Mol Gen Genet (1994) 243:600–604

SHORT COMMUNICATION

C. J. B. Hofmann · S. A. Rensing
M. M. Häuber · W. F. Martin · S. B. Müller
J. Couch · G. I. McFadden · G. L. Igloi
U.-G. Maier

The smallest known eukaryotic genomes encode a protein gene: towards an understanding of nucleomorph functions

Received: 26 October 1993 / Accepted: 15 December 1993

Abstract Cryptomonads are unicellular algae with plastids surrounded by four membranes. Between the two pairs of membranes lies a periplastidal compartment that harbours a DNA-containing organelle, termed the nucleomorph. The nucleomorph is the vestigial nucleus of a phototrophic, eukaryotic endosymbiont. Subcloning of parts of one nucleomorph chromosome revealed a gene coding for an Hsp70 protein. We demonstrate the expression of this nucleomorph protein-coding gene and present a model for protein transport from the host to the endosymbiont compartment.

Key words Cryptomonads · Periplastidal · Hsp70 Transport

Introduction

The endocytobiosis of phototrophic eukaryotic cells within eukaryotic host cells has led in the course of evolution to plastids surrounded by four membranes (Fig. 1) (see McFadden 1990, 1993; Douglas et al. 1991; Maier et al. 1991). Such plastids, called complex plastids (Sitte 1987), are typical for Chromophytes as well as for Chlorarachniophytes and Cryptophytes.

Cryptomonads represent an intermediate stage in the evolution of complex plastids (McFadden 1993; Sitte 1993). In this algal group, a periplastidal compartment harbouring 80S ribosomes and a DNA-containing organelle, the nucleomorph, is found between the two pairs of membranes (the periplastidal endoplasmic reticulum (ER) and the plastid envelope). We and others have demonstrated that the nucleomorph is the vestigial nucleus of a rhodophyte-like eukaryotic endosymbiont (Douglas et al. 1991; Maier et al. 1991; McFadden 1993).

In the cryptomonad *Pyrenomonas salina*, the nucleomorph genome consists of three linear chromosomes, 195, 225 and 240 kb in size (Eschbach et al. 1991). These three chromosomes contain rRNA gene clusters (Eschbach et al. 1991) whose transcripts are found in 80S ribosomes (McFadden 1990). These data suggest the presence of a functional genetic apparatus in the eukaryotic endosymbiont compartment, responsible for the expression of symbiont-specific protein genes. However, the nucleomorph genome is too small to encode all the necessary proteins. Thus, in addition to plastids and mitochondria, the nucleomorph represents a third genetically semi-autonomous organelle in these algae and possesses a chromosomal organization intermediate between that of plastids and nuclei. If this is true, most proteins that are located in the symbiont plasm or the plastid, must be transported from the host cytoplasm across two membranes or across four membranes in the case of the plastid.

We have sought to identify genes that are encoded by nucleomorph chromosomes and have investigated whether they are expressed, and, if so, what function they might serve in the cell. In order to elucidate functions carried out by the nucleomorph, we have subcloned large parts of chromosome II and searched for

Communicated by H. Saedler

C. J. B. Hofmann · S. A. Rensing · M. M. Häuber · S. B. Müller
U.-G. Maier (✉)
Institut für Biologie II/Zellbiologie, Schaenzlestr. 1,
D-79104 Freiburg, Germany

W. F. Martin
Institut für Genetik, Techn. Universität Braunschweig,
Konstantin-Uhde-Str. 5, D-38023 Braunschweig, Germany

J. Couch
Canadian Institute for Advanced Research,
Evolutionary Biology Program, Department of Botany,
University of British Columbia, Vancouver, BC, $V_6T_1Z_4$, Canada

G. I. McFadden
Plant Cell Biology Research Centre, School of Botany,
University of Melbourne, Parkville, VIC 3052, Australia

G. L. Igloi
Institut für Biologie III, Schaenzlestr. 1, D-79104 Freiburg,
Germany

This paper is dedicated to Prof. Dr. Peter Sitte on the occasion of his 65th birthday

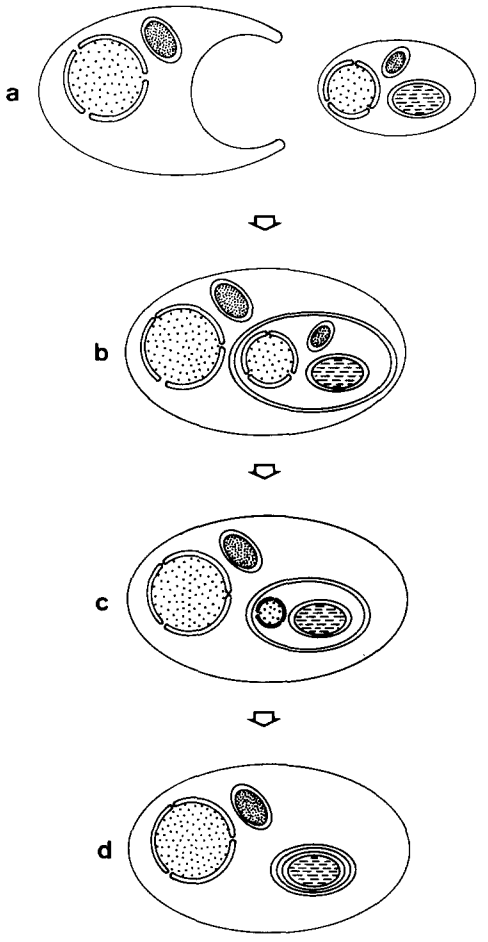

Fig. 1a–d Scheme of evolution of algae with complex plastids. **a** A phototrophic alga with nucleus (*dotted*), plastid (*hatched*) and mitochondria (*stippled*) is engulfed by a phagotrophic cell. **b, c** Continued endocytobiosis leads to loss of phototrophic cell functions, including the partial reduction of the nucleus to a nucleomorph. **d** Further loss of cell functions from the symbiont leads to an alga whose plastid is surrounded by four membranes, i.e. a complex plastid

open reading frames by shotgun sequencing. Here we present a characterization of the first identified protein-coding gene, *hsp*70, and discuss the possible function of Hsp70 in the periplastidal compartment.

Materials and methods

Cell cultivation and pulsed-field gel electrophoresis (PFGE) has been described previously (Eschbach et al. 1991). Cloning, manual sequencing, and hybridizations were done according to Sambrook et al. (1989), while automated sequencing was according to Igloi and Schiefermayr (1993). mRNA preparation and cDNA synthesis were performed as described (Martin et al. 1990), except that the algal cells were not ground prior to RNA extraction. The cDNA library was screened by plaque hybridization using a randomly labelled fragment spanning the 5' end of the nucleomorph *hsp*70 gene. Positively hybridizing clones were subcloned for sequencing. The initial analysis of the sequence was carried out in the databases using the BLAST search algorithm (Altschul et al. 1990). Detailed studies were done with the programm packages PC/GENE (version 6.60, IntelliGenetics) and HUSAR (Heidelberg Unix Sequence Analysis Resources). Alignments were performed using CLUSTAL V (Higgins et al. 1992).

Results and discussion

The nucleomorph of *Pyrenomonas salina* harbours three small chromosomes. They are numbered according to size: chromosome I is 240 kb, chromosome II 225 kb and chromosome III 195 kb long Maier (1992). These chromosomes were separated by PFGE in low-melting-point agarose. The gel region corresponding to chromosome II was excised and, after melting the agarose, the DNA was digested with various restriction enzymes. The resulting fragments were cloned and partly sequenced. Following double digestion with the restriction enzymes *Pst*I and *Sac*I, clones were isolated containing a gene, which was identified on the basis of database searches as coding for a 649 amino acid heat shock protein, *hsp*70 (Accession No. X72621). As in the case of other stress-regulated *hsp*70 genes (Gething and Sambrook 1992; Georgopoulos 1992), the nucleomorph-specific gene has no introns. The coding region has an A/T content of 65%, whereas the 5' and 3' regions of the nucleomorph-specific *hsp*70 are extremely A/T-rich (about 90%), as is the case for other nucleomorph-specific non-coding regions. This distribution of nucleotides, together with the lack of histone proteins in nucleomorph chromosomes (S. B. Müller, S.A. Rensing and U.-G. Maier, unpublished results) implies a rather primitive type of gene regulation in nucleomorph chromatin. The comparison of the nucleomorph Hsp70 with several other Hsp70 sequences (human, Hunt and Morimoto 1985; *Trypanosoma brucei*, Engmann et al. 1989; *Chlamydomonas reinhardtii*, Müller et al. 1992; maize, Rochester et al. 1986) revealed a high degree of amino acid sequence identity (Fig. 2). Hybridization experiments were performed as a control, showing that an hsp70 gene is actually located on nucleomorph chromosome II (Fig. 3). Under low stringency conditions an additional hybridization signal in the nucleus chromosomes was detected (not shown).

To demonstrate that the nucleomorph *hsp*70 gene is expressed, we constructed a cDNA library. Screening of 10^4 pfu with a fragment spanning the 5' terminus of the gene yielded 23 positive recombinant phages. A cDNA with the predicted nucleomorph-specific nucleotide sequence of the *hsp*70 gene, as well as the 3' non-coding region was detected by sequence analysis of some of these clones. The nucleomorph *hsp*70 gene is, therefore, actively transcribed *in vivo*, and we assume that it is translated by ribosomes in the periplastidal compartment.

The discovery of a nucleomorph-encoded *hsp*70 gene implies that the nucleomorph is not restricted to supplying ribosomal functions. In order to clarify the function(s) of the nucleomorph Hsp70 protein it will be important to determine whether or not heat shock induces transcription and whether the protein is located in the

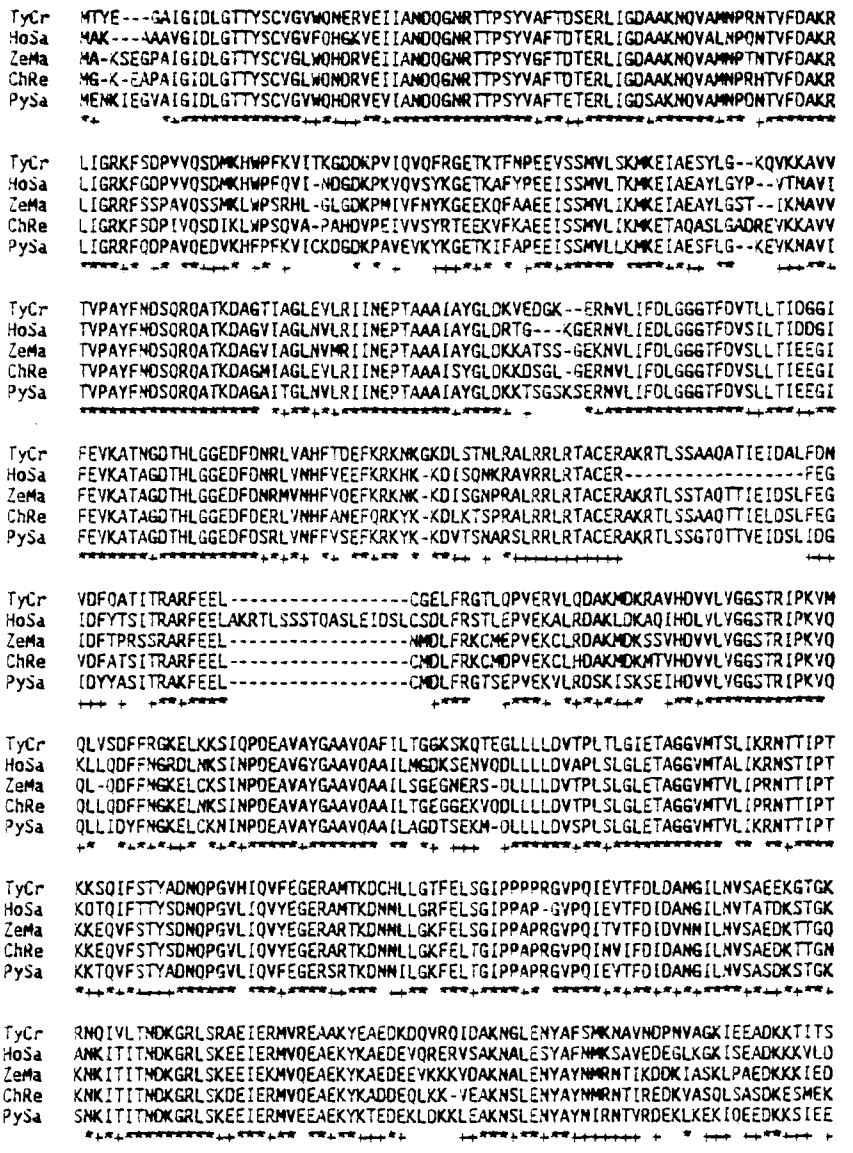

Fig. 2 Alignment of deduced amino acid sequences of *hsp*70 of *Trypanosoma brucei*, *Homo sapiens*, *Zea mays*, *Chlamydomonas reinhardtii* and *Pyrenomonas salina*. *Asterisks* indicate amino acid identities, *crosses* amino acid similarities

nucleomorph or in the periplastidal space. If it is in the latter compartment, it is possible that the nucleomorph-specific Hsp70 is involved in the transport/sorting of proteins that are imported into the periplastidal compartment. Several nuclear-encoded proteins that are located in the periplastidal space or the plastid have been detected using hybridization and immunolocalization techniques (our unpublished results). A close examination of the outer membrane of the periplastidal ER reveals that ribosomes attached to this membrane are not evenly distributed. Instead, the ribosomes are found in areas of the periplastidal ER clustered adjacent to the periplastidal cytoplasm (data not shown). This leads us to a possible model for protein import which is shown schematically in Fig. 4.

In a model that has been proposed for the transport of nuclear-encoded proteins into the plastid in some chromophytes, the proteins synthesized by ribosomes attached to the outer membrane pass during synthesis into the lumen of the periplastidal ER (Gibbs 1979). Vesicles containing the proteins then pinch off and fuse with the outer membrane of the plastid envelope. However, in contrast to chromophytes, cryptomonads contain a distinct eukaryotic plasm between the periplastidal ER and the plastid envelope. Since one Hsp70-related protein is involved in uncoating clathrin-coated vesicles (Chappell et al. 1986; Ungewickell 1985), it is possible that the nucleomorph Hsp70 is involved in such vesicular transport across the periplastidal space. However, our model involves not membrane vesicle fusion, but transport across the two membranes of the periplastidal ER in two steps. In comparison to protein transport through the inner mitochondrial membrane (Horst et al. 1993) two independent processes would be involved. The first membrane of the periplastidal ER could be penetrated by the processes of vectorial translation and the second membrane by a separate transport apparatus. Hsp70 could assist this transport process or act as a chaperone in the correct folding of the transported proteins.

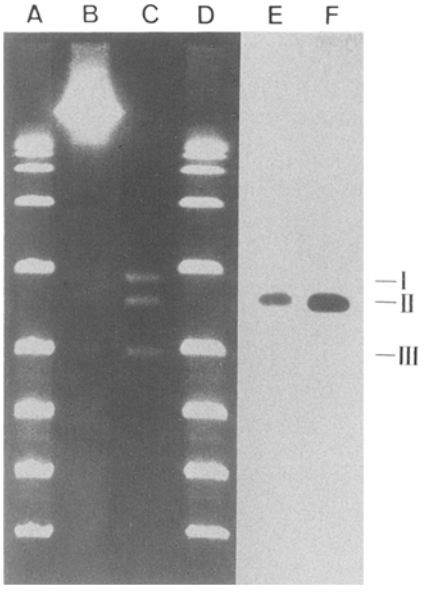

Fig. 3A–E Pulsed-field gel electrophoresis (lanes **A–D**) and Southern hybridization with an *hsp*70 probe (**E–F**) **A, D** Oligomers of phage lambda DNA. **B** Total DNA of *Pyrenomonas salina*. **D** nucleomorph DNA. Lanes **E** and **F** are hybridizations of samples **B** and **C** with an *hsp*70 probe. The nucleomorph chromosomes are indicated. Chromosome I has a length of 240 kb, chromosome II, 225 kb, and chromosome III, 195 kb

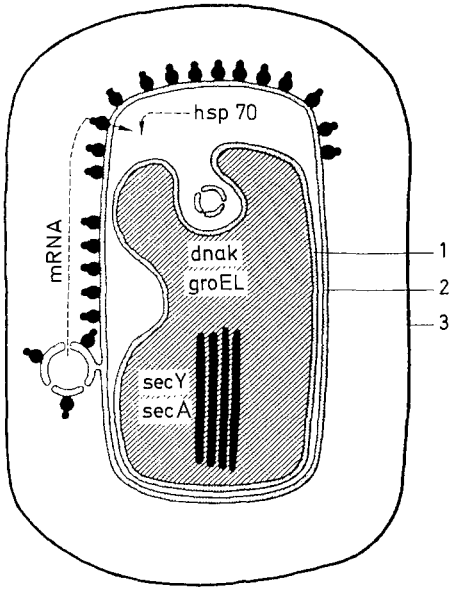

Fig. 4 Model for transport of proteins from the host compartment into the symbiont compartments. Genes for proteins destined for the symbiont, which are transcribed in the nucleus, are translated on ribosomes located at the outer membrane of the periplastidal ER (2). By a process of vectorial translation, the newly synthesized proteins are transported through the periplastidal ER into the symbiont cytoplasm where they are refolded. Hsp70 could be involved in the transport process and/or in the refolding of the transported proteins. For the subset of proteins, which are localized to the plastid, further components must be postulated. However, with DnaK, GroEL, SecA and SecY important factors of intracellular transport into the plastid have already been identified. 1, plastid envelope; 2, periplastidal ER; 3, cell membrane

Additional plastome-located genes, such as *secA* (Valentin 1993), *secY* (Douglas 1992), *cpn*60 (Maid et al. 1992) and *dnaK* (Wang and Liu, 1991) have been identified, whose translation products could be involved in sorting of plastid-located proteins. The discovery of the first nucleomorph-located protein gene has stimulated us to search for further nucleomorph-encoded protein genes. We have also carried out phylogenetic analyses using the Hsp70 sequence (Rensing and Maier 1994).

Acknowledgements The authors would like to thank Elfriede Schiefermayr and Klaus Scherzinger for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft and the Australian Research Council. G.McF. is the recipient of an Australian Research Council Senior Research Fellowship, SBM holds a stipend from the Land Baden Württemberg, FRG.

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