

# Composition and Structure of the 80 S Ribosome from the Green Alga *Chlamydomonas reinhardtii*: 80 S Ribosomes are Conserved in Plants and Animals

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We have conducted a proteomic analysis of the 80 S cytosolic ribosome from the eukaryotic green alga *Chlamydomonas reinhardtii*, and accompany this with a cryo-electron microscopy structure of the ribosome. Proteins homologous to all but one rat 40 S subunit protein, including a homolog of RACK1, and all but three rat 60 S subunit proteins were identified as components of the *C. reinhardtii* ribosome. Expressed Sequence Tag (EST) evidence and annotation of the completed *C. reinhardtii* genome identified genes for each of the four proteins not identified by proteomic analysis, showing that algae potentially have a complete set of orthologs to mammalian 80 S ribosomal proteins. Presented at 25 Å, the algal 80 S ribosome is very similar in structure to the yeast 80 S ribosome, with only minor distinguishable differences. These data show that, although separated by billions of years of evolution, cytosolic ribosomes from photosynthetic organisms are highly conserved with their yeast and animal counterparts.

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## Introduction

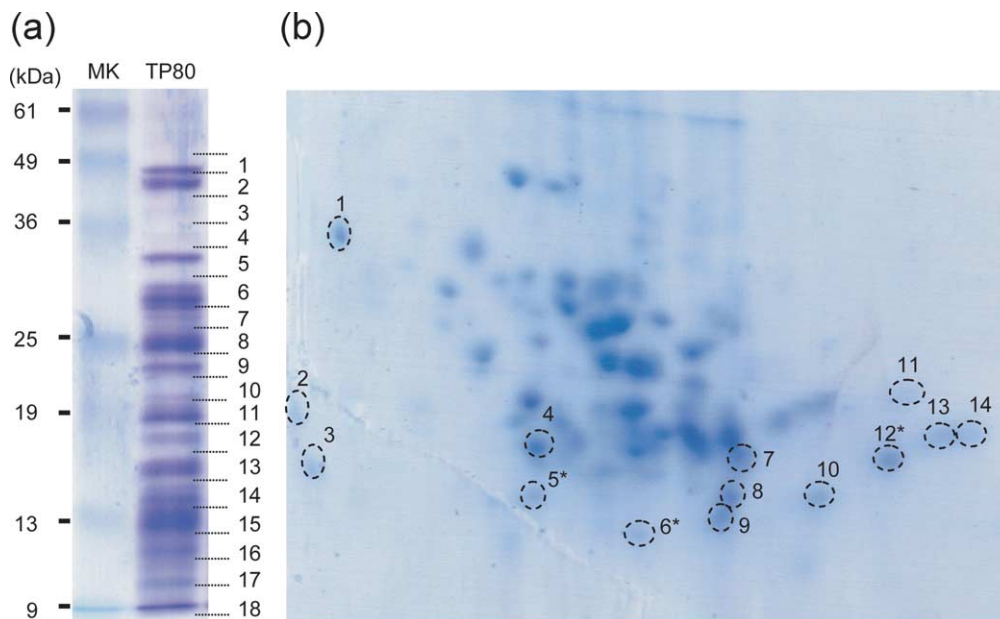
Ribosomes are the protein-synthesizing complex of the cell and are present in all organisms from bacteria to man to green plants. Ribosomes have two subunits; the smaller subunit, 30 S for the bacterial and 40 S for the eukaryotic ribosome, containing a single ribosomal RNA and 21–33 proteins (depending on the organism), 15 of which share homology between 30 S and 40 S ribosomal subunits.<sup>1</sup> The small ribosomal subunit is responsible for initial interactions with mRNA, and the correct positioning of the ribosome for translation start. mRNA decoding and tRNA fidelity verification is carried out by the small subunit.<sup>2</sup> The larger subunit of ribosomes, 50 S for the bacterial and 60 S for the eukaryotic ribosome, is composed of two or

three rRNAs and from 33 to 47 proteins, with 17 of these proteins conserved between 50 S and 60 S ribosomal subunits.<sup>1</sup> The large subunit houses the peptidyl transferase reaction center and the nascent polypeptide exit tunnel. Computational fitting of bacterial ribosome structures to protein density of a yeast cryo-electron microscopy (EM) map has allowed for delineation of a conserved ribosome core structure containing both protein and rRNA.<sup>3</sup>

Plant cells have three distinct ribosome species; 80 S ribosomes in their cytosol, 70 S-like ribosomes in their chloroplasts, and mitochondrial ribosomes. Based on two-dimensional gel electrophoresis, the number of proteins in the cytosolic 80 S ribosome of *Chlamydomonas reinhardtii* has been estimated at 96 (45 small subunit proteins, 48 large subunit proteins, and three proteins unique to the assembled ribosome), with molecular mass ranging from 12 kDa to 54 kDa.<sup>4</sup> Both the predicted number and molecular mass of these ribosomal proteins are greater than those found in yeast (77 proteins, <10,000–51,500 kDa<sup>5</sup>) or rat (82 proteins, 11,200–41,800 kDa<sup>6</sup>). These observations suggested that the *C. reinhardtii* cytosolic ribosome may have ribosomal proteins and/or post-translational

Abbreviations used: EM, electron microscopy; PSRP, plastid-specific ribosomal protein; LC-MS/MS, liquid chromatography/tandem mass spectrometry; ES, expansion segment; PRP, plastid ribosomal protein; EST, Expressed Sequence Tag.

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**Figure 1.** *C. reinhardtii* 80 S ribosomal proteins resolved using (a) SDS-PAGE and (b) 2D-PAGE. (a) Total proteins (TP80) extracted from 10 pmol of 80 S ribosomes were resolved and stained with CBB R-350. The gel was sliced into 18 pieces for in-gel enzymatic digestion and LC-MS/MS analysis, as indicated by the dotted lines. MK indicates a protein ladder. (b) TP80 (100 pmol) was resolved and stained with CBB R-350. First dimension (horizontal): urea-disc gel electrophoresis with 4% (w/v) polyacrylamide gel containing 8 M urea, pH 5.0; left, anode; right, cathode. Second dimension (vertical): SDS-PAGE with 10% (w/v) polyacrylamide gel containing 0.2% (w/v) SDS, pH 6.7; top, cathode; bottom, anode. Protein spots excised for N-terminal sequencing are indicated by dotted circles and numbered 1–14. Three spots (numbered 5, 6, and 12) corresponding to previously reported 80 S-specific proteins<sup>9</sup> are indicated by asterisks (see Discussion).

modifications specific to photosynthetic eukaryotes, a situation analogous to that found when chloroplast and bacterial ribosomes are compared. Chloroplast ribosomes from both algae and higher plants contain homologs of all of the bacterial 70 S ribosomal proteins, and they have a number of plastid-specific ribosomal proteins (PSRPs).<sup>7–9</sup> These PSRPs make up a substantial portion of the chloroplast ribosome and have been hypothesized to be involved in unique aspects of translation control in the organelle, including coordination with nuclear gene expression and light-activated translation.<sup>10</sup>

Recent studies have reported proteomic analyses of 80 S cytosolic ribosomes from *Arabidopsis thaliana*.<sup>11,12</sup> These studies have identified homologs for a majority of the proteins found in other 80 S ribosomes, the exceptions being confined to very small and/or basic proteins that are difficult to identify *via* proteomics. These studies also found a high degree of ribosomal protein heterogeneity, one study indicating that as many as 45% of the ribosomal proteins identified were present in 2D-PAGE as more than one spot.<sup>12</sup> One non-ribosomal protein that both of these studies were able to identify associated with the 80 S ribosome preparations was a homolog of RACK1, which is a G protein  $\beta$  subunit homolog consisting of seven WD40 repeats predicted to form a seven-bladed propeller structure, similar to that of  $G_{\beta}$ .<sup>13</sup> A wide range of functions have been ascribed to RACK1,<sup>13</sup>

and a large number of proteins that interact with RACK1 have been identified, although it is unclear how protein interactions with RACK1 affect change in the cell. Recently, it has been recognized that RACK1 associates with 80 S ribosomes and is likely best considered the 33rd protein of the 40 S subunit of cytosolic ribosomes.<sup>14</sup> RACK1 is involved in regulation of ribosome function,<sup>14,15</sup> and the location of this protein on the three-dimensional model of the 80 S ribosome has been identified,<sup>16</sup> in what was previously referred to as the “head lobe”.<sup>17</sup> Sengupta *et al.* were able to fit a  $G_{\beta}$  crystal structure to the difference density, yielding a model for the positioning of RACK1 on the 40 S subunit.<sup>16</sup> The knob of density assigned to this protein is present on all 80 S ribosome maps reported in the literature,<sup>16–20</sup> lending further support to the claim of RACK1 as the 33rd ribosomal protein of the small subunit.

Here, we present an in-depth analysis of the cytosolic 80 S ribosome from the photosynthetic green algae *C. reinhardtii*. We have isolated cytosolic ribosomes from this unicellular photosynthetic eukaryote and analyzed the complete proteome of the ribosome using highly sensitive mass-spectrometry technologies. Database mining of the newly completed *C. reinhardtii* nuclear genome sequence and annotation accompanied and complemented this proteomic analysis to identify a complete set of genes encoding homologs of all of the 80 S ribosomal proteins found in yeast and mammals.

As in other eukaryotes, a RACK1 homolog has been identified as an integral part of the algal 80 S ribosome. Single-particle analysis of cryo-EM images was used to calculate a 25 Å resolution three-dimensional structure of the *C. reinhardtii* 80 S ribosome. Our findings show that, though separated by billions of years of evolution, the cytosolic ribosomes from photosynthetic organisms are very similar in composition and structure to their mammalian and yeast counterparts.

## Results

### Protein identification

Cytoplasmic (80 S) ribosomes were isolated from wild-type, cell wall-less *C. reinhardtii* cells (strain cc3395) using multi-step sucrose gradient ultracentrifugation (see Materials and Methods). Proteins from isolated ribosomes were separated using SDS-PAGE, the gels sliced into 18 sections

(Figure 1(a)), and each gel section digested using trypsin or endoproteinase Lys-C. The peptide fragments that were generated were subjected to liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis (described in Materials and Methods). The SEQUEST algorithm using *C. reinhardtii* Expressed Sequence Tag (EST) and Swiss Prot databases was used to identify corresponding peptide sequences (each peptide identified and assigned can be found in Supplementary Table 1). In this study, we designate *C. reinhardtii* cytosolic ribosomal proteins using the rat nomenclature system<sup>1</sup> instead of the designation employed by Fleming *et al.* (i.e. cyS1-cyS45 for small subunit proteins and cyL1-cyL48 for large subunit proteins, numbers given in order of decreasing molecular mass<sup>4</sup>), since all of the proteins identified in this study are rat orthologs (as described below) and the rat nomenclature has been used for yeast and for *Arabidopsis* 80 S proteins.<sup>21,22</sup>

Homologs of 75 of the 80 rat 80 S ribosomal

**Table 1.** *C. reinhardtii* 40 S ribosomal protein genes

	Gene Accession no. <sup>a</sup>	Transcript ACE no. <sup>b</sup>	Protein		SDS-PAGE slice no.	2D-PAGE spot no.
			Calculated pI/MW	Observed MW		
<i>40 S proteins</i>						
Sa	C_130042	20021010.1783.1	5.07/30810.0	28,700–35,600	5,6	
S2	C_250114	20021010.698.1	10.20/30291.09	26,500–32,200	6,7	
S3	C_20102	20021010.6021.1	9.66/25848.20	24,000–28,700	7,8	
S3a	C_930034 <sup>c</sup>	20021010.7986.1	9.82/29331.19	26,500–32,200	6,7	
S4	RPSc4 <sup>c</sup>	20021010.2065.1	9.98/29483.62	26,500–32,200	6,7	
S5	C_290058	20021010.448.1	9.73/21638.10	15,000–22,200	10,12,13	
S6	C_220138 <sup>c</sup>	20021010.419.1	10.45/28435.18	28,700–32,200	6	
S7	C_1010036 <sup>c</sup>	20021010.4958.1	9.69/22114.44	18,300–46,800	2,10,11	
S8	C_270086	20021010.8174.1	10.43/23895.69	22,200–28,700	7,8,9	
S9	C_2010001	20021010.8536.1	10.44/22130.75	16,500–20,100	11,12	
S10	C_520039	20021010.1264.2	9.99/19307.89	10,100–16,500	13,14,15,17,18	4
S11	C_160238 <sup>c</sup>	20021010.7565.2	10.47/17781.66	15,000–16,500	13	
S12	C_2320001 <sup>c</sup>	20021010.653.1	4.83/15070.3	12,300–13,600	15	
S13	C_1050007 <sup>c</sup>	20021010.6274.1	10.51/17119.2	13,600–16,500	13,14	
S14	C_480013	20021010.3961.1	10.31/16301.71	11,200–15,000	14,15,16	
S15	C_3470003	20021010.2986.1	10.56/16629.49	12,300–15,000	14,15	
S15a	C_1490015 <sup>d</sup>	20021010.8662.1	10.05/14838.48	11,200–13,600	15,16	
S16		20021010.1283.1	10.47/15977.82	11,200–13,600	15,16	
S17	C_1010002 <sup>c</sup>	20021010.278.1	10.20/15900.45	11,200–15,000	14,15,16	
S18	C_270185 <sup>c</sup>	20021010.5056.2	10.60/17579.40	13,600–15,000	14	
S19	C_20042	20021010.5820.1	9.96/16997.68	12,300–13,600	15	
S20	C_60064	20021010.2202.1	9.88/13339.68	12,300–15,000	14,15	
S21	C_120069 <sup>c</sup>	20021010.2178.1	5.81/9104.2	N.A.		
S23	C_370016 <sup>c</sup>	20021010.9079.1	10.35/15676.39	12,300–15,000	14,15	
S24	C_1480038	20021010.9181.1	10.63/15121.75	13,600–15,000	14	
S25	C_1680017	20021010.8122.1	10.38/11817.98	10,100–11,200	17,18	
S26	C_560007	20021010.6938.1	11.23/13216.28	12,300–13,600	15	
S27A	C_380105	20021010.5393.1	9.10/9515.01	<10,100	18	
S27B	C_550074 <sup>c</sup>	20021010.7482.1	9.50/9595.24	<10,100	18	
S27a	C_270027	20021010.4311.1	9.86/9404.0	12,300–22,200	10,14,15	11
S28	C_490089 <sup>c</sup>	20021010.8417.1	11.16/7402.72	<10,100	18	
S29		20031215.160.2.	9.69/6492.42	<10,100	18	
		2.11				
S30		20021010.4106.1	12.31/6972.3	<10,100	18	
RACK1	C_430028 <sup>c</sup>	20021010.6103.1	7.59/35144.81	26,500–32,200	6,7	

<sup>a</sup> Applies to JGI database ver2.0 (<http://genome.jgi-psf.org/chlre2/chlre2.home.html>).

<sup>b</sup> Applies to Chlamy Center EST database (<http://www.chlamy.org>).

<sup>c</sup> Gene information under this accession number is incorrect in JGI ver2.0, but has been manually corrected and will show up correctly in ver3.0.

<sup>d</sup> Gene was not annotated in ver2.0, but gene is present and has been annotated for ver3.0.

**Table 2.** *C. reinhardtii* 60 S ribosomal protein genes

	Gene accession no. <sup>a</sup>	Transcript ACE no. <sup>b</sup>	Protein		SDS-PAGE slice no.	2D-PAGE spot no.
			Calculated pI/MW	Observed MW		
<i>60 S proteins</i>						
P0	c	20021010.934.3	6.07/34574.93	26,500–34,700	5,6,7	1
P1	C_1070009	20020630.6971.1	4.40/10875.23	10,100–11,200	17,18	
P2	C_1210002	20021010.2262.1	4.89/10879.30	10,100–12,300	16,17,18	2
L3	C_660005 <sup>d</sup>	20021010.1449.1	10.22/43508.8	43,300–46,800	2	
L4	C_2390008 <sup>d</sup>	20021010.9442.1	10.44/44910.56	46,800–49,600	1	
L5	C_240141 <sup>d</sup>	20021010.728.2	8.96/33672.51	32,200–35,600	5	
L6	C_280126	20021010.1844.1	10.32/24371.60	20,100–24,000	9,10	
L7	C_960038 <sup>d</sup>	20021010.2033.1	10.23/27575.55	24,000–26,500	8	
L7a	C_380026 <sup>d</sup>	20021010.7313.1	10.33/29194.60	20,100–32,200	6,7,10	
L8	C_910064 <sup>d</sup>	20021010.8836.2	10.73/27969.13	28,700–32,200	6	
L9	C_3830001	20021010.2452.1	9.90/21518.31	18,300–22,200	10,11	
L10	C_700007 <sup>d</sup>	20021010.8020.1	10.32/24183.0	22,200–28,700	7,8,9	
L10a	C_20112	20021010.8340.1	9.94/23944.48	24,000–26,500	8	
L11	C_410087 <sup>d</sup>	20021010.9108.1	10.00/20474.69	15,000–16,500	13	
L12	C_380016	20021010.3624.1	9.19/17604.60	15,000–16,500	13	
L13	C_3320003	20021010.2992.2	10.89/24483.68	12,300–28,700	7,8,9,10,14,15	
L13a	C_380137 <sup>d</sup>	20021010.8414.1	11.30/20727.65	16,500–20,100	11,12	
L14	C_870056	20021010.4027.1	10.34/15341.12	11,200–16,500	13,15,16	
L15	C_680018 <sup>d</sup>	20021010.7238.1	11.36/24162.20	22,200–24,000	9	
L17	C_930005 <sup>d</sup>	20021010.3.1	10.33/21145.35	16,500–20,100	11,12	
L18	C_490056 <sup>d</sup>	20021010.505.3	11.17/20929.91	18,300–22,200	10,11	
L18a	C_180091 <sup>d,e</sup>	20021010.3843.1	10.41/21371.7	16,500–16,500	11,12,13	
L19	C_50050 <sup>d</sup>	20021010.6392.1	11.28/23427.64	22,200–39,200	4,9	
L21	C_1030044	20021010.9424.1	10.78/18317.57	13,600–16,500	13,14	
L22	C_2370012 <sup>d</sup>	20021010.2212.1	9.54/14460.87	11,200–13,600	15,16	
L23	C_1790006 <sup>d</sup>	20021010.4834.1	10.20/15018.7	12,300–13,600	15	
L23a	C_3670002 <sup>d</sup>	20021010.2305.1	10.14/16399.35	12,300–15,000	14,15	
L24	RPL26 <sup>d</sup>	20021010.2072.1	11.01/17035.99	15,000–18,300	12,13	
L26	C_250105	20021010.4511.2	10.59/16473.2	15,000–16,500	13	
L27	C_170104 <sup>d</sup>	20021010.8598.2	10.38/15265.14	12,300–13,600	15	
L27a	C_20131 <sup>d</sup>	20021010.8882.1	10.43/16391.07	13,600–15,000	14	
L28	C_1610010 <sup>d</sup>	20021010.3034.1	10.42/13502.70	10,100–13,600	15,16,17,18	
L29	C_120106	20020630.3764.2	11.49/6497.55	N.A.		
L30	C_260095 <sup>d</sup>	20021010.111.1	9.58/12001.05	10,100–12,300	16,17,18	
L31	C_630066	20021010.8247.2	10.08/13254.35	13,600–15,000	14	
L32	C_290117	20021010.9561.3	11.18/15480.15	12,300–15,000	14,15	
L34	C_60053	20021010.2166.1	10.78/13576.19	12,300–13,600	15	
L35	C_240109 <sup>d</sup>	20021010.8729.1	11.35/14863.9	13,600–18,300	12,14	
L35a	C_800006 <sup>d</sup>	20021010.3469.1	10.65/12058.26	10,100–12,300	16,17	7,8
L36	C_1150032	20021010.6629.2	11.66/11126.52	10,100–12,300	16,17,18	12
L36a	C_90011	20021010.2082.1	10.31/11420.74	N.A.	13	
L37	C_130075	20021010.8356.1	11.41/10585.27	12,300–13,600	15	14
L37a	C_200204 <sup>d</sup>	20021010.3375.1	10.46/10277.1	<10,100	18	10
L38	C_1120050 <sup>d</sup>	20021010.1420.1	9.90/7817.25	<10,100	18	6
L39	C_220133	20021010.7102.1	12.24/6315.50	N.A.		
L40	C_760061 <sup>d</sup>	20021010.5520.1	10.32/6181.4	<10,100	18	
L41	d	20021010.2262.1	12.96/3337.15	N.A.		

<sup>a</sup> Applies to JGI database ver2.0 (<http://genome.jgi-psf.org/chlre2/chlre2.home.html>).

<sup>b</sup> Applies to Chlamy Center EST database (<http://www.chlamy.org>).

<sup>c</sup> Gene was not annotated in ver2.0, but gene is present and has been annotated for ver3.0.

<sup>d</sup> Gene information under this accession number is incorrect in JGI ver2.0, but has been manually corrected and will show up correctly in ver3.0.

<sup>e</sup> It is unclear whether the first two amino acids are encoded, or should be considered part of the 5' untranslated region.

proteins were identified by sequences of either tryptic or Lys-C peptides generated from *C. reinhardtii* cytosolic ribosomes. Supplementary Figures 1 and 2 indicate the peptides identified for each protein in the small and large ribosomal subunits, respectively. The observed molecular mass of proteins (from SDS-PAGE, Figure 1(a)) agreed reasonably with molecular mass calculated from reliable EST-contigs (Tables 1 and 2). Fifteen plastid ribosomal proteins (PRPs), S3, S7, S9, S12, S18, L1, L3, L4, L6, L9, L10, L12, L15, L18, and L31,

were also identified from our 80 S ribosome preparation (see Supplementary Table for peptides). These proteins indicate contamination of 80 S cytosolic ribosome preparation with some PRPs; a smaller number of peptides from each protein were detected as compared with our previous plastid ribosome proteomics, and identified ESTs are exactly as assigned to PRP genes.<sup>7,23</sup> Three Lys-C peptides belonging to non-ribosomal proteins (such as nuclear DNA-binding protein G2p-related protein, putative uridylyltransferase,

and putative RNA helicase) were also detected from 80 S ribosome fractions (listed in Supplementary Table 1). In each case, only a single peptide fragment was detected and assigned to an EST or gene, whereas 80 S ribosomal proteins were identified by a number of fragments (except a few small ribosomal proteins, which generated single detectable peptide ions). We therefore believe these are trace contaminants or may be substoichiometric ribosome-associating proteins.

Five rat orthologs, S21, L29, L36a, L39 and L41, were not identified by LC-MS/MS analyses. The theoretical molecular mass and isoelectric point of these missing proteins show that they are very small proteins and most are highly basic. Small and basic proteins sometimes escape mass spectrometric detection ( $m/z$  400–1400) because digestion by trypsin or Lys-C generates peptides too small to be identified using this method. Acidic proteins usually have less cleavage sites for trypsin or Lys-C digestion, and may escape identification. We therefore directly analyzed N-terminal sequences of 14 protein spots cut from a polyvinylidene fluoride (PVDF) membrane blot of 80 S proteins separated on 2D gels using Edman degradation without enzymatic digestion. Three acidic proteins (spots 1–3) and 11 basic and/or small proteins (spots 4–14) were sequenced (Figure 1(b)). Spots 1, 2, 4, 6, 7/8, 10, 11, 12, 13, and 14 gave clear N-terminal sequences corresponding to P0, P2, S10, L38, L35a, L37a, S27a, L36, L36a, and L37, respectively (and see Supplementary Figures 1 and 2). Spots 3, 4, and 9 did not allow Edman degradation, so these proteins are likely post-translationally modified and N-terminally blocked. A total of 76 rat orthologs were thus identified by LC-MS/MS analyses or Edman sequencing. Contrary to what had been predicted, no protein novel to the *C. reinhardtii* 80 S ribosome was identified (see Discussion). The four missing proteins not identified directly by proteomic analyses (S21, L29, L39, and L41) could be identified unambiguously from both EST and genomic databases, and are thus most likely protein components of the *C. reinhardtii* cytosolic ribosome. Full-length coding regions for each rat ribosomal protein ortholog were determined through the use of EST assemblies, and have been deposited with the *C. reinhardtii* genome database† (corrected annotations will not be viewable until release 3.0 of the database).

In most cases, peptide sequences obtained by LC-MS/MS or Edman degradation could be used to assign a single transcript to each 80 S protein. However, S27 was identified in two forms (designated S27A and S27B) that appear to be transcribed from two distinct genes. Pairwise comparison of amino acid sequences of S27A and S27B indicates that they share only 72% identity and 78% similarity, and identification of the two distinct forms in our 80 S preparation suggests ribosome

heterogeneity. Ribosome heterogeneity is supported also by the presence of many protein isoforms due to post-translational modification and/or processing. L35a was identified from two different spots (spots 7 and 8 in Figure 1(b)), therefore L35a is present in at least two forms with different isoelectric points. The tripeptide N-terminal extension (GKK-) of L37 is unique to *C. reinhardtii* compared with other L37 proteins (see Supplementary Figure 2), and Edman degradation of its N terminus (GXK-) indicated that the second Lys residue is modified by an uncharacterized group. As seen in Supplementary Table 1, S5, S7, S8, S10, S14, S17, S27a, P0, P2, L7a, L10, L10a, L13, L14, L18a, L19, L28, L30, L35, and L36 were identified from three or more separate gel slices, indicating that these proteins may be present in multiple isoforms or as multimers upon gel electrophoresis.

### The 80 S ribosomal proteins

All of the rat small subunit ribosomal protein orthologs, with the exception of S21, were identified through proteomic analyses (as discussed above). Putative genes encoding all 32 of the small subunit ribosomal proteins found in rat were identified in the completed genome sequence of *C. reinhardtii* and EST databases. Also identified on the small subunit of the ribosome was the *C. reinhardtii* RACK1 homolog. Of these 33 proteins, 24 show remarkable similarity to their yeast and human counterparts, averaging 55% identity of amino acid sequence among the three species. Of the other nine proteins, two share homology with yeast and are somewhat different from human, three have better homology with their human counterpart than with yeast, and four differ equally from both their yeast and human homologs. Table 3 indicates the percentage similarity that each small subunit protein shares with its orthologs from rat, yeast, and *Arabidopsis thaliana*.

All of the large subunit ribosomal proteins, with the exceptions of L29, L39 and L41 (as discussed above), were identified through our proteomic analyses. Putative genes encoding all 47 of the large subunit ribosomal proteins found in rat, including those not identified *via* proteomics, were found in the completed genome sequence of *C. reinhardtii*. Table 4 lists the percentage similarity that each large subunit protein shares with its orthologs from other species. 37 of these 47 proteins share a remarkable degree of identity with their yeast and human counterparts. Of the remaining ten, three share homology with yeast and differ from human, two are similar to human and different from yeast, and the five remaining proteins differ equally from both their yeast and mammalian homologs. It should be noted that L28, found in mammalian ribosomes but lacking in yeast, is present in the *C. reinhardtii* ribosome. Fleming *et al.* reported three low molecular mass proteins (corresponding to spots 5, 6, and 12 in Figure 1(b)) as missing from 40 S and 60 S ribosomal subunits

† <http://genome.jgi-psf.org/chlre2/chlre2.home.html>

**Table 3.** Similarity of *C. reinhardtii* 40 S ribosomal proteins to higher and lower eukaryotes, and higher plants

Protein	% Similarity		
	Rat <sup>a</sup>	Yeast	<i>Arabidopsis</i> <sup>b</sup>
<b>Sa</b>	78	75	77
<b>S2</b>	88	73	86
<b>S3</b>	88	81	93
<b>S3a</b>	77	78	90
<b>S4</b>	82	79	76
<b>S5</b>	90	78	93
<b>S6</b>	73	70	69
<b>S7</b>	77	67	83
<b>S8</b>	76	64	85
<b>S9</b>	88	83	92
<b>S10</b>	80	71	84
<b>S11</b>	77	74	75
<b>S12</b>	75	58	77
<b>S13</b>	83	82	88
<b>S14</b>	92	71	84
<b>S15</b>	66	74	79
<b>S15a</b>	88	85	92
<b>S16</b>	89	84	91
<b>S17</b>	81	80	80
<b>S18</b>	84	82	81
<b>S19</b>	68	68	80
<b>S20</b>	91	79	91
<b>S21</b>	67	68	80
<b>S23</b>	88	86	92
<b>S24</b>	66	77	88
<b>S25</b>	83	74	77
<b>S26</b>	77	75	74
<b>S27</b>	76	79	81
<b>S27a</b>	75	71	86
<b>S28</b>	86	86	85
<b>S29</b>	82	82	83
<b>S30</b>	65	59	77
<b>RACK1</b>	83	66	81
Average % similarity	80.0	75.1	83.3

Boldface indicates proteins identified through proteomic techniques and supported by EST evidence, italics indicate proteins for which only EST evidence exists.

<sup>a</sup> Equivalent to human as similarity among mammals is nearly 100%.

<sup>b</sup> *A. thaliana* proteins as predicted;<sup>12</sup> where possible the A isoform was used for comparison.

but found on the assembled 80 S ribosome.<sup>4</sup> Our results indicate that spots 6 and 12 are homologs of rat L38 and L36, respectively, and that at least these two proteins are part of the large ribosomal subunit.

As a whole, small differences are seen in proteins predicted to come both early and late in ribosome assembly,<sup>24</sup> in proteins with and without homologs in bacteria,<sup>1,25,26</sup> and in proteins with potential to contact RNAs, initiation factors,<sup>25,27</sup> elongation factors,<sup>24</sup> and the emerging nascent polypeptide chain.<sup>20,24</sup> Differences between 80 S proteins in algae and other species are not limited to one or the other of the subunits, nor have they been confined to proteins interacting with specific domains of the rRNAs. The unremarkable nature of this collection of differences in ribosomal proteins between *C. reinhardtii* and its higher and lower eukaryotic neighbors indicates that, though separated by billions of years of evolution, the composition of 80 S ribosomes is so similar in algae, yeast,

**Table 4.** Similarity of *C. reinhardtii* 60 S ribosomal proteins to higher and lower eukaryotes, and higher plants

Protein	% Similarity		
	Rat <sup>a</sup>	Yeast	<i>Arabidopsis</i> <sup>b</sup>
<b>P0</b>	59	57	57
<b>P1</b>	70	44	58
<b>P2</b>	80	65	51
<b>(P3)</b>			
<b>L3</b>	77	73	83
<b>L4</b>	68	65	73
<b>L5</b>	69	66	69
<b>L6</b>	66	62	61
<b>L7</b>	67	66	75
<b>L7a</b>	75	67	71
<b>L8</b>	81	78	85
<b>L9</b>	69	72	74
<b>L10</b>	76	70	83
<b>L10a</b>	71	68	74
<b>L11</b>	83	78	82
<b>L12</b>	82	77	87
<b>L13</b>	53	57	70
<b>L13a</b>	79	74	80
<b>L14</b>	62	39	71
<b>L15</b>	72	72	81
<b>L17</b>	79	68	77
<b>L18</b>	78	70	84
<b>L18a</b>	66	70	78
<b>L19</b>	73	71	81
<b>L21</b>	62	66	79
<b>L22</b>	62	47	62
<b>L23</b>	89	87	92
<b>L23a</b>	79	70	71
<b>L24</b>	65	62	80
<b>L26</b>	70	81	73
<b>L27</b>	65	66	69
<b>L27a</b>	69	76	50
<b>L28</b>	55	N/A	56
<b>L29</b>	86	83	84
<b>L30</b>	87	83	86
<b>L31</b>	68	73	64
<b>L32</b>	74	63	79
<b>L34</b>	55	61	63
<b>L35</b>	65	54	62
<b>L35a</b>	69	71	82
<b>L36</b>	75	67	84
<b>L36a</b>	82	85	83
<b>L37</b>	75	80	84
<b>L37a</b>	85	61	90
<b>L38</b>	63	56	87
<b>L39</b>	85	85	92
<b>L40</b>	89	87	91
<b>L41</b>	100	100	92
Average % similarity	73.0	69.4	75.7

Boldface indicates proteins identified through proteomic techniques and supported by EST evidence, italics indicate proteins for which only EST evidence exists. Parentheses indicate proteins not identified.

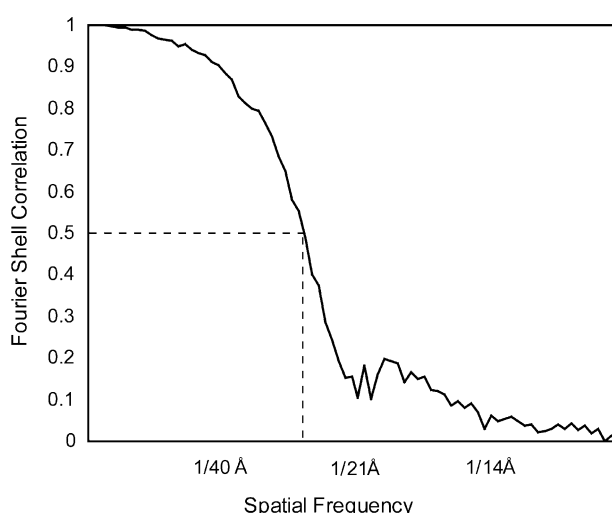
<sup>a</sup> Equivalent to human as similarity among mammals is nearly 100%.

<sup>b</sup> *A. thaliana* proteins as predicted;<sup>12</sup> where possible the A isoform was used for comparison.

and mammals that they probably also share similarities in assembly and functions attributed to their protein components.

### Acidic stalk proteins

Eukaryotic ribosomes have three proteins (P0, P1



**Figure 2.** Fourier shell correlation curve of three-dimensional reconstruction. The Fourier shell correlation 0.5 cut-off criterion was used to report the resolution of the map here.

and P2) identified as acidic stalk proteins, based on their unique (among the large subunit proteins) acidic pI values, and their assembly into a stalk structure, extending out from the body of the ribosome. P1 and P2 proteins are the only proteins known to exist on 80 S ribosomes in greater than stoichiometric amounts, each being present as a dimer that binds to P0. In higher plants, both monocotyledons and dicotyledons, an additional acidic stalk protein, P3, has been identified that shares some homology with both P1 and P2.<sup>28</sup> The stoichiometry of P1 and P2 proteins in ribosomes containing P3 is unknown. The *C. reinhardtii* 80 S ribosome contains P0, P1, and P2, but lacks the P3 protein and gene, confirming that P3 is found only in higher plants, and not in all photosynthetic organisms.

### rRNAs

Sequences of rRNA genes for the small ribosomal subunit 18 S rRNA (GenBank accession M32703), and both of the small rRNA components of the large ribosomal subunit (5 S, GenBank accession X02706, X02707; and 5.8 S GenBank accession X02708) are available. A complete sequence of the 25 S rRNA is not available from either GenBank or the JGI *C. reinhardtii* nuclear genome sequence database. However, a partial 25 S sequence is available from GenBank (accession AF183463) and two scaffolds with a high level of sequence similarity to yeast 25 S rRNA (scaffold\_1364, and scaffold\_305) could be identified from version 2.0 of the JGI database†.

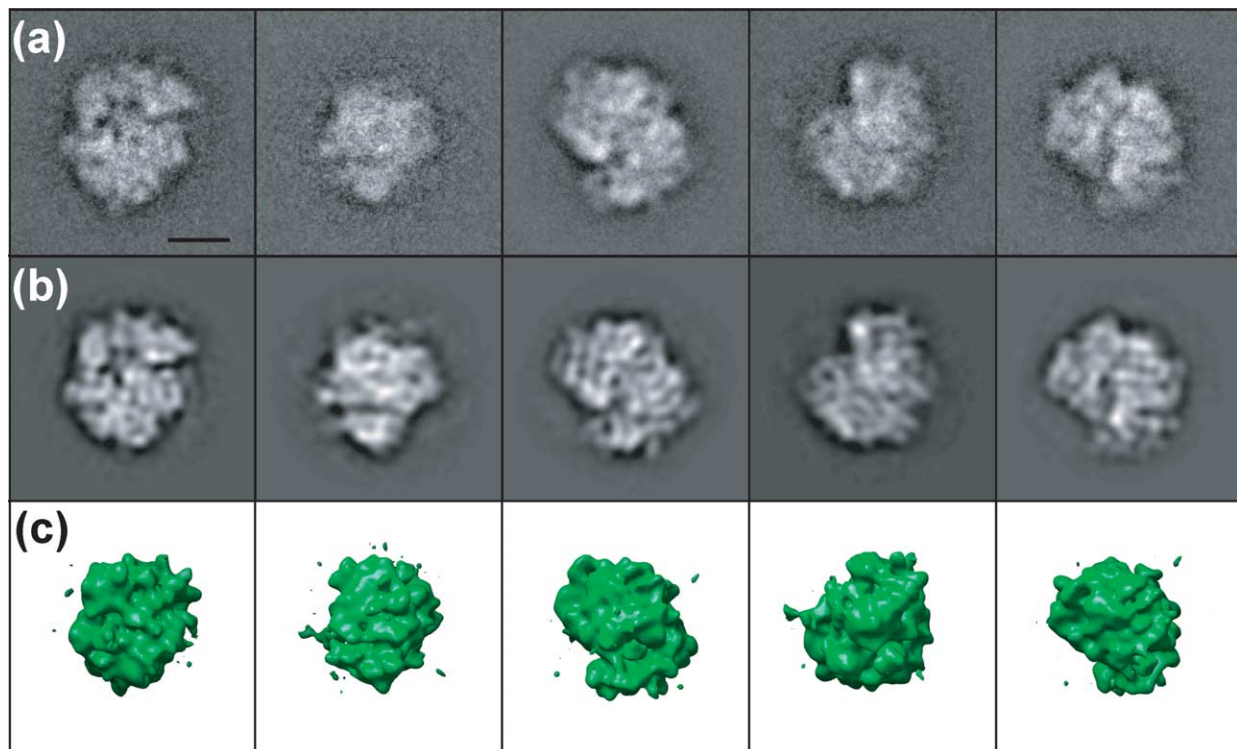
Alignment and assembly of these three pieces of genome sequence aligns to >98% of the yeast 25 S sequence. When these two sequences are aligned with rat and human 28 S rRNA sequences, it is clear that, excepting only the very ends of the *C. reinhardtii* 25 S rRNA where the sequence is incomplete, none of the expansion segments seen in rat or human is present in *C. reinhardtii* 25 S rRNA. This is consistent with alignment results of large subunit rRNAs from higher plants, like *A. thaliana*, which also lack expansion segments found in mammals.

### Structure determination

Single-particle analysis of cryo-EM images was used to calculate a three-dimensional map of the 80 S cytosolic ribosome from *C. reinhardtii* (see Materials and Methods). The Fourier shell correlation curve used to determine the final resolution of the map is shown in Figure 2. A 0.5 cutoff criterion was used, indicating a final resolution of 25 Å. Representative average images of particles in a number of orientations, along with views of the final map in these orientations are provided in Figure 3. The final map is compared to the yeast ribosome in Figure 4.<sup>29</sup> The structure of the 80 S cytosolic ribosome from *C. reinhardtii* is very similar to that of the yeast 80 S ribosome. These structural similarities extend to both the small and large subunits, and to all parts of the ribosome observed from any orientation, with only a few details to set the two structures apart at this resolution. With only minor differences in protein or rRNA composition between the yeast and *C. reinhardtii* ribosomes, it was not anticipated that large visible differences would be observed in a cryo-EM reconstruction at moderate resolution.

One visible difference between the *C. reinhardtii* and yeast ribosomes is the lack of an extended expansion segment (ES) 27 on the *C. reinhardtii* ribosome surface. This helical segment of rRNA has been identified from the comparison of high-resolution maps of both the 80 S and 70 S ribosomes separated computationally into RNA and protein components.<sup>3</sup> This RNA helix normally occupies one of two regions on the surface of the ribosome, and in the yeast map can actually be seen extended in both conformations (Figure 4(b)). ES27 is necessary for ribosome function,<sup>30</sup> and shows movement from one preferred conformation to another upon binding of the Sec61 channel complex.<sup>31</sup> The *C. reinhardtii* ribosome map lacks extended density in either of these regions, indicating that this portion of 25 S rRNA does not have a stable, preferred conformation in *C. reinhardtii* under the buffering conditions used. There is a small knob of density in the area where this helix would connect to the main body of the ribosome, which implies that the helix is extended from the body of the ribosome, but is likely too flexible to be resolved in this structure. MFold predictions of secondary structure in this region of the

† <http://genome.jgi-psf.org/chlre2/chlre2.info.html>



**Figure 3.** Structure of the *C. reinhardtii* 80 S ribosome. (a) Average images of ice particles representing five different orientations of the ribosome. (b) Projections from the starting model for refinement matching the five particle orientations. (c) Structure of the *C. reinhardtii* 80 S ribosome oriented at the same angle as the particle averages. The scale bar represents 100 Å.

*C. reinhardtii* rRNA indicate a helical arrangement similar to that in the yeast ES27 (not shown).<sup>32</sup>

There are a number of additional small differences visible on the surface of the ribosome; most having to do with the shift in position of finger-like protrusions of density, and all of these occur in areas of the ribosome that have been defined as eukaryotic ribosome-specific protein density.<sup>3</sup> Since it is not clear which proteins are involved in formation of these structures, it is unknown what effect a change in orientation of the structures might have on ribosome function. It seems likely that the differences between the *C. reinhardtii* ribosome and the yeast ribosome structures represent the flexibility of proteins bound on the surface of the ribosome. None of these appears to be in areas that are important for contact with any ribosome interacting factors.

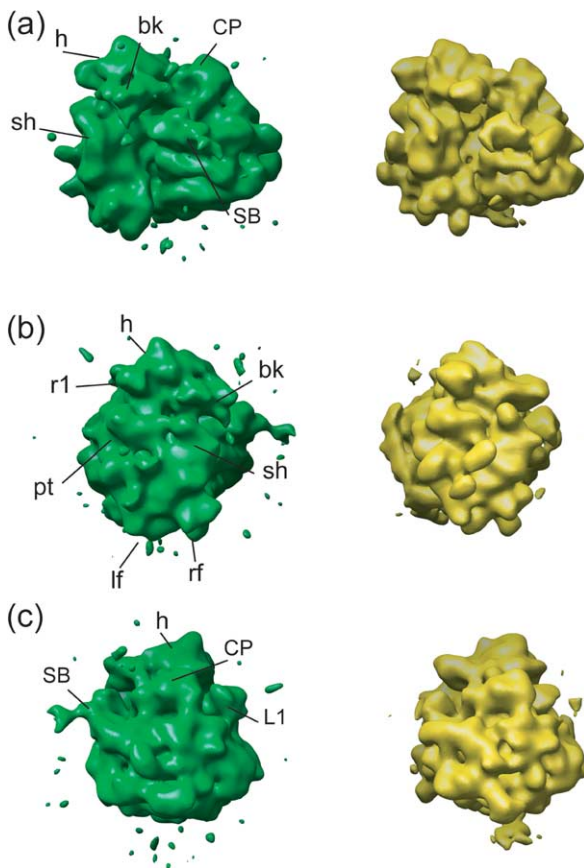
## Discussion

This is the first determination of the proteome and three-dimensional structure of an 80 S ribosome from a photosynthetic organism. The *C. reinhardtii* 80 S ribosome is well-conserved with other 80 S ribosomes, from human to yeast to higher plants, in both composition and structure. Predicted proteins from the *A. thaliana* ribosome share the highest level of homology (average 67% amino acid identity) with *C. reinhardtii* (Tables 1 and 2). Algal

and yeast proteins share a smaller percentage homology than do algal and mammalian homologs. The small differences observed between ribosomal proteins of algae and other species are present in both subunits, and in proteins that interact with rRNAs, mRNA and translation factors. The dispersal of these small differences over the entire ribosome suggests that the function of these proteins in the context of the ribosome is maintained, and that the 80 S cytoplasmic ribosome of algae is essentially identical in composition with that of the 80 S ribosomes of mammals and yeast.

As evidenced in Figures 4 and 5, the overall structure of the algal and yeast ribosomes is highly conserved. The yeast ribosome structure has been shown to be similar to those from rat<sup>17</sup> and rabbit<sup>18</sup> (with the main differences belonging to rRNA ESs), and shares a degree of structural similarity with bacterial ribosomes.<sup>3</sup> The small differences in structure between the *C. reinhardtii* and yeast 80 S ribosomes can be attributed to minor protein differences and to small conformational differences between the two ribosomes (see Results).

The remarkable similarity of the 80 S ribosome to its ancestral neighbors is in marked contrast to that of the chloroplast ribosome from *C. reinhardtii*. Using a similar proteomic and bioinformatics approach, we have recently identified all of the protein components of the chloroplast 70 S-like ribosome.<sup>23</sup> While homologs for most of the bacterial 70 S ribosomal proteins were identified,



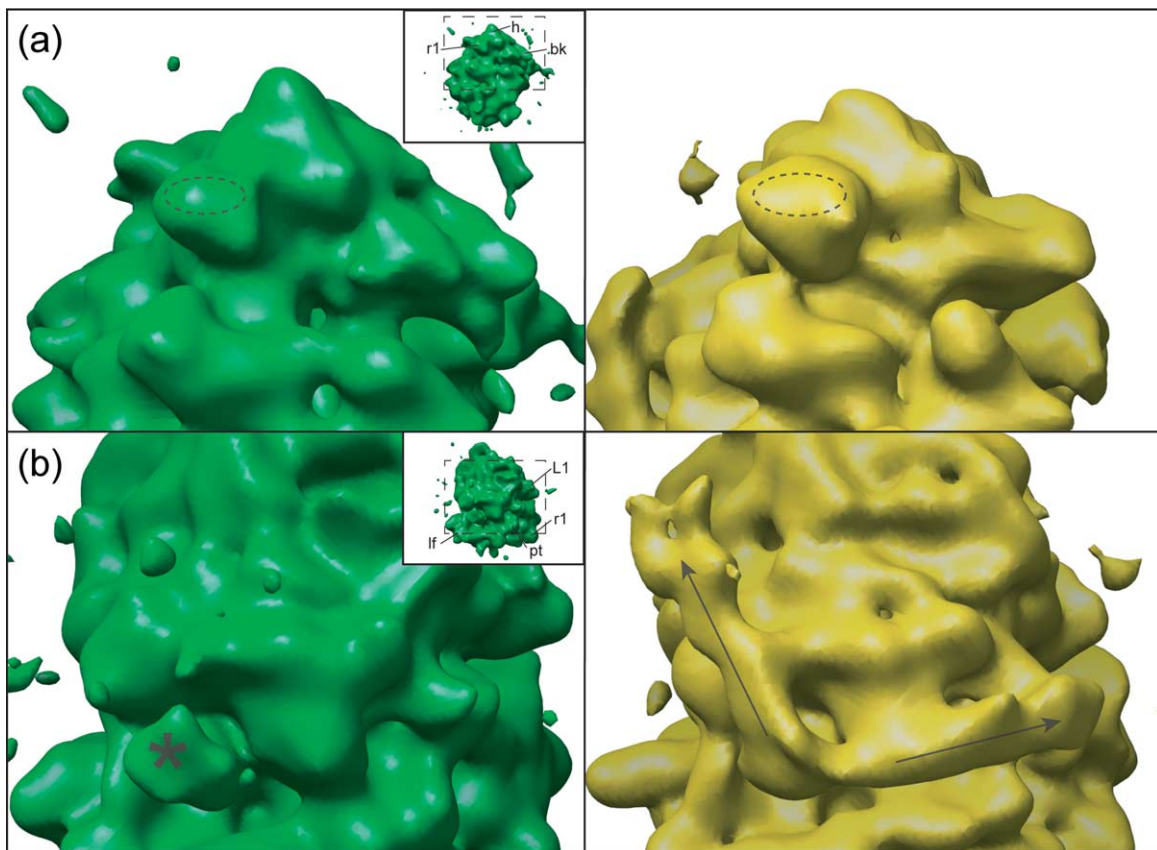
**Figure 4.** Comparison of *C. reinhardtii* and *S. cerevisiae*<sup>29</sup> ribosomes. (a) Side view of ribosome, with the 40 S subunit on the left and the 60 S subunit on the right. (b) Views from the solvent side of the 40 S subunit, and (c) from the solvent side of the 60 S subunit. Algal ribosome is shown in green, yeast in gold. Indicated landmarks: bk, beak; h, head; lf, left foot; pt, platform; r1, RACK1; rf, right foot; sh, shoulder; CP, central protuberance; L1, L1 protuberance; SB, stalk base.

seven additional proteins were found, only four of which have homologs identified in chloroplast ribosomes of higher plants.<sup>8,9</sup> In addition, three of the identified bacterial protein homologs have large additional chloroplast-unique domains; these additional proteins and domains in total add approximately 10% mass to the chloroplast ribosome as compared with a bacterial 70 S ribosome. In algae and higher plants, chloroplast proteins are encoded by both the nuclear and chloroplast genomes, and many function only in large complexes like the photosystems and the chloroplast ribosome. This requires that gene expression from nuclear and chloroplast genomes be tightly coupled. One might assume that the constraints of coordinated gene expression would impact the functionality, hence composition and structure, of both the cytosolic and chloroplast ribosomes in a similar manner. While the chloroplast ribosome has diverged significantly from its prokaryotic origins and among various photosynthetic organisms, the

data presented here show that in plants the cytosolic ribosome has remained remarkably unchanged from its evolutionary neighbors.

Although the protein component of the *C. reinhardtii* 80 S ribosome is virtually identical with that of yeast, mammals, and higher plants, it should be noted that the gene organization and post-translational modifications of these proteins is quite different in algae compared to other organisms. In mammals, ribosomal proteins are generally encoded by a single active gene, but mammalian genomes contain a large number of silent pseudogenes (from 145 pseudogenes for L21 down to three pseudogenes for L14).<sup>33</sup> In yeast, many of the ribosomal protein genes are duplicated, and in all cases, both gene copies are transcribed and produce functionally indistinguishable, identical, or virtually identical proteins.<sup>27</sup> In *Arabidopsis*, none of the ribosomal protein genes is single-copy and most are encoded by three or four expressed genes.<sup>21</sup> For at least 33 different ribosomal proteins, recent proteomic studies have revealed the presence of proteins from more than one of these genes being incorporated to *A. thaliana* ribosomes.<sup>11</sup> The 80 S ribosomal proteins of *C. reinhardtii*, with the exception of only S27 (two distinct genes encoding isoforms S27A and S27B), are encoded by single functional genes (summarized in Tables 1 and 2). S27 is one of the C2-C2-type zinc finger motif-containing ribosomal proteins, and this motif was found in both S27A and S27B. The presence of two distinct S27 mRNAs has been reported in rat<sup>34</sup> and channel catfish.<sup>35</sup> By disruption of one of these S27 genes, the extra-ribosomal function of S27 isoforms has been postulated, including rRNA processing in yeast,<sup>36</sup> and mRNA surveillance in *Arabidopsis*.<sup>37</sup> Our finding, that both S27 isoforms are incorporated into ribosomes suggests the presence of ribosome heterogeneity in algal cells. Furthermore, we identified a number of protein isoforms probably generated by post-translational modification or processing of 80 S ribosomal proteins in this alga, e.g. S5, L35a, and L37 (as described in Results). Any physiological roles of these protein isoforms and post-translational modifications are unclear, and are under investigation. These post-translational modifications may contribute to ribosome heterogeneity, and different types of 80 S ribosomes may function in different subcellular locations or translate different mRNA species. Further studies will be needed to test these hypotheses.

The algal homolog of RACK1 was identified as a part of the 80 S ribosome in *C. reinhardtii*. This protein has been described as Cblp, originally identified as a G protein  $\beta$  subunit-like polypeptide that was expressed constitutively throughout various environmental stresses and life-stages.<sup>38</sup> The RACK1 homolog of *C. reinhardtii* was identified as part of both the 40 S ribosomal subunit and 80 S ribosome (Table 1 and data not shown). As seen in Figure 5(a), our cryo-EM map of the algal 80 S ribosome contains the characteristic knob of RACK1



**Figure 5.** Views of the 80 S ribosome. *C. reinhardtii* 80 S shown in green, *S. cerevisiae*<sup>29</sup> shown in gold. Insets indicate the orientation of the ribosome map, dashed boxes denote the area highlighted. Indicated landmarks are as in Figure 4. (a) Close-up view of RACK1 position on the head region of the 40 S subunit. Dashed ellipse indicates relative position of RACK1 protein positioned upright against the head of the small subunit. (b) View of expansion segment (ES) 27 on 60 S subunit. Arrows indicate preferred positions of ES27 helix in yeast, while an asterisk denotes base density in algae.

density. RACK1 has been shown to function at a central point in a number of important signaling pathways and response mechanisms in the cell.<sup>13</sup> Future work will need to be done to determine which of the functions attributed to RACK1 is/are dependent on its association with the ribosome, and which might be extra-ribosomal function(s). There is accumulating evidence that ribosomal proteins have important functions away from their association with the ribosome,<sup>39–44</sup> and RACK1 may be an excellent example of this behavior.

The data presented here show that plant cytoplasmic ribosomes are highly conserved in both protein composition and structure with 80 S ribosomes from animals and other lower eukaryotes. Given that plants and animals separated three billion years ago, it seems remarkable that 80 S ribosomes have remained so similar, especially in light of the radical divergence of organelle ribosomes (both mitochondrial and chloroplast) over the same period. The genes encoding 80 S ribosomal proteins have clearly undergone divergent evolutionary paths in different eukaryotic species, in most yielding multiple coding and pseudogenes (discussed above) and thus providing substantial opportunity for protein divergence. That the essential function of a ribosome can be maintained

even in the face of large structural and compositional change is evidenced by the mitochondrial ribosome,<sup>45</sup> which has only a skeletal resemblance to cytoplasmic ribosomes, yet accomplishes the same decoding of mRNA and assembly of peptides. The degree of conservation of 80 S ribosome structure and composition between plants and animals suggests to us two possibilities. First, that there may be tight constraints on 80 S ribosome structure and composition that preclude divergence of the ribosomal proteins. It is intuitive that some fraction of the 80 S ribosomal proteins would have strong pressure to remain unchanged, due to their interactions with other proteins or cellular components essential for translation. This reasoning would not extend to each of the 80 proteins of the ribosome, at least with our current understanding of ribosome function and yet, with only a few exceptions, each 80 S protein remains highly similar from algae to mammals to yeast. In only four proteins (S10, L6, L14, and L29) is there greater than 20% difference between the yeast and mammalian ribosomal protein homologs, which amounts to less than a 3% difference in overall ribosomal protein composition.

The other possibility that might account for the observed degree of conservation of 80 S ribosomes is that the structure and composition of the

ribosome was already ideal, or at least very complete, when plants and animals diverged, and thus there was limited evolutionary pressure for change. Eukaryotic nuclear genomes are generally large, and there appears to be little selective pressure, or response to it, for them to be reduced in size. So perhaps the 80 S ribosome is the complete, complex version of a ribosome, containing all of the components that would ever be needed for translation, and these proteins remain unchanged simply because there is no pressure to reduce the complexity of the system. Organelle genomes, on the other hand, are small and are under strong selective pressure to be reduced in size and complexity. This, combined with the possibilities for mutation upon gene transfer to the nucleus, might help to explain the radical changes seen in organellar ribosomes compared with their evolutionary ancestors. It seems most likely to us that a combination of the above factors has resulted in the conservation seen between plant, animal, and yeast 80 S ribosomes. The necessary interactions of the 80 S ribosome with other partners, including translation factors, coding and regulating RNAs, and docking/localization proteins may place constraints on the structure and composition that, coupled with limited pressure to reduce protein size or complexity, results in the highly conserved 80 S ribosome.

## Materials and Methods

### Ribosome purification

*C. reinhardtii* (var. cc3395) cultures grown to mid-log phase were disrupted using a nitrogen bomb at 600 psi (1 psi  $\approx$  6.9 kPa), and then cytosolic 80 S ribosomes for proteome analysis were prepared by sucrose gradient ultracentrifugation, as described.<sup>23</sup> For cryo-EM imaging, polyamine-containing buffer (25 mM Tris-HCl (pH 8.0), 25 mM KCl, 5 mM MgCl<sub>2</sub>, 5 mM DTT, 0.05 mM spermine, 2 mM spermidine) was used during ribosome preparation instead of conventional buffer (25 mM Tris-HCl (pH 8.0), 25 mM KCl, 25 mM MgCl<sub>2</sub>, 5 mM DTT). Cytosolic ribosomes were further separated from chloroplast ribosomes through a second gradient. Total cytosolic ribosomal proteins (TP80) were extracted from purified ribosomes by an acetic acid-extraction method as described.<sup>8</sup>

### Proteomics

SDS-PAGE was performed as described by Laemmli,<sup>46</sup> using 1.5 mm thick 12% (w/v) polyacrylamide gel. Molecular mass markers used were BENCHMARK™ Prestained Protein Ladder (Life Technologies, Inc.). The 2D-PAGE and electroblotting were carried out as described.<sup>8</sup> Proteins separated by SDS-PAGE and 2D-PAGE were visualized by staining with Coomassie brilliant blue R-350 (Amersham Biosciences). Protein bands from SDS-PAGE or spots from 2D-PAGE were excised manually and transferred to 96-well plates. The plates were transferred to a Perkin Elmer Multiprobe-II liquid handling robot for destaining,<sup>47</sup> and for in-gel digestion. Robotic in-gel digestion with trypsin or endoproteinase Lys-C was performed as described.<sup>23</sup>

N-terminal protein sequencing was done with an Applied Biosystems model 492 protein sequencer.

Following digestion, peptides were extracted from the gel pieces with 5% (v/v) formic acid/5% (v/v) acetonitrile. A microbore HPLC system (Surveyor, ThermoFinnigan, San Jose, CA) was modified to operate at capillary flow-rates using a simple T-piece flow-splitter. Columns (6 cm  $\times$  100  $\mu$ m I.D.) were prepared by packing 100  $\text{\AA}$ , 5  $\mu$ m Zorbax C18 resin at 500 psi pressure into columns with integrated electrospray tips made from fused silica, pulled to a 5  $\mu$ m tip using a laser puller (Sutter Instrument Co., Novato, CA). Peptides were eluted in a gradient using buffer A (5% acetonitrile, 0.1% formic acid) and buffer B (90% acetonitrile, 0.1% formic acid), at a flow-rate of 400 nl/minute. Following an initial wash with buffer A for ten minutes, peptides were eluted with a linear gradient from 0–100% buffer B over 30 minutes. Samples were introduced onto the analytical column using a Surveyor autosampler (Surveyor, ThermoFinnigan, San Jose, CA). The HPLC column eluent was taken directly into the electrospray ionization source of a ThermoFinnigan LCQ-Deca XP Plus ion trap mass spectrometer (ThermoFinnigan, San Jose, CA). Spectra were scanned over the range 400–1500 mass units. Automated peak recognition, dynamic exclusion, and daughter ion scanning of the top three most intense ions were performed using the Xcalibur software as described.<sup>48</sup>

MS/MS data were analyzed using SEQUEST, a computer program that allows the correlation of experimental data with theoretical spectra generated from known protein sequences,<sup>49</sup> and the data were filtered and organized by DTASelect.<sup>50</sup> In this study, the criteria for a preliminary positive peptide identification for a doubly-charged peptide were a correlation factor ( $X_{\text{corr}}$ ) greater than 2.5, a delta cross-correlation factor ( $\Delta X_{\text{corr}}$ ) greater than 0.08, a minimum of one lysine peptide cleavage, and a high preliminary scoring.<sup>51</sup> For triply charged peptides, the correlation factor threshold was set at 3.5. Identical protein identification criteria have been used successfully in other studies.<sup>7,51,52</sup> All matched peptides were confirmed by visual examination of the spectra. All spectra were searched against a composite database containing SWISS-PROT and FASTA-format open reading frames (ORFs) generated from *C. reinhardtii* ESTs. Full-length coding regions for each of the ribosomal proteins have been annotated correctly to the JGI *C. reinhardtii* database v2.0. Annotations that are incorrect as viewed in ver2.0 are indicated as such in Tables 1 and 2, and will be corrected upon release of ver3.0 of this database.

### Single-particle reconstruction

Ribosomes for imaging were prepared as described above and frozen onto holey grids. Micrographs containing particles used in the final reconstruction were collected on a Philips CM200-FEG electron microscope operating at 120 keV. Negatives were digitized using a Zeiss SCAI scanner, and data were utilized at 2.71  $\text{\AA}$ /pixel. BOXER was used for manual particle picking. Defocus values for the data used ranged from 0.9  $\mu$ m to 1.8  $\mu$ m under-focus, and images were corrected for the contrast transfer function. Contrast transfer function estimates were obtained using CTFIT. Data processing utilized functions of both EMAN† and SPIDER‡ software

† <http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc/>  
‡ [http://www.wadsworth.org/spider\\_doc/spider/docs/master.html](http://www.wadsworth.org/spider_doc/spider/docs/master.html)

packages. Molecular graphics images were produced using the UCSF Chimera package from the Computer Graphics Laboratory, University of California, San Francisco.<sup>53</sup>

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## Supplementary Data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jmb.2005.06.022](https://doi.org/10.1016/j.jmb.2005.06.022)

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