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cDNA cloning of a Sec61 homologue from the cryptomonad alga *Pyrenomonas salina*

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Abstract. Sec61 is an endoplasmic reticulum transmembrane protein involved in the process of translocation of proteins across this membrane. To-date, the only cloned genes for Sec61 are derived from mammals and yeast. In this paper, we present the first full-length cDNA from a sec*61* gene of a plant cell. Comparison of the predicted protein sequence with all known Sec61 proteins, as well as with the bacterial/plastome-encoded homologue SecY, demonstrates a high degree of similarity among the SecY/Sec61 family.

Key words: Cryptomonads – Sec61 – ER proteins – Transport mechanisms

Introduction

Cryptomonads are unicellular, biflagellate algae. In contrast to the chloroplasts of higher plants and green and red algae, cryptomonad plastids are surrounded by four membranes. These so-called complex plastids are relicts of a symbiotic event between an organotrophic eukaryote and a photosynthetic eucyte (Tomas and Cox 1973; Gibbs 1981; Whatley 1981). Subsequently, reduction of the photosynthetic endosymbiont took place, leading in the case of cryptomonads to an intermediate form in the evolution of complex plastids (Maier et al. 1991; McFadden 1993). In this algal group, inner and outer membrane pairs are separated by a periplastidal compartment, harbouring 80*S*-type ribosomes as well as the vestigial nucleus of the eukaryotic endosymbiont, the so-called nucleomorph (Greenwood 1974; Greenwood et al. 1977; Morrall and Greenwood 1978; McKerracher and Gibbs 1982; McFadden 1990; Sitte and Baltes 1990). The nucleomorph of *Pyrenomonas salina* consists of three linear chromosomes totalling 660 kb (Eschbach et al. 1991). These contain protein-coding genes which are expressed (Hofmann et al. 1994). The chimaeric nature of cryptomonads makes them a unique system for the study of protein sorting for two reasons:

(1) As in other organisms, gene products derived from different genetic compartments can be assembled at their intracellular site of action. However, in cryptomonads the third semiautonomous organelle, the nucleomorph, has a potential capacity to encode gene products which take part in these kind of processes.
(2) Nuclear-encoded plastidal proteins must traverse at least four membranes to reach their final destination.

Although little is known about protein transport in algae, some components of the machinery for transport into the plastidal compartment, as well as factors involved in protein refolding, have already been identified (reviewed by Douglas 1992). In cryptomonads, intracellular communication between host and eukaryotic endocytobiont must take place not only over the plastidal envelope, but also across the barrier of the enclosing membranes of the periplastidal ER. The outermost membrane has been shown to be joined with the ER-membrane system of the eukaryotic host and to be studded with ribosomes (Hofmann et al. 1994). Until now, no transport components dealing with these membranes have been characterized.

In higher eukaryotes, the secretory pathway is initiated by cotranslational transport across the ER-membrane. Ribosomes involved in this process are initially free in the cytoplasm. The translation of an N-terminal signal sequence of a nascent polypeptide is recognized by the signal recognition particle (SRP). Subsequent events include the binding of the signal sequence by the SRP and complexing with the SRP receptor. Thereafter, the signal sequence dissociates from the SRP, and the nascent polypeptide chain is translocated across the ER membrane (for a review see Simon 1993). Over the past few years, candidates for this translocation machinery have been identified; for example, the *Saccharomyces cerevisiae* Sec61, Sec62 and Sec63 proteins (Deshaies and Shekman 1987; Rothblatt et al. 1989; Deshaies et al. 1991) which were found by genetic selection methods. Chemical cross-linking studies in yeast revealed binding of the Sec61, Sec62

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and BiP proteins to nascent translocating polypeptides (Müsch et al. 1992; Sanders et al. 1992). In mammalian ER-membranes the Sec61 complex consists of three proteins, the α-subunit presenting the homologue of Sec61p of yeast (Hartmann et al. 1994). Sec61 is a transmembrane protein and is thought to be the functional eukaryotic homologue of bacterial SecY (Ito et al. 1983; Brundage et al. 1990).

In this report we describe the characterization of the first isolated plant nucleus-encoded sec61 cDNA from the cryptomonad, *P. salina*. The deduced amino-acid sequence is compared to other Sec61 sequences. The possible role of Sec61 in protein transport in cryptomonads is discussed.

Materials and methods

Cell culture and harvesting of the algal cells. P. salina was cultured and harvested as previously described (Hansmann and Eschbach 1990).

RNA isolation. Harvested algal cells were treated with guanidinium hydrochloride. HES buffer (10 mM HEPES/pH 7.5; 1 mM EDTA/pH 8; 0.1% SDS) was added to the lysed cells, followed by phenol-extraction and ethanol-precipitation. To obtain poly $(A)^+$ RNA, total RNA was purified twice with oligo-dT cellulose (Pharmacia LKB, type 7); this purification was carried out according to the manufacturer's instructions.

Construction of a cDNA library. The cDNA was constructed with a Pharmacia kit (Pharmacia LKB) as described previously (Martin et al. 1990).

Pulsed-field gel electrophoresis. Preparation of nucleomorph and total cell chromosomes, as well as pulsed-field gel electrophoresis conditions, were as described in Eschbach et al. (1991).

Northern- and Southern-blot hybridization studies. Chromosomes, λ-phage DNA and RNA were transferred to Nylon membranes (Amersham Buchler, Hybond N) or nitrocellulose filters (Schleicher and Schüll BA85) according to Sambrook et al. (1989). The heterologous probe, coding for an acidic domain, from *Zea mays* HMG-protein (Grasser and Feix 1991) and the homologous probe from the full-length *P. salina* pSMH1 clone were labelled with α^{32}P-dATP (3 000 Ci/mmol; Amersham Buchler) by random primed labelling. Hybridization was carried out at 58 °C with two subsequent washes either in 0.2×SSC at 68 °C for the homologous probe or 2×SSC at 60 °C for the heterologous probe.

Subcloning and DNA sequencing. The 1770-bp cDNA *Not*I insert was subcloned into *Not*I-digested pBluescript SK, leading to pSMH1. This clone was sequenced according to the method of Sanger et al. (1977), using ^{35}S-dATP (600 Ci/mmol, Amersham Buchler, FRG) and a T7 sequencing kit (Pharmacia LKB).

Alignment and further characterization. Retrieval of the Sec61 and SecY sequences was carried out with the IRX information retrieval system from HUSAR (Heidelberg Unix Sequence Analysis Resources). The following (deduced) amino-acid sequences have been used (abbreviations in brackets, EMBL accession numbers cited): *P. salina* Sec61 (PYSA), X77805; *Saccharomyces cerevisiae* Sec61, (YEAST), X62340 (Stirling et al. 1992); *Canis familiaris* (DOG), M96629 (Görlich et al. 1992); *Rattus rattus* (RAT), M96630 (Görlich et al. 1992); *Pyrenomonas salina* SecY, X74773 (Rensing and Maier 1994); *Escherichia coli* SecY, X01563 (Cerretti et al. 1983). The sequences were aligned with the program CLUSTAL V (Higgins et al. 1992) using a PAM250 matrix, leading to a total length of 495 positions. The consensus sequence was calculated with the program CONSENSE 1.1 [S. Rensing, available from EMBL file server (NETSERV@/EMBL-Heidelberg.de) or rensing@sun1.biologie.uni-freiburg.de]. The amino acids S, R, H, G, K, Q, N, D and E were regarded as hydrophylic, whereas the amino acids C, F, Y, I, L, M, W and V were regarded as hydrophobic, the amino acids H, R and K as positively charged and the amino acids D and E as negatively charged in accordance with their Froemmel values (Frömmel 1984). Putative transmembrane domains were predicted with the program RAOARGOS (PC/GENE 6.60, IntelliGenetics Inc., Mountain View, Calif.).

Results and discussion

Interest in transport processes in cryptomonads led us to isolate ER-protein-coding genes from *P. salina*. Analysis of several ER proteins revealed that many of these possess a poorly-conserved acidic domain. In the hope of detecting different ER-protein cDNAs on the basis of this criterion alone, we screened 3.3×10^5 recombinant cDNA clones from the *P. salina* library at low stringency with a hybridization probe encoding a nonspecific, non-ER related, acidic domain from a maize HMG protein (Grasser and Feix 1991). Acidic domains are a common feature of several protein classes, e. g., ER-proteins and DNA-binding proteins such as HMGs. In one experiment we isolated a cDNA clone, λSMH1, the insert of which contained an open reading frame of 1 482 bp for a peptide with a calculated molecular weight of 54.2 kDa. Northern-blot analysis of *P. salina* total RNA reveals one transcript 1 800 nt in length which hybridizes to the probe used (data not shown). This ORF was identified by database comparison as the first Sec61 homologue from algae.

An alignment of known Sec61 protein sequences was constructed to analyze the conserved portions of the Sec61 sequence and the putative membrane-spanning domains (see Fig. 1). The putative transmembrane residues are well conserved, whereas the cytoplasmic and extraplasmatic domains are weakly conserved in part. The pSMH1-encoded protein shows 67% and 76% amino-acid similarity to its homologues from yeast (Stirling et al. 1992) and rat (Görlich et al. 1992), respectively. Overall approximate similarity scores between all so-far-available members of the protein family are: 70% among Sec61, 60% between bacterial and plastome-encoded SecY, and 35% between Sec61 and SecY. The homology of Sec61 and SecY was shown in a recent phylogenetic analysis (Rensing and Maier 1994).

Figure 2 shows a comparison of the hydropathy plots of the *E. coli* (Akiyama and Ito 1987) and *P. salina* SecY (Rensing and Maier 1994), as well as the rat and *P. salina* Sec61 amino-acid sequences. As indicated by bars at the bottom of the respective figures, all proteins possess several putative membrane-spanning domains. In the *P. salina* Sec61 sequence the computer program is only capable of detecting five membrane-spanning regions. This is due to the fact that at least some of the transmembrane domains contain negatively-charged amino-acid residues, which is in good agreement with the suspicion that those transmembrane domains building the hydrophilic channel should contain charged amino-acid residues at the positions exposed to the lumen of the channel. Taken together, the

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YEAST   MSSN--RVLDLFKPFESFLPEVIAPERKVPYNQKLIWTGVSLLIFLILGQ
RAT     MA---IKFLEVIKPFCVILPEIQKPERKIQFKEKVLWTAITLFIFLVCCQ
DOG     MA---IKFLEVIKPFCVILPEIQKPERKIQFKEKVLWTAITLFIFLVCCQ
PYSA    MGTSGVRFLTLVKPFMFALPEVSSATKKIPVNEKLLWTSIVVFLYLVCCQ
        M.....+FL.♦♦KPF...LPE♦..PERKI.♦*EK♦LWT.I.LFIFLVCCQ

YEAST   IPLYGIVSSETSDPLYWLRAMLASNRGTLLELGVSPIITSSMIFQFLQGT
RAT     IPLFGIMSSDSADPFYWMRVILASNRGTLMELGISPIVTSGLIMQLLAGA
DOG     IPLFGIMSSDSADPFYWMRVILASNRGTLMELGISPIVTSGLIMQLLAGA
PYSA    IPLYGITNTKSSDPFYWMRVILASNKGTLMELGISPIVTSGLVMQLLAGS
        IPL♦GI.SS*S.DPFYWMRVILASNRGTLMELGISPIVTSGLIMQLLAG.

YEAST   QLLQIRPESKQDRELFQIAQKVCAIILILGQALVVVMTGNYGAPSDLGLP
RAT     KIIEVGDTPK-DRALFNGAQKLFGMIITIGQSIVYVMTGMYGDPSEMGAG
DOG     KIIEVGDTPK-DRALFNGAQKLFGMIITIGQSIVYVMTGMYGDPSEMGAG
PYSA    KIIDVDQGTKEDKTLFQGAQKLLGILITIGESVAYVLSGMYGDVKDLGAG
        KII*V*...K.DR.LF*GAQKL♦G♦IITIGQS♦VYVMTGMYGDPS-♦GAG

YEAST   ICLLLIFQLMFASLIVMLLDELLSKGYGLGSGISLFTATNIAEQIFWRAF
RAT     ICLLITIQLFVAGLIVLLLDELLQKGYGLGSGISLFIATNICETIVWKAF
DOG     ICLLITIQLFVAGLIVLLLDELLQKGYGLGSGISLFIATNICETIVWKAF
PYSA    NAILIIVQLFTSGIIVICLDELLQKGYGIGSAISLFIATNVCESIVWKSF
        ICLLI.♦QLF.AGLIV♦LLDELLQKGYGLGSGISLFIATNICE.IVWKAF

YEAST   APTTVNSGRGKEFEGAVIAFFHLLAVRKDKKRALVEAFYRTNLPNMFQVL
RAT     SPTTVNTGRGMEFEGAIIALFHLLATRTDKVRALREAFYRQNLPNLMNLI
DOG     SPTTVNTGRGMEFEGAIIALFHLLATRTDKVRALREAFYRQNLPNLMNLI
PYSA    SPTTINTGRGTEFEGALVALFQLMITKTDKVRALQEAFYRQNLPNVTNLL
        SPTTVNTGRG.EFEGA♦IALFHLLATRTDKVRAL.EAFYRQNLPN♦.NL♦

YEAST   MTVAIFLFVLYLQGFRYELPIRSTKVRGQIG-IYPIKLFYTSNTPIMLQS
RAT     ATIFVFAVVIYFQGFRVDLPIKSARYRGQYNT-YPIKLFYTSNIPIILQS
DOG     ATIFVFAVVIYFQGFRVDLPIKSARYRGQYNT-YPIKLFYTSNIPIILQS
PYSA    ATVLVFVLVVYFQGFQVELPITPAKSKGMAGQFYPIKLFYTSNMPIILQT
        AT♦.VF.♦V♦YFQGFRV-LPI.SA+.RGQ.*..YPIKLFYTSN.PIILQS

YEAST   ALTSNIFLISQILFQKYPTNPLIRLIGVWG-IRPGTQGPQMALSGLAYYI
RAT     ALVSNLYVISQMLSARFSGNLLVSLLGTWSDTSSGGPARAYPVGGLCYYL
DOG     ALVSNLYVISQMLSARFSGNLLVSLLGTWSDTSSGGPARAYPVGGLCHYL
PYSA    ALVSNLYFISQILYKRYPENIIIHILGRWEEPEMSVSGQMRPVGGIAYYI
        ALVSNLY♦ISQ♦L..R♦..N.L♦*LLG.W*..*.G......PVGGL.YY♦

YEAST   QPLMSLSEALLDPIKTIVYITFVLGSCAVFSKTWIEISGTSPRDIAKQFK
RAT     SPPESFGSVLEDPVHAVVYIVFMLGSCAFFSKTWIEVSGSSAKDVAKQLK
DOG     SPPESFGSVLEDPVHAVVYIVFMLGSCAFFSKTWIEVSGSSAKDVAKQLK
PYSA    SPLNSFAEIVSDPVHALLYIIFILASCALFSKTWIQVSGTSASDVSKQLR
        SP..SF.*.L.DPVHA♦VYI.F♦LGSCA♦FSKTWIEVSG.SA*DVAKQLK

YEAST   DQGMVINGKRETSIYRELKKIIPTAAAFGGATIGALSVGSDLLGTLGSGA
RAT     EQQMVMRGHRETSMVHELNRYIPTAAAFGGLCIGALSVLADFLGAIGSGT
DOG     EQQMVMRGHRETSMVHELNRYIPTAAAFGGLCIGALSVLADFLGAIGSGT
PYSA    DQQMVMKGFRASSMQRELNRYIPTAAAFGGMCIGALSIVADFMGAIGSGT
        -QQMVM*G.RETSM.+ELNRYIPTAAAFGG.CIGALSV.ADFLGAIGSGT

YEAST   SILMATTTIYGYYEA--AAKEGGF--------TKNLVPGFSDLM-
RAT     GILLAVTIIYQYFEIFV---------------KEQSEVGSMGALLF
DOG     GILLAVTIIYQYFEIFV---------------KEQSEVGSMGALLF
PYSA    GILLAVTTIYQSWETILLAVTTIYQSWETIRKESRDTDAL-KMFG
        GILLAVT.IYQY♦E..................KE**.V..♦..L♦.
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Fig. 1. Sec61 amino-acid sequence alignment. Consensus-length: 495 characters; predicted transmembrane helices found by computer analysis are *double underlined*; putative transmembrane domains by comparison with the *E. coli* SecY sequence are *singly underlined*. Consensus sequence: character to show 75% (three sequences) conservation is |, 100% (four sequences) conservation is shown by ||. *Letters* are shown if the respective amino acid is found in ≥75% (three sequences), otherwise signs for hydrophilic (*), hydrophobic (♦), negatively-charged (–), and positively-charged (+) amino acids are shown; nonconserved positions are marked by *dots* (.). For amino-acid classification see section Materials and methods. (*Pyrenomonas salina* EMBL assession number: X77805)

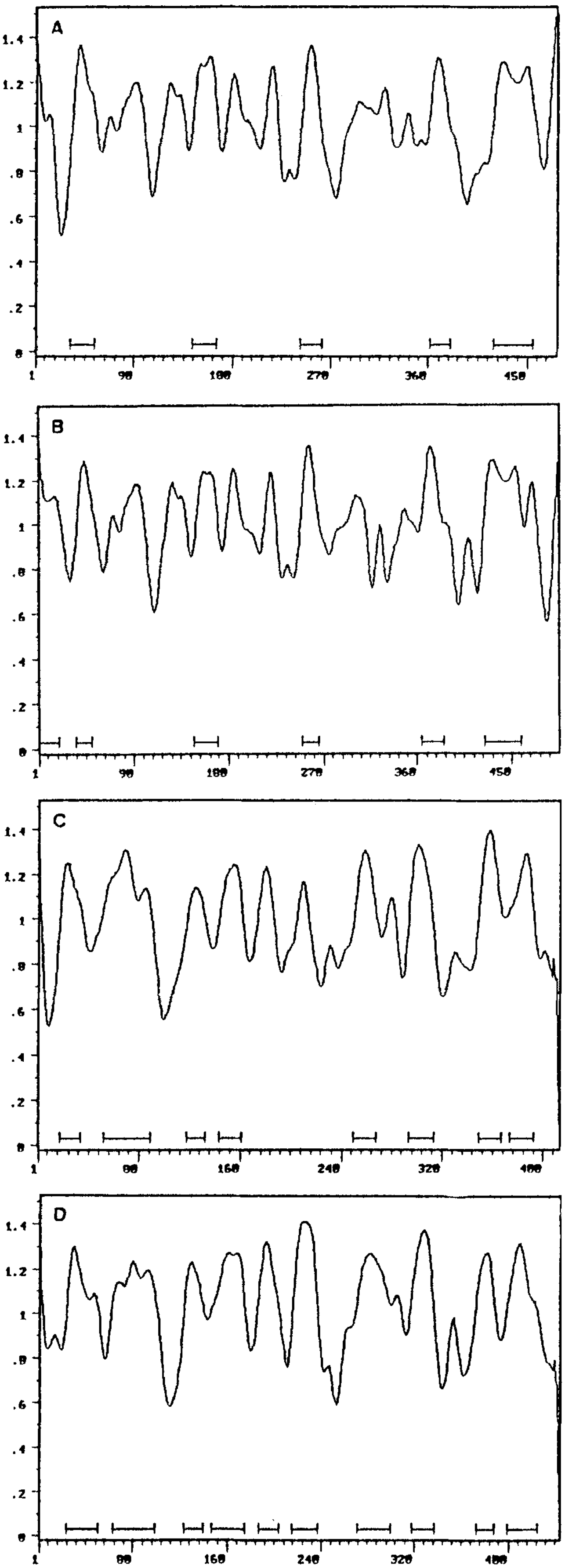

Fig. 2 A–D. Comparison of hydropathy plots. Hydropathy plots of two Sec61 and two SecY amino-acid sequences predicted by the program RAOARGOS (see Materials and methods). The minimal peak value is set to 1.11 and the minimal length of a transmembrane helix is set to 14. **A** Sec61 rat; **B** Sec61 *P. salina*; **C** SecY *P. salina*; **D** SecY *E. coli*

amino-acid sequence similarity between the determined transmembrane domains in SecY and the homologous positions of *P. salina* Sec61, as well as the similar hydropathy plot profiles, indicate that the *P. salina* Sec61 may span the membrane ten times with the N-terminus located in the cytoplasm, as was predicted for other members of this protein family (Akiyama and Ito 1987; Hartmann et al. 1989; Stirling et al. 1992; Flachmann et al. 1993). A comparison of the transmembrane spanning domains of Sec61 and SecY are shown in Rensing and Maier (1994).

Since there are two different nuclei in cryptomonad cells – one belonging to the host and one located in the periplastidal compartment, being derived from the eukaryotic endosymbiont – we were interested in the chromosomal localization of the *sec*61 gene. Utilizing pulsed-field gel electrophoresis of total and nucleomorph DNA preparations, we separated the chromosomes of the two different eukaryotic genomes. When DNA was transferred to a Nylon membrane and hybridized with radiolabelled *sec*61 cDNA, *sec*61 was exclusively restricted to the host nuclear chromosome fraction (data not shown).

In cryptomonads, nuclear-encoded proteins which function in the plastid or the periplastidal compartment must be transported to their intracellular site of action. Since the outermost membrane surrounding the complex plastid is connected to the ER-system of the host, and is likewise fitted with attached ribosomes (Hofmann et al. 1994), in addition to the transport across the rough ER-membrane we postulate a possible role for Sec61 in transport mechanisms over the outermost eukaryote symbiont membrane. When we synthesized Sec61 in a baculovirus insect expression system, the protein migrated in SDS-PAGE with the mobility corresponding to a 44-kDa molecule (data not shown). This is in contrast to the predicted actual molecular weight of 54 kDa and may be due to the hydrophobic nature of the protein. A similar, anomalously-fast migration of the Sec61 was shown in yeast (Deshaies et al. 1991). In order to learn more about the function of Sec61 in cryptomonads, we plan to try to produce antibodies against the overexpressed protein for use in immunolocalization studies with ultra-thin sections of *P. salina*.

As we have also isolated the plastome-encoded *sec*Y gene from *P. salina* (Rensing and Maier 1994), this is the first organism from which both the *sec*Y and *sec*61 genes are available for studying sorting mechanisms across eukaryotic and 'prokaryotic' membranes within the same cell.

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References

Akiyama Y, Ito K (1987) Topology analysis of the SecY protein involved in protein export in *Escherichia coli*. EMBO J 6: 3465–3470

Brundage L, Hendrick JP, Schiebel E, Driessen AJM, Wickner W (1990) The purified *E. coli* integral membrane protein SecY/E is sufficient for reconstitution of SecA-dependent precursor protein translocation. Cell 62:649–657

Cerretti DP, Dean D, Davis GR, Bedwell DM, Nomura M (1983) The spc ribosomal protein operon of *Escherichia coli*: sequence and cotranscription of the ribosomal protein genes and a protein export gene. Nucleic Acids Res 11:2599–2616

Deshaies RJ, Shekman R (1987) A yeast mutant defective at an early stage in import of secretory protein precursors into the endoplasmic reticulum. J Cell Biol 105:633–645

Deshaies RJ, Sanders SL, Feldheim DA, Shekman R (1991) Yeast Sec proteins involved in translocation into the endoplasmic reticulum are assembled into a membrane-bound multi-subunit-complex. Nature 349:806–808

Douglas SE (1992) Eukaryote-eukaryote endosymbiosis: insights from studies of a cryptomonad alga. BioSystems 28:57–68

Eschbach S, Hofmann CJB, Maier U-G, Sitte P, Hansmann P (1991) A eukaryotic genome of 660 kb: electrophoretic karyotype of nucleomorph and cell nucleus of the cryptomonad alga, *Pyrenomonas salina*. Nucleic Acids Res 19:1779–1781

Flachmann R, Michalowski CB, Löffelhardt W, Bohnert HJ (1993) SecY, an integral subunit of the bacterial preprotein translocase, is encoded by a plastid genome. J Biol Chem 268:7514–7519

Frömmel C (1984) The apolar surface area of amino acids and its empirical correlation with hydrophobic free energy. J Theor Biol 111:247–260

Gibbs SP (1981) The chloroplast of some algal groups may have evolved from endosymbiotic eukaryotic algae. Ann NY Acad Sci 361:193–208

Görlich D, Prehn S, Hartmann E, Kalies K-U, Rapoport TA (1992) A mammalian homolog of Sec61p and SecY is associated with ribosomes and nascent polypeptides during translocation. Cell 71:489–503

Grasser KD, Feix G (1991) Isolation and characterization of maize cDNAs encoding a high-mobility-group protein displaying a HMG-box. Nucleic Acids Res 19:2573–2577

Greenwood AD (1974) The Cryptophyta in relation to phylogeny and photosynthesis. In: Sanders JV, Goodchild DJ (eds) 8th Int Congr Electron Microscopy, Canberra 2:566–567

Greenwood AD, Griffith HB, Santore UJ (1977) Chloroplasts and cell compartments in cryptophyceae. Br Phycol J 12:119

Hansmann P, Eschbach S (1990) Isolation and preliminary characterization of the nucleus and the nucleomorph of a cryptomonad, *Pyrenomonas salina*. Eur J Cell Biol 52:373–378

Hartmann E, Rapoport TA, Lodish HF (1989) Predicting the orientation of eukaryotic membrane-spanning proteins. Proc Natl Acad Sci USA 86:5786–5790

Hartmann E, Sommer T, Prehn S, Görlich D, Jentsch S, Rapoport TA (1994) Evolutionary conservation of the protein translocation complex. Nature 367:654–657

Higgins DG, Bleasby AJ, Fuchs R (1992) CLUSTAL V: Improved software for multiple sequence alignment. CABIOS 8:189–191

Hofmann CJB, Rensing SA, Häuber MM, Martin WF, Müller SB, Couch J, McFadden GI, Igloi GL, Maier U-G (1994) Smallest known eukaryotic genomes encode a protein: towards an understanding of nucleomorph functions. Mol Gen Genet 243:600–604

Ito K, Wittekind M, Nomura M, Shiba K, Yura T, Miura A, Nashimoto H (1983) A temperature-sensitive mutant of *E. coli* exhibiting slow processing of exported proteins. Cell 31:789–797

Maier U-G, Hofmann CJB, Eschbach S, Wolters J, Igloi GL (1991) Demonstration of a nucleomorph-encoded eukaryotic small subunit ribosomal RNA in cryptomonads. Mol Gen Genet 230:155–160

Martin W, Lagrange T, Li Y-F, Bisanz-Seyer C, Mache R (1990) Hypothesis for the evolutionary origin of the chloroplast ribosomal protein L21 of spinach. Curr Genet 18:553–556

McFadden GI (1990) Evidence that cryptomonad chloroplasts evolved from photosynthetic eukaryotic endosymbionts. J Cell Sci 95:303–308

McFadden GI (1993) Second-hand chloroplasts: evolution of cryptomonad algae. Adv Bot Res 19:190–239

McKerracher L, Gibbs SP (1982) Cell and nucleomorph division in the alga *Cryptomonas*. Can J Bot 60:2440–2452

Morrall S, Greenwood AD (1978) Ultrastructure of the nucleomorph division in species of cryptophyceae and its evolutionary implications. J Cell Sci 54:311–328

Müsch A, Wiedmann M, Rapoport TA (1992) Yeast Sec proteins interact with polypeptides transversing the endoplasmic reticulum membrane. Cell 69:343–352

Rensing SA, Maier U-G (1994) The SecY protein family: comparative analysis and phylogenetic relationships. Mol Phyl Evol (in press)

Rothblatt JA, Deshaies RJ, Sanders SL, Daum G, Shekman R (1989) Multiple genes are required for proper insertion of secretory proteins into the endoplasmic reticulum in yeast. J Cell Biol 109: 2641–2652

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning; a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sanders SL, Whitfield KM, Vogel JP, Rose MD, Shekman RW (1992) Sec61p and BiP directly facilitate polypeptide translocation into the ER. Cell 69:353–365

Sanger F, Nichlen S, Coulson AR (1977) DNA sequencing with chain-termination inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Simon S (1993) Translocation of proteins across the endoplasmic reticulum. Curr Opinion Cell Biol 5:581–588

Sitte P, Baltes S (1990) Morphometric analysis of two cryptomonads. Quantitative evaluation of fine-structural changes in an endocytobiotic system. In: Nardon P, Gianinazzi-Pearson V, Grenier AM, Margulis L, Smith DC (eds) Endocytobiology IV. INRA, Paris: pp 229–233

Stirling CJ, Rothblatt J, Hosobuchi M, Deshaies R, Shekman R (1992) Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. Mol Biol Cell 3:129–142

Tomas RN, Cox ER (1973) Observations on the symbiosis of *Pteridinium balticum* and its intracellular algae. J Phycol 9:304–323

Whatley JM (1981) Chloroplast evolution – ancient and modern. Ann NY Acad Sci 361:154–165

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