

A *secY* homologue is found in the plastid genome of *Cryptomonas* Φ

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An open reading frame with significant similarity to the *secY* gene of *Escherichia coli* has been found within a ribosomal protein operon on the plastid genome of the chlorophyll *c*-containing alga *Cryptomonas* Φ . The gene encodes a protein of 420 amino acids (molecular weight 46,906 daltons) and contains ten potential membrane-spanning domains, as in the *E. coli* homologue. This report of a *secY* homologue in a plastid genome provides preliminary evidence that a prokaryotic-like protein export system may be operating in plastids.

Algae; Plastid; Ribosomal protein operon; Secretion; Chloroplast endoplasmic reticulum

1. INTRODUCTION

In Gram-negative Eubacteria, at least six proteins (SecA, B, D, E, F and Y) are required for targeting and protein translocation across the plasma membrane (see [1]). Many of these proteins have been isolated and their genes cloned and sequenced. The cytosolic SecB protein functions as a chaperonin and as part of a receptor cascade [2], the membrane-associated SecA protein functions as an ATPase [3] and the integral membrane proteins SecY and SecE mediate translocation by interacting with SecA [4]. Integral membrane proteins SecD and SecF may be required for the later stages of protein export or for interaction of precursors with signal peptidases [5]. Recently, *sec* homologues have also been found in the Gram-positive Eubacterium, *Bacillus subtilis* [6–8].

In land plants and green algae, translocation across plastid membranes (see [9], [10–12]) and targeting of nuclear-encoded proteins to the plastid envelope membrane of [13,14] have been studied. Cleavage sites for plastid transit peptides [15] and thylakoid lumen leader peptides [16,17] have also been investigated. However, protein targeting and translocation has not been investigated in the rhodophyte, chromophyte or cryptophyte algae.

Chromophyte and cryptophyte algae present especially interesting systems for studying protein translocation since the plastids of these organisms are surrounded by an extra pair of membranes, termed the chloroplast endoplasmic reticulum, or CER [18]. The outermost membrane of the CER, which bears eukaryotic ribosomes and is usually continuous with the nuclear

membrane, is probably derived from the endomembrane system of the host, while the innermost membrane is probably derived from the plasma membrane of the eukaryotic endosymbiont which gave rise to the plastid [19,20]. Cryptomonads are unique among the CER-containing algae in retaining a nucleomorph (the vestigial nucleus of the eukaryotic endosymbiont) in the periplastidal space between the CER and plastid envelope [21].

Presumably in an alga as complex as *Cryptomonas* Φ , gene products encoded by the nuclear or nucleomorph genomes that are targeted to the plastid, contain distinctive N-terminal extensions which serve as signals in intracellular sorting and assist these proteins to traverse the additional membranes of the CER. Those gene products destined for the thylakoid of the plastid may contain additional signals. However, sequence analysis of *Cryptomonas* Φ plastid *cpeB* gene, which encodes the β subunit of phycoerythrin (a lumenal protein), does not predict an N-terminal extension [22]. On the other hand, the sequences of genomic clones of *cpeA* from the cryptomonad *Chroomonas*, do predict a leader sequence [23]. The mechanism of protein translocation across either the thylakoid, plastid or CER membranes is unknown although Gibbs [24] has suggested that vesicles carrying nucleus-encoded proteins destined for the plastid bud off from the inner CER membrane and then fuse with the outer plastid envelope membrane.

In both *B. subtilis* and *E. coli*, the integral membrane protein SecY is encoded at the promoter distal region of the *spc* ribosomal protein operon. Although the *secY* gene is not present on any of the three land plant plastid genomes which have been completely sequenced [25–27], it is possible that it might reside on the plastid genome of a less advanced alga. It appears that gene transfer from the plastid progenitor to the host genome has not proceeded as far in some algae as in land plants

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[28,29] and in cryptomonads in particular, there appear to be many genes which are encoded on the plastid rather than the nuclear genome [30,31]. For example, sequence analysis of the *str* operon from *Cryptomonas* Φ shows the presence of ribosomal protein genes which are not found on plastid genomes of land plants [32]. In order to see if a *secY* homologue is present on the plastid genome of *Cryptomonas* Φ , the promoter distal region of the *spc* ribosomal protein operon was sequenced.

2. MATERIALS AND METHODS

Isolation of plastid DNA from *Cryptomonas* Φ and construction of a clone bank has been described [33]. Ribosomal protein operons were mapped on the plastid genome by hybridisation with heterologous probes and by sequencing [32]. The promoter distal region of the *spc* operon which potentially would encode *secY* was located on two adjacent 1.9 kb *Sa*II fragments. Both strands of the DNA from this region were completely sequenced using the dideoxy chain termination method [34] and synthetic oligonucleotide primers. Analysis of the DNA sequence and the derived amino acid sequence was performed using the DNA Strider version 1.1 program [35] and databank searching was performed using the FastP program [36] on a Macintosh II computer (Apple Computers).

3. RESULTS AND DISCUSSION

A physical map of the plastid genome of *Cryptomonas* Φ is shown in Fig. 1a. The positions of the *S10*, *spc*, *alpha* and *str* ribosomal protein operons are indicated by solid bars. The promoter distal region of the *spc* operon is shown in Fig. 1b and the locations of coding regions are indicated by stippled bars. An open reading

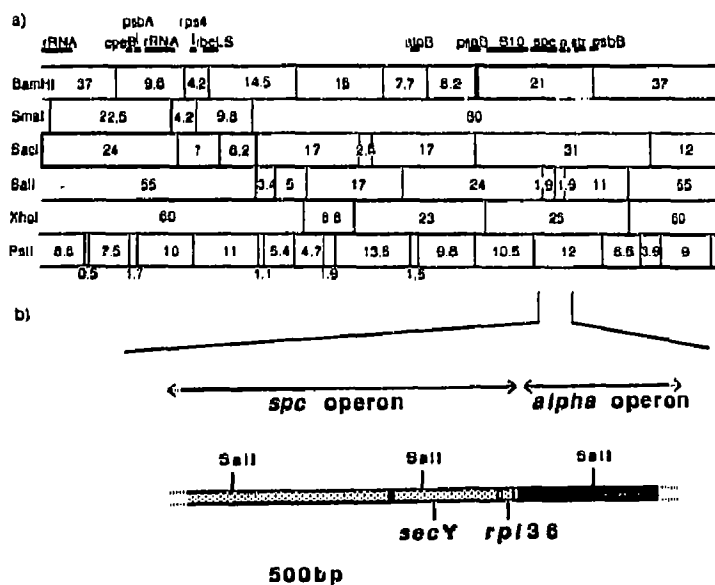


Fig. 1. (a) Physical map of the plastid genome of *Cryptomonas* Φ . The locations of the *S10*, *spc*, *alpha* and *str* ribosomal protein operons and other coding regions are indicated by solid bars. (b) Region of the plastid genome containing the *secY* gene. Coding regions within the *spc* and *alpha* ribosomal protein operons are denoted by light and dark stippling, respectively.

frame of 1,260 nucleotides with similarity to *secY* from *E. coli* was found which spanned the two 1.9 kb *Sa*II fragments. The nucleotide sequence of *secY* and the flanking upstream and downstream regions, including the 5'-terminus of *rp136* (the gene encoding ribosomal protein L36), are shown in Fig. 2. The derived amino acid sequence (420 residues, 46,906 Da) is given below the nucleotide sequence. As with other *Cryptomonas* Φ plastid genes [33,35], there is very little space between the two genes (only 27 nucleotides).

An alignment of the *secY* gene products from *Cryptomonas* Φ , *B. subtilis* and *E. coli* is shown in Fig. 3. The two proteins are essentially colinear, with the exception of N- and C-terminal extensions in the *E. coli* sequence relative to those from *Cryptomonas* Φ and *B. subtilis*. There are several other places where small insertions or deletions occur. Excluding the N- and C-terminal extensions, the amino acid sequences from *Cryptomonas* Φ and *E. coli* are 38.7% identical and 60.1% similar (when conservative replacements are included). The similarity to the SecY sequence from *B. subtilis* is only slightly lower (35.3 and 60.7%, respectively).

In *E. coli*, SecY is an integral membrane protein which contains ten transmembrane segments, five periplasmically exposed regions and six cytoplasmically exposed regions [37]. It is part of the membrane-bound translocase which also contains the SecE [4] and possibly SecD and SecF proteins [5]. The hydropathic profiles of the SecY proteins from *E. coli*, *B. subtilis* and *Cryptomonas* Φ are virtually superimposable (Fig. 4) and the ten putative membrane-spanning domains are present in all three molecules.

The fact that the *secY* gene is found within the transcriptionally active *spc* ribosomal operon (Wang, Liu and Douglas, in preparation) indicates that it is not an inactive pseudogene and that the SecY polypeptide is functional in the cell. Also, the conservation in primary amino acid sequence and, more importantly, the hydropathy profile, argues for a functional gene product. The discovery of a gene for a component of a prokaryotic protein export system in a plastid genome is intriguing and may shed light on possible mechanisms of protein translocation in plant cells. Thus far, no *sec* genes have been reported in any plant system, possibly because they are present in nuclear genomes rather than the extensively studied plastid genomes. The plastid genome of *Cryptomonas* Φ contains genes not found on other plant plastid genomes and therefore is a useful system for studies of plastid functions which are normally dependent on the products of nuclear-encoded genes. The possibility that other *sec* genes are also plastid-encoded in *Cryptomonas* Φ is currently under investigation.

The subcellular location of the SecY from *Cryptomonas* Φ is unknown. The inner membrane of the plastid envelope is the homologue of the plasma membrane of *E. coli* and it is possible that SecY is involved

aat gat cgt aac cta gac ctt cat tga tgt ttt ttt cgt gtt ata aga ctt cta aat tga gat tag aaa tct tat aat cat ata 84
 ttt acc aat acg ttt atg aat acc tca ata aaa tct att aaa aaa caa gat tta aaa gat cgg ata gta ttt acc ttc ttt tta 168
 met asn thr ser ile lys ser ile lys lys gln asp leu lys asp arg ile val phe thr leu phe leu
 secY
 att gtc atg tct cgt tta ggt aca ttt ctg ccc ata cgg gga gtt gat cat gat gct ttt tat caa agt ata ata agt aat cca 252
 ile val met ser arg leu gly thr phe leu pro ile pro gly val asp his asp ala phe tyr gln ser ile ile ser asn pro
 tta gtt aat ttt cta aat gta ttt tct gca ggt cgg ttt gct tgg atc ggt gtt ttt gct tta ggt ata gtt cct tac ata aat 336
 leu val asn phe leu asp val phe ser gly gly gly phe ala ser ile gly val phe ala leu gly ile val pro tyr ile asp
 gct tca att att gta caa tta gct act aat tgg atc cgg agt tta gaa aag tta caa aaa caa caa gct caa tta ggt cga caa 420
 ala ser ile ile val gln leu ala thr asn ser ile pro ser leu glu lys leu gln lys glu glu gly glu leu gly arg gln
 aaa ata gtt caa ctt aca aga tat gtc gca tta gtc tgg gct ttg att caa agt att gga gta tca ttt tgg gta cga cct tat 504
 lys ile val gln leu thr arg tyr val ala leu val trp ala leu ile gln ser ile gly val ser phe trp val arg pro tyr
 gta ttt aac tgg gat tta aac ttt gtt ttc gct atg agc tta acc tta act ata ggt tgg atg tta ata atg tgg ttt tca gaa 588
 val phe asn trp asp leu asn phe val phe ala met ser leu thr leu thr ile gly ser met leu ile met trp phe ser glu
 caa ata act caa aaa cga ata ggt aat ggt cct tca cta ctt att ttt att aat att att tct gga tta cct aaa ttg tta caa 672
 gln ile thr glu lys gly ile gly asn gly pro ser leu leu ile phe ile asp ile ile ser gly leu pro lys leu leu gln
 tca caa att caa tcc act cgt ctt aat att caa gca tta gat ata ttt gta ctt gtt ttc att ttt tca gtc atc ata att ggg 756
 ser gln ile gln ser thr arg leu asn ile gln ala leu asp ile phe val leu val phe ile phe ser val met ile ile gly
 att att ttt ata caa gaa ggt ata aaa cga att cct atc att tct gca cgg caa ctt ggt aaa ggg caa atg gat aat aaa aca 840
 ile ile phe ile gln glu gly ile lys arg ile pro ile ile ser ala arg gln leu gly lys gly gln met asp asn lys thr
 agt tat tta cct ttg aaa ctg aat caa agc ggt gta atg cca att ata ttt gcc tct gct gtt tta gtc tta cca gct tat tta 924
 ser tyr leu pro leu lys leu asn gln ser gly val met pro ile ile phe ala ser ala val leu val leu pro ala tyr leu
 gcc caa ctg gta tgg aat gaa caa tta aga aca gtc tta cat ttg ttt gat ggt acc agt aat aat aaa tta ctt tat tta tta 1008
 ala gln leu val ser asn glu gln leu arg thr val leu his leu phe asp gly thr ser asn asn lys leu leu tyr leu leu
 ttc tat ttt aca tta att tta ttc ttt agt tat ttt tat aca tct tta ata ttg aat cca aat gat gta tcc aaa aat ctg aaa 1092
 phe tyr phe thr leu ile leu phe phe ser tyr phe tyr thr ser leu ile leu asn pro asn asp val ser lys asn leu lys
 aaa atg gag tct agt att tat ggt gtt cga cca ggt aaa gct act aca gaa tat tta caa aaa aca ttg aat cga cta aca ttt 1176
 lys met glu ser ser ile tyr gly val arg pro gly lys ala thr thr glu tyr leu gln lys thr leu asn arg leu thr phe
 tta gga gct tta ttc ttg gct ttt ata gct att gtt cct aat att att gaa aca tta act aat tta tct gta ttt aaa ggt tta 1260
 leu gly ala leu phe leu ala phe ile ala ile val pro asn ile ile glu thr leu thr asn leu ser val phe lys gly leu
 ggt ggt acc tca tta tta ata att gtt gcc gta caa gtt gac acc tct aag caa att caa act tat ctg att tca aaa aat tat 1344
 gly gly thr ser leu leu ile ile val gly val gln val asp thr ser lys gln ile gln thr tyr leu ile ser lys asn tyr
 gaa act ata gta cgt taa ctt aaa ttt aaa taa ata tat taa ttc atg aaa gta gta agt tca att ggt agt tta aaa aat cgt 1428
 glu thr ile val arg och met lys val val ser ser ile gly ser leu lys asn arg
 rpl36

Fig. 2. Nucleotide sequence of the *secY* gene and flanking regions from *Cryptomonas* Φ . The deduced amino acid sequence is shown beneath the nucleotide sequence. The positions of putative transmembrane segments (see Fig. 4b) are indicated by roman numerals I–X.

B. subti HNYV-----DIAME I LPT LHL IVD- RIGAP I VPT VYAE- --ALDAGECHVT D L L T F G E A L T G F S I F M E I T
 Crypto HNTS I K S I F K Q L E A I V D- L P L I V M K L O T F L P I N V D N D- --A P T O R I I E N P L V E I I N T S G G A F S I G V P A L G I V
 B. coli HARGG L D P G A N G G L G- E L E N L P L Y L A L G- R I G S F I P I G I D A V L A E L L E G G A G T I I T H P E R S G A L I A A L P A L G I A
 B. subti P Y T A S I I L L L Q D V V P T M S E K G S V C A R E L A O P T A T P T V L S F I O A L O N S T E R N L- A N G H L I R E S S E V T T L I A L V L G G
 Crypto P T S A S I I V L A T H I P E L S E L G N G S L G I G I V L T A L V A L I G S I S E S T- --S V N V Y V T M O L N T V E N S I T L T I G
 B. coli P T S A S I I L L L Q D V V P T L- A E I R E G E S G A R I S Q Y I N T S L V A L P O S T G I A S L N F M F C H G L V I M P F A C T V T A V O L V G S
 B. subti T A F L N K L G S I T S G N G S I S I I F A G I V S I F E T I- C Q I Y E T G V C H S Q G L F K I V E V A L L V I A L L A V E S V I F I O D A V N I A I
 Crypto S H L I N T F S Q I T S G S I G N G S L L I P I M I S O L P E L L Q S I G S T A L M I- --G A L D I V V L T F I E V H I G I I P I O G S I R A I P I
 B. coli T T E L A R L A G Q I T S G S I G N G S I S I I F A G I V S I F E T I- C Q I Y E T G V C H S Q G L F K I V E V A L L V I A L L A V E S V I F I O D A V N I A I
 B. subti Q T A S G T S P A S G G S I T H L P L E V M A G V I P I P A V A F L I T P G I A S P F O T H V I E N- --I Q M P D T A P F V G A I T V A L I I A P T I F
 Crypto I S A N- Q L G G G H S E T P L P L G S G Q S I P A V A V A L P A T L A G L V S R G L P V L N- A T C T S E R H A L L Y L F P T S L I P T P T
 B. coli K T A N P G S G A R V T A N G S T H L P L E V M A G V I P I P A S A S I I F A G I V S I F E T I- C Q I Y E T G V C H S Q G L F K I V E V A L L V I A L L A V E S V I F I O D A V N I A I
 B. subti T A F V V P P G H A D L K H Q G S I P G V N G S T G A T S I L T L T P V S I P L A V I S I L P I P I O A C L P Q S A Q I G S T E L L I V E V A L
 Crypto T T S L I A P G V S E H L K H Q S S I Y G V R G A P T E T L Q T L N L T P L G A L P L A F I A I V P H I I E T L T H L S V T G L G S T L L I V E V V V
 B. coli V T A L T P H E T A N L K H S G A P V I G S T A T I D E V H T L S L A L L A T E L L I E S L E P A D A N E V P T I- F O S T E L L I V E V V V
 B. subti S T N K L A S G L V E N T M E T H E N
 Crypto S T S K I Q T L I S A N T S I V A
 B. coli S T N K V S T M E T H E N S T E A L E A N N A G T G A

Fig. 3. Alignment of SecY proteins from *Cryptomonas* Φ , *Bacillus subtilis* and *Escherichia coli*. Colons indicate identities and dots indicate conservative replacements according to Schwartz and Dayhoff [38].

in protein translocation across this membrane. However, most of the protein translocation in plastids is in the reverse direction, i.e. from the cytoplasm into the plastid. SecY may be involved in protein translocation across the thylakoid membranes or even across the CER, although the latter is unlikely given that the outer membrane of this pair is derived from the host endomembrane system rather than the endosymbiont. Possible mechanisms of protein targeting and translocation in *Cryptomonas* Φ are currently under investigation.

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REFERENCES

- [1] Bieker, K.L., Phillips, G.J. and Silhavy, T.J. (1990) J. Bioenerg. Biomemb. 22, 291–310.

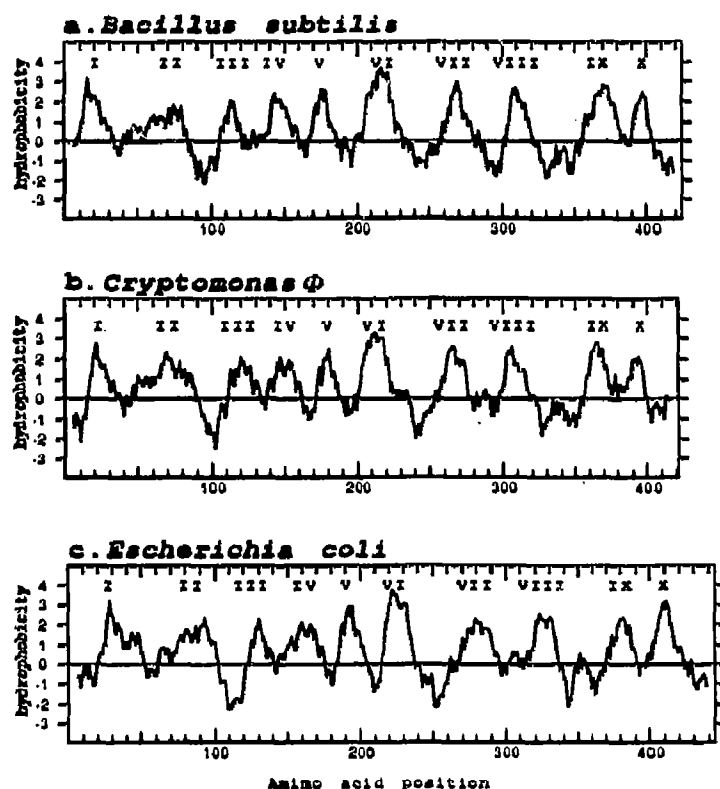


Fig. 4. Hydropathy plots of SecY proteins from *Cryptomonas* Φ , *Bacillus subtilis* and *Escherichia coli*. Plots were obtained using the Kyte-Doolittle option of DNA Strider [35]. The positions of putative transmembrane segments are indicated by roman numerals I–X.

- [2] Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J.P. and Wickner, W. (1990) *Cell* 63, 269–279.
- [3] Lill, R., Dowham, W. and Wickner, W. (1990) *Cell* 60, 271–280.
- [4] Brundage, L., Hendrick, J.P., Schiebel, E., Dreissen, A.J.M. and Wickner, W. (1990) *Cell* 62, 649–657.
- [5] Gardel, C., Johnson, K., Jacq, A. and Beckwith, J. (1990) *EMBO J.* 9, 3209–3216.
- [6] Nakamura, K., Nakamura, A., Takamatsu, H., Yoshikawa, H. and Yamane, K. (1990) *J. Biochem.* 107, 603–607.
- [7] Suh, J.W., Boylan, S.A., Thomas, S.M., Dolan, K.M., Oliver, D.B. and Price, C.W. (1990) *Mol. Microbiol.* 4, 305–314.
- [8] Overhoff, G., Klein, M., Speis, M. and Freudl, R. (1991) *Mol. Gen. Genet.* 228, 417–423.
- [9] Schmidt, G.W. and Mishkind, M.L. (1986) *Annu. Rev. Biochem.* 55, 879–912.
- [10] Keegstra, K. and Olsen, L.J. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 471–501.
- [11] Schnell, D.J., Blobel, G. and Pain, D. (1990) *J. Cell Biol.* 111, 1825–1838.
- [12] Franzen, L.-G., Rochaix, J.-D. and von Heijne, G. (1990) *FEBS Lett.* 260, 165–168.
- [13] von Heijne, G. and Nishikawa, K. (1991) *FEBS Lett.* 278, 1–3.
- [14] Li, H.-M., Moore, T. and Keegstra, K. (1991) *Plant Cell* 3, 709–717.
- [15] Gavel, Y. and von Heijne, G. (1990) *FEBS Lett.* 261, 455–458.
- [16] Last, D.I. and Gray, J.C. (1989) *Plant Mol. Biol.* 12, 655–666.
- [17] Howe, C.J. and Wallace, T.P. (1990) *Nucleic Acids Res.* 18, 3417.
- [18] Gibbs, S.P. (1981) *Int. Rev. Cytol.* 72, 49–99.
- [19] Gibbs, S.P. (1981) *Annu. N.Y. Acad. Sci.* 361, 193–208.
- [20] Douglas, S.E., Murphy, C.A., Spencer, D.F. and Gray, M.W. (1991) *Nature* 350, 148–151.
- [21] Greenwood, A.D., Griffiths, H.B. and Santore, U.J. (1977) *Brit. Phycol. J.* 12, 119.
- [22] Reith, M. and Douglas, S.E. (1990) *Plant Mol. Biol.* 15, 585–592.
- [23] Jenkins, J., Hiller, R.G., Speirs, J. and Godovac-Zimmermann, J. (1990) *FEBS Lett.* 273, 191–194.
- [24] Gibbs, S.P. (1979) *J. Cell Sci.* 35, 253–266.
- [25] Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H. and Ozeki, H. (1986) *Nature* 322, 572–574.
- [26] Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.-Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. and Sugiura, M. (1986) *EMBO J.* 5, 2043–2049.
- [27] Hiratsuka, J., Shimada, H., Whittier, R., Ishibashi, T., Sakamoto, M., Mori, M., Kondo, C., Honji, Y., Sun, C.-R., Meng, B.-Y., Li, Y.-Q., Kanno, A., Nishizawa, Y., Hirai, A., Shinozaki, K. and Sugiura, M. (1989) *Mol. Gen. Genet.* 217, 185–194.
- [28] Baldauf, S.L. and Palmer, J.D. (1990) *Nature* 344, 262–265.
- [29] Gantt, J.S., Baldauf, S.L., Calic, P.J., Weeden, N.F. and Palmer, J.D. (1991) *EMBO J.* 10, 3073–3078.
- [30] Douglas, S.E. and Durnford, D.G. (1989) *Plant Mol. Biol.* 13, 13–20.
- [31] Wang, S. and Liu, X.-Q. (1992) *Proc. Natl. Acad. Sci. USA* (in press).
- [32] Douglas, S.E. (1991) *Curr. Genet.* 19, 289–294.
- [33] Douglas, S.E. (1988) *Curr. Genet.* 14, 591–598.
- [34] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
- [35] Marck, C. (1988) *Nucleic Acids Res.* 16, 1829–1836.
- [36] Pearson, W.R. and Lipman, D.J. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2444–2448.
- [37] Akiyama, Y. and Ito, K. (1987) *EMBO J.* 6, 3465–3470.
- [38] Schwartz, R.M. and Dayhoff, M.O. (1978) in: *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3 (M.O. Dayhoff, ed.) pp. 353–358, National Biomedical Research Foundation, Washington.