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Isolation, characterization and chromosomal mapping of an actin gene from the primitive red alga *Cyanidioschyzon merolae*

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Abstract Based on the results of cytological studies, it has been assumed that *Cyanidioschyzon merolae* does not contain actin genes. However, Southern hybridization of *C. merolae* cell-nuclear DNA with a yeast actin-gene probe has suggested the presence of an actin gene in the *C. merolae* genome. In the present study, an actin gene was isolated from a *C. merolae* genomic library using a yeast actin-gene probe. The *C. merolae* actin gene has no intron. The predicted actin is composed of 377 amino acids and has an estimated molecular mass of 42 003 Da. Southern hybridization indicated that the *C. merolae* genome contains only one actin gene. This gene is transcribed at a size of 2.4 kb. When Southern hybridization was performed with *C. merolae* chromosomes separated by pulsed-field gel electrophoresis, a band appeared on unseparated chromosomes XI and XII. A phylogenetic tree based on known eucaryote actin-gene sequences revealed that *C. merolae* diverged after the division of Protozoa, but before the division of Fungi, Animalia and Chlorophyta.

Key words Actin gene · *Cyanidioschyzon merolae* · Pulsed-field gel electrophoresis (PFGE) · Phylogenetic tree

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Introduction

Cell division is one of the most important and fundamental characteristics of organisms. In studies of cytokinesis in eukaryotic cells, animal cells such as sea urchin eggs and newt eggs have been principally employed (Schroeder 1968; 1970; Perry et al. 1971; Mabuchi 1986). It is now widely accepted that the contractile rings in cytokinesis are composed primarily of actin filaments (Perry et al. 1971; Schroeder 1973; Sanger and Sanger 1980; Cao and Wang 1990). However, the contractile rings in animal cells are large and composed of numerous filaments. Therefore, they are unsuitable for analyzing the complete structure of the contractile rings and the mechanism of their contraction. An organism with a smaller and simpler contractile ring would be ideal for this purpose. *Cyanidium caldarium* strain RK-1 and *Cyanidioschyzon merolae*, which belong to the Cyanidiophyceae of the Bangiophyceae (Seckbach 1992), have been examined previously. Cytological studies have revealed that they are two of the most primitive eukaryotes (Nagashima et al. 1984; Seckbach 1992; Suzuki et al. 1992). They are very small (1.5–3 μm in cell diameter) unicellular eukaryotes, and are about 1/1000 the diameter of newt eggs (Selman and Perry 1970). Therefore, the entire structure of the cell can be easily observed by three-dimensional reconstruction using serial-section electron microscopy. In a dividing *C. caldarium* strain RK-1 cell, a contractile ring can be identified (Suzuki et al. 1995). It has the following features in common with those of animal cells: (1) Cytochalasin B, an inhibitor of a polymerization of actin filaments, inhibits cell division (Table 1; Mita and Kuroiwa 1988). (2) The contractile ring can be detected by FITC-phalloidin (Table 1; Suzuki et al. 1995). (3) A ring-like structure can be found inside the plasma membrane at the division furrow (Table 1; Suzuki et al. 1995). (4) A single band is detected by immunoblotting using the anti-actin antibody (Table 1; Suzuki et al. 1995). In addition, two electron-dense bands have been observed at the leading edge of the cleavage furrow (Suzuki et al. 1995). The inner band is composed of a bundle of ap-

Table 1 Comparison of the features of *C. caldarium* strain RK-1 and *C. merolae*

Feature	*Cyanidium caldarium* strain RK-1	*Cyanidioschyzon merolae*	References
Observation of contractile ring by electron microscopy	+	–	Kuroiwa et al. (1994) Suzuki et al. (1995)
Detection by phalloidin staining	+	–	Mita and Kuroiwa (1988) Suzuki et al. (1995)
Inhibition of cell division by cytochalasin B	+	–	Mita and Kuroiwa (1988) Suzuki unpublished data
Detection of actin protein in immunoblotting	+	–	Suzuki et al. (1995)

proximately 20 actin-like filaments which are arranged in the form of a raft (Suzuki et al. 1995). This simple structure for the contractile ring may be beneficial for analyzing the structure and the mechanism of the contractile ring.

In contrast, cytokinesis of *C. merolae* shows unusual features. When *C. merolae* cells were used in experiments (1) to (4) above, negative results were observed (Table 1; Kuroiwa et al. 1994; Suzuki et al. 1995; Suzuki unpublished data). Therefore, *C. merolae* may undergo a different type of cell division which does not involve actin filaments, or else the actin of *C. merolae* may differ slightly from typical actin. Alternatively, the actin gene may not exist in the *C. merolae* genome.

Recent studies have revealed that, in addition to being involved in cytokinesis, actin is also involved in the division of plastids. In *C. merolae* and *C. caldarium* strain RK-1, a ring-like structure composed of many filaments was first found in the cytoplasm near the constricted isthmus of a dividing chloroplast (Mita et al. 1986; Mita and Kuroiwa 1988; Kuroiwa et al. 1993; Suzuki et al. 1994). This structure seems to contract and eventually cause the chloroplast to divide. Therefore, it was called a plastid-dividing ring (PD ring; Mita et al. 1986). The PD ring is thought to be composed of actin-like filaments (Mita and Kuroiwa 1988). An additional ring structure, similar to the PD ring, can be found around dividing mitochondria of *C. merolae* and has been named the mitochondria-dividing ring (MD ring; Kuroiwa et al. 1993). The MD ring is believed to be essential for the division of mitochondria (mitochondriokinesis; Kuroiwa et al. 1993). The MD ring is also thought to be composed of actin-like filaments.

Therefore, actin is a key protein for understanding cytokinesis and organellekinesis. The actin of *C. merolae* is particularly interesting because of its unusual cytokinesis. First, we examined whether the *C. merolae* genome contained an actin gene. Next, the actin gene was isolated and characterized. Using pulsed-field gel electrophoresis (PFGE), the chromosome on which the actin gene exists was also identified. Finally, the phylogenetic position of the primitive red alga *C. merolae* was determined using the nucleotide sequences of known actin genes.

Materials and methods

DNA extraction. C. merolae cells were cultured in Allen's medium (Allen 1959). Cells were harvested, frozen, and ground to a powder with a liquid nitrogen-cooled mortar and pestle. Total DNA was then extracted with a phenol series, phenol/chloroform, and finally chloroform. Cell-nuclear DNA was purified by ultracentrifugation as described elsewhere (Suzuki et al. 1992).

Construction and screening of genomic libraries. C. merolae DNA was partially digested with *Mbo*I, and 15–20-kbp fragments were ligated to *Bam*HI-digested λDash II vector arms (λDash II *Bam*HI vector kit, Stratagene, Calif.). Ligated DNA was packaged in vitro using a cDNA cloning system λgt10 kit (Amersham International plc, UK). To isolate candidates which might include the *C. merolae* actin gene, the genomic library was screened using the 1.0-kbp *Xho*I-*Hin*dIII fragment, which contains most of the yeast actin gene (Ng and Abelson 1980), as a probe. The DNA probe was labeled with [α-^{32}P] dCTP using a Megaprime DNA labeling system (Amersham). Pre-hybridization and hybridization were performed at 42 °C in a hybridization solution (6×SSC, 5×Denhardt's solution, 0.5% SDS and 100 μg/ml of denatured salmon testis DNA) without a probe for 1 h and with a probe for 16 h. Post-hybridization was carried out by washing the membrane with a washing buffer series (2.0, 1.0, 0.5, 0.1×SSC, each containing 0.1% SDS) at 42 °C for 20 min per buffer (low-stringency conditions). Screening of the *C. merolae* genomic library resulted in six positive candidates. Phage DNAs were isolated from these six candidates by the liquid lysate method (Sambrook et al. 1989). When phage DNAs were digested with *Eco*RI and hybridized with the yeast actin-gene probe under low-stringency conditions, only a 2.6-kbp *Eco*RI fragment hybridized in each case. This fragment was subcloned into the *Eco*RI site of the plasmid vector pBluescript SKII+(Stratagene) to facilitate further analysis.

DNA sequencing. Deletion, sequencing and sequence analysis were performed according to the methods of Takano et al. (1994).

Southern hybridization. The *C. merolae* actin-gene probe used corresponded to most of the actin coding region, including nucleotides 215 to 1360 of the *C. merolae* actin gene shown in Fig. 1. Ten micrograms of cell-nuclear DNA digested with an appropriate restriction enzyme were separated on a 1.0% agarose gel in 1×TAE buffer [40 mM Tris-acetate and 1 mM EDTA (pH 8.0)] for 15 h at 20 V. DNA was transferred to a nylon membrane filter using a VacuGene vacuum blotting system (Pharmacia LKB Biotechnology, Sweden) and hybridized with a ^{32}P-labeled *C. merolae* actin-gene probe under low-stringency conditions.

The chromosomal location of an actin gene. Sample plugs for PFGE were prepared according to procedures described previously (Takahashi et al. 1993). The counter-clamped homogeneous electric fields (CHEF) Mapper Pulsed Field Electrophoresis System (Bio-Rad, USA) was used. Under all conditions, 1.0% agarose NA gels (Phar-

macia LKB Biotechnology) were used. DNA in sample plugs was electrophoresed at 14 °C in circulating 0.5 × TBE buffer [90 mM Tris-borate, 2 mM EDTA (pH 8.0)] at 6 V/cm with an angle of 120°. Under normal conditions, electrophoresis was performed with pulse intervals of 60 s for 15 h, and then continued with pulse intervals of 90 s for 9 h. To separate chromosomes of more than 1000 kbp, between 600 kbp and 1000 kbp, and less than 600 kbp, electrophoresis was performed using the auto-algorithm mode of the CHEF Mapper System to separate DNAs between 900 kbp and 1100 kbp (51 h 16 min, pulse intervals 101.2–144.03 s), between 650 kbp and 770 kbp (47 h 37 min, pulse intervals 70.64–74.32 s), and between 370 kbp and 650 kbp (41 h 14 min, pulse intervals 43.28–58.93 s), respectively. Gels were stained in 0.5 mg/ml of ethidium bromide (EtBr) for 1 h and then photographed under UV illumination. The separated chromosomes were transferred to a nylon membrane filter by the same method used for Southern hybridization. Intact chromosomal DNAs from *S. cerevisiae* in agarose plugs (Bio-Rad) were used as size markers. Using the 2.6 kbp *Eco*RI fragment containing the *C. merolae* actin gene as a probe, Southern hybridization was performed under almost same conditions as in low-stringency conditions except that hybridization buffer additionally contains 50% formamide and washing was carried at 65°C (high-stringency conditions).

Phylogenetic analysis with actin-gene sequences. The nucleotide sequence of an open reading frame (ORF) of the *C. merolae* actin gene was used together with those of various eukaryotes. Only the first and second positions of codons were used in the analysis due to mutational saturation at the third positions (Hightower and Meagher 1986; Bhattacharya et al. 1991). They were aligned by Clustal V software (Higgins et al. 1991) without changing the default conditions. A phylogenetic tree was inferred with the maximum parsimony method using the PAUP software package (ver. 3.1.1; Swofford 1993) with a heuristic search procedure. Monophyly of groups was assessed with the bootstrap method (100 replications; Felsenstein 1985). The nucleotide sequence of the actin gene of *Trypanosoma brucei* was used as an outgroup (Amar et al. 1988).

Results and discussion

Existence of an actin gene in the *C. merolae* genome

To determine whether the *C. merolae* genome contained an actin gene, *C. merolae* cell-nuclear DNA was digested with appropriate restriction enzymes, electrophoresed, blotted and hybridized with the yeast actin-gene probe. A single band appeared in all restriction patterns (data not shown). This result suggested that the *C. merolae* genome contained an actin gene.

Cloning of the *C. merolae* actin gene

To isolate the actin gene from *C. merolae'* a genomic library was constructed. Screening of the *C. merolae* genomic library resulted in six positive candidates. When phage DNAs of these candidates were digested with *Eco*RI and hybridized with the yeast actin-gene probe, only a 2.6-kbp *Eco*RI fragment was hybridized in each case. This fragment was subcloned into the plasmid vector and DNA sequences were determined (Fig. 1). As a result, an ORF was found in the fragment. It encoded a protein which was composed of 377 amino acids, and with an estimated molecular mass of 42 003 Da. The number of amino acids and molecular mass agreed well with those of typical actin. When the derived amino-acid sequence was compared to the database, it showed a high degree of identity with various actins: between 58.6% (ciliated protozoa *Euplotes crassus* actin; Harper and Jahn 1989) and 84.9% (*Homo sapiens* γ-actin; Erba et al. 1988). It showed 78.8% identity with *S. cerevisiae* actin (Ng and Abelson 1980). These results suggested that *C. merolae* has a typical actin gene. However, despite this high level of identity, *C. merolae* actin was not identified by FITC-phalloidin (Suzuki et al. 1995). In typical actin, phalloidin is bound in the vicinity of Glu-117, Met-119, and Met-355 (Vandekerckhove et al. 1985). These amino-acid residues exist at corresponding positions in *C. merolae* actin; i.e. residues Glu-118, Met-120, and Met-357. As suggested by Miki et al. (1987), a region between amino acids 1 and 68 may also be involved in the binding of phalloidin to actin. Therefore, certain replacements in this region, or the insertion of a single Gln-234 residue, may change the higher structure of the molecule necessary for phalloidin binding.

A comparison of the derived amino-acid sequence of *C. merolae* actin to other known actins also revealed that the *C. merolae* actin gene may have no intron. Other lower eukaryotes, such as *Dictyostelium discoideum* (Romans and Firtel 1985), *Oxytricha fallax* (Kaine and Spear 1982), *Tetrahymena pyriformis* (Hirono et al. 1987) and *Schizosaccharomyces pombe* (Mertins and Gallwitz 1987), also have no intron.

Examination of the gene family of the *C. merolae* actin gene

To determine whether the actin gene forms a gene family in *C. merolae*, as in many previously examined eukaryotes, *C. merolae* DNA was digested with appropriate restriction enzymes, electrophoresed, blotted and hybridized with a *C. merolae* actin-gene probe (Fig. 2). A single band appeared in the restriction digests of *Bam*HI, *Eco*RI, *Pst*I, *Sac*I, and *Xba*I. In contrast, *Hin*dIII-digested genomic DNA produced two bands, perhaps because the *Hin*dIII site is located near the end of the actin gene (Fig. 1). This finding, together with the lack of different actin genes in the genomic library, suggests that the *C. merolae* genome contains a single actin gene. Lower eukaryotes, such as *S. cerevisiae* (Ng and Abelson 1980), *S. pombe* (Mertins and Gallwitz 1987), *T. pyriformis* (Hirono et al. 1987), *Volvox carteri* (Cresnar et al. 1990), *Aspergillus nidulans* (Fidel et al. 1988), *Costaria costata* (Bhattacharya et al. 1991), *Achlya bisexualis* (Bhattacharya et al. 1991) and *Phytophthora megasperma* (Dudler 1990), also have only one actin gene. These results suggest that lower eukaryotes, including *C. merolae*, need not have multiple actin genes, each with a different function, as is found, for example, tissue-specific and developmental gene expression (Fyrberg et al. 1981, 1983; Minty et al. 1982; Melloul et al. 1984; Hightower and Meagher 1986; Meagher and McLean 1990; Mounier and Prudhomme 1991).

To examine whether this actin gene is transcribed, Northern hybridization was performed. It revealed that *C. me-*

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   1  ACGCTCCACGTGTCCAGGTTTATCCAGGGCGGCCATTGGCGCGCTGTCCCGTCGGTTTTTGCTTCAAACGCATCGTTTGGCGAAAATGGCGCTCGTTGCACGCTTCACTGCGCGGGAGAG  120
 121  CGTTCGTTCATAACTGACGCACCGCCCCAGAGTATACAAAGCAGTTCGCAAAGCAAAGAGCGTTGACTGGACTTGACTATGACAGAGGAGGAAATAACAGCTCTTGTGATCGATAATGGT  240
   1                                                                                  M  T  E  E  E  I  T  A  L  V  I  D  N  G   14

 241  TCTGGGATGGTGAAAGCGGGTTTCGCGGGAGACGACGCGCCCCGTGCAGTTTTCCCATCCATAGTGGGACGTCCGCGACACCAAGCAGTCATGGTTGGCATGGGGCAAAAAGACTCTTAT  360
  15   S  G  M  V  K  A  G  F  A  G  D  D  A  P  R  A  V  F  P  S  I  V  G  R  P  R  H  Q  A  V  M  V  G  M  G  Q  K  D  S  Y   54

 361  GTGGGCGACGAAGCGCAGTCGAAACGCGGTATCCTATCGCTCAAGTACCCGATCGAGCACGGCATCGTAACGAACTGGGATGATATGGAAAAGATCTGGTACCACACTTTTTACAACGAG  480
  55   V  G  D  E  A  Q  S  K  R  G  I  L  S  L  K  Y  P  I  E  H  G  I  V  T  N  W  D  D  M  E  K  I  W  Y  H  T  F  Y  N  E   94

 481  TTGCGCATCTCCCCCGAGGATCATCCAGTGTTGTTAACCGAAGCACCTTTGAACCCGAAAGCCAACAGGGAAAAAATGACCCAGATCATGTTCGAGACGTTCAACGTTCCCGCGATGTAC  600
  95   L  R  I  S  P  E  D  H  P  V  L  L  T  E  A  P  L  N  P  K  A  N  R  E  K  M  T  Q  I  M  F  E  T  F  N  V  P  A  M  Y  134

 601  GTCGCGATCCAGGCAGTATTGTCGTTGTATGCTTCGGGGCGCACCACGGGTATTGTCGTCGACAGCGGCGACGGTGTGACGCACACTGTGCCCATCTACGAAGGCTATGCCTTGCCGCAT  720
 135   V  A  I  Q  A  V  L  S  L  Y  A  S  G  R  T  T  G  I  V  V  D  S  G  D  G  V  T  H  T  V  P  I  Y  E  G  Y  A  L  P  H  174

 721  GCTATCATGCGCATAGATCTGGCTGGACGAGACCTAACCGACTATCTCGCCAAGATCCTTACAGAGCGCGGATATTCATTCACGACGACAGCCGAACGAGAGATCGTGCGAGACATCAAG  840
 175   A  I  M  R  I  D  L  A  G  R  D  L  T  D  Y  L  A  K  I  L  T  E  R  G  Y  S  F  T  T  T  A  E  R  E  I  V  R  D  I  K  214

 841  GAAAAATGTTGTTATGTGGCACAGGACTACGATCATGAGTTGGAGATTGCCTCATCGCAGCCGGCGAAAATCGATAAGCAGTATGAACTCCCCGACGGACAAATCATCACGATTGGGAGT  960
 215   E  K  C  C  Y  V  A  Q  D  Y  D  H  E  L  E  I  A  S  S  Q  P  A  K  I  D  K  Q  Y  E  L  P  D  G  Q  I  I  T  I  G  S  254

 961  GAGCGTTTCAGATGCCCGGAGGTGCTCTTCCAGCCGTCCTTGATCGGTATGGAGGGCGAGGGTATTCATAATGTTGCTTATCAGAGCATCATGAAATGCGATGTCGACATCCGGAAAGAT 1080
 255   E  R  F  R  C  P  E  V  L  F  Q  P  S  L  I  G  M  E  G  E  G  I  H  N  V  A  Y  Q  S  I  M  K  C  D  V  D  I  R  K  D  294

                                                                                                          HindIII
1081  CTGTACGCAAACGTGGTTCTCAGCGGCGGCACGACGATGTTCCCAGGCATCGCGGATCGGATGCAGCGGGAACTCGCTAGTGTTGCACCATCTTCGGTGAAGATCAAGCTTGTAGCGCCA 1200
 295   L  Y  A  N  V  V  L  S  G  G  T  T  M  F  P  G  I  A  D  R  M  Q  R  E  L  A  S  V  A  P  S  S  V  K  I  K  L  V  A  P  334

1201  GCGGAGCGCAAATATAGCGTGTGGATCGGCGGCAGCATTTTGGCCTCGTTGAGCACTTTTCAGCAGATGTGGATTAGTAAGGCGGAGTATGACGAGTTCGGACCCTCTGTGGTACACCGC 1320
 335   A  E  R  K  Y  S  V  W  I  G  G  S  I  L  A  S  L  S  T  F  Q  Q  M  W  I  S  K  A  E  Y  D  E  F  G  P  S  V  V  H  R  374

                EcoRI
1321  AAATGTTTCTGAGGCAGACGCTTGAAGCGCTGGAGAATTC                                                                              1360
 375   K  C  F  *                                                                                                            377
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Fig. 1 The nucleotide sequence of the *C. merolae* actin gene and its 5′ and 3′ flanking regions. The derived amino-acid sequence is located below the nucleotide sequence. Nucleotide and amino-acid numbers are indicated on both sides of each line. An *asterisk* under codon TGA indicates a stop codon. AAGCTT and GAATTC in the *open boxes* indicate the *Hin*dIII and *Eco*RI sites, respectively. This sequence data will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with the accession number D32140

rolae actin gene is transcribed at a size of 2.4 kb (data not shown). A minor 2.0-kb transcript is also generated in *C. merolae* (data not shown), but it is not yet clear whether this minor band is a degradation product of the major band or an un-degraded 2.0-kb transcript.

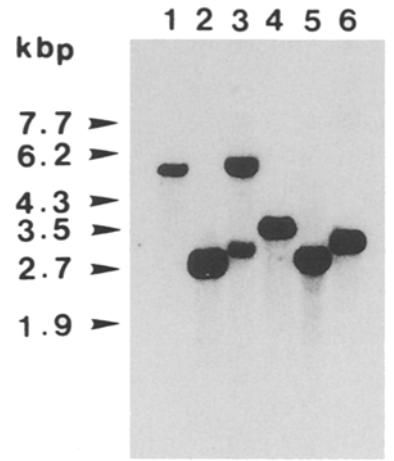

Fig. 2 Genomic Southern hybridization with the *C. merolae* actin-gene probe. In each case, 10 μg of cell-nuclear DNA from *C. merolae* was digested with *Bam*HI (*1*), *Eco*RI (*2*), *Hin*dIII (*3*), *Pst*I (*4*), *Sac*I (*5*), and *Xba*I (*6*), and then Southern hybridization was performed in each restriction digest under low-stringency conditions. λDNA digested with *Sty*I was used for size markers

Mapping of the actin gene onto *C. merolae* chromosomes

Recently, the chromosomal DNA molecules of *C. merolae* have been resolved into 15 bands (Maleszka 1993; Takahashi et al. 1993). In our previous paper (Takahashi et al. 1993) we mentioned that an increase in the genome size may occur as a result of further separation of some non-stoichiometric bands. Further separation of *C. merolae* chromosomes revealed that two of the bands (bands X and XIII in our previous paper) can each be separated into two additional bands. These 17 bands were thus re-numbered and the genome size re-calculated to be about 14.2 Mbp (Fig. 3, Table 2). This value is about three times that in *Escherichia coli* (Kohara et al. 1987), but is still one of the smallest among the eukaryotes. When Southern hybridization was performed with chromosomal DNAs separated under normal conditions, the hybridized band was detected at the middle position (Fig. 3, panel 4). To clarify which chromosome hybridized with the *C. merolae* actin-gene probe, we separated the middle position and re-hybridized it. Only one hybridized band appeared near chromosomes XI and XII (Fig. 3, panel 6). However, it is difficult to determine which band was hybridized with the actin-gene probe, since chromosomes XI and XII are too close to be separated even under the best conditions.

Phylogenetic position of *C. merolae*

Based on known actin-gene sequences, a phylogenetic tree was constructed with the actin gene of *T. brucei* as an outgroup (Fig. 4). The branching pattern was fairly reason-

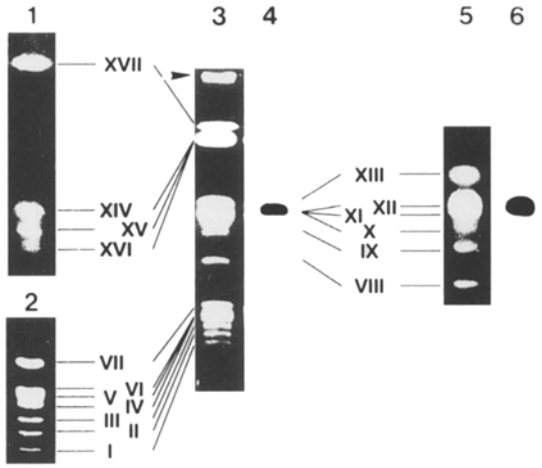

Fig. 3 Southern hybridization analysis of chromosomes separated by PFGE with the *C. merolae* actin-gene probe. The *arrowhead* indicates the position of the well. EtBr staining patterns of previously separated *C. merolae* chromosomes were made under the following conditions: normal conditions (*3*) and auto-algorithm conditions for separation of DNAs between 900 kbp and 1100 kbp (*1*), between 370 kbp and 650 kbp (*2*), and between 650 kbp and 770 kbp (*5*). Panels (*4*) and (*6*) show hybridization patterns with the *C. merolae* actin-gene probe to the chromosomes separated in panels (*3*) and (*5*), respectively

Table 2 Designations and estimated sizes of *C. merolae* chromosomes. Note: Since chromosomes with asterisks are newly separated bands, genome size and chromosome numbers have been revised

Chromosome	kbp
I	420
II	440
III	470
IV	510
V	520
VI	540
VII	590
VIII	720
IX	790
X*	820
XI	830
XII	835
XIII	920
XIV*	1150
XV	1250
XVI	1320
XVII	2050
Total	14175

able: the Fungi, Animalia, and Chlorophyta each formed a group. In a previous paper (Bhattacharya et al. 1991) almost the same result was obtained. In our tree, *C. merolae*, which belongs to the Rhodophyta, diverged after the divergence of Protozoa, but before the divergence of Fungi, Animalia and Chlorophyta, indicating a polyphyletic origin for the Chlorophyta and Rhodophyta. This result agrees with the phylogenetic inferences based on plastid-encoded *rbcL* (the gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) sequences (Douglas et al.

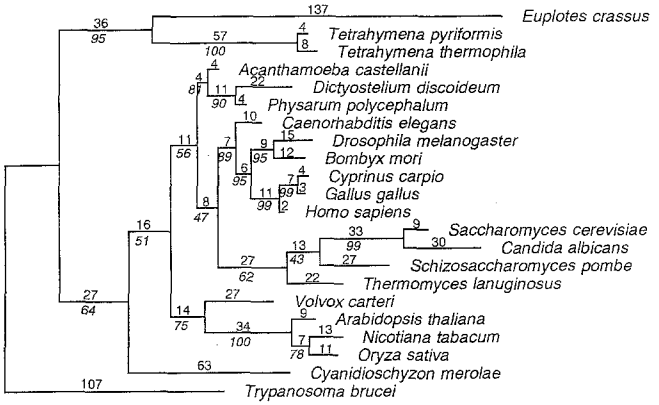

Fig. 4 Phylogenetic analysis of actin-coding regions from *C. merolae* and other major eukaryotes. Only the first and second positions of the codons of actin genes were used. Maximum-parsimony analysis of actin coding regions using a heuristic procedure (PAUP, ver. 3.1.1; Swofford 1993) identified a single parsimonious phylogram. The actin gene of *T. brucei* was used as an outgroup. The numbers of steps between nodes are shown above the branches, and bootstrap values, based on 100 replications, are shown below the branches (*italic numbers*). Other sequences than that of *C. merolae* actin gene are from the following sources: *E. crassus*, Harper and Jahn (1989); *T. pyriformis*, Hirono et al. (1987); *T. thermophila*, Cupples and Pearlman (1986); *T. brucei*, Amar et al. (1988); *A. castellanii*, Nellen and Gallwitz (1982); *D. discoideum*, Romans and Firtel (1985); *P. polycephalum*, Nader et al. (1986); *C. elegans*, Krause et al. (1989); *D. melanogaster*, Sanchez et al. (1983); *B. mori*, Mounier et al. (1987); *C. carpio*, Liu et al. (1990); *G. gallus*, Wang and Morais (1992); *H. sapiens*, Erba et al. (1988); *S. cerevisiae*, Gallwitz and Sures (1980); *C. albicans*, Losberger and Ernst (1989); *S. pombe*, Mertins and Gallwitz (1987); *T. lanuginosus*, Wildeman (1988); *V. carteri*, Cresnar et al. (1990); *A. thaliana*, Narin et al. (1988); *N. tabacum*, Thangavelu et al. (1993); *O. sativa*, McElroy et al. (1990)

1990; Valentin and Zetsche 1990; Morden and Golden 1991; Ohta et al. unpublished data). In contrast, trees derived from 16*s* rRNA sequences (Douglas and Turner 1991) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene sequences (Liaud et al. 1994) suggested a monophyletic origin for the Chlorophyta and Rhodophyta. Further studies, involving, for example, the isolation and sequencing of the 16*s* rDNA of *C. merolae* and the construction of a phylogenetic tree, may be necessary. The isolation and sequencing of actin genes from other Rhodophyta and the construction of a phylogenetic tree, may also be needed. This would more accurately determine the position of the Rhodophyta, including that of *C. merolae*.

The tree shown in Fig. 4 also suggested that *C. merolae* is one of the earliest-diverged eukaryotes. As mentioned in the Introduction, cytological studies support the primitive standing of this alga. As for molecular studies, they have only just begun. The *trpA* gene, which is the gene for the a subunit of tryptophan synthase, and the *trxM* gene, which is the gene for thioredoxin *m*, have been found on the plastid genome of *C. merolae* (Ohta et al., unpublished data). In other plants, proteins derived from these genes act in the plastid, but the genes themselves are encoded by the nuclear genome of the cell. This is the first example of these genes being encoded on a plastid genome. The exis-

tence of the *trpA* and *trxM* genes on the plastid genome indicates the very primitive standing of *C. merolae*. Moreover, the genome size of *C. merolae* was determined using PFGE, and showed that it has one of the smallest genome sizes among eukaryotes, and is thus one of the most primitive eukaryotes.

Studies of cytokinesis in such a primitive eukaryote may provide new information about a primitive type of cytokinesis which links cytokinesis in prokaryotes to that in higher eukaryotes.

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