REVIEW ARTICLE

Mitochondrial ribosomal proteins (MRPs) of yeast

Hanns-Rüdiger GRAACK*[1] and Brigitte WITTMANN-LIEBOLD†

*Institut für Genetik, Freie Universität Berlin, AG Kress, Arnimallee 7, D-14195 Berlin, Federal Republic of Germany, and †Max-Delbrück-Centrum für Molekulare Medizin, AG Proteinchemie, Robert-Rössle-Strasse 10, D-13125 Berlin-Buch, Federal Republic of Germany

Mitochondrial ribosomal proteins (MRPs) are the counterparts in that organelle of the cytoplasmic ribosomal proteins in the host. Although the MRPs fulfil similar functions in protein biosynthesis, they are distinct in number, features and primary structures from the latter. Most progress in the eludication of the properties of individual MRPs, and in the characterization of the corresponding genes, has been made in baker's yeast (*Saccharomyces cerevisiae*). To date, 50 different MRPs have been determined, although biochemical data and mutational analysis propose a total number which is substantially higher. Surprisingly, only a minority of the MRPs that have been characterized show significant sequence similarities to known ribosomal proteins from other sources, thus limiting the deduction of their functions by simple comparison of amino acid sequences. Further, individual MRPs have been characterized functionally by mutational studies, and the regulation of expression of MRP genes has been described. The interaction of the mitochondrial ribosomes with transcription factors specific for individual mitochondrial mRNAs, and the communication between mitochondria and the nucleus for the co-ordinated expression of ribosomal constituents, are other aspects of current MRP research. Although the mitochondrial translational system is still far from being described completely, the yeast MRP system serves as a model for other organisms, including that of humans.

INTRODUCTION

Eukaryotic cells that depend on the consumption of oxygen for the production of metabolic energy (ATP) contain mitochondria, the organelles where the final steps of carbon catabolic metabolism and hydrogen oxidation take place. Mitochondria are thought to be descendants of the α subdivision of ancient purple bacteria which became incorporated into the pre-eukaryotic cell by endosymbiosis during evolution [1]. Despite the fact that mitochondria are now completely genetically dependent on nuclear genome expression for maintenance of their function, they still keep some molecular relicts of their former unicellular independency. Mitochondria contain their own DNA, coding for a few genes which are essential for mitochondrial and cellular metabolic functions. The mitochondrial inner membrane, which is assumed to represent the former outer membrane of the endosymbiont, has a unique lipid composition. Mitochondria possess their own translational machinery for the expression of their genes, and they use an alternative genetic code [2]. Nearly all constitutents of the mitochondrial protein-synthesis apparatus are unique and distinct from their cytoplasmic counterparts. This is also valid for the mitochondrial ribosome (mitoribosome). Although the general features of these ribosomes are comparable with those of bacterial or eukaryotic cytoplasmic ribosomes, they do not share any of their constitutents with the cytoplasmic ribosomes of their host [3]. This has been established by isolation of mitochondrial (mt) ribosomal constituents from pure mitochondria, followed by comparison of the biochemical properties of the mt rRNA and mitochondrial ribosomal proteins (MRPs) with those of the cytoplasmic ribosomal constitutents ([4,5]; for a review, see [3]).

Mitochondrial ribosomes from different species display a wide variation in their features and in the number and properties of their constituents. Their sedimentation-coefficient (*s*) values vary between 80 S in ciliatae, 70–74 S in fungi, 77–78 S in higher plants, and 55 S in metazoans such as shark and cow [3,6]. However, the total molecular mass of the mitoribosomes is at least as large as that of bacterial ribosomes. Their lower *s* values as compared with the homogeneous groups of eubacterial (70 S) or eukaryotic cytoplasmic (80 S) ribosomes reflect differences in the protein/RNA ratio. Whereas *Escherichia coli* ribosomes contain proteins and RNAs in a mass ratio of 1:2, in mitoribosomes this ratio varies from approx. 1:1 in yeast up to 2:1 in bovine mitoribosomes [4,5]. Most mitoribosomes of animals and fungi contain only two RNA molecules; in yeast mitoribosomes rRNAs of 15 S and 21 S are found [7]. In plant mitoribosomes an additional 5 S RNA is present.

Since a larger portion of the molecular mass of the mitoribosome is provided by proteins, it is not surprising that the actual number of different MRPs exceeds that in bacteria, and in general it is similar to, or higher than, that in eukaryotic cytoplasmic ribosomes. *E. coli* contains 55 ribosomal (r-)proteins [8], whereas the corresponding number of eukaryotic cytoplasmic r-proteins varies from 75 to 76 in yeast [5,9] to 70 in rat [10]. In yeast mitoribosomes, 68–77 MRPs have been differentiated by two-dimensional (2D) PAGE [5,11,12], and in bovine and rat mitoribosomes 85 and 86 MRPs have been counted respectively [4,10]. However, calculation of the numbers is compromised by differences in staining and migration of the individual proteins, depending on the staining and PAGE methods applied. In yeast, 50 MRPs have been identified in the large subunit, including four pairs of proteins encoded by the same gene [13]. The authors [13]

Abbreviations used: MRP, mitochondrial ribosomal protein from different sources; mt, mitochondrial; ORF, open reading frame; PTF, peptidyltransferase; r-protein(s), ribosomal protein(s); YmL or YmS, yeast mitochondrial ribosomal protein of the large or small ribosomal subunit respectively; 1D and 2D, one- and two-dimensional; MMP, mitochondrial matrix protease; MIP, mitochondrial intermediate peptidase; DHFR, (Chinese-hamster) dehydrofolate reductase.

[1] To whom correspondence should be addressed.

Table 1 MRPs of yeast

pI values were taken from the literature or calculated from the mature form of the MRP using the program ISOELECTRIC (Genetics Computer Group) [63]; values in parentheses are deduced from complete protein sequences if the processed form is not known; Protein family name: families of similar r-proteins have been assigned according to the respective *E. coli* r-proteins of the large (L*xx*) or small (S*xx*) subunit unless stated otherwise; If no protein counterpart could be identified in the data banks, protein families have been termed according to the corresponding MRP; abbreviation: n.d., not determined. Key to superscript letters: [a,b]The molecular mass calculated from the deduced amino acid sequence is given as [a]. The molecular mass of the complete (unprocessed) ORF is given in parentheses if the N-terminal signal peptide has not been determined; [b]Molecular mass estimated by 1D SDS or 2D PAGE. [c]If MRPs and/or their genes have been termed differently in different publications, they are named according to the first determination of the respective sequence. In the case of amino acid sequences determined by direct biochemical methods, e.g. peptide microsequencing, incomplete sequences have also been assigned. Genes or ORFs which have been detected and termed by data resulting from the yeast genome project without any mitochondrial ribosomal location and function are given as synonyms. Protein names based on the YmL and YmS nomenclature are termed as described in [11,13]. [d]YmL5 and YmL7 of [11] are two different modified forms of the same protein [13]. [e]Protein shows an elongated spot in the 2D PAGE owing to probable modification [13]. [f]Size of signal peptide deduced from properties of the N-terminal sequence. [g]Gene contains intron(s). [h]Sequence has been submitted to Swissprot databank [13]. [i]MRP-L27 has been mapped to chromosome 10 by [35] and to chromosome 2R by [36]. [j]The initiator methionine is cleaved off, hence this is not a cleavable signal sequence for mitochondrial import. [k]Only the shortest allele reported is considered; for other allelic forms see [64]. [l]YmL11 shows two different N-termini separated by four amino acid residues as found by amino acid sequencing [24]; the elongated form is presented. [m]Two proteins have been assigned to a single gene, see YmL10/YmL18, YmL14/YmL24, YmL17/YmL30, YmL34/YmL38. [n]Two mRNAs of different lengths have been detected; depending on the translational start site two different N-termini are possible. [p]This name is preliminary, since no 2D PAGE position according to [11] has yet been established.

Protein name[c]	Gene name[c]	Synonym	Accession number	Chromosomal gene location	Amino acid residues		Mol. mass of mature protein		Essential for mitochondrial function?	pI	Protein family name	Reference(s)
					Total	Signal peptide	Da[a]	kDa[b]				
(a) Large subunit												
YmL2	*MRP7*	MRP7p	P12687	14L	371	27	40 092	40	Yes	10.61	L27	[24,25,26]
YmL3	*MRP-L3*	YM9711.14	S54026	13R	390	59	37 179	36	n.d.	9.60	YmL3	[11,13]
YmL4	*MRP-L4*	L9753.1	P36517	12R	319	14	35 295	35	Yes	6.91	YmL4	[11,27]
YmL5/7[d]	*MRP-L7*	YDR237w	S54533	4R	292	19	31 754	29	n.d.	10.36	L5	[13,24]
YmL6[e]	*YML6*[g]	YML025c	SC8337	13	286	26	29 040	30	n.d.	10.24	L4	[13,24]
YmL8	*MRP-L8*	HRD238	P22353	10L	238	(1)[j]	26 822	28.5	Yes	10.60	L17/S13	[24,28,29]
YmL9	*MRP-L9*		P31334	7	269	19	27 553	27.5	Yes	10.92	L3	[11,30]
YmL10[m]	*MRP-L10*	YNL284c	Z71560	14	272[m]	47	29 856	28.5	n.d.	11.10	L15[m]	[13,24]
YmL11[l]	*MRP-L11*	YDL202w	Z74250	4	249	31	24 804	25	n.d.	10.30	YmL11	[13,24]
YmL13	*MRP-L13*	YK105	Q02204	11	275	86	21 619	21	No	9.01	YmL13	[24,31]
YmL14[m]	*MRP-L14*	YMR193w	S50921	13R	258[m]	21	27 532	31	n.d.	10.85	L28[m]	[13,24]
YmL15	*MRP-L15*		S72159	12	253	28	24 929	27	n.d.	9.55	YmL15	[13,24]; this study
YmL16[e]	*MRP-L6*	MRP-L6p	S46764	8R	214	16	21 634	23	Yes	10.48	L6	[24,32]
YmL17[m]	*MRP-L17*	YNL252c	S63225	14L	281[m]	19	30 253	15	n.d.	9.32	YmL17[m]	[13]
YmL18[m]	*MRP-L10*	YNL284c	Z71560	14	272[m]	47	29 856	24	n.d.	11.10	L15[m]	[13,24]
YmL19	*MRP-L19*	YNL185c	Z71461	14	158	n.d.	(16 670)	16	n.d.	(10.68)	L11	[13,24]
YmL20	*MRP-L20*	YKR405	P22354	11R	195	18	20 626	19	Yes	11.00	YmL20	[28,33]
YmL23[e]	*MRP-L23*	YOR150w	Z75058	15	164	n.d.	(18 463)	15	n.d.	(10.91)	L13	[13,24]
YmL24[m]	*MRP-L14*	YMR193w	S50921	13R	258[m]	21	27 532	17	n.d.	n.d.	L28[m]	[13,24]
YmL25[e]	*YMR26*		P23369	7	156	(1)[j]	18 368	18	Yes	10.87	YmL25	[24,34]
YmL27	*MRP-L27*		S77888	10; 2R[i]	146	16	14 798	15	Yes	10.90	YmL27	[24,35,36]
YmL28	*MRP-L28*	YDR462w	M88597	4R	147	26	14 387	14.5	n.d.	11.11	YmL28	[13,24]
YmL30[m]	*MRP-L17*	YNL252c	S63225	14	281[m]	19	30 926	16	n.d.	n.d.	YmL17[m]	[13,24]
YmL31	*MRP-L31*		X15099	11	131	12	14 246	14.5	Yes	11.44	YmL31	[11,35,37]
YmL32	*MRP-L32*	YCR041	R5BY32	3R	183	71	13 374	11	n.d.	10.68	YmL32	[13,24,38]
YmL33	*MRP-L33*		D90217	13	99	(1)[j]	11 012	11	Yes	11.04	L30/L16	[24,39]
YmL34[m]	*MRP-L38*	YKL170w	S38000	11L	138	57	8 812	12.5	n.d.	10.27	L14[m]	[13,24,40]
YmL35[e]	*MRP-L35*	YDR322w	U32517	4	367	n.d.	(42 824)	42	n.d.	(10.34)	YmL35	[13,24]
YmL36	*MRP-L36*		S44701	2R	196	33	18 552	8.5	n.d.	10.34	YmL36	[13,24]
YmL37	*MRP-L37*		S46149	2R	105	24	9 185	13.5	n.d.	9.30	YmL37	[13,24]
YmL38[m]	*MRP-L38*		S38000	11L	138	None	14 904	n.d.	n.d.	10.57	L14[m]	[13,24,40]
YmL39	*MRP-L39*		S55110	13L	70	(1)[j]	7 841	11	n.d.	11.54	L33	[13,24]
YmL40	*MRP-L40*	YPL173w	Z73529	16	297	n.d.	(33 749)	36	n.d.	(10.26)	Potato S4	[13,24]
YmL41	*MRP20*	MRP20	M81969	4	263/253[n]	45/35[n]	25 711	32	Yes	10.31	L23	[24,41]
YmL44	*MRP-L44*[g]	YMR44	JQ0369	13	98	None	11 476	13	n.d.	10.38	YmL44	[24,42]
YmL45	*MRP-L45*	YGL125w	Z72647	7	599	38	63 936	26	n.d.	n.d.	YmL45	[13]
YmL47	*RML16*	RML16p	P38064	2L	232	41[f]	(26 517)	24	Yes	(11.12)	L16	[14,43,44]
YmL49[e]	*MRP-L49*	YJL096w	S50297	10L	224	n.d.	(25 402)	15	n.d.	(11.37)	YmL49	[13,45]
MRP49	*MRP49*		M81697	11L	137	19[f]	(16 020)	16	No	10.15	MRP49	[40,41]
	YELO50c	YELO50cp	U18779	5	393	n.d.	(43 785)	n.d.	Yes	(11.50)	L2	[46]
(b) Small subunit												
var1[k]	*var1*		P02381	mt	396	None	46 786	40	Yes	10.73	var1	[2,47,48]
MRP1	*MRP1*		M15160	4	321	n.d.	(36 628)	37	Yes	(9.81)	MRP1	[49,50]
MRP2	*MRP2*		R3by14	16R	115	15[f]	(13 538)	14	Yes	(11.70)	S14	[49,51]
YmS2	*MRP-S2*	YHR075c	U10556	8	400	35	40 770	40	n.d.	8.51	YmS2	[13]
MRP4	*MRP4*	YSCMRP4A	M82841	8	394	n.d.	(44 159)	43	Yes	(9.59)	S2	[52]
MRP-S9	*MRP-S9*		P38120	2R	278	n.d.	(31 924)	n.d.	Yes	(11.07)	S9	[53]
MRP13	*MRP13*	YmS-A	M22109	7	324	10	34 676	35	No	10.60	MRP13	[13,54]
YmS16	*MRP17*	MRP17	P28778	11R	131	None	17 343	17	Yes	10.66	YmS16	[13,55]
YmS18	*MRP-S18*	YNL306w	Z71582	14	217	59	17 734	21	n.d.	10.63	YmS18	[13]
MRPS28p	*MRPS28*		P21771	4	286	33	29 467	28	Yes	10.59	S15	[56]
NAM9	*NAM9*		M60730	14R	485	34[f]	(56 509)	53	Yes	(10.22)	S4	[57,58]
PET123	*PET123*		P17558	15R	318	n.d.	(41 650)	39	Yes	(10.61)	PET123	[59–61]
YmS-T[p]	*YmS-T*[p]		?[h]	4	?[h]	(1)[j]	10 559	14	n.d.	n.d.	YmS-T	[13]
(c) Subunit not determined												
YMR-31	*YMR-31*		P19955	6R	123	8	12 792	12.5	n.d.	10.09	YMR-31	[42,62]

assume that at least 10 more MRPs remain to be found in the large subunit. Taken together, the yeast mitoribosome appears to contain at least 90 proteins, and the actual number may exceed 100. In metazoans the preliminary results suggest an even higher number. From this point of view the mitoribosome is one of the most complex multi-protein–RNA systems of the cell, and thus deserves to be looked at more closely.

The complexity of mitoribosomes is also reflected at the molecular-genetic level. The biosynthesis of mitoribosomes requires co-ordinated expression of both mitochondrial and nuclear genes. In all cases so far reported the mt rRNAs are encoded by the mt genome, whereas, in contrast, the genes for MRPs are mostly found in the nuclear genome. Whereas in plants a considerable number of MRP genes (varying according to the species) is located on the mt DNA [14], in most protozoa and fungi only a few, or no, MRPs [3] are encoded by the mt DNA. However, the encoding of 27 MRPs by the mt DNA has recently been reported in the case of the protozoon *Reclinomonas americana* [15]. All of these could be identified by comparison with known ribosomal protein sequences. The MRPs encoded by nuclear genes are synthesized on cytoplasmic ribosomes and imported into the mitochondria [16]. Thus they depend on the mitochondrial protein-import mechanisms for their proper insertion and final location and function. Most MRPs so far investigated contain N-terminal signal sequences for mitochondrial import, which are cleaved off during or after import.

How the expression of mitochondrial- and nuclear-encoded mt ribosomal constituents is co-ordinated still remains one of the enigmas of mitochondrial research. The stoichiometric synthesis of all individual mt ribosomal components must be strictly controlled to avoid a huge waste of metabolic energy. So far, the investigation of expression of individual mt ribosomal components has revealed various control mechanisms working in parallel (see below), but a general mechanism, if one exists, tightly linked to the metabolic status of the cell, has still to be eludicated.

Several features of MRP function can be distinguished, e.g. by the investigation of naturally occurring or artificially induced mutants, or by the binding of antibiotics, metabolic products, or of RNA and other proteins. MRP import into the mitochondria and the assembly of ribosomal subunits have been studied. Other functions can be deduced from the sequence similarity between individual MRPs and bacterial r-proteins with known properties. However, the direct assignment of MRP functions within the mitoribosome is limited, since no *in vitro* protein-synthesis system composed solely of mitochondrially specified components translating *mitochondrial* mRNAs is available today. Mitoribosomes isolated and reconstituted from their subunits are able to synthesize peptides from artificial RNA [e. g. poly(U)], if they are supplied with soluble factors from bacteria [17,18]. Nevertheless, *in vitro* translation of mitochondrial mRNAs seems to depend on many more features specific for the mitochondrial translational system which are not available in an artificial system ([19]; for a review, see [20]). The presence of translational activators specific for individual mt mRNAs, or the association of mitoribosomes with the mitochondrial inner membrane, might be indispensible for proper mitoribosomal function [20–22].

To date, most of the information about MRPs has been obtained from yeast MRPs. Since yeast is a facultative anaerobic organism, it does not strictly depend on proper mitochondrial function, as is the case with most other eukaryotes. Mitochondrial mutants can be studied easily, and appropriate classical and molecular-genetic approaches, including targeted one-step gene disruption, are well established. Last, but not least, the yeast genome sequencing project has revealed many new insights into the genetics and molecular biology of yeast MRP genes. The first complete genome information for a eukaryotic organism facilitates the identification of MRP genes, e.g. by comparison with only partial peptide-sequence information. This supports the assignment of MRPs which are not similar to other known (ribosomal) proteins. This will be helpful particularly for the identification of MRPs among the unknown open reading frames (ORFs) detected by other genome-sequencing projects such as the Human Genome Project.

IDENTIFICATION AND CHARACTERIZATION OF MRPs

Once it was recognized that mitochondrial ribosomes differ from their cytoplasmic counterparts, it took some time to characterize the mt ribosomal components and to distinguish them from the cytoplasmic ones [5,12]. Ribosomes were isolated from purified mitochondria, and the proteins derived from isolated ribosomal subunits were analysed by 2D PAGE techniques showing differences between the mt and the cytoplasmic ribosomal protein mixtures in composition, number, molecular masses and isoelectric points (pI) of the individual proteins [5,11,23]. The first MRP to be identified was the var1 protein and its mitochondrially localized gene (Table 1). This protein attracted attention as a mt translational product with very unusual features (see below; for a review, see [65]). The *var1* gene shows unusual conversion mechanisms as well as divergences in the mt genetic code [2,66,67,68]. The tight association of var1 with the small subunit of the mitoribosome was shown by radioactive labelling of mt translational products and comparison with mt ribosomal constituents [47,48].

Since var1 is the only MRP coded for by the mt DNA, it follows that all other MRPs are encoded by nuclear genes. Nuclear genes of MRPs are assumed preferentially to complement pleiotropic mutations affecting several or all of the mt translational products. Mutants unable to synthesize mt proteins lose their mt DNA with high frequency, changing from ρ^+ to ρ^0 or ρ^- ([69]; see also Table 4 below for an explanation of the ρ designations). Several such mutants have been cloned by applying genetic and immunological methods, for example, *MRP1* and *MRP2* [49]. The association of the proteins with the small ribosomal subunit was determined immunologically [51,59]. The genes for MRP4, MRP17, PET123, and the *MRP-L6* gene coding for YmL16 (MRP-L6p), were similarly cloned.

Other MRPs were identified by their sequence similarity as compared with *E. coli* r-proteins. The NAM9 protein is similar to the EcoS4 and the yeast cytoplasmic S13 proteins, MRP-S9 is a member of the EcoS9 family [53], and the YmL47/*RML16* is an EcoL16 counterpart [43]. However, this assignment by sequence comparison seems to be limited by the fact that most MRPs are *not* similar to any known r-protein (Table 1). The unambigous confirmation of the mt ribosomal assignment has to be provided by other methods such as the purification and amino acid sequencing of mature proteins, which has been done for RML16p/YmL47 and YmL16 [13], or by immunological detection in isolated ribosomal subunits. Some other genes have been identified in a similar way, by immunological screening of expression libraries with antibodies against MRPs, namely *MRP7* [25], *MRP13* [54], *MRP20* [41] and *MRP49* [41].

Nearly all of the other MRPs and their corresponding genes listed in Table 1 were identified by direct biochemical methods: MRPs were isolated from ribosomal subunits, and were separated and assigned by one-dimensional (1D) and 2D PAGE (Figure 1). The proteins were purified by HPLC and/or 2D blotting, subjected to partial proteolytic digestion, and the resulting peptides as well as the mature MRPs were analysed by N-

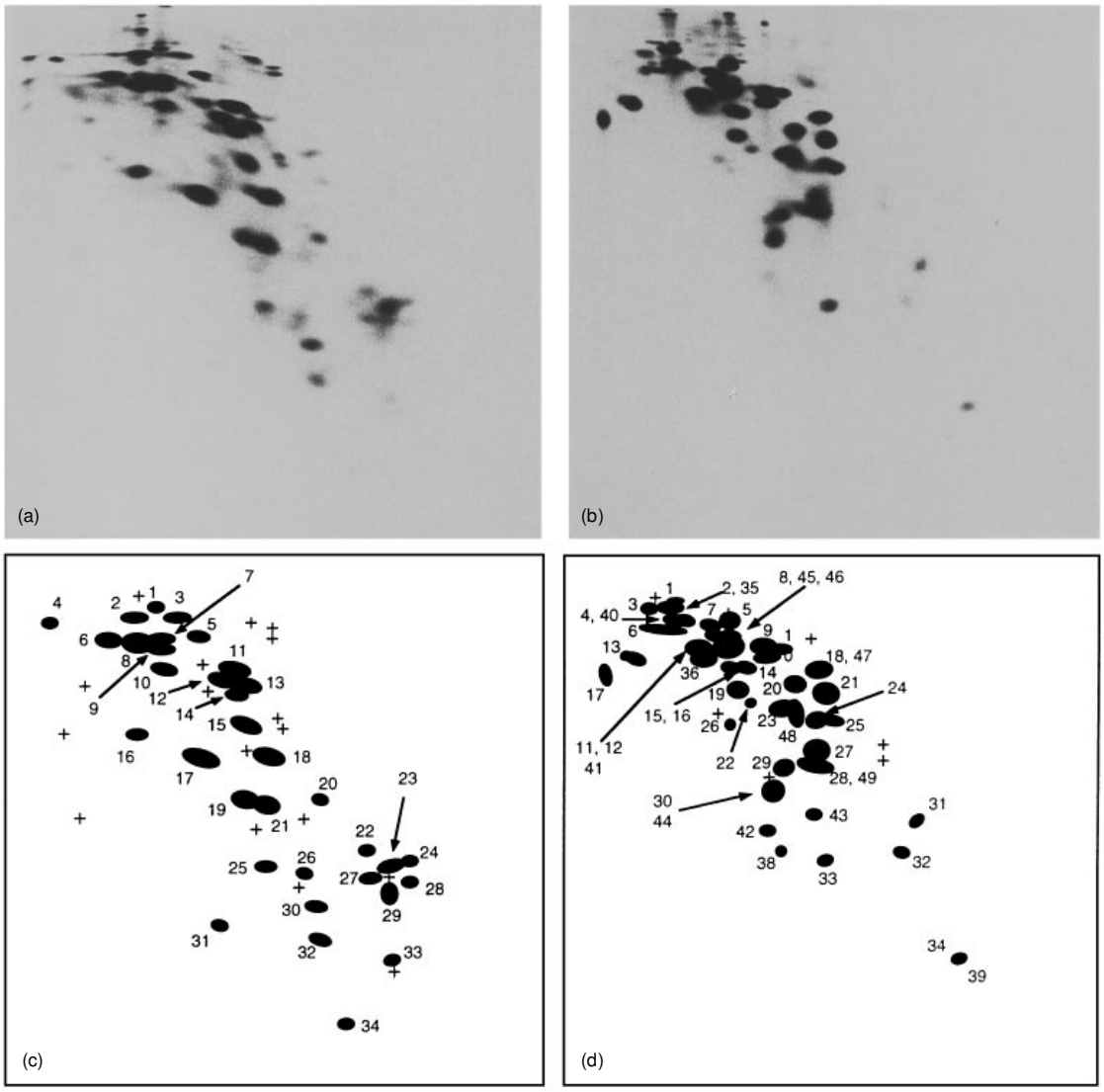

Figure 1 2D PAGE of mitochondrial ribosomal proteins

(**a**) 2D separation of yeast small subunit (37 S) MRPs; (**b**) two-dimensional separation of yeast large subunit (50 S) MRPs; (**c**) schematic drawing of (**a**); (**d**) schematic drawing of (**b**). Faintly visible spots in (**a**) and (**b**) have been marked with ' + ' in (**c**) and (**d**). The primary assignment of (**c**) and (**d**) in [11] follows the mode of [70] and was later extended in (**d**) as described in [13,24].

terminal amino acid sequencing. The resulting amino-acid-sequence data were used for subsequent gene cloning, or for identification of the corresponding ORFs determined by the yeast genome sequencing project ([13]; see the other references of Table 1). Comparison of the N-terminal amino acid sequences with the ORFs deduced from nucleotide sequences revealed extensions and cleavage sites for the proposed signal sequences for mt import (Table 2). In some cases the postulated cleavage site predicted by analysis of the deduced amino acid sequence could be confirmed by N-terminal sequencing of the mature MRP. This applies to YmL2/*MRP7* [24–26], YmL16/*MRP-L6* [24,32], YmL41/*MRP20* [24,41], MRP13/YmS-A [13,54], and YmS16/*MRP17* [13,55] (see Tables 1 and 2). The molecular mass of the mature MRP can be calculated and compared with that obtained by experimental methods. Failure to obtain an N-terminal sequence is suggestive of a putative N-terminal block of the mature protein, as was reported for YmL19 and YmL23 [24]. In the case of YmL16/MRP-L6, two different C-termini were reported [32] and by the yeast genome project (accession no. S46764), the latter extending the protein by nine amino acid residues. Sequencing of a C-terminal peptide demonstrated that the elongated form is the 'correct' one [13,32].

However, the limitations of this 'computational science' simply by comparison of sequences are obvious. If differences between the calculated and the experimentally obtained molecular masses exceed the methodological limitations, then errors in the nucleotide sequence as well as proteolytic events or modifications of the mature MRPs have to be considered. This becomes a matter for discussion in cases where two distinct proteins with different molecular masses have been isolated which are encoded by the same gene (Table 1; see below). The ORF for YmL15 could not be found in the yeast genome sequencing project owing to a

Table 2 Putative N-terminal signal sequences for import of MRPs

Amino acid sequences are given in the single-letter code according to the references given in Table 1. Positions of the extension of the signal peptide are given. The putative cleavage site is marked with the dash (–). Arginine (R) residues in position –2 or –10 preferentiable for proteolytic processing during mitochondrial import are emboldened. A motif [24] of at least three amino acid residues, an aromatic amino acid [preferentially phenylalanine (F)] surrounded by positively charged (K, H or R) and/or hyroxylated amino acid residues is underlined. Notes: [a]YmL5 and YmL7 of [11] are two different modified forms of the same protein [13]. [b]Two proteins have been assigned to a single gene (see Table 1). [c]Two different possible translation start codons have been determined; amino acid residues beginning at the first start codon are given in lower-case letters. The last column lists the cleavage class [71].

Protein	Sequence	Cleavage class
	-80 -70 -60 -50 -40 -30 -20 -10 +10	
Large subunit		
YmL2	MWNPILLDTSSFSFQKHVGVFLQVRN - ATKRAAGSRT	R-2
YmL3	MGIVLKRAIAAGMKPFPNSTWHWSRTIRPFSQHLSSTCFLQQSSRFTSKRYLHLSTLTQ - QEKRFLPESE	R-10
YmL4	MWKRSFHSQGGPLR - ARTKFTKPKP	R-no
YmL5/7[a]	MQRFSLVTHRSFSHSCVKP - KSACSLVKPV	R-10
YmL6	MTIKRNLVKTLQSIRYQATTATAHAE - STLNPLPVTV	R-no
YmL8	M - TVGIARKLSR	-
YmL9	MSKFLQGSIFSISKLHVRY - SSTRPFLVAP	R-2
YmL10/YmL18[b]	MKAERQTGLRNSFTTVIGRKLINTFVPSMMLTSLFKSPVLAFQSYRY - VSILGQLKPS	R-2
YmL11	MLQLRFMPGWVPRNGFFGLKETIGTVHKRFY - ALASEQPSRK	R-3
YmL13	MGRRKGGHSMRMSSLLKLHCIRPLPQRSVWLSGYKQKARCIHSSAANGDFMSWFKRKKQEEHQEPVKDTKQLIKDIEEGTNEASSQ - SSSNNKNRLE	R-no
YmL14/YmL24[b]	MQKIFRPFQLTRGFTSSVKNF - RQWRLIETRK	R-10
YmL15	MENSMMFISRSLRRPVTALNCNLQSVRT - VIYLHKGPRI	R-2
YmL16	MSFIQRRLLSQTLFLR - SQVGSLPLYI	R-10
YmL17/YmL30[b]	MKVNLMLKRGLATATATAS - SAPPKIKVGV	R-no
YmL20	MIGRGVCCRSFHTAGSAW - KQFGFPKTQV	R-10
YmL25	M - SYKQYFDSLP	-
YmL27	MKGSPISQFSKTSINA - LTRPWKKYRD	R-no
YmL28	MLAQTFKKPHRAVLEQVSGTTVFIRN - KRTKSKSSLS	R-2
YmL31	MFGPFKLTSPVA - GGLLWKIPWR	R-no
YmL32	MNSLIFGKQLAFHKIVPTTAIGWLVPLGNPSLQIPGQKQLGSIHRWLREKLQQDHKDTEDKDFFSNNGILL - AVPKKKVSHQ	R-no
YmL33	M - VFYKVTLSRS	-
YmL34[b]	MIFLKSVIKVIDNSGAQLAECIKVIRKGSPKSPAMVGDRIVCVIQKAKPLTQNITGT - ANTNRVKKGD	R-no
YmL36	MPPERTGTNANEHTFCSLLMLKSIFAKRFASTG - SYPGSTRITL	R-no
YmL37	MLARSLGYRLISTSRILYNKPTVK - SVVSSCPAGT	R-10
YmL38[b]	- MIFLKSVIKV	-
YmL39	M - VKVKSKNSVI	-
YmL41[c]	mprltvgtknMLYPLQKTLAVGSCKPEQVPIRSLASVVESSSKIL - DKSGSDREVD	R-no
YmL44	- MITKYFSKVI	-
YmL45	MKITEKLEQHRQTSGKPTYSFEYFVPKTTQGVQNLYD - MDRMYEASLP	R-no
YmL47	MFPYLTRMNLSIKMGGLTLKESSPNAFLNNTTIARRFKHEY - PRFKIVKQKQ	R-no
MRP49	MSKVAQQLKFLNKISATTR - LPQILVDPKK	R-no
Small subunit		
MRP2	MGNFRFPIKTKLPPG - FINARILRDN	R-no
YmS2	MSDDLRRKIALSQFERAKNVLDATFQEAYEDDEND - GDALGSLPSF	R-no
MRP13	MFRFTVWRRF - ASTGEAKAKL	R-2
YmS16	- MLYELIGLVR	-
YmS18	MLLQPVWKGCRWTQFVRPIRRWNSTGTNRGVPFSFKDISNQEDITNISYPSSSDSVLTK - SNGSSEVYKP	R-no
MRPS28p	MSIVGRNAILNLRISLCPLFMGKRSFVSSPVSN - SAKAVKFLKA	R-10
NAM9	MPRKANLLKSLARGRVRTSFNKYNLFNLYKKGGV - DLKSKSLYQQ	R-no
YmS-T	M - SGKPPVYRLP	-
Subunit not determined		
YMR-31	MIATPRL - AKSAYEPMIK	R-2

sequencing error. By subsequent sequencing of a PCR-amplified DNA fragment from chromosomal yeast DNA, the correct sequence could be established and an ORF for YmL15 could be predicted that fits the biochemical data ([13]; the present study). Further, the ORF for YmS-T was not listed in the yeast genome data [13], and the biochemical data for YmL45 (molecular mass, 2D-PAGE position) are far from matching the features of YmL45, as deduced from the ORF identified in the yeast genome [13]. This ORF predicts a molecular mass of 64 kDa, as opposed to 26 kDa determined by 2D PAGE [13]. The deduced pI of the predicted protein is quite acidic (5.39) and does not fit at all with the 2D-PAGE position of YmL45 (Figure 1). However, the mature YmL45 was shown by N-terminal sequencing to be at least partly identical with the identified ORF [13]. The reason for this result might be a DNA sequencing mistake covering a putative intron, or an unknown proteolytic cleavage of the YmL45 precursor protein.

Recently some MRP genes have been determined by a computer search of yeast ORFs which show significant sequence similarity to eubacterial r-proteins. Among these are the counterparts of EcoL1, EcoL2, EcoL7/L12 and EcoL34 [46]. However, only the yeast gene specifying the EcoL2 counterpart has so far been proved to encode a true MRP [46]. Other MRPs have not been found, of which 2D-PAGE data, molecular mass or partial-amino-acid-sequence data have accumulated (Table 3). However, the data are not sufficient at present to identify the corresponding genes, and they have therefore not been included in Table 1.

Table 3 Putative MRPs so far insufficiently characterized

(a) Proteins which have been characterized biochemically. (b) Yeast gene products identified by sequence similarity to eubacterial r-proteins lacking mt protein confirmation [46].

(a)

Protein	2D PAGE position	Molecular mass of the mature protein (kDa)	HPLC data*	Partial amino acid sequence obtained	Reference(s)
YmL1	+	35	+	+	[24]
YmL12	+	32	+	+	[24]
YmL21	+	23	+	+	[13]
YmL22	+	?	+	−	[11,24]
YmL29	+	14	+	+	[13]
YmL42	+	?	−	−	[13]
YmL43	+	?	+	−	[13]
YmL46	+	?	+	−	[13]
YmL48	+	?	+	−	[13]
YmS6	+	35	−	+	[13]
YmS-B	−	18	−	+	[13]
MRP3	−	60	−	−	[72]

(b)

Protein name	GenBank accession number	Sequence similarity to eubacterial r-protein [46]
YD9727.11	Z48758	L1
YGL068w	Z72591	L7/L12
YD9727.10	Z48758	L34

* Information about peptide profiles after Lys-C digestion or positional information in HPLC separations of total MRP mixtures available (see respective references).

A further question is whether other proteins which interact with mt ribosomes more or less tightly should be considered as MRPs. For example, PET54, PET122 and PET494 are specific activators of *COX3* mRNA translation. They interact with small-subunit MRPs, but they are not, in themselves, constituents of the small ribosomal subunit (for a review, see [73]). This is also true for the PET127 protein. A mutation of PET127 suppresses *PET122* mutations by changing the accuracy of translational initiation [55]. Deletion of *PET127* leads to a stabilization of unstable mutant mt mRNAs of *COX2* and *COX3* [74]. However, the observed effect is not linked to the mt translation itself. PET127p, which is located at the mt inner membrane, seems to be a general effector of mt mRNA processing and mRNA stability rather than a true MRP [74].

So far, 36 and 13 genes have been unambiguously identified as coding for yeast MRPs of the large and small ribosomal subunits respectively. Approx. 50 proteins from the large subunits and 35 proteins from the small subunit have been detected by biochemical methods. It is therefore probable that the number of identified yeast MRP genes will increase in the near future.

At this point it is appropriate to discuss the nomenclature for MRPs used in this review and in several original articles. Owing to the involvement of different laboratories, some confusion has arisen concerning the use of the term 'MRP' for protein and gene names; 'YMR', 'PET' and 'NAM' are even more confusing. Recently T. Mason and B. Baum suggested 'RML*xx*' and 'RMS*yy*' for **R**ibosomal **M**itochondrial protein **L**arge or **S**mall subunit respectively, with the number *xx* or *yy* indicating the related eubacterial counterpart [46]. However, this nomenclature ignores the majority of MRPs which are not related to any eubacterial r-protein. This nomenclature is also unable to describe an MRP that shows sequence similarity to *two* different eubacterial r-proteins (YmL8, YmL33; see Table 1). It is further confusing if an mt *large* subunit r-protein shows sequence similarity to a *small* subunit r-protein from another organism (YmL40; see Table 1). Accordingly we prefer the term YmL*xx* or YmS*yy* for **Y**east **m**itochondrial ribosomal **L**arge or **S**mall subunit protein respectively, for the mt r-*proteins* according to a 2D-PAGE map which identifies an MRP unambiguously. The genes are termed *MRP-Lxx* or *MRP-Sxx*, corresponding to the L*xx* or S*xx* numbering of the protein name. This nomenclature includes all MRPs, regardless as to whether any related r-proteins in other classes of organisms exist.

BIOCHEMICAL PROPERTIES OF MRPs

MPRs, although a distinct class of r-proteins, are in general similar to other r-proteins. MRPs are relatively small, their molecular masses varying from 60 kDa to less than 10 kDa, with a statistical average size of 25 kDa [5]. This is comparable with the yeast cytoplasmic r-proteins, which are of similar molecular mass [23]. The data obtained by 2D PAGE are in good agreement with the results of 1D SDS/PAGE and the molecular masses deduced from cloned MRP genes (Tables 1 and 3). r-Proteins are assumed to be more or less basic, owing to their interactions with the rRNA molecules. However, MRPs are less basic than their cytoplasmic counterparts from the same organism [10,75]. This reflects the larger proportion of mt r-proteins, which are involved less in protein–RNA and more in protein–protein interactions. The calculated pI values are given in Table 1 and show the yeast MRPs to be slightly but significantly basic. Only YmL4 and YmS2 have pI lower than 9.0. From the 2D-PAGE map (Figure 1), YmL13 and YmL17 are supposed to be the 'most acidic' MRPs. Their calculated pI values of 9.01 and 9.32 respectively

are consistent with this proposal in comparison with the other identified MRPs. On the other hand it should be noted here that the 2D-PAGE system used did not separate highly acidic r-proteins [11].

MRPs which are imported into the mitochondria should contain some peptide signal information for mitochondrial delivery. The multi-step process of mt protein import and the properties of the proteins to be imported have been eludicated in detail during recent years [76–78]. One might assume that a group of proteins, present in mitochondria in stoichiometric amounts and assembled into one multi-subunit RNA–protein complex, would have similar properties with regard to the signalling of their own mt import. However, yeast MRPs show a great diversity; many have typical cleavable signal sequences, whereas others lack these altogether (Tables 1 and 2). Both the longest and the shortest signal sequences have been found among the signal sequences identified. When signal peptides are cleaved off, they do not follow a common import and cleavage mechanism. Some MRPs seem to be processed solely by the mt matrix protease (MMP), but others show a two-step cleavage mechanism which also involves mitochondrial intermediate peptidase (MIP) [79]. For MRPS28p a two-step mechanism has been demonstrated in detail [56,71]. All different classes of substrates for MPP and MIP have been found, namely R-2, R-3, R-10, as well as R-none, according to [71] (see Table 2). It is striking that most of the longest signal peptides determined so far (longer than 30 amino acid residues) belong to the R-no cleavage class (Table 2).

Although true cleavable signal sequences can be assigned, they might not be necessary for proper import at all. YmL20 possesses a cleavable signal peptide of 18 amino acid residues [28]. The protein containing this N-terminal peptide is transported properly into mitochondria *in vitro* [80]. In a fusion protein this signal peptide directs Chinese-hamster dehydrofolate reductase (DHFR) into mitochondria. At the same time, YmL20, lacking the N-terminal signal peptide, is also properly imported [80]. YmL8 has no cleavable signal peptide, and only the initiator methionine is cleaved off post-translationally [28]. It seems likely that the peptide information for mt import may also be hidden in the mature protein. Deletion mutants lacking the N-terminal 40 amino acid residues and/or 52 amino acids of the C-terminus are properly imported [80]. The N-terminal 86 amino acids of YmL8 direct DHFR into the mitochondria, whereas the C-terminal 52 amino acid residues do not. Sufficient import information must thus be localized between amino acids 41 and 86, since this peptide directs both the truncated YmL8 as well as the YmL8–DHFR hybrid protein into mitochondria [80]. Similar features might be expected of other MRPs lacking a cleavable signal peptide (Table 2).

Other proteolytic modifications are still a matter of discussion, since at least some of them seem to be linked to proteolytic artifacts during protein-purification procedures. YmL11 has been isolated in two forms differing by four amino acid residues at the N-terminus [24]. In four cases, two proteins could be identified that were being translated from the same gene but which differed in size by at least 10 kDa (Table 1). It has not yet been proved whether these are two forms of an individual protein generated by different post-translational modifications or whether they are artifacts caused by harsh preparation methods, as has been proposed (for example) for the YmL17/YmL30 protein pair [13]. In contrast, the variant forms of the var1 protein depend on different alleles of the mt *var1* gene. By multiple insertions of short in-frame nucleotide elements, multiple allelic forms differing in up to 26 amino acid residues can be formed [64]. In heterozygous crosses, non-parental progeny arise, shifting the shorter alleles to the longer ones [67,68]. The mechanism for these genetic modifications is not yet clear; neither a crossing-over nor a mechanism similar to intron splicing is involved [68]. No mt translational product is necessary for this reaction [67].

Detailed analyses of biochemical properties of MRPs are rare, and most properties have been deduced from amino acid sequences. An example is YmL4, where a proline-rich N-terminal region, a putative nuclear localization signal and four hydrophobic C-terminal domains have been reported [27]. The latter have been speculated to be responsible for the hydrophobic features of YmL4, since the protein is the last to be eluted on reversed-phase HPLC under acidic conditions [11].

FUNCTIONS OF MRPs

The function of mt ribosomes is the biosynthesis of a very small number of proteins encoded by the mt DNA. So why does the mt ribosome contain so many more proteins than, for example, the *E. coli* ribosome, which translates thousands of different messages? Additionally, translation and other factors specific for individual mt mRNA species provide more-or-less proper processing, translation and release of these few mt transcripts [20,21,74]. The fundamental process of protein biosynthesis is thus manifested in mitochondria in a variation which is much less well understood than the translational process on eubacterial or eukaryotic ribosomes in general. For many of the factors and MRPs there are no homologous counterparts in cytoplasmic ribosomes. The eludication of the functions of the diverse proteins specific for mt translation remains a lengthy and difficult process which is only at its beginning. So far, several non-ribosomal factors influencing mt protein biosynthesis have been characterized (for a review, see [20]), but direct links to the functions of MRPs have been studied only rudimentarily [49,50]. It has been suggested that mt ribosomes are more-or-less associated with the inner side of the mt inner membrane [20,21]. This makes reasonable sense, since specific translational factors are located on the mt inner membrane [20]. Most mt translational products are hydrophobic inner-membrane proteins. On the other hand, mt ribosomes can be isolated in the same way as classical ribosomes on sucrose gradients, without any remnants of membrane lipids attached to them. However, so long as the determination of functional links between MRPs, other relevant factors, the mt inner membrane and mRNAs remains nebulous, we will not be able to give a comprehensive model of the very specific way in which mitochondria have kept this part of their ancient independent metabolism alive.

Ribosomes of all species contain a core of conserved r-proteins [81,82]. Owing to the principal functional steps of the protein biosynthetic pathway, these represent a 'minimal ribosome', conserved in structure and function. Additional non-conserved r-proteins are considered to maintain functions specific for the respective species or organelle that might be dispensable for the general biosynthetic process. So far more MRPs have been described that are not homologous with known r-proteins than those which do have counterparts in yeast cytoplasmic ribosomes or in *E. coli*. Of the 50 MRPs whose amino acid sequences have been determined completely (excluding protein pairs), only 21 show significant sequence similarities to *E. coli* r-proteins (Table 1). Most of the MRPs are considerably larger than their eubacterial counterparts containing N-terminal and/or C-terminal extensions, as well as insertions relative to the corresponding *E. coli* proteins. A total of 29 MRPs are not similar to any of the *E. coli* or yeast cytoplasmic r-proteins (Table 1). Thus it is not possible to deduce functions for these proteins by comparison with their well-studied bacterial counterparts. The uniqueness of these MRPs does not imply that they are dis-

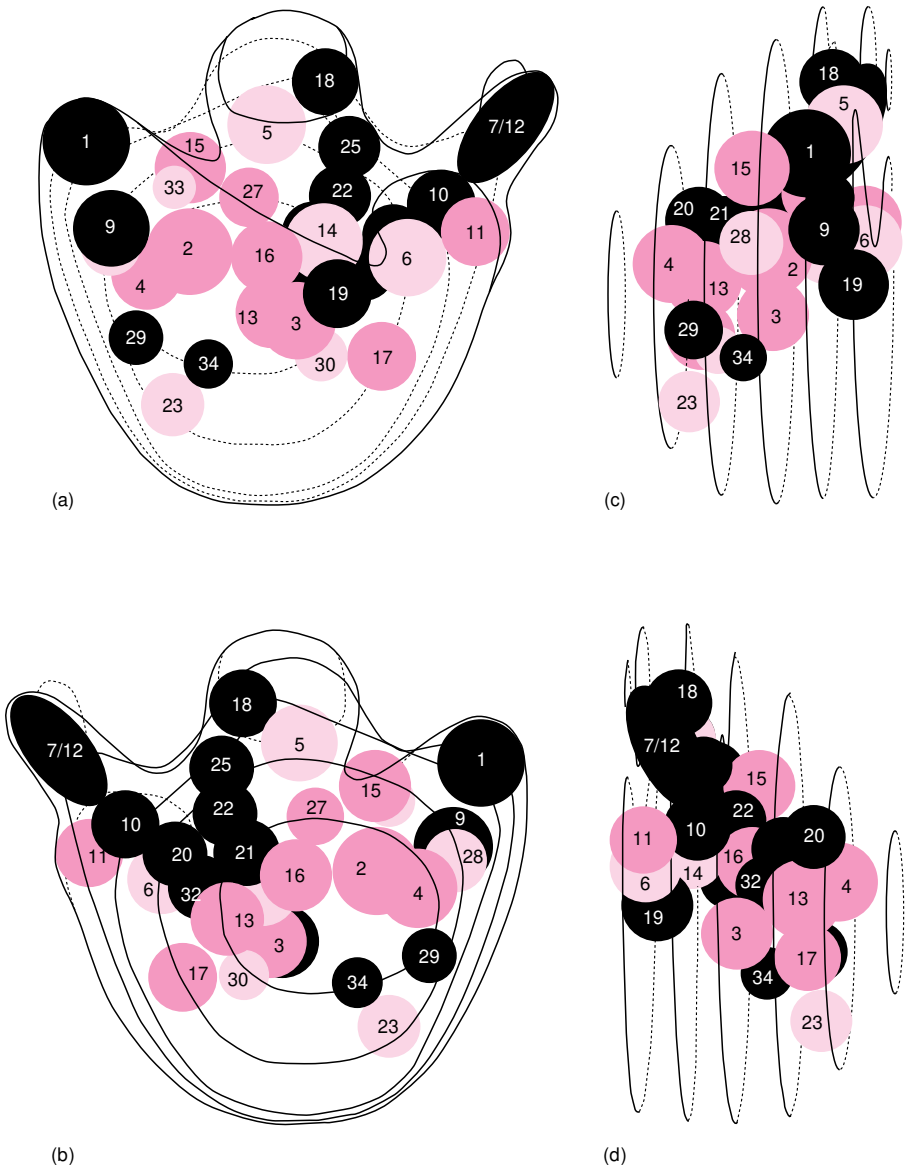

Figure 2 Protein locations of *E. coli* r-proteins in the large ribosomal subunit

Proteins are represented by circles and are numbered according to the *E. coli* ribosome [86]. Proteins in light pink have similar counterparts in the yeast mitochondrial ribosome; proteins labelled in dark pink have similar counterparts in the yeast mitoribosome and belong to the minimal PTF activity [84]. (**a**) Front view; (**b**) back view; (**c**) lateral view, from the right; (**d**) lateral view, from the left.

pensable for mt ribosomal function. Out of 25 MRPs which have been tested by gene disruption or other mutations, only three are dispensable for mitochondrial function. The remaining 22 are essential, regardless as to whether they are similar to *E. coli* r-proteins or not. Mitochondria seem to be more rigorous in their dependence on individual proteins, since even MRPs whose bacterial counterparts seem not to be essential for bacterial ribosomal function are indispensable in yeast mitochondria (YmL2 versus EcoL27 [83] and YmL33 versus EcoL30 [39]). On the other hand, the dispensable MRPs are all unique to mitochondria (YmL13, MRP13 and MRP49; Table 1).

The peptidyltransferase (PTF) activity is one of the best-characterized domains of the ribosome. About 16 out of the 34 large-subunit r-proteins have been implicated in PTF activity in *E. coli* [84]. The MRP counterparts have been identified for many of these *E. coli* proteins (Figure 2; see also [46] for a review). However, little is known about the function of the MRPs from direct experimental data. The functional domain structure of YmL2 (MRP7p) has been studied intensively [44,46]. The N-terminus of YmL2 shows strong sequence similarity to EcoL27, and thus a function in the PTF activity can be assumed (Figure 2). It was shown by several deletion and insertion mutants that YmL2 is rather tolerant against sequence variation. In *mrp7* mutants YmL2 can be replaced by the corresponding MRP7p from *Kluyveromyces lactis*. Large deletions within the N-terminus as well as deletion of the C-terminal part of YmL2, cause *pet*$^-$ mutants. This is astonishing, since EcoL27 is not absolutely indispensable for ribosomal function [46,83,84]. Smaller deletions

in the C-terminal part of YmL2 cause several types of cold- or heat-sensitive phenotypes. Although a *trans* expression of both the N-terminal peptide (amino acid residues 1–85) and the MRP-specific C-terminal region (amino acid residues 86–344) does not restore YmL2 function, both peptides could be localized on large mitoribosomal subunits, thus showing independent binding sites for contact with other mitoribosomal constituents [44]. EcoL27 is a constituent of the central protuberance of the large ribosomal subunit (Figure 2), but it does not bind 23 S RNA. In complete ribosomes EcoL27 has been cross-linked to EcoS9 by chemical reagents, suggesting a close proximity of these proteins.

Introduction of small in-frame deletions into the MRP counterpart of EcoL2 (Table 1) has revealed the necessity of the deleted amino acid residues (nos. 336–342) for mt function. A single point mutation of the neighbouring His^{343} does not affect mt protein biosynthesis [46]. This is astonishing, since His^{343} is the MRP equivalent of a histidine residue that is absolutely conserved among the members of the L2 r-protein family, which has been shown to be directly involved in PTF activity [87]. Another important protein for the PTF is L3. The counterparts of EcoL3 from other sources, including the MRP YmL9, are mostly similar to one another in their C-termini [30], suggesting a functional importance of this domain. L3 has been chemically cross-linked to L19, whose counterpart in the mt ribosome has not yet been identified [88].

Rat mt ribosomal subunits form particles similar to bacterial ribosomal subunits [89], as known from electron-microscopic studies. Similar functions in assembly by similar constituents of the mt ribosomal subunits can be anticipated. The *E. coli* r-proteins L3, L4, L13, L20 and L24 are prominent early-assembly proteins essential for correct formation of the large ribosomal subunit (Figure 3b; [82]). A similar way of assembly may be assumed for their MRP counterparts, namely YmL9, YmL6 and YmL23 respectively. YmL47, the product of the *RML16* gene, is similar to EcoL16. Although EcoL16 is one of the minimal set of PTF r-proteins under certain conditions, *E. coli* ribosomes lacking EcoL16 synthesize polyphenylalanine from poly(U) artificial mRNA. Thus the function of EcoL16 seems to have more of a structural than a catalytic nature [43].

YmL41/MRP20 is similar to EcoL23 and to its yeast cytoplasmic counterpart, L25. The latter two are primary RNA-binding proteins that act early in subunit assembly (Figure 3a), recognizing similar regions within the 23 S rRNA. mt 21 S rRNA contains corresponding structures which might bind to YmL41/MRP20. The yeast cytoplasmic L25 is not dispensable, whereas for EcoL23 this has not been clearly determined. The L23 equivalent of *Haloarcula marismortui* has been chemically cross-linked to L29 [90], the MRP counterpart of which has not yet been identified. Another rRNA-binding protein which is important for assembly of the large ribosomal subunit is EcoL17, whose MRP counterpart is YmL8 (Figure 3a).

Although fewer MRPs of the small subunit have been characterized, much more is known about their structure and function as a result of intensive mutational analysis. The var1 protein is known to function in assembly of the small ribosomal subunit [2]. In the presence of inhibitors of mt protein synthesis, 30 S particles instead of 37 S subunits accumulate as a result of the lack of mitochondrially synthesized var1 [91]. Without var1, three to five proteins are not assembled into the small ribosomal subunit, suggesting that var1 acts in a late step of the assembly. MRP2 is one of the MRPs missing in var1-depleted small subunits [17,51,92], whereas proteins PET123, MRP1, MRP4 and MRPS28p are not affected [51]. However, no significant sequence similarities between var1 and any other cytoplasmic r-protein have been detected. It is interesting to note that, in some organisms, var1-like MRPs have been detected encoded on the mt DNA (*Williopsis mrakii* [93]; *Torulopsis glabrata* [94]) or in the nucleus (*Coprinus cinereus* [95]). In contrast, in *Neurospora crassa*, a single MRP has been shown to be encoded by mt DNA which is analogous to var1 in function, but is not similar in sequence [2,51,96].

The *E. coli* counterpart EcoS15 of MRPS28p acts early in the assembly of the small ribosomal subunit (Figure 3). MRPS28p faciliates the binding of other MRPs to the small mt ribosomal subunit [97], mirroring the function of EcoS15; it might therefore bind to the universal helix 22 of the 15 S RNA. The protein domain structure of MRPS28p has been studied in detail. The mature MRPS28p consists of an N-terminal peptide of 117 amino acids, a central region of 89 residues similar to EcoS15, and a C-terminal extension of 48 amino acids which are dispensable for function [97]. Both the N-terminal domain and the EcoS15-related central peptide region are necessary for maintaining proper mt function. If both peptides are supplied in *trans*, they mutually facilitate incorporation of each other into the small subunit and are able to restore ribosomal function [97]. The central region of MRPS28p can replace EcoS15 in active *E. coli* ribosomes, albeit less efficiently, and the N-terminal peptide of MRPS28p, which is unique to the mt r-protein, enhances the replacement of the central peptide in functional *E. coli* ribosomes [98].

The counterpart of EcoS2, MRP4, seems not to be necessary for ribosomal subunit assembly. EcoS2 has been suggested to be involved in tRNA binding [52]. MRP4 and other members of the S2 family show a significant sequence similarity to the mammalian 68 kDa high-affinity laminin receptor. The functional relevance of this observation is not clear [52].

NAM9 belongs to the EcoS4 family. As with most MRPs, the degree of sequence similarity of NAM9 to its eubacterial and chloroplast counterparts is higher than that to its yeast cytoplasmic counterpart, S13. Since EcoS4 is involved in the control of translational fidelity, a similar function can be suggested for NAM9. The *nam9-1* mutation, which suppresses specific mutant *ochre* codons in mt mRNA derived from a single base substitution, supports this assumption [57,58].

Truncated mutations of the COX3-specific translational activator PET122 are suppressed by mutated PET123 and/or MRP1 [61]. Mutants of MRP1 are not able to by-pass the complete PET122 function or to rescue total deletions of PET122. The MRP1 mutants themselves cause no intrinsic mutant phenotype in haploid cells. The PET122 truncation suppressing PET123 mutation causes intrinsic heat-sensitive respiration in haploid cells. If both MRP1 and PET123 mutations are combined in a haploid, the resultant cells are respiratory-deficient at all temperatures, thus suggesting a functional interaction between PET123 and MRP1 [59]. Specific C-terminal truncations of PET122 can also be suppressed by MRP17 and PET123 [55]. Since PET122 has been shown to associate with the mt inner membrane, a close functional proximity of all three MRPs to the membrane can be proposed, in agreement with the observation that mt ribosomes are localized on the inner surface of the mt inner membrane [21,22]. In *N. crassa* an MRP has been identified (MRP3) that is associated with the mt inner membrane as well as with the small subunit of the ribososome [72]. The amount of MRP3 within the mt inner membrane exceeds that bound to ribosomes by a factor of 50, although the functional relevance of this observation is not clear. Astonishingly, a computer search has identified sequence similarity between MRP3 and the C-terminal domain of the dihydrolipoamide acetyltransferase subunit (E2p) of the pyruvate dehydrogenase complex of *E. coli*. *N. crassa* MRP3 is closely related to the E2p subunit from yeast,

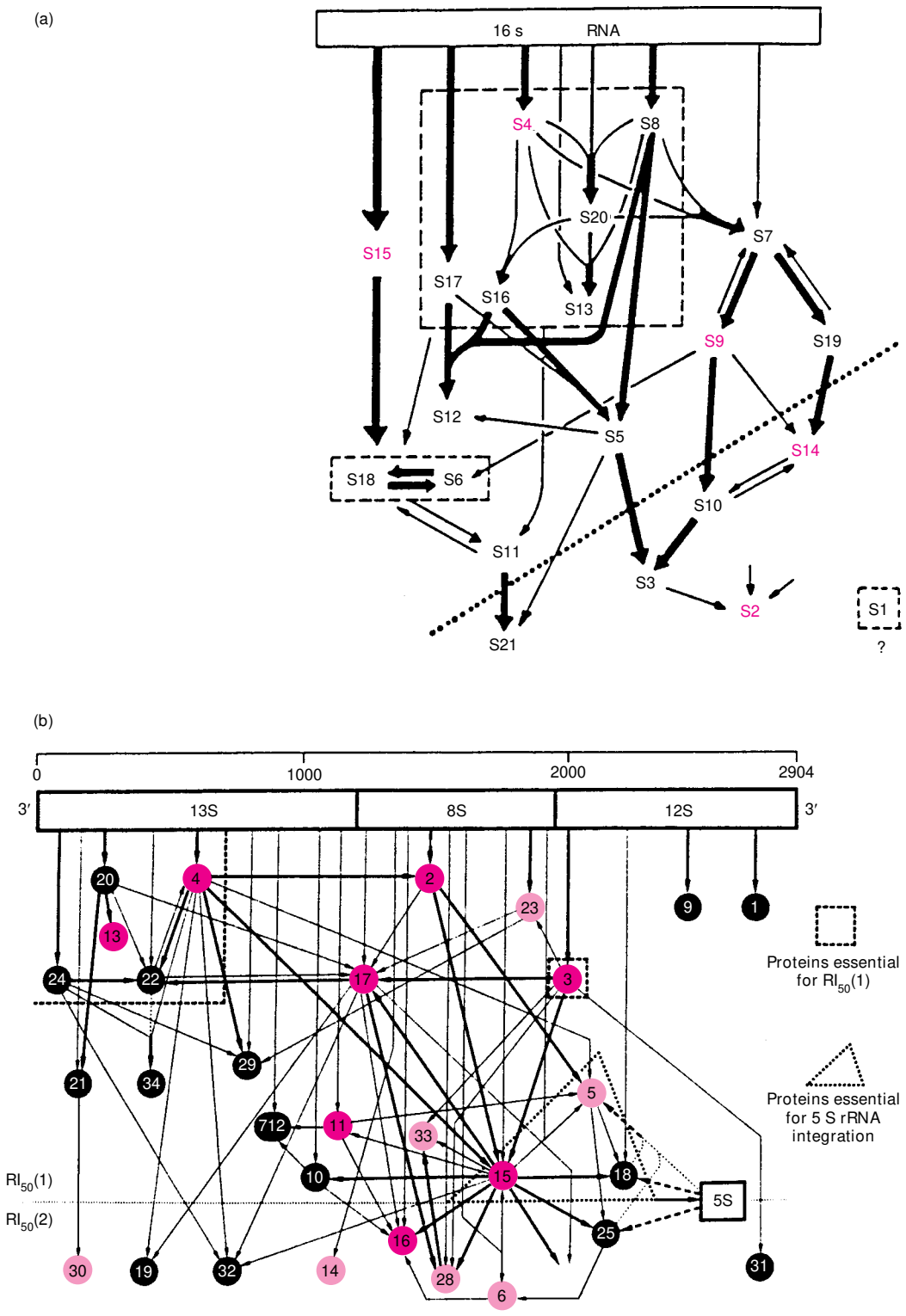

Figure 3 Assembly map of the *E. coli* ribosomal subunits

Shown are the numbers of the proteins in *E. coli*. Those in red indicate that similar yeast MRPs have been identified. (**a**) Assembly map of the *E. coli* small ribosomal subunit, as taken from [81]. (**b**) Assembly map of the *E. coli* large ribosomal subunit as given in [82]. Proteins of which similar proteins in yeast mitoribosomes have been found, and which belong to the minimal PTF activity according to [84] are labelled in red. Proteins are coloured pink if similar proteins in yeast mitoribosomes have been found. Reproduced from [81,82] with the permission of the authors and the American Society for Biochemistry and Molecular Biology.

showing 54 % identical residues [99]. A protein immunologically related to MRP3 has been detected in yeast mt ribosomal and in membrane fractions, although the corresponding gene has not been identified [72].

Little attention has been paid to clarifying the binding of MRPs to antibiotics, whereas this was studied intensively on prokaryotic ribosomes [100,101]. For instance YmL2/MRP7p and MRP2 can be assumed to bind spiramycin in a similar manner to their *E. coli* counterparts, EcoL27 and EcoS14 respectively [86]. The synthesis of var1 and all other mt translational products is inhibited up to 99 % by erythromycin [91]. Chloramphenicol also affects mt protein biosynthesis, although the translational inhibition of various mRNAs varies.

The genetic interactions from the nucleus to the mitochondria have been well studied [16,73,102]. In recent years the first evidence has been obtained for a molecular communication *from* the mitochondria *to* the nucleus. A putative feedback mechanism concerning the response of the mitochondria to nuclear information or molecular signals was proposed, and several pathways of mitochondrial signalling were suggested, e.g. by the direct transfer of proteins or RNA from the mitochondria to the cytoplasm, or by nuclear effects based on metabolic events in the mitochondria. However, the data presently available do not allow a simple mechanism to be deduced. A possible mt translation process, e.g. via MRPs, may be involved. Since cytoplasmic r-proteins often fulfil additional non-ribosomal functions (for a review, see [103]), hypothetical bifunctional properties may be proposed for MRPs as well. MRP3 of *N. crassa* and yeast are examples of a bilocalized activity [72]. Other MRPs which are not dispensable for yeast growing on *fermentable* carbon sources thus imply that there is a second, cytoplasmically derived, function, as shown by gene disruption of nuclear MRP genes. If *MRP-L8* is disrupted by an insertion in the 3′ untranslated region, growth on glucose media is hampered in haploid strains [28]. Gene disruption of *MRP-S9* also causes decreased growth on glucose and fructose, and the growth on maltose and galactose is even slower [53]. Under de-repressing conditions, no activity of cytoplasmic gluconeogenetic enzymes such as fructose-1,6-bisphosphatase or phosphoenolpyruvate carboxykinase is detected [53]. More striking are the effects of a gene disruption of *MRP-L4*. Cells grow extremely slowly on glucose media, and enlarged and elongated cells are observed in the exponential phase of growth. In the stationary phase, growing cells contain one or two gigantic vacuoles [27]. Interestingly, for YmL4 a putative nuclear localization signal, as well as a proline-rich region, have been described [27]. The latter motif is a common feature of transcription-enhancing factors [104].

In *N. crassa* the mRNA level of the nuclear-encoded MRP Cyt-21 increases fivefold if mt protein biosynthesis is inhibited by chloramphenicol [9]. This result gives a direct hint that mitochondria 'report' to the nucleus if a nuclear gene product is required. The authors of [9] discuss whether a protein message is sent from the mitochondria to the nucleus or whether the nuclear gene-expression machinery is triggered by the impaired mt metabolic state [9]. If the latter were to be the case, any gene disruption targeting an mt housekeeping nuclear gene should give rise to such a nuclear answer. This has been shown for the expression of the yeast *CIT2* gene, encoding a peroxisomal isoform of citrate synthase. If the mt metabolic state is being altered, e.g. in ρ^0 cells, transcription of *CIT2* is increased 6–30-fold, whereas the expression of the mt isoform of citrate synthase is unaffected [105]. This retrograde regulation is mediated by two proteins, RTG1 and RTG2, the first of which is a putative basic helix–loop–helix transcription factor that binds to the promotor of *CIT2* recognizing a UAS_r (upstream activation site element) [105]. Neither factor is essential for viability or respiratory competence, but cells lacking RTG1 or RTG2 become auxotrophic for glutamic or aspartic acid and cannot use acetate as a sole carbon source. This suggests that both the tricarboxylic acid and the glyoxylate cycle are affected, thus pinpointing a communication mechanism between mitochondria, peroxisomes and nucleus [105].

Other cases of mitochondrial–cytoplasmic/nuclear interaction are much less well understood. A mt translation initiation codon mutation in the *COX3* mRNA is partially suppressed by a spontaneous nuclear mutation. This mutation causes cold-sensitivity when the organism grows on fermentable carbon sources and was proved to affect *RPS18A*, one of the two copies of the yeast cytoplasmic S18 r-protein gene. Yeast S18 is the counterpart to EcoS17 and human S11. EcoS17 is involved in providing translational fidelity and is essential for the small-ribosomal-subunit assembly (Figure 3a). In the *RPS18A* mutant the functional activity of the cytoplasmic small ribosomal subunit is reduced. This decrease in *cytoplasmic* small ribosomal subunit activity suppresses an *mt* initiation codon mutation [106]. A comparable phenomenon has been observed in *Podospora anserina*. A mutation in a cytoplasmic r-protein (S12) causes premature senescence of growing filaments by site-specific deletions of mt DNA. However, the cytoplasmic r-protein is not detected immunologically in mitochondria, but rather a protein of higher molecular mass can be identified by antibodies in the mitochondria, putatively the corresponding S12 MRP. The pathway of early mt DNA deletion corresponding to the cytoplasmic S12 r-protein is completely unknown [107]. Both cases describe effects, caused by alterations in cytoplasmic ribosomal function, that seem to affect mt protein biosynthesis by altering translational fidelity.

MRP GENES: THEIR EXPRESSION AND REGULATION

Assembly of functionally active mt ribosomes depends on the coordinated expression of mitochondrially localized and nuclear genes. Investigation of expression of nuclear and mt MRP genes has revealed different mechanisms of expression control; hence a general mechanism involved in the stoichiometric production of mt ribosomal constitutents cannot be postulated.

var1 is transcribed as a long polycistronic mRNA, which also spans the ATPase9 (*oli*1) and the $tRNA^{Ser}_{UCN}$ genes [108]. The long untranslated 5′ region of *var1* is unusual compared with mammalian mt mRNAs, which are compactly organized on mt DNA, leaving almost no gaps of untranslated sequences [109]. The expression of *var1* is regulated at the level of polycistronic mRNA processing and by its turnover rate [108]. In addition to the variation in the genetic code mentioned above (ATA codes for Met) TGA codes for Trp and CTX codes for Ser in the *var1* mRNA. These codons are similarly translated in human, bovine and murine mitochondria.

Compared with the *var1* gene the nuclear-localized genes for all other MRPs are quite normal. They are scattered around the genome (Table 1), leaving no relict of an ancient bacterial polycistronic genetic organization. No MRP genes are clustered adjacent to each other on the chromosome, with the exception of the MRP13 gene, which occurs together with the cytoplasmic r-protein gene, rp39A [54]. MRP genes are present only once in the haploid yeast genome. This is in contrast with genes for cytoplasmic r-proteins, which often appear in at least two copies differing slightly from one another [110]. The codon usage of MRP genes is similar to that of other weakly expressed yeast genes [111], but can be distinguished significantly from them [112]. Only very few genes contain introns (Table 1), which again

Table 4 Expression of MRP genes under different metabolic and genetic conditions

Expression of MRP genes is listed schematically. mt genetic status: ρ^+, fully intact mitochondria; ρ^0, mt DNA missing, no mt respiration and protein synthesis; ρ^-, mt DNA partially deleted, but the respective mt rRNAs (15 S for small subunit MRPs, 21 S for large subunit MRPs) are still retained; conditions of expression: a, glucose repression; b, glucose de-repression; c, overexpression by transformation with multicopy plasmid containing the respective MRP gene; RNA, MRP mRNA level; Prot., MRP protein level; ⇑, level is elevated, strongly (⇑ ⇑); ⇒, level remains stable; ⇓, level is decreased.

mt genetic status ...	ρ^+						ρ^0						ρ^-				
Expression conditions ...	a		b		c		a		b		c		a		b		
MRP	RNA	Prot.	RNA	Prot.	RNA	Prot.	RNA	Prot.	RNA	Prot.	RNA	Prot.	RNA	Prot.	RNA	Prot.	Ref.
YmL2/MRP7	⇒	9 × ⇓			20 × ⇑	⇒	⇒	9 × ⇓	⇒	⇑							[25]
YmL13	⇓		⇑														[31]
YmL16/MRP-L6p	⇓		2 × ⇑														[32]
YmL25/YMR26	⇓		⇑		10 × ⇑	⇑		⇑		10 × ⇑							[34]
YmL33	⇒		⇑				⇑		⇑								[39]
YmL41/MRP20	⇓	⇓	⇑	⇑ ⇑			⇑	⇓	⇑	⇓			2 × ⇑	2 × ⇑	20 × ⇑	20 × ⇑	[41]
YmL47/RML16p	⇓	⇓	⇑	⇑			⇒	⇓	⇑	⇓			⇓	⇓	⇑	⇑	[43]
MRP49	⇓	⇓	⇑	⇑			⇑	⇓	⇑	⇓			⇓	⇓	⇑	⇑	[41]
MRP1	⇓		⇑		100 × ⇑	55 × ⇑		⇒									[49]
MRP2	⇓		⇑				⇒	⇓									[49]
MRP13	⇓	⇓	⇑		⇑	⇑	⇑	⇓	⇑	⇓			⇑	⇑			[54]
PET123								⇓						⇓			[60]

is in contrast with the yeast cytoplasmic r-protein genes, where introns are relatively common [110]. Nearly all MRP genes show the common features for expression of yeast genes, such as TATA boxes, correct nucleotide environments of the initiation codon and transcription termination signals, as well as polyadenylation signals. Some putative binding sites for expression factors have been determined by sequence comparison, but none has been proven by a detailed study. Perfect binding sites for HAP2/HAP3 transcription factors have been identified in the 5′ untranslated region of *MRP-L4* [27], *MRP-L13* [31] and *MRP2* [41]. If a single mismatch is tolerated, binding might be possible also in the promoter regions of *MRP7*, *MRP13*, *YMR26*, *MRP20*, and *MRP49* [41]. As mentioned before, the presence or depletion of different *cis* signals may reflect different ways of regulating expression, as summarized in Table 4. MRP expression depends mainly on glucose repression and de-repression, which decreases or elevates mRNA and protein levels respectively, although the levels of change of mRNA and/or protein are different (Table 4). YmL2/*MRP7* has a unique feature, since the mRNA level remains stable under glucose repression, whereas the amount of protein decreases 9-fold [25]. Under de-repressing conditions the mRNA level is increased severalfold, depending on the MRP gene and the de-repressing medium. Interest has focused on the co-ordinated expression, e.g. the fate of MRPs in the ribosomal-assembly process. In the expression of yeast cytoplasmic r-proteins the most efficient and rigid regulation concerns the rapid degradation of r-proteins that are not assembled into ribosomal subunits [110]. This mechanism is also found with MRPs, but is not of general validity. MRP2, MRP13, PET123, YmL41/MRP20, YmL47/RML16p and MRP49 are unstable if no mt rRNA is accessible in ρ^0 cells (Table 4). In contrast YmL2/MRP7 and MRP1 remain unaffected in ρ^0 cells. If MRPs are over-expressed by the introduction of multicopy vectors bearing additional MRP genes into yeast cells, the YmL2/MRP7 level remains stable as well, although the level of *MRP7* transcript is increased 20-fold. In contrast, other MRPs which are unstable in ρ^0 strains, show elevated levels of mRNA and protein in this case (MRP1, MRP13, YmL25/YMR26). This mechanism also differs from the situation with yeast cytoplasmic r-proteins, where additional gene copies are compensated for by an excess turnover of additional mRNA and/or additional protein [110].

In general, two main mechanisms of MRP expression regulation can be observed. (i) MRP genes are subjected to catabolite repression and de-repression at both the mRNA and protein level. Although no detailed study has been performed on common promoter structures of MRP genes or binding of *trans* factors, the above mechanism can be postulated summarizing the studies on expression control of individual MRP genes (Table 4). Exceptions (the differing YmL2/MRP7 expression) might reflect putative multiple functions of MRPs. (ii) Independently of catabolite-controlled expression, proteolytic mechanisms putatively localized in mitochondria prevent the accumulation of large amounts of MRPs that are not assembled into functional ribosomal subunits by rapid degradation of free proteins. Exceptions are also known for this mechanism (see Table 4).

EVOLUTIONARY ASPECTS

Today, ancient evolutionary events cannot be reconstructed, but they can be deduced from 'molecular fossils' preserved in living organisms. Ribosomal proteins fulfill the three basic conditions which serve as a basis for phylogenetic considerations, namely their ubiquitous occurrence, moderate but significant differences between species, and the presence of sufficient mutatable positions.

The evolution of MRPs includes at least two major events: first, the endosymbiotic event which obviously took place very early in living history, and, secondly, the successive transfer of the MRP genes from the mt DNA to the nucleus. This process can be elucidated in a stepwise manner today, since the genes of specific MRPs show all the different stages of a successful gene transfer from mitochondria to the nucleus in different plant species [113]. The adoption of a new signal peptide for re-

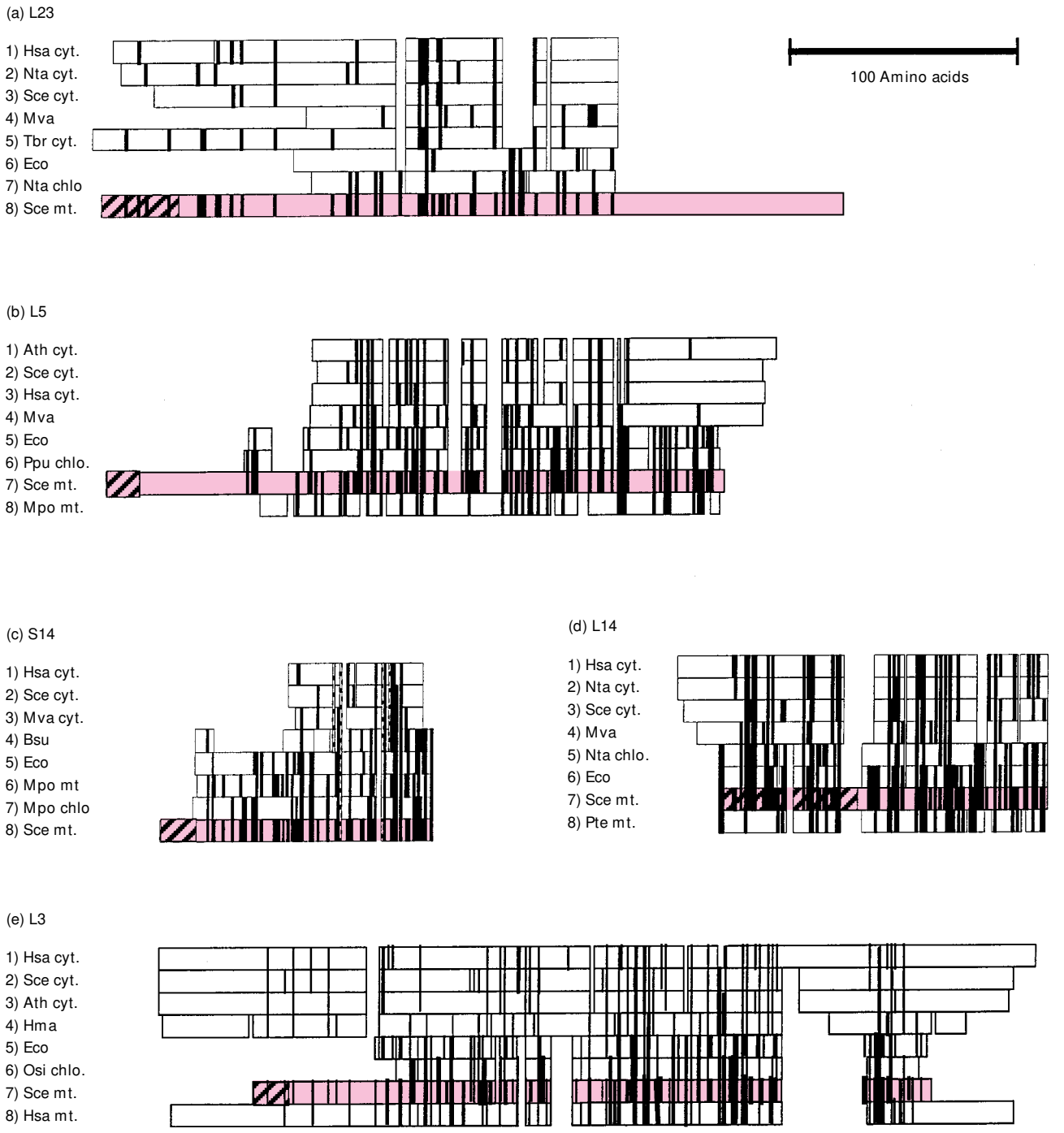

Figure 4 Schematic sequence alignment of ribosomal proteins

r-Protein sequences from various sources were aligned by using the PILEUP program [63]. Accession numbers are from the SWISSPROT databank. Sequence extensions are given in boxes, and gaps are introduced for the best alignment if necessary. The respective yeast MRPs are highlighted in pink, and signal peptides are hatched. Amino acid residues in other r-proteins identical with the MRP sequence are given as black lines or boxes. (**a**) EcoL23 r-protein family: (1) *Homo sapiens* cytoplasmic (sw:rl2b-human); (2) *Nicotiana tabacum* (tobacco plant) cytoplasmic (sw:rl2b-tobac); (3) *S. cerevisiae* cytoplasmic (sw:rl25-yeast); (4) *Methanococcus vannielii* (sw:rL23-metva); (5) *Trypanosoma brucei* cytoplasmic (sw:s41653); (6) *E. coli* (sw:rl23-ecoli); (7) *N. tabacum* chloroplast (sw:rk23-tobac); (8) *S. cerevisiae* mitochondrial YmL41/MRP20 (sw:rm41-yeast). (**b**) EcoL5 r-protein family: (1) *Arabidopsis thaliana* (thale cress) cytoplasmic (sw:rl11-arath); (2) *S. cerevisiae* cytoplasmic (sw:rl16-yeast); (3) *H. sapiens* cytoplasmic (sw:rL11-human); (4) *M. vannielii* (sw:rl5-metva); (5) *E. coli* (sw:rl5-ecoli); (6) *Porphyra purpurea* chloroplast (sw:rk5-porpu); (7) *S. cerevisiae* mitochondrial YmL7 (sw:rm07-yeast); (8) *Marchantia polymorpha* mitochondrion (sw:rm05-marpo). (**c**) EcoS14 r-protein family: conserved cysteine residues of a putative zinc-finger motif are dotted. (1) *H. sapiens* cytoplasmic (sw:rs29-human); (2) *S. cerevisiae* cytoplasmic (sw:rs29a-yeast); (3) *Methanococcus vannielii* (sw:rs14-metva); (4) *Bacillus subtilis* (sw:rs14-bacsu); (5) *E. coli* (sw:rs14-ecoli); (6) *M. polymorpha* mitochondrial (sw:rr14-marpo); (7) *M. polymorpha* chloroplast (sw:rt14-marpo); (8) *S. cerevisiae* mitochondrial MRP2 (sw:rt02-yeast). (**d**) EcoL14 r-protein family: (1) *H. sapiens* cytoplasmic (sw:rl17-human); (2) *N. tabacum* cytoplasmic (sw:rl17-tobac); (3) *S. cerevisiae* cytoplasmic (sw:rL1a-yeast); (4) *M. vannielii* (sw:rl14-metva); (5) *N. tabacum* chloroplast (sw:rk14-tobac); (6) *E. coli* (sw:rl14-ecoli); (7) *S. cerevisiae* mitochondrial YmL34/YmL38 (sw:rm38-yeast); the N-terminal sequence of YmL38 which is missing in YmL34 is hatched.; (8) *Paramecium tetraurelia* mitochondrial (sw:rm14-parte). (**e**) EcoL3 r-protein family: (1) *H. sapiens* cytoplasmic (sw:rL3-human); (2) *S. cerevisiae* cytoplasmic (sw:rL3-yeast); (3) *A. thaliana* cytoplasmic (sw:rl3a-arath); (4) *Halobacterium marismortui* (sw:rl3-halma); (5) *E. coli* (sw:rl3-ecoli); (6) *Odontella sinensis* chloroplast (sw:rk3-odosi); (7) *S. cerevisiae* mitochondrial YmL9 (sw:rm09-yeast); (8) *H. sapiens* mitochondrial (sw:rm03-human).

transport of the cytoplasmically synthesized MRP into the mitochondria does not seem to be a major evolutionary transition. The random insertion of a complete coding sequence into nuclear DNA has a good chance of acquiring an N-terminal extension suitable for mt import [114]. Other MRPs which contain effective mitochondrial localization signals within their respective sequences obviously have no need for an additional signal peptide at all. A more severe problem is the translational switch of an MRP gene that has been transfered to an expression system using a slightly altered genetic code. It remains unclear whether the mitochondria were already using an alternative genetic code (and mRNA-editing mechanisms) before the MRP genes left the mt DNA. Comparable studies on plant MRPs encoded by mt or nuclear DNA in different species may help to resolve this problem. The corresponding positions of codons differentially translated in mitochondria and cytoplasm should be analysed for their respective DNA and amino acid compositions.

If r-proteins from different origins, e.g. eubacteria, eukaryotic cytoplasm, mitochondria and chloroplast, are compared, they can be grouped according to their sequence similarity [115] or alternatively by sequence extension. Eukaryotic cytoplasmic r-proteins are commonly elongated as compared with eubacterial r-proteins. MRPs can be even longer than their cytosolic equivalents, showing N- and/or C-terminal extensions. This might reflect additional functions attributible either to extended protein–protein interactions in the mitoribosome or to bifunctionality of the MRPs. Other MRPs are similar in size to eukaryotic cytoplasmic r-proteins, or smaller. How these additional sequences have been acquired during gene transfer remains speculative, although models for the acquisition of sequences by exon shuffling or gene fusion during transfer have been discussed [28]. The latter could provide an explanation in the case of YmL8, which shows significant sequence similarities to EcoL17 *and* EcoS13 [24,28], both r-proteins being encoded within the same operon in *E. coli* [116]. However, this theory cannot explain whether the sequence of a large subunit MRP (YmL40) is related to that of the small subunit r-proteins S4, S7 and S4 of potato, yeast and human respectively [13]. In *Arabidopsis thaliana* the adoption of a ribonucleoprotein-binding domain by the nuclear-encoded *rps19* MRP gene has been shown. This additional function of rps19 replaces rps13 both structurally and functionally in the mitoribosome [117].

Here we present a few examples of aligned r-protein families that are typical for the MRPs and their sequence relationship to other r-protein classes. The families were selected on the basis that the r-protein sequences of (nearly) all the groups from different organellar or cellular origins are available (Figure 4). The EcoL23 r-protein family can be divided into three major groups by length (Figure 4a). The smallest proteins are of bacterial, archaeal and chloroplast origin. The sequence similarities to the yeast MRP YmL41/MRP20 are rather low if only the alignments of identical amino acids are considered. The second group includes the eukaryotic cytoplasmic EcoL23 counterparts (from plants, animals and fungi), which are twice the size of EcoL23. It is interesting to note that identical amino acids are found in the N-termini of these proteins, although the eubacterial-like sequences are lacking this region. The yeast MRP has an additional C-terminus of close to 100 amino acids which has no counterpart in the other r-proteins.

Furthermore, in the EcoL5 r-protein family, eukaryotic cytoplasmic and archaebacterial as well as bacterial, chloroplast, and plant mt r-proteins represent rather homogeneous groups (Figure 4b). The two MRPs presented here are quite different from each other. The plant MRP resembles the bacterial r-protein in size, but also shows gaps corresponding to the eukaryotic r-protein group. The highest degree of sequence similarity is to yeast MRP YmL5/7.

The EcoS14 r-protein family (Figure 4c) is more heterogeneous in length, although the degree of sequence similarity among its members is higher than that in the EcoL23 and EcoL5 alignments. A striking difference is shown by the *B. subtilis* S14 class, which does not correspond either to the eubacterial or to the eukaryotic/archaebacterial groups (no. 4 in Figure 4c). An interesting detail is the gradual conservation of a zinc-finger binding motif, $CX_2C\text{-}X_n\text{-}CX_2C$, that is putatively functional in archaebacteria, eukaryota and *Bacillus subtilis*, but which lacks two or three of its cysteine residues in the yeast MRP, *E. coli* and in plant organellar r-proteins. In the chloroplast r-protein (no. 7 of Figure 4c) the first cysteine residue is replaced by a histidine residue.

Proteins of the EcoL14 family are quite similar to each other (Figure 4d). The eukaryotic cytoplasmic and archaebacterial r-proteins are slightly elongated at their N-termini. Eubacteria, chloroplast and plant mt r-proteins are also relatively homogeneous. The yeast MRP is differentiated from them in that it lacks specific gaps, as well as by an internal elongation. The latter interestingly represents the C-terminus of the N-terminal peptide which is missing in the YmL34 protein version deduced from the *MRP-L38* gene (see Table 1). This internal elongation and a putative *in vivo* cleavage in YmL34 suggest a possible *trans* function of the N- and C-termini of the YmL38 protein.

EcoL3 is one of the important r-proteins involved in PTF activity. The functionally important sequences and putative amino acid residues may be tentatively deduced from an alignment of the corresponding r-proteins (Figure 4e). The EcoL3 r-proteins contain surprisingly many identical amino acid residues among all family members. The sorting of groups among them again differs from the examples shown above. Clearly the eukaryotic cytoplasmic proteins can be separated from all the others by the extremely large gaps. The other EcoL3 r-proteins, from eubacteria, archaebacteria, chloroplasts and mitochondria, form a more or less homogeneous group. The MRPs of yeast and human are elongated at their N- and C-termini. Most of the conserved amino residues are found in the C-termini of the proteins considered, suggesting a common functional domain of the C-terminal part of the respective proteins.

For all of the r-protein families discussed here, it is obvious that identical amino acids in *all* r-proteins are often proline or glycine residues, suggesting that bending of the amino acid chain might be an important feature which is not replaceable by other secondary or tertiary structural elements. Positively charged amino acids (arginine, lysine and histidine) are frequently replaced by each other and hydrophobic residues are replaced by other hydrophobic amino acids of similar size (e.g. isoleucine versus leucine, alanine and valine).

However, sequence comparison does not provide an answer as to where the new proteins arose in cases where no counterparts in cytoplasmic ribosomes are yet available. Are these additional r-proteins comparable with the 'ancient ribosome', or do mitoribosomes preserve the most complete set of r-proteins in the specialized environment of the mitochondrial endosymbiont, whereas other ribosomes have specialized by eliminating unnecessary proteins? It seems hard to believe that the yeast mitoribosome has adopted at least 28 new MRPs, while at the same time the number of translatable messages has decreased to a handful. As more sequences of (mitochondrial) r-proteins are discovered, and complete sequences of several different organisms become known, more light will be shed on the evolutionary development and origin of these proteins.

CONCLUSIONS AND FUTURE PROSPECTS

Mitoribosomes are an example of a highly complex organelle for the biosynthesis of proteins. An analogous translation is performed in the cytosol by a simpler machinery. We still do not understand the functional role of the many more MRPs as compared with the other ribosomes. At present we are on the way to characterizing the different constituents of the mitoribosome, e.g. that of yeast, but we are far from understanding the functional implications of their existence. Because of the experimental possibilities and the support from the yeast genome project, *S. cerevisiae* will provide the first complete set of MRP data. The yeast data will help to identify MRPs and their genes in other genome-sequencing projects where biochemical data for proteins are much more difficult to obtain. Several ORFs may not be recognized as MRPs for the moment, since they have no counterparts in other ribosomal systems. MRP functions might possibly best be studied in an organism for which the MRPs are not essential. Since it summarizes all data on MRPs known to date, this review will also be useful for the human genome sequencing project. However, the difficulties encountered with an 'automatic' identification by computer search demonstrate the limits of the efforts that are being made to understand an organism simply by genome sequencing. Also in that context, yeast and its MRPs are simple examples for more highly developed organisms and their studies.

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