGCAGTTTTCTTGATCTATCCAATTGGTCAGGGAAGTTTTTCTGATGGTATGCTCTGAGAATCTCTGGTACTTTCAATTTCATGATTGCATGCCGAG ProValAlaAlaAlaThrAlaValPheLeuIleTyrProIleGlyGlnGlySerPheSerAspGlyMetProLeuGlyIleSerGlyThrPheAsnPheMetIleValPheGlnAlaGlu
1081	CACAACATTCTTATGCACCCATTTCATATGTTAGGCGTAGCTGGTGTATTCGGTGGTTCCCTATTCAGTGCATGGTTCCTTGGTAACTTCCAGTTGATCAGGGĂAACCACAGAĂ HisAsnileleuMethisProPhehisMetLeuGlyValAlaGlyValPheGlyGlySerLeuPheSerAlaMethisGlySerLeuValThrSerSerLeuIleArgGluThrThrGlu
1201	AATGAATCTGCTAACGAAGGTTACAGATTCGGTCAAGAGGAAGAAACTTACAATATCGTAGCCGCTCATGGTTATTTTGGCCGATTGATT
1321	TCGTTACACTTCTTCTTAGCTGCTTGGCCTGTAGTAGGTÄTCTGGTTCACTGCTTTAGGTATCAGCACTÄTGGCCTTTAÅCCTAAACGGTTTCAACCAATCCGTAGTTGATAGC SerLeuHisPhePheLeuAlaAlaTrpProValValGlyIleTrpPheThrAlaLeuGlyIleSerThrMetAlaPheAsnLeuAsnGlyPheAsnPheAsnGlnSerValValAspSer
144 <b>1</b>	CAAGGCCGGGTAATTAATACTTGGGGTGATATCATTAATCGTGGTAATCTTGGTATGGAAGTTATGCATGAACGTAATGCTCACAACTTCCCTCTAGACCTAGCTGCTATTGAAGCTCCA GlnGlyArgValIleAsnThrTrpAlaAspIleIleAsnArgAlaAsnLeuGlyMetGluValMetHisGluArgAsnAlaHisAsnPheProLeuAspLeuAlaAlaIleGluAlaPro
45.44	

1681	GGATGTAGCCAAGTGGATTAAGGCGGTGGATTGTGAATCCACTATGCGCGGGTTCAATTCCCGCCGTTCGCCCACACTCGAATCGCTTTTTCTATAAATACGAGACATATAAGTCATACA
1801	AGTAAAGAGATCTATTCATTGATAAGAAAAAAGAAAAAAAGTGAACGGGGTTTGGATTGATGATAAACTAGAATCCTGGGTCGCGAACAGTGATTCATGAGGAAGAAAAAAATTC
1921	ORF/40 TTGGTTCAGCTATCCGCCTTAACGACAGAAAAAAGAATTCTATTGAGTCTGACTCATAGTGATCATTTCTCAAAGAATGACTCTGGTTATCAAATGATTGAACAACCGGGAGCAATTTAT Metilegluginproglyalailetyr
2041	TTACGATACTTAGTTGACATTCATCAAAAAGCATCTAATGAATTATGAGTTCAATACCTCCTGTTTAGCAGAAAGACGGATATTCCTTGCTCATTCTCAGACAATCACTTATTCACAAACT LeuArgTyrLeuValAspIleHisGlnLysHisLeuHetAsnTyrGluPheAsnThrSerCysLeuAlaGluArgArgIlePheLeuAlaHisSerGlnThrIleThrTyrSerGlnThr
2161	TCGTATGGGACTAATAGTTTTCATTTCCCATCTCATGGAAAACCTTTTTCGCTCCGCTTAGCCTTATCCCCCTCTAGGGGTATTTTAGTGATAGGTTCTATAGGAACTGGACGATCCTAT SerTyrGlyThrAsnSerPheHisPheProSerHisGlyLysProPheSerLeuArgLeuAlaLeuSerProSerArgGlyIleLeuValIleGlySerIleGlyThrGlyArgSerTyr
2281	TTGGTAAAATACCTAGCGACAAACTCCTATGTTCCTTTCATTACGGTATTCCGAACAAGTTCCGGGATAAGAATCCTCAATTTATTGATGATAATGGATATTGATAATAGTAACAATATT LeuVallysTyrLeuAlathrAsnSerTyrValProPheileThrValPheProAsnLysPheleuAspLysAsnProGlnPheileAspAspIleAspIleAspileAspi
2401	GATGCTAGTGACGATATCGATATGGATAATAGTGACAATATTGATGATGATGATGATGGTGGACCTTGCTACAGAGCTGGAACTGCTCACTTGGATGAATGCGCTAACTATGGATAGGGAG AspAlaSerAspAspIleAspMetAspAsnSerAspAsnIleAspAspAspIieAspArgAspLeuAlaThrGluLeuGluLeuLeuThrTrpMetAspAlaLeuThrHetAspArgGlu
2521	ATGAAGGCGGAAATAGCCCGATTGTTTATCACCCTTCAATTCGAATTAGCAAGAGCAATGTCTCCTTGCATAATATGGATCCCCAACATTCATGATCTGGATGTGAATGAGTCGAATTAC MetLysAlaGluIleAlaArgLeuPheIleThrLeuGInPheGluLeuAlaArgAlaMetSerProCysIleIleTrpIleProAsnIleHisAspLeuAspValAsnGluSerAsnTyr
2641	TTATCCCTCCGGGCTATTAGTGAACCATCTCTCCAGGGATTGTGAAAGATGTTCTCCCTATAAATATTCTTGTTATTGCTTCGACTCATATTCCCCAAAAAGTGGATCCCCCTCTAATAGCT LeuSerLeuGlyLeuLeuValAsnHisLeuSerArgAspCysGluArgCysSerProIleAsnIleLeuValIleAlaSerThrHisIleProGlnLysValAspProAlaLeuIleAla
2761	CCGAAAAAATTAAATACGTGCATTAAGTTACGAAGGCTTCTTATTCCACAACAACGAAAGTACTTTTTCACTCTTTCATATACTAGGGGATTTCACTTGGAAAATAAAATGTTCCATACT ProlyslysleuAsnThrCysIlelysleuArgArgLeuLeuIleProGlnGlnArgLysTyrPhePheThrLeuSerTyrThrArgGlyPheHisLeuGluAsnLysMetPheHisThr
2881	AACGGATTCGGGTCCATAACCATGGGGCCCAATGCACGAGATATTGTAGCACTTACCAATGAGGTCCTATCAATTAGTATTACACAGAAGAAATCAATTATAGACACTAATACGA AsnGlyPheGlySerileThrMetGlyProAsnAlaargAspileValAlaLeuThrAsnGluValLeuSerileSerileThrGlnLysLysSerileIleAspThrAsnThrIleArg
3001	TCCGCTCTTCATAGACAAACTTGGGATTTGCAATCCCAGGTAAGATTGGTTCAGGATCATGAGATCCTTTTCTATCAGATAGGAAGGGCTGTAGCACAAAATGTACTTATAAGTAATTTA SerAlaleuHisArgGlnThrTrpAspleuGinSerGlnValArgLeuValGlnAspHisGluileLeuPheTyrGlnIleGlyArgAlaValAlaGlnAsnValLeuIleSerAsnLeu
3121	AGTAATTGCCCCATAGATCCTATATCTATCTATCTGAAGAATAAATCATGTAACGAGGGGGATTCTTATTTGTACAAATGGTACTTCGAACTAGGAACTGGAAGAATTGACGATA SerAsnCysProIleAspProIleSerIleTyrLeuLysAsnLysSerCysAsnGluGlyAspSerTyrLeuTyrLysTrpTyrPheGluLeuGlyThrSerMetLysLysLeuThrIle
3241	CTTCTTTATCTTTTGAGTTGTACTGCCGGATCGGTCGCTCAAGATCTTTGGTCTTTACCCGGACCCGATGAAAAAACGGGATCACTTCCTATGGACTCGTTGAGAATGATTCTGATCTA LeuLeuTyrLeuLeuSerCysThrAlaGlySerValAlaGlnAspLeuTrpSerLeuProGlyProAspGluLysAsnGlyIleThrSerTyrGlyLeuValGluAsnAspSerAspLeu
3361	GTTCATGGCCTATTAGAAGTAGAAGGCGCTCTGGTGGGATCTTCACGGACAGAAAAAAATTGCAGTAAGTTTGAGAATGATCGAGTGACATTGCTTCTTCGGCCCGAACCGAGGAATCCC ValhisGlyLeuLeuGluValGluGlyAlaLeuValGlySerSerArgThrGluLysAsnCysSerLysPheGluAsnAspArgValThrLeuLeuLeuArgProGluProArgAsnPro
3481	TTAGAGAGGATGCAAAACGGATCTTGTTCTATCCTTGATCAGAGATTTCTCTCTGAAAAATACGAATCGGAGTTTGAAGAAGGCGCCCCTGCCCCGCAACAGATAGAGGAGGATTTATTC LeuGluArgMetGlnAsnGlySerCysSerIleLeuAspGlnArgPheLeuSerGluLysTyrGluSerGluPheGluGluGlyAlaLeuAlaProGlnGinIleGluGluAspLeuPhe
3601	AATCACATAGTTTGGGCTCCTACAATATGGCGCCCTTGGGCGTTTCTTTGTATCGAAAGGCCCAATGAATTGGGATTTTCCTATTGGTCCAGGTCATTTCGGGGCAAGCCGATCTTGTAT AsnHisIleValTrpAlaProThrIleTrpArgProTrpAlaPheLeuCysIleGluArgProAsnGluLeuGlyPheSerTyrTrpSerArgSerPheArgGlyLysArgIleLeuTyr
3721	GATGAAGAGGATGAGCTTGAAGAGAATGATTCGGAGTTCTTGCAGAGTGGAACCATGCAGTACAAGACACGAGATAGAT
3841	TGGGACCCTGCAGATCCACTATTITTCTATTCAAAGATCGGCCCCCTGGCTCTGTGTTTTCACGTCTAGAATTATTTGCAGATGAAGAGATGTCAAAGGGGCTTCTTACTTCCCAAACT TrpAspProAlaAspProLeuPhePheLeuPheLysAspArgProProGlySerValPheSerArgLeuGluLeuPheAlaAspGluGluHetSerLysGlyLeuLeuThrSerGlnThr
3961	TCCCAAATTGAGCATCTATTTAGATATAAATATACACGCTGGTTTATCAACAAGACGCAAGAAAAGCACTTCGAATTTTTGATTCATCGTCAGAGATGTCTTAGAACCAATAGTTCATTA SerGlnIleGluHisLeuPheArgTyrLysTyrThrArgTrpPheIleAsnLysThrGlnGluLysHisPheGluPheLeuIleHisArgGlnArgCysLeuArgThrAsnSerSerLeu
4081	TCTAATAGATCTTTCCGTTCTAATACTCTATCCGAGAGTTATCAGTATTTATCAAAATCTGTTCCTATCTAACGGAACACTATTGGATCAAATGACAAAGACTTTGTTGAGAAAAAAGATGG SerAsnArgSerPheArgSerAsnThrLeuSerGluSerTyrGlnTyrLeuSerAsnLeuPheLeuSerAsnGlyThrLeuLeuAspGlnMetThrLysThrLeuLeuArgLysArgTrp
4201	CTTTTCCCGGATGAAAATGAAAATGGATTCATGTAACAGGAGAAAAGATTTCCCATTCCTTTCCTTAGCCGCAAAGATATGTGGCCATGAAAGAGGGGATTCAGTGGAACAGAATTGACT LeuPheProAspGluMetLysIleGlyPheMet-c-
4321	"-35" "-10" ORF77 GGGGGGTAGAGTCGTGGAAACGCTTGTTTCTTCCATATT <u>TTGGAC</u> CTTAGCTCCGTGGAACTA <u>TATGTT</u> ACTGCTGAAACACAGAAGAATTTAAATCTTGGATCAAAACACTATGTATG
4441	ATGGTATGAACTGCCTAAACAAGAATTCTTGAACAGCAAACAACCGGTTCATATATTCACGACCAAGAAGTACTGGATTCTCTTTCGGATAGGCCCTGAAAGGAGGAAGGCTGGAAT TrpTyrGluLeuProLysGlnGluPheLeuAsnSerLysGlnProValHisilePheThrThrLysLysTyrTrpileLeuPheArgileGlyProGluArgArgArgLysAlaGlyMet
4561	GCCAAAGGCGTCTATTATTTCATTCACCCGACCCAATCCAGTTTGGGAACGTCCAGTGCCAAAGTCACTGACTG
4681	
4801	
4921	
5041	ATCTATCTAGTGTAATGTATGGAATATATGAAAAAGGGTGGAGTATTTATATAGATCGGTCATGTCATATAGGACCCAGTTGGACATCCCAATTGCTTCGATTTGAATTATCCGGAGAATGC MetGluTyrMetLysArgValGluTyrLeuTyrArgSerValMetSerTyrArgThrGlnLeuAspIleGlnLeuLeuArgPheGluLeuSerGlyGluCys
5161	CTTATAGATATATCAAAAAGATGGACAATGAAACCTCTTTCTCGATTCACTCAAAGAGGTGAATAGGGTTCCAAAGAGGAGATATGTAAAAAGAAGGTTCGATTGCGTCTATTCCTAATCC Leu[leAsplleSerlysArgTrpThrilelysProleuSerArgPheThrGlnargGlyGlu-a-

# 5281 TAAATGGAATGTAACGACGTAGGGATTAGGGATCC

Fig. 1. Nucleotide and deduced amino-acid sequences of a 5.3-kb region of the plastid DNA from C. reflexa. Putative promoters are underlined (-35 region and -10 region). Opposite arrows indicate potential termination sequences

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Fig. 2. Comparative gene arrangement of *N. tabacum* (NT) and *C. reflexa* (CR). A 12.4-kb area from tobacco corresponds with a colinear 5.3-kb region in the ptDNA of *C. reflexa*. Data for the tobacco gene map is derived from Shinozaki et al. (1986) and Sugiura (1992)

### Results

### Gene arrangement in Cuscuta reflexa

The nucleotide sequence of a 5.3-kb region of the *C*. reflexa plastid genome was determined (Fig. 1). The gene arrangement, presented in Fig. 2, is the same as in the autotrophic higher plant *N*. tabacum, a closely related angiosperm (*Nicotiana* and *Cuscuta* both belong to the order Solanales). The cloned area contains genes for the D1 protein (32-kDA protein; *psbA*), tRNA<sup>His</sup> (*trnH*), ORF740, ORF77, tRNA<sup>Leu</sup> (*trnL*), and ORF55, and is colinear with a 12.4-kb region of tobacco ptDNA (Fig. 2). The difference in size is due to several deletions, totalling approximately 7.1 kb, in the ptDNA of *C*. reflexa.

# Deletions upstream of the psbA gene affect trnK

The *psbA* gene in *C. reflexa* ptDNA is highly conserved as compared to other higher plants. The homology to corresponding genes is in the range of 94% (e.g., *N. tabacum*, Sugita and Sugiura 1984). Neither the coding



Fig. 3. Southern blots of *Nicotiana* (*NT*) and *Cuscuta* (*CR*) total cellular DNA (digested with *Bam*H1, *Eco*RI, *Hin*dIII) hybridized against two gene probes derived from a tobacco-homologous subclone (pTB28). Blots illustrate the absence of the 5'-ORF 2280 (*probe 1*) and *rpl2/rpl23* genes (*probe 2*) from *Cuscuta* total cellular DNA. Stringency conditions:  $2 \times 20 \text{ min } 1 \times \text{SSC}$ , 0.5% SDS at  $25^{\circ}\text{C}$ 

region nor the regulating sequences (promoter, terminator) are remarkably different. Upstream of the *psbA* gene the 3'-end of an ORF 509A homologue is located (Fig. 2). In *N. tabacum*, this ORF 509A is present in an intron between the two exons of tRNA<sup>Lys</sup> (*trnK*). The 3'-exon of tRNA<sup>Lys</sup> (UUU) seems to be lost in *C. reflexa* due to a deletion of about 180 bp. No statement about the 5'-exon is possible as yet (Fig. 2).

# rpl2 and rpl23 have been deleted in the plastid DNA of C. reflexa

Downstream from *psbA*, a large deletion of about 6.5-kb can be observed. The deleted area starts with the first base of the large inverted repeat A (IRA) when compared to tobacco. This deletion comprises two ribosomal protein genes, *rpl2* and *rpl23*, the gene for tRNA<sup>IIe</sup> (*trnI*), as well as 1540 amino-acid residues of an ORF 2280 homologue. In order to find out whether these genes have been translocated in *C. reflexa*, heterologous hybridization experiments were carried out. Neither gene probes for *rpl2* and *rpl23* nor one for the 5'-end of ORF 2280 hybridized to total DNA from *C. reflexa* (Fig. 3).



**Fig. 4.** Transcript of the *psbA* gene from *I. purpurea* (*IP*) and *C. re-flexa* (*CR*). In Northern blots a *Cuscuta*-specific gene probe hybridized with total cellular RNA of approximately 1.3 kb in size. Stringency conditions:  $2 \times 20$  min 6 M Urea,  $1 \times SSC$ , 0.4% SDS at  $42 \,^{\circ}C$ 

# ORF 740, ORF 77 and ORF 55 downstream from the psbA gene

As a result of this large deletion the open reading frame in *C. reflexa* homologous to ORF 2280 (tobacco, Sugiura 1992) and ORF 2131 (spinach, Zhou et al. 1988) is reduced to 740 amino acids. The overall degree of aminoacid identity is in the range of 90%.

There are two other ORFs between the *psbA* gene and *trnL* in the ptDNA of *C. reflexa* (Fig. 1). ORF 77 shows homology (approximately 87% identical residues) to ORF 70 of tobacco in a 54 amino-acid region. ORF 55 shows no homology to any known gene and may have been formed spontaneously due to several base substitutions. Two other ORFs (ORF 92, ORF 115) found in the chloroplast genome of tobacco between ORF 2280 and *trnL* are lost in *C. reflexa* ptDNA.

# tRNAs downstream from the psbA gene

In the chloroplast genome of *C. reflexa* only two tRNAs, tRNA<sup>His</sup> (GUG) and tRNA<sup>Leu</sup> (CAA); are located downstream from the *psbA* gene; tRNA<sup>IIe</sup> (CAU) is deleted. The *trnH* gene shows identical nucleotides at 94.7% of positions compared to the *trnH* from *N. tabacum* while *trnL* is identical (100%). The *trnI* (CAU) gene located between *rpl23* and ORF 2280 of *N. tabacum* ptDNA is lost in the corresponding region of the *Cuscuta* chloroplast genome but another *trnI* (GAU) gene has been detected in the 16S-23S spacer where it is 100% homologous to tobacco (Gerd Haberhausen, unpublished results).

# Transcript level of the psbA gene

We used a *psbA*-specific gene probe from *C*. *reflexa* to investigate the transcript level in Northern experiments using total cellular RNA from *C*. *reflexa* and *I*. *purpurea*. For this purpose, the same organs (stems) were chosen to

obtain more reliable results. Surprisingly similar amounts of mRNA specific for *psbA* could be detected in both *C*. *reflexa* and *I. purpurea* (Fig. 4). No hybridization signals for ORF740 were found in *C. reflexa* (data not shown).

# Discussion

Sequence analysis of a 5.3-kb region of the chloroplast genome from the holoparasitic flowering plant C. reflexa reveals a genomic organization that differs to a large extent from the corresponding region in the non-parasitic plant N. tabacum. This is surprising since examination of another region in the ptDNA of C. reflexa revealed that neither the gene content nor the nucleotide sequence of six plastid genes are significantly different from those of N. tabacum (Haberhausen et al. 1992). The ptDNA organization discussed here includes one gene (psbA) that shows no remarkable changes at the nucleotide sequence level in either its coding region or its the regulatory sequences. Also the transcription of the psbA gene in C. reflexa is comparable to that of an autotrophic plant (I. *purpurea*). In contrast to this, we found open reading frames (ORF 740, ORF 77) that could still be functional but with the grade of homology to corresponding genes decreased due to several deletions/insertions. Furthermore, some genes in C. reflexa ptDNA seem to be completely lost (rpl2, rpl23, trnI, trnK). Astonishingly, all lost genes are part of the translation apparatus. This raises the question of whether plastid-encoded genes in Cuscuta can be translated into proteins. In relation to this, two question scenarios are conceivable: (1) the missing genes have migrated into the nucleus and the gene products must be imported into the plastids from the cytoplasm, or (2) the genes are lost completely, which implies that the translation apparatus in plastids of C. reflexa is nonfunctional. The first of these two possibilities has been described for another ribosomal protein (rpl22) in some legumes by Gantt et al. (1991) and is the most recent case of gene transfer reported up to now. A number of similar events are known, due to a massive evolutionary transfer of genes from former endosymbionts to the nucleus of the host cell (Gray 1983; Palmer 1990).

In spinach, the *rpl23* gene seems to have become a pseudogene (Thomas et al. 1988) by splitting into two overlapping reading frames. As a consequence of this no rpl23 gene product is detectable. Results from several other plants examined indicate either a loss of requirement for the CL23 protein in chloroplasts or a transfer of the rpl23 gene to nuclear genome (Zurawski and Clegg 1987). On the other hand, ribosomal protein CL23 is functional in Marchantia polymorpha (Ohyama et al. 1986) and tobacco (Yokoi et al. 1991), which is more closely related to *Cuscuta* than is spinach. Here we hypothesize for a total loss of rpl2 and rpl23, based on the fact that no Rubisco protein (encoded by the plastid *rbcL* gene) is detectable in C. reflexa by immunological methods (Haberhausen et al. 1992). If one assumes total loss of the rpl2 and rpl23 genes then we are confronted with the interesting phenomenon that, on the one hand, several photosynthesis-related genes are present in *C. reflexa* in a relatively unaltered and hence functional form. On the other hand, the translational machinery of the plastids appears to be non-functional. Clear evidence regarding this question cannot be given at present. However, the homology of the area examined, especially the flanking sequences in non-coding regions close to the deletion, points to the fact that the deletion (transfer or loss) is a relatively recent event in the ptDNA of *C. reflexa*. Interestingly, the deletion starts exactly with the beginning of the large inverted repeat (IRA, IRB) as compared to tobacco, probably indicating a 'hot spot region' in plastid DNA.

It seems that the ptDNA in *C. reflexa* is not reduced to the same extent as in other parasitic plants (e.g., *Epifa*gus or *Conopholis*). On the genetic level this species may be regarded as a transition state between photosynthetic plants and those which are fully adapted to a parasitic life. Therefore, we believe that *C. reflexa* is an excellent model for studying the evolutionary and molecular development of the chloroplast genome.

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