Genetic Resources

The Chloroplast Genome of a Chlorophyll *a+c-*Containing Alga, *Odontella sinensis*

Klaus V. Kowallik, Bettina Stoebe, Ina Schaffran, Peter Kroth-Pancic, and Ulrich Freier

E-mail: kowallik@uni-duesseldorf.de Institut für Botanik der Heinrich-Heine-Universität Düsseldorf, D 40225 Düsseldorf, Germany

Key Words: chloroplast genome, Chromophyta, diatom, Odontella sinensis

Abstract: The chloroplast genome of a marine centric diatom, *Odontella sinensis*, was cloned and sequenced. The circular genome is 119,704 bp in length (AC = Z67753;). It contains an inverted repeat sequence of 7,725 bp separating two single-copy regions of 38,908 and 65,346 bp, respectively, and 174 genes and open reading frames, of which nine are duplicated within the inverted repeat segments.

hloroplast genomes from algae not belonging to the chlorophyll *a+b*-containing lineage have only recently been characterized by their entire nucleotide sequences. These include the plastid genome of *Porphyra purpurea* (191,028 bp; Reith & Munholland, 1995) and the cyanelle genome of the enigmatic unicellular flagellate *Cyanophora paradoxa* (135,599 bp; Stirewalt et al., 1995). To date, chloroplast genes from chlorophyll *a+c*-containing algae have been sequenced arbitrarily. They included genes that are known from land plant chloroplast genomes (*e.g., psaA; psbA, -C, -D; atpB, -E, -I, -H, -F, -A; rbcL; petB, -D;* cf. Kowallik, 1994), but also some that are novel or known to be nuclear in land plants (cf. Valentin et al., 1993).

In order to establish evolutionary traits among non-green plastids, the chloroplast genome of the centric diatom *Odontella sinensis* was cloned and sequenced. It contains 119,704 bp. An inverted repeat (IR) segment of 7,725 bp, containing the rRNA operon, separates a small single-copy (SSC) region of 38,908 bp from a large single-copy (LSC) region of 65,346

Abbreviations: IR, inverted repeat; LSC, large single-copy region; nt, nucleotide; SSC, small single-copy region.



Fig. 1. Gene map of the circular chloroplast genome of *Odontella sinensis.* Genes transcribed clockwise are shown inside the circle, those transcribed counter-clockwise, are shown outside the circle. The inverted repeats are indicated by bold-type segments. Counting of the sequence starts at the right-hand inverted repeat (IRa) and continues clockwise.

bp (Fig. 1). The *Odontella* chloroplast genome reveals an overall coding capacity of 84.3 percent and has a (G+C)-content of 31.8 percent, which is equivalent to calculations based on the density of purified plastid DNA by equilibrium density sedimentation in gradients of CsCl (Kowallik, 1989).

To date, 174 genes and open reading frames (minimum of 25 amino acid residues) have been determined, of which nine are duplicated within the IR (Table I). No intron sequences or *ndh* genes have been found. Four pairs of genes show a 3'-5' overlap (rpl4/rpl23, 8 nt; ycf24/ycf16, 1 nt; atpF/atpD, 4 nt; psbD/psbC, 53 nt). All three stop codons are

Table I. List of genes and ORFs of the *Odontella sinensis* **chloroplast genome.** The gene list proceeds clockwise from the LSC boundary of the right-hand inverted repeat (IRa). Genes are listed together with their putative gene products. ORFs with a (G+C)-content less than 30% (ORFs 25, 26a, 26b, 27, 29a, 29b, 41, 44, 46) are designated (A+T)-rich and may be non-coding. Numerical positions include the first nucleotide of the translation initiation codon as well as the last nucleotide of the stop codon. Transcription initiation and termination sites of tRNAs and rRNAs were determined according to multiple alignments and secondary structure models. Genes marked "(–)" are on the complementary strand.

Gene	Gene Product	Coding Sites	
	start IRa		1
trnP	tRNA-Pro(ugg)	267	340
ORF355		478	1545
rrn16	16S ribosomal RNA	2210	3694
trnI	tRNA-Ile(gau)	3761	3835
trnA	tRNA-Ala(ugc)	3838	3910
rrn23	23S ribosomal RNA	3976	6866
rrn5	5S ribosomal RNA	6938	7063
ycf32	ORF36	7318	7428
rpl32	50S ribosomal protein L32	7563	7739
,	end IRa	7725	
psaC	PSI, Fe-S polypeptide SU VII, 9 kDa	7825	8073
ycf5	ORF312 (-)	8139	9077
ORF46	(A+T)-rich	9287	9427
rps6	30S ribosomal protein S6	9482	9772
trnN	tRNA-Asn(guu)	9852	9922
thiG	involved in thiamine biosynthesis	9944	10729
clpC	Clp protease, caseinolytic-like (–)	10894	13551
trnC	tRNA-Cys(gca)	13780	13850
trnL	tRNA-Leu(uaa)	13921	14006
rps10	30S ribosomal protein S10 (–)	14207	14518
tufA	elongation factor Tu (–)	14531	15760
rps7	30S ribosomal protein S7 ()	15831	16307
rps12	30S ribosomal protein S12 (–)	16341	16721
rpl31	50S ribosomal protein L31 (–)	16773	16991
rps9	30S ribosomal protein S9 (-)	17005	17421
rpl13	50S ribosomal protein L13 (–)	17445	17864
rpoA	RNA polymerase α-chain (-)	17893	18831
rps11	30S ribosomal protein S11 (–)	18883	19275
rps13	30S ribosomal protein S13 (-)	19320	19691
rpl36	50S ribosomal protein L36 (–)	19713	19826
secY	preprotein-translocase subunit Y (–)	19866	21143
rps5	30S ribosomal protein S5 (-)	21166	21723
rpl18	50S ribosomal protein L18 (-)	21765	22172
rpl6	50S ribosomal protein L6 (-)	22219	22758
rps8	30S ribosomal protein S8 (–)	22777	23175

Chloroplast Genome of Odontella sinensis

rvl5	50S ribosomal protein L5 (–)	23209	23925
rpl24	50S ribosomal protein L24 (–)	23957	24190
rol14	50S ribosomal protein L14 (-)	24191	24556
rvs17	30S ribosomal protein S17 (–)	24569	24823
rnl29	50S ribosomal protein L29 (-)	24868	25137
rpl16	50S ribosomal protein L16 (-)	25144	25557
rns3	30S ribosomal protein S3 (–)	25609	26253
rpl22	50S ribosomal protein L22 (–)	26295	26642
ORF148	(-)	26691	271.37
rns19	30S ribosomal protein S19 (-)	27149	27427
rpl12	50S ribosomal protein L2 (-)	27472	28299
rnl23	50S ribosomal protein L23 (–)	28324	28632
rol4	50S ribosomal protein L4 (-)	28625	29272
rnl3	50S ribosomal protein L3 (-)	29320	29910
dnaK	Hsp70-type chaperone	30213	32057
oroFL	chaperonin 60 kDa (-)	32125	33711
trnR	tRNA-Arg(acg)	33891	33964
trnO	tRNA-Gln(uug)	34020	34089
nshW	PSII protein W 13 kDa	34326	34673
ucf40	ORE73 homologous to Parnhura ORE71 (-)	34997	35218
trnH	tRNA-His(gug)	35294	35368
rns4	30S ribosomal protein S4	35435	35955
rps1 rns16	30S ribosomal protein S16	36238	36/177
vcf35	ORF128	36607	26002
nshA	PSII D1 reaction-center protein (_)	37330	38/101
1014 1014	ORE382 homologous to Parultura ORE437 ()	37339	20700
ycf46	ORE497 homologous to Paruliura ORE491 ()	30700	41202
ycj±0 rn/34	50S ribosomal protein L 34 (-)	41404	41292
ser A	preprotein-trapslocase subunit (-)	41404	41000
rnl27	50S ribosomal protein L 27 (_)	44272	44447
rn/21	50S ribosomal protein L21 (-)	44272	44020
vcf30	former trsE(rhcR homolog) (-)	44040	44003
ycj30 truI	tRNA-Lou(upg) ()	45509	40430
11111	(ag) (-)	40484	40004
	end IRb	46634	
rpl32′	50S ribosomal protein L32' (–)	46632	46796
ycf32	ORF36 (–)	46931	47041
rrn5	5S ribosomal RNA (–)	47296	47421
rrn23	23S ribosomal RNA (–)	47493	50383
trnA	tRNA-Ala(ugc) (–)	50449	50521
trnI	tRNA-Ile(gau) (–)	50524	50598
rrn16	16S ribosomal RNA (–)	50665	52149
ORF355	(-)	52814	53881
trnP	tRNA-Pro(ugg) (–)	54019	54092
	start IRb		54358
аср	acyl carrier protein (–)	54453	54695
ycf45	ORF455, homologous to Porphyra ORF565 (-)	54953	56320
rpl20	50S ribosomal protein L20 (–)	56561	56905

rpl35	50S ribosomal protein L35 (-)	56915	57109
ycf42	ORF204, homologous to Porphyra ORF199 (-)	57112	57726
psaE	PSI, subunit IV (–)	57835	58050
, ycf25	ORF 644, homologous to E. coli		
5.5	cell-division protein FtsH	58339	60273
rps14	30S ribosomal protein S14	60428	60730
vsaM	PSI, protein M	60884	60976
chlI	chlorophyll biosynthesis, probable		
	magnesium-chelatase subunit	61054	62115
ycf47	ORF74, homologous to Porphyra ORF71	62143	62367
trnM	tRNA-Met(cau)	62586	62659
vsaD	PSI, ferredoxin-binding protein II	62744	63163
trnS	tRNA-Ser(uga)	63199	63286
vetB	cvtochrome b6	63341	63988
vetD	cytochrome b6/f complex, subunit IV	64035	64517
trnR	tRNA-Arg(ucu) ()	64774	64845
trnD	tRNA-Asp(guc) (-)	65093	65167
trnS	tRNA-Ser(gcu) (–)	65253	65340
trnfM	tRNA-fMet(cau) (-)	65380	65452
vcf33	ORF64	65614	65808
ORF29a	(A+T)-rich (–)	65920	66009
ORF41	(A+T)-rich (-)	66057	66182
trnY	tRNA-Tyr(gua) (-)	66350	66431
trnV	tRNA-Val(uac)	66555	66626
trnT	tRNA-Thr(1101)	66647	66717
rhcS	Rubisco small subunit (-)	66864	67283
rbcl	Rubisco, large subunit (_)	67323	68795
ucf74	ORF486	69086	70546
ycf16	ORF251	70546	71301
atnl	ATP synthese CE subunit IV	70340	72157
atnH	ATP synthese CF_ subunit III	71427	72475
atnC	ATP synthese CF subunit II	72587	72057
atnE	ΔTP synthese CF subunit I	72101	73660
atuD	ATP synthese CF ₀ subunit δ	73657	74000
atn A	ATP synthase CF subunit o	73037	75770
nchR	PSIL CP47 chlorophull apoprotoin	74200	73777
psob nchT	PSIL protoin T 2 (Da(ucf8)	70040	77377
psul	$\begin{array}{c} \text{PSII, protein I, S KDa(ycjo)} \\ \text{PSII, protein N(c)} \end{array}$	77029	77002
psolv nehu	PSIL phosphonrotoin 10 kDa	777066	79160
psorr		77900	70109
9010 Nof21	ORF29 ORF49	78200	70544
<i>ycj51</i>	OKF42 DCL suburit IV = $VD_{-}()$	70504	70012
psuj mar	PSI, suburit IX, 5 KDa (-)	78575	78700
psur	PSI, subunit III, plastocyanin-binding (–)	78732	79289
ps01	OPE112 have been to Devile OPE111 ()	79654	/9//0
ycj41	ORF115, nomologous to Porphyra ORF111 (~)	79855	80196
ycj59 dwO	O(Kr) = 0	81201	ð1171 82077
GXQ	DCL suburit XL()	812UI 82171	82076
psaL 	PSI, SUDUNIT AI (-)	82171	82623
ycr/	UKF31	82800	82895

ycf4	ORF181	82917	83462
trnG	tRNA-Gly(ucc)	83508	83578
psbE	cytochrome b559 α-chain	83634	83888
psbF	cytochrome b559 β-chain	83901	84032
psbL	PSII, protein L	84068	84184
psbl	PSII, protein J	84209	84328
ORF26a	(A+T)-rich (–)	84426	84506
ORF380		84538	85680
ORF25	(A+T)-rich (–)	85745	85822
trnE	tRNA-Glu(uuc) (–)	85882	85953
trnG	tRNA-Gly(gcc) (–)	85995	86066
ycf9	ORF61 (-)	86186	86371
ycf12	ORF34 (–)	86419	86523
trnK	tRNA-Lys(uuu)	86730	86801
ORF44	(A+T)-rich	86932	87066
psbC	PSII, CP43 chlorophyll apoprotein ()	87163	88578
psbD	PSII, D2 reaction-center protein (-)	88526	89581
psal	PSI, subunit VIII (–)	89824	89940
psbK	PSII, protein K	90146	90280
petG	cytocĥrome b6/f complex, subunit V	90417	90530
rpl12	50S ribosomal protein L12 (-)	90778	91161
rpl1	50S ribosomal protein L1 (–)	91234	91926
rpl11	50S ribosomal protein L11 (-)	91949	92374
ÓRF27	(A+T)-rich $(-)$	92456	92539
trnW	tRNA-Trp(cca) (-)	92660	92732
dnaB	DNA-replication helicase	92815	94182
trnF	tRNA-Pĥe(gaa) (–)	94184	94256
psbX	PSII, protein X, 4.1 kDa	94593	94709
ORF99	-	94783	95082
psbV	PSII, cytochrome c ₅₅₀ (<i>petK</i>)	95139	95630
ORF29b	(A+T)-rich (–)	95659	95748
rpl19	50S ribosomal protein L19	96389	96751
petF	ferredoxin	96895	97194
petA	apocytochrome f (–)	97278	98222
ycf43	ORF263, homologous to Porphyra ORF254 (-)	98256	99047
atpE	ATP synthase CF ₁ subunit ε (–)	99151	99552
atpB	ATP synthase CF ₁ subunit β (–)	99566	100993
ycf3	ORF179 (–)	101162	101701
rps18	30S ribosomal protein S18 (-)	101792	102010
rpl33	50S ribosomal protein L33 (-)	102014	102208
rps20	30S ribosomal protein S20	102420	102701
rpoB	RNA polymerase β-chain	102865	107004
rpoC1	RNA polymerase β' -chain	107028	109559
rpoC2	RNA polymerase β''-chain	109599	114044
ORF26b	(A+T)-rich	114058	114138
rps2	30S ribosomal protein S2	114159	114848
psaB	PSI, P700 apoprotein A2 (-)	114970	117171
psaA	PSI, P700 apoprotein A1 (-)	117351	119609

used, with a preference for TAA. Four genes start with GTG instead of ATG (*rps3*, *rps13*, *rpl23*, *rbcS*).

Identified genes are named according to Hallick and Bottomley (1983) and Hallick (1989). Open reading frames shared by homologous sequences of other chloroplast genomes are designated as *ycf* (Hallick and Bairoch, 1994), genes for tRNAs are labelled using the one-letter code of their respective amino acids. Open reading frames unique to *Odontella* are designated as *ORF*, followed by the number of encoded amino acid residues. Genes shared with the *Porphyra purpurea* chloroplast genome and the cyanelle genome of *Cyanophora paradoxa*, but unknown from land-plant chloroplast genomes, include *acp*, *atpD*, *atpG*, *dnaK*, *groEL*, *petF*, *psaE*, *psaF*, *psbV*, *psbX*, *rbcS*, *tufA*, *secY*, and *ycf16*, *-24*, *-30*, *-31*, *-32*, *-33*, *-35*, *-39*. In addition, several genes for ribosomal subunits (*rpl1*, *-3*, *-6*, *-11*, *-12*, *-18*, *-34*, *-35*, and *rps5*, *-6*, *-10*, *-13*, *-17*, *20*) appear to be unique among plastid genomes of non-green algae. Whereas eight open reading frames (*ycf40-47*) have counterparts in the *Porphyra* chloroplast genome, none of these ORFs were found in the cyanelle genome.

A detailed description of the sequence and its phylogenetic implications will appear elsewhere.

Acknowledgment: This work was supported by the Deutsche Forschungsgemeinschaft (Molekulare Evolution der Pflanzen, grants Ko 439/ 6-1 and 439/6-2).

References

- Hallick, R.B. 1989. Proposals for the naming of chloroplast genes. II. Update to the nomenclature of genes for thylakoid membrane polypeptides. Plant Mol. Biol. Reptr. 7:266-275.
- Hallick, R.B. and A. Bairoch. 1994. Proposals for the naming of chloroplast genes. III. Nomenclature for open reading frames encoded in chloroplast genomes. Plant Mol. Biol. Reptr. 12:29-30.
- Hallick, R.B. and W. Bottomley. 1983. Proposals for the naming of chloroplast genes. Plant Mol. Biol. Reptr. 1:38-43.
- Kowallik, K.V. 1989. Molecular aspects and phylogenetic implications of plastid genomes of certain chromophytes, pp.101-124 in *The Chromophyte Algae: Problems and Perspectives* (Green J.C., B.S.C. Leadbeater, and W.L. Diver, eds.). The Systematics Association Special Volume 38. Clarendon Press, Oxford.
- Kowallik, K.V. 1993. Origin and evolution of plastids from chlorophyll a+c-containing algae: Suggested ancestral relationship to red and green algal plastids, pp. 223-263 in Origins of Plastids (Lewin, R.A., ed.). Chapman and Hall, New York and London.
- Reith, M., and J. Munholland. 1995. Complete nucleotide sequence of the Porphyra purpurea chloroplast genome. Plant Mol. Biol. Reptr. 13:338-340.
- Stirewalt, V.S., C. B. Michalowski, W. Löffelhardt, H. J. Bohnert, and D. A. Bryant. 1995. Nucleotide sequence of the cyanelle genome from *Cyanophora paradoxa*. Plant Mol. Biol. Reptr. 13:327-332.
- Valentin, K., R.A. Cattolico and K. Zetsche. 1993. Phylogenetic origin of the plastids, pp. 193-212 in Origins of Plastids (Lewin, R.A., ed.). Chapman and Hall, New York and London.