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On the last common ancestor and early evolution of eukaryotes: reconstructing the history of mitochondrial ribosomes

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Abstract

Understanding early evolution is a major challenge for the post-genomic era. A promising way to tackle this issue is to analyze the evolutionary history of key cellular systems through phylogenomic approaches. The current availability of genomic data from representatives of diverse lineages (especially eukaryotes), together with the ever growing number of proteomic characterizations now provides ample material to apply this type of analyses to trace back the origin and evolution of the three domains of life. Here, we have reconstructed the composition of the ancestral mitochondrial ribosome in the Last Eukaryotic Common Ancestor (LECA) and investigated its subsequent evolution in six major eukaryotic supergroups. We infer that LECA possessed a mitochondrial ribosome that was already much larger than its bacterial ancestor, with 19 additional specific proteins, indicating that a certain amount of time occurred between initial endosymbiosis at the origin of the mitochondrion and the diversification of present-day eukaryotic lineages, involving the loss of different sets of ribosomal protein-coding genes, their transfer to the host genome, as well as the acquisition of many novel components. This chaotic history for a such fundamental cellular machinery is puzzling, especially when compared to cytosolic, bacterial or chloroplastic ribosomes, which are much more stable. Intriguingly, archaeal ribosomes also show a very dynamic nature, with multiple independent losses among lineages.

Keywords: Phylogenomics; Ribosome; Mitochondria; LECA; Archaea; Eukaryotes

1. Introduction

The place of eukaryotes in the tree of life and the very origin of this domain remains one of the most intriguing issues in evolution and is subject of lively debate (Gribaldo et al., 2010). In particular, their sistership with Archaea in rooted universal trees has led to the common assumption that Eukaryotes arose from a common ancestor with Archaea (Brown and Doolittle, 1995; Gogarten et al., 1989; Gribaldo and Cammarano, 1998; Iwabe et al., 1989). Alternatively, it has been proposed that Eukaryotes derive from a *bona fide* archaeon and should therefore emerge from within the Archaea in universal trees (see (Martin, 2005) and references therein). Much effort is made to elucidate the relationships between Archaea and Eucarya in order to test these two hypotheses using classical phylogenetic approaches (Ciccarelli et al., 2006; Cox et al., 2008; Foster et al., 2009; Gribaldo et al., 2010; Harris et al., 2003; Rivera and Lake, 2004; Yutin et al., 2008). However, an alternative way to tackle the issue is to try tracing back the nature of the Last Eukaryotic Common Ancestor (also called LECA), as this can provide important insights into the early evolution of this domain of life and on its origin (Koonin, 2010). Over the last 15 years,

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the explosive increase of genomic data from eukaryotes, especially protists, coupled to the improvement of molecular phylogeny tools and approaches such as multigene analyses (Burki et al., 2008; Delsuc et al., 2005; Hackett et al., 2007; Hampl et al., 2009; Rodriguez-Ezpeleta et al., 2005; Rodriguez-Ezpeleta et al., 2007) have profoundly changed our vision of the evolution of the domain of life to which we belong. Taken together, these results suggest a rapid divergence of present-day eukaryotic lineages into seven major supergroups (Fig. 1): Opisthokonta (including Metazoa, Choanoflagellata and Fungi), Archaeplastida (including Glaucophyta, red algae, green algae and land plants), Rhizaria (including Radiolaria, Foraminifera, amoeboid flagellates), Alveolata (including Apicomplexa and Ciliata), Heterokonta (including glaucophyta, diatoms, golden and brown algae), Amoebozoa (including amoebas and slime molds), Excavata (including Diplomonads and Parabasalia, Jacobids, Heterolobosea, Euglenozoa/Kinetoplastids). Additionally, the position of major algal lineages (Haptophyta and Cryptophyta) remains uncertain, but a grouping with Rhizaria, Alveolata and Heterokonta has been recently suggested (Burki et al., 2009). Regarding the order of emergence of these major eukaryotic groups, it has been proposed that the first dichotomy from LECA occurred between a lineage of uniciliates that gave rise to Opisthokonta and Amoebozoa (Unikonts) and a lineage of biciliate cells that gave rise to the five other supergroups (Bikonts) (Stechmann and Cavalier-Smith, 2002, 2003).

Even if important progresses on the phylogeny of eukaryotes have been made, the early steps in the evolution of this domain of life remain still largely unknown. Fortunately, the current number of complete genomes from nearly all major supergroups now allows the application of phylogenomics approaches to study the early evolution of eukaryotic cells (i.e. their metabolisms, protein complexes, cellular processes, etc.). Briefly, these approaches consist in investigating the taxonomic distribution and the phylogeny of homologues of the components of a particular cellular system in main representatives of the studied group for which complete genome sequences are available (Eisen and Fraser, 2003). Taken together, these data allow deciphering the evolutionary history of the system and therefore a better understanding of the evolutionary history of the organisms carrying it. Such strategy was successfully applied for instance to study the origin and evolution of nuclear pore complexes (Bapteste et al., 2005; Mans et al., 2004), cell division structures such as the midbody (Eme et al., 2009), membrane-trafficking systems (Dacks and Field, 2007), and unique metabolisms such as the pathway for sterol biosynthesis (Desmond and Gribaldo, 2009). As a starting point, phylogenomic approaches rely on the knowledge of the composition of the cellular system under investigation in at least one representative. These data are becoming increasingly available following the development of proteomics approaches, especially for eukaryotes. To illustrate the power of phylogenomic approaches, we present here a case study with the mitochondrial ribosome.

It is known that the evolution of mitochondria has been and is still very dynamic (Bullerwell and Gray, 2004; Burger et al., 2003; Lang et al., 1999). For example, a number of genes coding for ancestral mitochondrial proteins of alphaproteobacterial origin have been lost, while others have been transferred to the host nucleus, their products being expressed in the cytoplasm and imported into the mitochondrion by a dedicated transport system (Lang et al., 1999). These losses/ translocations have been very different in the different eukaryotic lineages. For example, three protein-coding genes only have been retained in the mitochondrial genome of Plasmodium falciparum while nearly 100 still reside in the mitochondrial genome of the Jacobid Reclinomonas americana (Gray et al., 2004). However, even extremely reduced mitochondrial genomes encode at least a few genes such as those coding for the components of respiration and coupled oxidative phosphorylation, or for transfer and ribosomal RNAs (Gray et al., 2004). The protein-coding genes that still reside in the mitochondrion are transcribed, and the resulting messenger RNAs are translated by the ribosomes (hereafter referred to as mitoribosomes) of alphaproteobacterial ancestry.

Mitoribosomes have undergone important changes during the evolution of the alphaproteobacterial symbiont into an established organelle (Burger and Lang, 2003; Gray et al., 2001). In particular, regions of ribosomal RNA components have been lost/deleted and have been compensated by the recruitment of eukaryotic-specific ribosomal proteins (O'Brien, 2003; Suzuki et al., 2001). These proteins are not homologous to cytosolic ribosomal proteins and represent de novo eukaryotic innovations, and will be hereafter referred to as Ek-MRPs (for Eukaryotic Mitochondrial Ribosomal Proteins). As shown by the few available proteomic characterizations, mammals and yeast mitoribosomes comprise approximately 70-80 MRPs (Gan et al., 2002; Graack and Wittmann-Liebold, 1998; Koc et al., 2001a; Koc et al., 2001b; O'Brien et al., 1999; O'Brien et al., 2000; Towpik, 2005), of which around 20 are eukaryotic-specific (Koc et al., 2001b). By contrast, only 57 ribosomal proteins have been identified in Bacteria (Lecompte et al., 2002), 54 of which are present in alphaproteobacteria and were therefore also likely present in the very first ancestor of mitochondria (these 54 mitochondrial ribosomal proteins of alphaproteobacterial ancestry will be hereafter referred to as Bt-MRPs, for Bacterial Mitochondrial Ribosomal Proteins). More specifically, a proteomic analysis of the mammalian mitoribosome has identified 48 MRPs in the large subunit (LSU) and 29 MRPs in the small subunit (SSU) (Koc et al., 2001a,b). A proteomic analysis of the mitoribosome of the yeast Saccharomyces cerevisiae has identified 44 MRPs in the LSU and 33 MRPs in the SSU, including 24 new components not present in the mammalian mitoribosome (Graack and Wittmann-Liebold, 1998; Saveanu et al., 2001). A similar analysis of the LSU of mitoribosome from a second fungus (Neurospora crassa) has confirmed the presence of most of the MRPs found in S. cerevisiae but failed to identify new ones (Gan et al., 2002). These analyses highlight great differences in the mitoribosome composition of these organisms despite the fact that they all belong to the supergroup of Opisthokonta. Two analyses performed on members of a second eukaryotic major group, the



Fig. 1. Reference phylogenetic tree of Eukaryotes used to infer the evolution of the sets of MRPs during the evolutionary history of this domain.

Excavates, have shown that the inclusion of novel MRPs appears to be a general tendency in the evolution of mitoribosomes. In fact, a partial proteomic analysis of the mitoribosome of the kinetoplastid *Leishmania tarentolae* identified 13 LSU MRPs, and 39 SSU MRPs of which 17 are unique to kinetoplastids (Maslov et al., 2006, 2007). More recently, a proteomic characterization of a mitoribosome was obtained from a second representative of kinetoplastids, *Trypanosoma brucei* (Zikova et al., 2008). All MRPs characterized in *L. tarentolae* were confirmed, as well as 81 additional ones, reaching the impressive number of 133 MRPs (77 in LSU, 56 in SSU) identified with high confidence. Importantly only 38 of these have homologues to other eukaryotic MRPs, while 95 (51 LSU, 44 SSU) appear unique to Kinetoplastids (i.e. no homologues exist in other eukaryotic lineages).

Smits et al. (Smits et al., 2007) have recently investigated the distribution of MRP homologues in a sample of 17 eukaryotic genomes representatives of five supergroups. They inferred a mitochondrial ribosome in LECA that contained already 16 novel Ek-MRPs with respect to its alphaproteobacterial ancestor, and reconstructed its subsequent evolution that involved large variations of protein content (both acquisitions and losses) in different lineages. Based on bioinformatic predictions, they also identified a number of previously unidentified MRPs homologues that may be responsible for mitochondrial-related disorders in humans (Smits et al., 2007). Here, we have extended this study by analyzing a much larger taxonomic sampling consisting of 38 complete nuclear and mitochondrial genomes including in particular representatives from a sixth major eukaryotic phylum (Heterokonts). This allowed to identify 22 MRPs previously undetected in a number of eukaryotic genomes and therefore to define with more precision the evolutionary history of the mitoribosome over eukaryotic evolution. This has been characterized by a very dynamic process, which appears to be still ongoing, and which contrasts with the overall stasis of cytosolic ribosomes. Curiously, archaeal ribosomes also display a similar chaotic history, involving mainly loss of proteins.

2. Material and methods

2.1. Dataset construction

Homologues of each of the 336 MRPs identified in the proteomic characterizations of mitochondrial ribosomes of mammals (Koc et al., 2001a,b), yeast (Graack and Wittmann-Liebold, 1998; Saveanu et al., 2001), and kinetoplastids (Graack and Wittmann-Liebold, 1998; Koc et al., 2001a,b; Maslov et al., 2006, 2007; Saveanu et al., 2001) as well as homologues of the 54 alphaproteobacterial ribosomal proteins (Bt-MRP) were searched using Blastp (Altschul et al., 1997) and default parameters on the RefSeq database at the NCBI. At this preliminary stage, no global e-value cutoff was used to filter Blastp results, but we carefully examined these to select to select unambiguous homologues. These were aligned using MUSCLE 3.6 (Edgar, 2004). The MUST package (Philippe, 1993) was used to refine multiple alignments and to build preliminary neighbor-joining trees in order to clearly identify mitochondrial sequences from their cytoplasmic or chloroplastic homologues. MRP alignments containing only the mitochondrial homologues were then used to build HMM profiles using the HMMER 2.3.2 tools (http://hmmer.janelia. org/). These profiles were then used to identify additional homologues (in particular very divergent ones) in a local sequence databank containing 38 complete nuclear and mitochondrial genomes representing a broad taxonomic sampling of eukaryotes, 50 bacteria and 6 archaea (a complete list is provided in Supplementary Table 1). Genomes were retrieved from the NCBI (ftp://ftp.ncbi.nih.gov/genomes/), except for Ostreococcus tauri (Palenik et al., 2007), Phythophtora ramorum (Tyler et al., 2006), Thalassiosira pseudonana (Armbrust et al., 2004) and Naegleria gruberi which were retrieved from the JGI (http://genome.jgi-psf.org/euk_ home.html), and Cyanidioschyzon merolae (Matsuzaki et al., 2004) which was retrieved from a dedicated website (http:// merolae.biol.s.u-tokyo.ac.jp). The sequences of Naegleria were produced by the US Department of Energy Joint Genome Institute in collaboration with the user community (http:// www.jgi.doe.gov/). The complete procedure (i.e. alignment reconstruction and refinement, reconstruction of preliminary neighbor-joining trees, construction of new HMM profiles, and search for additional mitochondrial homologues) was repeated until no additional homologues could be retrieved in the local database. Gene absences were systematically and carefully verified by additional searches on the corresponding complete genome sequences by tBLASTn.

2.2. Phylogenetic reconstructions

For final phylogenetic analyses, ambiguously aligned positions were manually removed using MUST. Bayesian phylogenetic trees were constructed using MrBayes 3.2 (Ronquist and Huelsenbeck, 2003). We used a mixed model of amino acid substitution and a Γ law (four discrete categories plus a proportion of invariant sites) to take into account among site rate variation. MrBayes was run with four chains for 1 million generations and trees were sampled every 100 generations. To construct the consensus tree, the first 1500 trees were discarded as "burnin".

2.3. Inference of ancestral mitoribosome composition

Based on homologues present in analyzed eukaryotic genomes we inferred the pattern of gains and losses of MRPs along eukaryotic evolution, using a reference eukaryotic phylogenetic tree rooted between Bikonts and Unikonts. Our inference is based on a parsimony criterion minimizing the number of convergent translocation of Bt-MRPs coding genes from mitochondria to host nucleus and the number of convergent losses. We also assumed (i) that no convergent recruitment of Ek-MRPs occurred during the evolution of eukaryotes, (ii) that no gene reverse-translocation occurred (i.e. from the nucleus to mitochondria) and (iii) that no HGT of MRPs coding genes occurred between eukaryotic lineages. In practice, given that it is known that 54 Bt-MRPs were present in the ancestor of mitochondria, to infer the presence of a given Bt-MRP in the ancestor of a eukaryotic lineage it sufficed that the protein be present in at least one representative of its offspring. By contrast, we could infer the presence of a given Ek-MRPs in the ancestor of a eukaryotic group only when homologues of the protein were present in at least one present-day representative from both sister lineages arising

from this ancestor. Finally, the absence of a given protein in a eukaryotic phylum was inferred only when no homologues were found in any of its present-day representatives. To avoid biases linked to our taxonomic sampling, we performed additional similarity searches in public databases to verify that no homologue of the protein was known in representatives of the phylum that were not included in our taxonomic sampling.

3. Results and discussion

We identified 22 MRPs in various eukaryotes that were previously unreported (Smits et al., 2007), both of alphaproteobacterial ancestry (Bt-MRPs) and eukaryotic-specific (Ek-MRPs) (Table 1, indicated by red boxes with X symbols). This probably results on the larger spectrum of homologues that was used to build the HMM model, likely improving detection (especially for small proteins). Moreover, our phylogenetic analysis indicates that a number of proteins previously proposed to be MRPs (Smits et al., 2007) are in fact cytoplasmic copies (see below). It should be noted that Ppe1 was reported to function as a nuclear or cytosolic phosphatase methylesterase in yeast and human whereas it was designated previously as an MRP of the small subunit (SSU) of the yeast mitoribosome (see (Smits et al., 2007) and reference therein). In addition, whereas MRPs are systematically absent in amitochondriate protists, a homologue of this protein is present in the genomes of two of them (Giardia and Trichomonas, Table 1b). Either this is a unique case of recruitment of a former MRP in amitochondriates, or else this protein was misannotated and does not function in the mitoribosome, as already suggested (Smits et al., 2007). In the absence of confirmation by proteomic analysis, we considered Ppe1 as a bona fide MRP in our analysis. Other than this exception, there was no ambiguity on the function of all other proteins analyzed.

3.1. Early events following endosymbiosis (before LECA)

Alphaproteobacteria harbor 54 ribosomal proteins (Bt-MRPs, 33 in the LSU and 21 in the SSU, Table 1) that were therefore also likely present in the alphaproteobacterial ancestor of mitochondria. We analyzed the fate of these ribosomal proteins from the time of the endosymbiosis event up to LECA. Despite a large eukaryotic taxonomic sampling, we confirm the absence of S20 in all the eukaryotic genomes (both nuclear and mitochondrial) analyzed (Table 1b), suggesting that this protein was lost very early in eukaryotic evolution (Fig. 2).

We also sought to reconstruct the events of gene translocation to the host nucleus prior to LECA. Based on the hypothesis that gene translocation occurred only in one direction (from the mitochondrion to the host nucleus), the 27 Bt-MRPs (15 LSU and 12 SSU) that are encoded in the mitochondrial genome of at least one species (Table 1) can be inferred to have been also encoded in the mitochondrial genome of LECA, i.e. before the diversification of present-day eukaryotic lineages (Fig. 2). It is noteworthy that all these 27 Bt-MRPs are still encoded in the Table 1

Taxonomic distribution of homologues of the 109 proteins from the LSU (a) and 189 from the SSU (b) of the ribosome in the 38 analyzed eukaryotic lineages. The first column refers to the names of mammalian MRPs, the second column indicates the corresponding names in yeast. MRPs of bacterial origin (Bt-MRPs) are indicated by a grey background whereas eukaryotic-specific MRPs (Ek-MRPs) have a white background. Yellow/dark gray background corresponds to MRPs from genomes analyzed for the first time. MRPs with a name beginning by Tb and Lmj designate MRPs characterized in by proteomic analysis in the mitoribosomes of *T. brucei* and *L. major* respectively. The presence of an MRP homologue is indicated by an X when it is encoded in the nuclear genomes and by an M if the corresponding gene is located in the mitochondrial genome. Differences with the results from Smits et al. (Smits et al., 2007) are highlighted by red squares. Question marks indicate ambiguities. The * in one protein of *P. ramorum* indicates that the protein was not found in this organism whereas this is found in close relatives. Accordingly, the protein is inferred to be present in ancestors of this lineage. Question marks indicate cases where assessment of homology was ambiguous.

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L3	Mrp19	x	x	х	х	х	х	x	x	х	х	x	x	х			x	x	x	х	x	x	x	x		x	x	x	×	х			x	x	x	x	x	x	
L4	Yml6	x	x	х	х	x	х	x	x	х	x	х	x	х			х	x	x	x	x	x	x	х		x	х	x	×	х			×	x	x	x	x	×	
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L6	Mrp16								х	х	х	х	х	х			м	X	х		м	м	М	М		×	х	х	м	М								м	М
L9	Mrp150	X	X	х	х	х	х	X		х	х		X	х			×	X	х		х		×	х		x	х	х	×	х			×	х	х	х	×	×	
L10	Mrpl11	X	X	X	X	X	X	X	X	X	X	X	X	X												×	X	X											M
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L15	MrpI10/MrpI18	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x		x	x	x	x	x			x	x	x	x	x	x	
L16	Mrpl16	м	x	х	х	х	х	х	x	х	х	х	х	х			м	м	х	х	м	м	м	м		x	х	х	м	м			х	x	х	х	х	м	м
L17	Mrp18	x	x	х	х	х	х	х	x	х	х	х	х	х			х	x	х	х		х	х	х		х	х	х	х	х			х	х	х	х	х	х	
L18		х	х	х	х	х	х	х										х	х			х																	М
L19	lmg1	X	X	х	х	х	х	X	х	х	х	х	х	х				X	х	х	х	x	×	х		х	х	х	×	х									М
L20			X	х	х	х	х	х									х	X	×	x	X	м	×	X		x	х	x	x	х			×	х	X	х	x	×	М
L21	MrpI49	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		X	X	X	×	X			X	X	X	X	X	X	
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L25	mprio			~	~	~	~		~	~	~						x	x	x	x				~		X	x	x	x	x			^	^		~		~	
L27	Mrpl2/Mrp7	x	x	х	х	х	х	X	x	х	x	x	x	х			х	x	x	x	x	x	x	x		х	x	x	x	x			x	x	x	x	x	x	м
L28	Mrpl24/Mrpl14	x	x	х	х	х	х	х	x	х	х	х		х			х	x	x			x	×	х		x	х	х	×	х			×	x	х	х	x	×	
L29/L47	Mrp14	x	x	х	×	х	х	x	х	х	х	×	x	х			х	x	×	х	х	x	×	х		×	x	х	×	х			×	х	×	х	×	×	
L30	Mrpl33	X	X	х	х	х	х	x	х	х	х	×	х	х			х	X	×	х	х	x											×	х	×	х	×		
L31	Mrpl36								×	х	×			х																									М
L32	Mrp132		X	x	x	X	X	X	x	х	×	×	×	x			×	X	×	x	X	×																	М
L33	Mrp/39	×	×	×	×	×	×	×	×	~	×	×	×	×			×	×	×	x	×	×	×	×		×		×	×	×			×	×	×	×	×	×	
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L37			X	x	x	x	x	х																	-		_	-							3 - V				
L38	Mrpl35	x	x	х	х	х	х	x	х	х	х	х	х	х																			x	X	х	X	х	х	
L39			x	х	х	х	х	х																															
L40	Mrpl28		x	х	х	х	х	х	х	х	х	х	х	х																									
L41	Mrpl27	x	X	х	х	х	х	x	х	х	х	х	х	х			х	X	х			x	x	X		х	х		×	х									
L42			X	x	x	X	X	X																															
L43	Mrp151	X	×	×	×	×	×	×	×	×	×	x	x	x			x	×	x	х		x	X	x		x	X	X	×				x	x	X	x	x	x	
L44	Mba1	x	x	x	x	x	x	x	x	x	x	x	x	^			х	x	х	x	х																		
L46	Mrpl17/Mrpl30	x	x	x	x	x	x	x	x	x	x	x	x	х			x	x	x	x	x	x	x	x		x	х	х	x	х			х	х	х	х	х	х	
L48			x	х	х	х	х	x																															
L49	Img2	х	x	х	х	х	х	x	х	х	х	х	х	х			х					х	x	х		х	х	х	X*	х			х	х	х	х	х	х	
L50		х	x	х	х	х		х																															
L51			x	х	х	х	х	х																															
L52		x	X	х	×	x	x	×									х																×	×	х	×	х	×	
L53	Mrp144		X	x	x	X	X	X	X	X	X	X	×	~			X	X	X	X	X	x		~			~	~	X	X								~	
155	Mrp137		×	×	×	×	×	×	X	x	X	X	X	X			х	×	X	x	X		X	×		*	X	X	X	X								X	
L56			Â	^	×	×	×	x									х																						
	Mrpl13				î	~					х						~																						
	Mrpl15								x	×	х	x	x	x																									
	Mrpl20								x	x	х	х	x	х																									
	Mrpl25								х	х	х	х	х	х																									
	Mrpl31								х	х	х	×	х	х																									
	Mhr1							1	Х	х	х	х																											

(continued on next page)

Table 1 (continued)

								Opis	sthok	onta							Amo	ebozoa		An	chaep	olasi	tida			Alve	olata			Heterol	konta				E	Excave	ata			
	M. brevicollis		D. melanogaster	T. castaneum	H. sapiens	M. musculus	m. muscunas	D. rerio	c. crogars	A. fumigatus	N. crassa	S. Cerevisiae	S. pombe	C. neoformans	U. maydis	E. cuniculi	E. histolytica	D. discoideum	A. thaliana	O. sativa	C reinhardtii		O. tauri C. merolae	P. tetraurelia	T. thermophila	C. parvum	P. falciparum	P. yoelii	T. annulata	P. ramorum	T. pseudonana	G. lamblia	T. vaginalis	T. cruzi	T. brucei	L. infantum	L. major	L. braziliensis	N. gruberi	mito. Reclinomonas
Tb927.1.1160																																5		х	х	х	х	х		
Tb927.3.820																																		х	х	х	х	х		
ТЬ927.4.2720																																		х	х	х	х	х		
Tb927.4.4610																																		х	х	х	х	х		
Tb927.5.2070																																		х	х	х	х	х		
Tb927.5.3870																			1															х	х	х	х	х		
Tb927.5.4120																																		х	х		х	х		
Tb927.6.1440																																		х	х	х	х	х		
Tb927.6.2480																																		х	х	x	х	х		
Tb927.6.3600																																		x	х	x	х	х		
Tb927.6.3930 LmjF30.2660																																		x	х	x	х	х		
ТЬ927.6.4080																																		X	x	X	X	x		
Tb927.6.4200																																		x	x	X	X	x		
Tb927.7.1640																																		X	X	X	X	X		
Tb927.7.2630																																		×	×	×	×	×		
Tb927.7.2990																																		Ŷ	Ŷ	Ŷ	Ŷ	Ŷ		
Tb927.7.3430																																		Ŷ	Ŷ	Ŷ	Ŷ	Ŷ		
Tb927.7.3510																																		x	x	x	x	x		
Tb927.7.6800																																		x	x	x	x	x		
Tb927.7.7010																																		x	x	x	x	x		
Tb927.8.1880																																		х	х	x	х	х		
Tb927.8.2760																																		х	х	х	х	х		
Tb927.8.3170																																		х	х	x	х	х		
Tb927.8.3300																																		х	х	x	х	х	х	
Tb09.160.2000																																		х	х	х	х	х		
Tb09.160.2250																																		х	х	х	х	х		
Tb09.211.0810																																		х	х	х	х	х		
Tb09.211.3800																																		х	х	х	х	х		
Tb09.211.4580																																		х	х	х	х	х		
Tb10.26.0600																																		х	х	х	х	х		
Tb10.26.1000																																		х	х		х	х		
Tb10.389.1710																																		х	х	х	х	х		
Tb10.6k15.3290																			1															х	х	х	х	х		
Tb10.70.0780																																		х	х	х	х	х		
Tb10.70.5970																																		х	х	x	х	х		
Tb10.70.7950																																		x	X	X	X	X		
Tb10.70.7960																																		X	X	X	X	X		
TE11.01.0500																																		X	×	X	X	X		
Tb11.01.1215																																		×	×	x	×	X		
Tb11.01.1600																																		× ×	Ŷ	×	× ×	~		
Th11 01 1840																																		×	Ŷ	Ŷ	Ŷ	×		
Th11 01 2340																																		×	Ŷ	×	Ŷ	×		
Tb11.01.3500																																		x	x	x	x	x		
Tb11.01.7140																																		x	x	x	x	x		
Tb11.01.8760																																		x	x	x	x	x		
Tb11.02.3230																																		x	х	x	х	x		
Tb11.02.3670																																		x	х	x	х	x		
Tb11.02.3800		I							1										I					1										x	х	x	х	x		
Tb11.55.0016																																		х	х	х	х	х		

(continued on next page)

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Table 1 (continued)

							(Opisth	okont	a						Amo	ebozoa		Arch	aepla	stida			A	veolata	8		Heter	okonta				E	cava	ta			
b		M. brevicollis	D. melanogaster	T. castaneum	H. sapiens	M. musculus	D. rerio	C. elegans	A. fumigatus	N. crassa	S. cerevisiae	S. pombe	C. neoformans	U. maydis	E. cuniculi	E. histolytica	D. discoideum	A. thaliana	O. sativa	C. reinhardtii	O. tauri	C. merolae	P. tetraurelia	T. thermophila	P. falciparum	P. yoelii	T. annulata	P. ramorum	T. pseudonana	G. lamblia	T. vaginalis	T. cruzi	T. brucei	L. infantum	L. major	L. braziliensis	N. gruberi	mito. Reclinomonas
S2	Mrp4	×	×	×	x	x	x	x	×	x	x	×	×	x			м	x	м	x	M	x						м	м								м	M
S3/S24	Var1	M	x	x	x	x	x	x		^	M	^	^	M			M	M	M	^	M	M	м	м				M	M								M	M
S4	Nam9/Mna6	м	Â	~	~	~	~	~	x	x	x	x	x	x			M	M	м		M	м						м	M								м	M
S5	Mrps5	×	x	x	x	x	x	x	x	x	x	x	x	x			x	x	×		×	x	x	x	x	x	x	x	×			×	x	x	x	x	×	
S6	Mrp17	x	x	x	x	x	х	x	x	x	x	x	x	x				x	x	х	x		x	x	x	x	x	x				x	x	x	x	x	×	
S7	Rsm7	x	x	х	х	х	х	х	x	х	х	x	x	x			м	м	м	x	м	x						м	м								м	м
S8	Mrps8	м							x	х	х	x	x	x			м				м	м			х	x	х	м	м			x	x	x	x	х	м	м
S9	Mrps9	x	х	х	х	х	х	х	x	х	х	х	х	х			x	х	х	х	×	x	x	x	х	х	х	х	х			×	x	×	х	х	х	
S10	Rsm10	x	x	х	х	х	х	х	x	х	х	х	х	х			х	x	х		м	-			X	x	х	м	м								м	м
S11	Mrps18		х	х	х	х	х	х	x	х	х	х	×	х				x	х	х	м	м			Х	х	х	м	м			×	x	×	х	х	м	м
S12	Ynr036c	м	х	х	х	х	х	х	х	х	х	х	×	х			м	м	М	х	м	м	м	м	х	x	х	м	м					м	М	М	м	М
S13	Sws2	м							х	х	х	х	х	х			м		м	х	м		м	м				м	м								м	М
S14	Mrp2	м	×	х	х	х	х	х	×	х	х	×	×	X			м	X	X	×	м	M	м	м	Х	X	X	м	м								м	М
S15	Mrps28		×	x	х	х	х	х	X	х	х	×	×	×			×	X	X	×	×	×	×	×	х	X	X	×	×			×	x	×	x		×	
S16	Mrps16	×	X	×	X	×	X	×	×	X	×	×	×	X				×	×		×	×	×	×	X	X	X	×	×			×	×	×	X	X	×	
S17	Mrps17	×	X	X	X	X	X	X	X	X	X	X	×	X			X	X	X	×	X		X	X	×	×	×	X	X			×	×	×	X	X	X	
518	Rsm18		×	×	×	×	×	×	×	×	×	×	×	×			Å	×	×	×	×	×	×	×	_			×	×			×	×	×	×	×	×	
\$20	RSIIII9	IVI							^	^	^	^	^	^			IM	^	IVI	^	IVI			101				IVI	M								IVI	IVI
S21	Mrp21/Mrp50		x	x	x	x	x	x	x	x	x	x	x	x				x	x																			
S22	impt initpoo	х	x	x	x	x	x	x	^	~	~	~	~	~				~	~	-			-			-						-						
S23	Rsm25		x	х	х	х	х	х	x	х	х	х	х	х				X	х																			
S25	Mrp49	х	x	х	х	х	х	х	х	х	х																										х	
S26			x	х	х	х	х	х																														
S27			х	х	х	х	х	х																														
S28			х	х	х	х	х	х																														
S29	Rsm23	х	х	х	х	х	х	х	х	х	х	х	х	х			х	x	х	х	х	x	x	x	х	х	х	х	х			х	х	х	х	х	х	
S30			х	х	х	х	х	х																														
S31			x	х	х	х	х	×																														
S33	Rsm27	X	X	х	х	х	х	х	X	х	х	х	х	х			×	X	х		×																	
S34			X	x	x	x	x	X						X			×	X	x	х	×	x	×	×								×	x	X	х	x	x	
\$35	Rsm24	X	X	X	X	x	×	х	X	X	x	x	x	X			×	X	X		x	×	X	X	x	x	x											
530	Pem22	×	Ŷ	~	~	×	Ŷ	×	Ŷ	×	Ŷ	×	Ŷ	Ŷ			Ŷ		×	×	×	v	×	ŷ	v	×	×	v	×			×	v		v	×	~	
	Pne1	Ŷ	x	×	x	x	x	×	x	x	x	x	x	x			Ŷ	x	x	x	x	Ŷ	x	Ŷ	Ŷ	Ŷ	^	x	Ŷ	X	x	x	Ŷ	×	x	x	x	
	Mrps35								x	x	x	x	x	x					?		?																	
	Mrp51								x	x	х	x	x	x																								
	Mrp13										х																											
	Mrp1										х	х	х	х																								
	Rsm26								x	х	х	х																										
	Pet123								х	х	х																											
	Mrp10		х	х	х	х	х	х	х	х	х	х	х	х			х	x	х		х																	
	Rsm28										х																											
TL007 / 000-	Mrp8										х																					v	v					
10927.1.2990	1 miE27 4005																															×	×	X	×	Y		
Th027 3 2260	LmiE25 2160																															×	Ŷ	×	×	×		
Tb927.3.5240	LmiF29.0430																															x	x	x	x	x		
Tb927.3.770	LmjF27.0180																															x	x	x	x	x		
Tb927.3.970																																x	x	x	x	x		
Tb927.4.3690																																x	x	x	x	x		
Tb927.5.1510	LmjF15.0080																															x	x	х	х	x		
Tb927.5.1790	LmjF15.0410																															х	х	х	х	х		
Tb927.5.3640																																х	х	х	х	х		
Tb927.5.4040	LmjF33.0010																															х	х	х	х	х		
Tb927.6.2080	LmjF30.0650																															х	х	х	х	х		
Tb927.6.2180	LmjF30.0740																															×	х	х	х	x		
Tb927.6.4080																																Х	х	х	х	х		

(continued on next page)

Table 1 (continued)

							Op	oistho	konta	3						Amo	ebozo	ba		Arch	aepla	astida	3	_		Alve	olata			Heterok	onta				E	xcava	ata			
	M. brevicollis	D melanocaster	T castaneum	H sanians	A minority	w. muscuius	D. rerio	C. elegans	A. fumigatus	N. crassa	S. cerevisiae	S. pombe	C. neoformans	U. maydis	E. cuniculi	E. histolytica	D. discoideum		A. thaliana	O. sativa	C. reinhardtii	O. tauri	C. merolae	P. tetraurelia	T. thermophila	C. parvum	P. falciparum	P. yoelii	T. annulata	P. ramorum	I. pseudonana	G. lamblia	T. vaginalis	T. cruzi	T. brucei	L. infantum	L. major	L. braziliensis	N. gruberi	mito. Reclinomonas
Tb927.6.4560 LmjF30.3220																																		х	х	х	х	X		
Tb927.6.4580																																		х	х	х	х	x		
Tb927.6.4930 LmjF30.3530																																		х	х	х	х	x		
Tb927.7.2620 LmjF22.0830								1																										х	х	х	х	x		
Tb927.7.3050 LmjF22.0990																																		х	х	х	х	x		
Tb927.7.3240 LmjF22.1220								l																										х	х	х	х	x		
Tb927.8.1430																																		х	х	х	х	x		
Tb927.8.4550																																		х	х	х	х	x		
Tb927.8.4860																																		х	х	х	х	x		
Tb927.8.5200 LmjF16.1230																																		х	х	х	х	x		
Tb09.160.3800 LmjF15.1400																																		х	х	х	х	x		
Tb09.160.4750 LmjF15.0780																																		х	х	х	х	×		
Tb09.211.2430 LmjF35.3940								l																										х	х	х	х	×		
Tb09.211.3000 LmjF35.3490								l																										х	х	х	х	x		
Tb09.211.4510 LmjF35.2230																																		х	х	х	х	x		
Tb10.26.0050																																		х	х	х	х	x		
Tb10.26.0750																																		х	х	х	х	x		
Tb10.61.0190																																		х	х	х	х	x		
Tb10.61.0690																																		х	х	х	х	x		
Tb10.61.3110								1																										х	х		х	x		
Tb10.70.3840 LmjF03.0630								1																										х	х	х	х	x		
Tb10.70.4220 LmjF03.0220																																		х	х	х		x		
Tb11.01.1910																																		х	х	х	х	x		
Tb11.01.2170 LmjF36.4120																																		х	х	х	х	x		
Tb11.01.3300 LmjF28.2180																																		х	х	х	х	x		
Tb11.01.3710																																		х	х	х	х	x		
Tb11.01.7510																																		х	х	х	х	x		
Tb11.02.0130 LmjF33.2510																																		х	х	х	х	×		
Tb11.02.3180 LmjF24.0830																																		х	х	х	х	×		
Tb11.47.0024 LmjF27.0630		1																																х	х	х	х	x		

mitochondrial genome of the Jacobid *Reclinomonas americana*, of which nine are not found in any other mitochondrial genome, underscoring the uniqueness of the organelle in this protist. Indeed, if the mitochondrial genome sequence of *Reclinomonas* were not included in the analysis, only 18 Bt-MRPs (seven LSU and 11 SSU) instead of 27 would have been inferred as encoded in the mitochondrial genome of LECA. The presence of 27 Bt-MRPs in the mitochondrial genome of LECA is therefore a minimum estimate and it cannot be excluded that it will increase when additional mitochondrial genome sequences from a wider range of protists become available.

On the contrary, the 26 Bt-MRPs that are not encoded in any currently available mitochondrial genome (including that of *Reclinomonas*) might have been transferred to the host nuclear genome prior to the divergence of major eukaryotic supergroups (Table 1 and Fig. 2). This observation may be explained by two hypotheses: (i) considerable time elapsed between the endo-symbiosis and the diversification of present-day eukaryotic lineages or (ii) the translocation of Bt-MRPs to the host nucleus occurred rapidly after endosymbiosis. A third possibility that cannot be totally ruled out is the independent and convergent translocation of the genes coding for these Bt-MRPs to the nucleus after the divergence of present-day eukaryotic lineages. We therefore sought to gather additional clues on the order and the timing of transfer from the ancestral mitochondrial genome

to the host nucleus by looking at bacterial endosymbionts involved in genomic reduction processes than might be similar to that undergone by the ancestor of mitochondria (Tamames et al., 2007). This is the case for four organisms belonging to distantly related lineages: the gammaproteobacteria Candidatus Carsonella ruddii PV (endosymbiont of psyllids; 0.16 Mb and 182 protein-coding genes (Nakabachi et al., 2006)) and Buchnera aphidicola str. Cc (endosymbiont of the cedar aphid, Cinara cedri; 0.42 Mb and 357 coding genes (Perez-Brocal et al., 2006)), the alphaproteobacterium Candidatus Hodgkinia cicadicola Dsem (symbiont of the cicada Diceroprocta semicincta; 0.14 Mb and 169 coding genes (McCutcheon et al., 2009)) and the flavobacterium Candidatus Sulcia muelleri DMIN (gut symbiont of the Blue-Green Sharpshooter and several other leafhopper species; 0.24 Mb and 226 coding genes, unpublished). We found that, except for *B. aphidicola*, these endosymbiont genomes lack a large number of ribosomal protein-coding genes (Table 2). Interestingly, the number of ribosomal proteins in these symbionts correlates well with the size of their genomes. More precisely, the two smallest genomes (Candidatus C. ruddii and in Candidatus H. cicadicola) encode only 40 ribosomal proteins, whereas 48 are present in Candidatus S. muelleri (Table 2). It should be noted that, despite an important reduction of its genome (0.42 Mb and 357 proteincoding genes), no losses of ribosomal proteins are observed in



Fig. 2. Inference of the set of MRPs present in the last eukaryotic common ancestor and its subsequent evolution in Unikonts and Bikonts. MRP acquisitions are indicated by green arrows, whereas losses are highlighted by red arrows. Proteins of alphaproteobacterial origin encoded in mitochondria are indicated in bold. Bt-MRPs predicted to be encoded in mitochondria are indicated in red, whereas those encoded in the nucleus are indicated in black. Ek-MRPs are indicated in blue italic font. Lineages that have lost their mitochondrion are marked with crosses; in these cases no ribosomal proteins are detected in their genomes, to the exception of Ppe1 (but see text for discussion).

B. aphidicola (Table 2). This strongly suggests that ribosomal proteins are likely among the last to be lost during the process of genome reduction after the endosymbiotic event. The flavobacterium Candidatus Sulcia muelleri has lost six ribosomal proteins, and all of these are also missing in Candidatus Carsonella ruddii and Candidatus Hodgkinia cicadicola. The absence of ribosomal proteins in the three extremely reduced endosymbiont genomes is puzzling and has been discussed previously (Tamames et al., 2007). In fact, since these losses are not compatible with a viable ribosome, it has been put forward that the genes coding for the missing components (as other missing genes involved in important functions) have been transferred to the host genomes, and their products, like in the case of mitochondria, are now imported into the symbionts (Nakabachi et al., 2006). However, a recent analysis of the genome of Cinara cedri has shown that the genome reduction of its symbiont, B. aphidicola, has not been accompanied by gene translocation to the host nuclear genome (Nikoh et al., 2010). Alternatively, it has been proposed that key missing proteins in reduced endosymbionts might be complemented by the mitochondrial proteins of the host (Moya et al., 2008; Tamames et al., 2007). In agreement with this hypothesis, mitochondrial homologues of all the missing ribosomal proteins in these endosymbionts are encoded in the current available genomes of insects (Tables 1 and 2, and Fig. 3) and thus probably also in the genome of their hosts. However, the two Bt-MRPs (S20 and L25) that have been lost early in metazoan evolution (see below) should be considered as definitely missing from the ribosomes of two of these three symbionts (i.e. *Candidatus* Carsonella ruddii and *Candidatus* Hodgkinia cicadicola), because these cannot be re-imported from the host genome. It has been also proposed that the loss of key proteins may be complemented by proteins coming from co-endosymbionts (Moya et al., 2008). This is a very interesting hypothesis to test because it would require a dedicated export/import system between two endosymbionts.

Interestingly, five of the ribosomal proteins that have been lost from the genomes of these endosymbionts are also missing from the inferred mitochondrial genome of LECA (Table 2). Moreover, three additional protein losses are shared between the two smallest genomes (Candidatus Carsonella ruddii and Candidatus Hodgkinia cicadicola) and the mitochondrion (Table 2). These results suggest that these proteins may be among the first to be lost/translocated during the establishment of endosymbiosis. It is therefore possible that the same proteins had already been translocated to the host genome of LECA after mitochondrial endosymbiosis. Moreover, given that the loss/translocation of ribosomal proteins appears to be a late event in endosymbiosis (as testified by their presence in the least reduced genome of Buchnera), it is possible that in LECA also the mitochondrial endosymbiosis was already at a late stage.

Table 2

Comparison of the 54 Bt-MRPs with the set of proteins inferred to have been encoded in the mitochondrial genome of LECA (indicated by an M) and the ribosomal proteins present in extremely reduced genomes of four bacterial endosymbionts of insects: the gammaproteobacteria *Candidatus* Carsonella ruddii PV (0.16 Mb, endosymbiont of psyllids) and *Buchnera aphidicola* str. Cc (0.42 Mb, endosymbiont of the cedar aphid, Cinara cedri), the alphaproteobacterium *Candidatus* Hodgkinia cicadicola Dsem (0.14 Mb, symbiont of the cicada Diceroprocta semicincta) and the flavobacterium *Candidatus* Sulcia muelleri DMIN (0.24 Mb, gut symbiont of the Blue-Green Sharpshooter and several other leafhopper species).

LU YP, 802562 YP, 802560 YP, 802580 YP, 802587 YP, 802583 YP, 802587 YP, 802583 YP, 802583 YP, 802583 YP, 802587 YP, 802583 YP, 903584325 M 1.0 YP, 802563 YP, 802580 YP, 802586 YP, 903584228 M<		Candidatus carsonella ruddii	Buchnera aphidicola Cc	Candidatus Hodgkinia cicadicola Dsem	Candidatus Sulcia muelleri DMIN	LECA
LiYP,802502YP,802503YP,802503YP,802503YP,802503YP,802531YP,00353714YP,00353761MIL3YP,802552YP,802876YP,00338713YP,00353761YP,00353761YP<00353761	LSU					
12 YF,802550 YF,802855 YF,00308714 YF,00343364 M 1.4 YF,802551 YF,902885 YF,00308712 YF,00343362 M 1.5 YF,802581 YF,903875 YF,00308722 YF,00343362 M 1.6 YF,802580 YF,00308723 YF,00354334 M 1.6 YF,802560 YF,902985 YF,00308723 YF,00354326 M 1.0 YF,802561 YF,9020867 YF,003086703 YF,00354328 M 1.11 YF,802564 YF,9020867 YF,00308673 YF,00354328 M 1.15 YF,802574 YF,802864 YF,003087703 YF,003543370 M 1.16 YF,802574 YF,802864 YF,003087703 YF,00354370 M 1.17 YF,802574 YF,802861 YF,003087703 YF,00354372 M 1.16 YF,802581 YF,90308776 YF,90354376 YF,903543375 M 1.20 YF,802581 YF,802681 YF,00308776 YF,90354336 M	L1	YP_802562	YP_802600		YP_003543227	М
1.3 YP.802582 YP.802887 YP.0038712 YP.00543361 1.5 YP.802543 YP.903876 YP.0038712 YP.00543371 M 1.6 YP.802560 YP.9033873 YP.0038722 YP.00543371 M 1.70.12 YP.802560 YP.9033873 YP.0038723 YP.00354326 M 1.71.12 YP.802561 YP.0038703 YP.00354326 M 1.13 YP.802561 YP.0038773 YP.00354326 M 1.13 YP.802573 YP.0038773 YP.00354326 M 1.14 YP.802573 YP.9038673 YP.00354376 M 1.15 YP.802574 YP.0038773 YP.00354376 M 1.16 YP.802579 YP.0038773 YP.00354375 M 1.17 YP.802579 YP.0038774 YP.00543375 M 1.18 YP.802579 YP.0038774 YP.00543366 YP.00543375 M 1.20 YP.802581 YP.0038774 YP.00543366 YP.00543266 YP.00543266 YP.00543	L2	YP_802550	YP_802885	YP_003038714	YP_003543364	М
1.4 YP.802581 YP.802587 YP.00308772 YP.003543302 1.5 YP.802540 YP.802576 YP.00308722 YP.003543374 M 1.6 YP.802560 YP.802578 YP.00308725 YP.00354326 M 1.0 YP.802561 YP.8035703 YP.00354326 M 1.0 YP.802561 YP.90208703 YP.00354328 M 1.11 YP.802564 YP.90358702 YP.00354328 M 1.13 YP.802584 YP.90358703 YP.00354328 M 1.14 YP.802584 YP.90358720 YP.003543370 M 1.15 YP.802584 YP.90358723 YP.00354337 M 1.16 YP.802584 YP.9035873 YP.00358733 YP.00354337 M 1.17 YP.802581 YP.9035874 YP.00354337 M 1.16 YP.802584 YP.00368774 YP.00354337 M 1.20 YP.802580 YP.00368774 YP.00354336 M 1.21 YP.802581 YP.802586 YP.00354328 M 1.22 YP.802547 YP	L3	YP_802552	YP_802888	YP_003038712	YP_003543361	
L5YP,802543YP,802576YP,00303722YP,00543371ML7L12YP.802560YP,802573YP,00303725YP,00543371ML7L12YP.802561YP,00303703YP,00543226ML10YP.802561YP,00303703YP,00543226ML11YP.802563YP,802587YP,00303703YP,0054326ML13YP,802563YP,9025873YP,00303703YP,00543270ML14YP,802587YP,0030370YP,00543370ML15YP,802587YP,0030372YP,00303728YP,00543376ML16YP.802581YP,0030372YP,00303738YP,00543375ML17YP.802581YP,0030372YP,00303734YP,00543375ML18YP.802580YP,0030374YP,00543375ML19YP,802581YP,00303743YP,00543277ML20YP,802580YP,00303762YP,0054327ML21YP,802580YP,0033762YP,00543261YL23YP,802593YP,802583YP,00303762YP,0054321ML24YP,802593YP,802584YP,00534394ML25YP,802594YP,902543YP,00534376ML28YP,802543YP,9033773YP,90354326ML30YP,802543YP,802543YP,00335781YP,0054337ML31YP,802543YP,802543YP,00335773YP,0054337ML32YP,802546YP,900335773YP,90354334M <td>L4</td> <td>YP_802551</td> <td>YP_802887</td> <td>YP_003038713</td> <td>YP_003543362</td> <td></td>	L4	YP_802551	YP_802887	YP_003038713	YP_003543362	
L6 YP.802540 YP.802573 YP.00338725 YP.00343734 M L9 YP.802561 YP.802912 YP.00338704 YP.00343726 M L0 YP.802563 YP.802901 YP.00338702 YP.00343226 M L11 YP.802564 YP.802564 YP.00338702 YP.00343254 M L14 YP.802544 YP.802566 YP.003038702 YP.00343377 M L14 YP.802546 YP.802586 YP.003038722 YP.00343377 M L16 YP.802546 YP.802861 YP.00308718 YP.00343737 M L16 YP.802537 YP.802872 YP.00308776 YP.00343737 M L19 YP.802501 YP.00383724 YP.00354326 M L20 YP.802583 YP.90354326 YP.00354326 M L21 YP.802543 YP.802877 YP.00383716 YP.00354326 M L21 YP.802543 YP.802877 YP.00354324 YP.00354324 M L22 YP.80	L5	YP_802543	YP_802876	YP_003038722	YP_003543371	М
L7L/L 2 VP_802598 VP_00383704 VP_00343226 L0 VP_802561 VP_8025912 L1 VP_802563 VP_802501 VP_00338703 VP_00343228 M L13 VP_802563 VP_802561 VP_00338702 VP_00343236 M L14 VP_802544 VP_802587 VP_00308723 VP_00343373 M L15 VP_802546 VP_802581 VP_00308726 VP_00343375 M L17 VP.802539 VP_802861 VP_00308726 VP_00343375 M L17 VP.802530 VP_802872 VP_00308733 VP_00343375 M L10 VP_80287 VP_00308734 VP_00343376 M L21 VP_80287 VP_00343740 VP_00343376 M L21 VP_802876 VP_00343412 M M L22 VP_802487 VP_802876 VP_00343412 M L23 VP_802548 VP_00343414 M M L24 VP_802543 VP_802546 VP_0	L6	YP_802540	YP_802873	YP_003038725	YP_003543374	М
1.0 YP_802561 YP_802563 YP_802563 YP_802563 YP_802563 YP_802563 YP_802563 YP_802564 YP 1.14 YP_802564 YP_8025872 YP_00338702 YP_00354325 M 1.15 YP_802544 YP_802586 YP_003038702 YP_003543370 M 1.15 YP_802546 YP_802586 YP_003038712 YP_003543377 M 1.16 YP_802537 YP_802582 YP_003038726 YP_003543376 M 1.17 YP_802537 YP_802581 YP_003038734 YP_00354327 M 1.20 YP_802581 YP_003038734 YP_00354329 M 1.21 YP_802583 YP_80383 YP_003038716 YP_00354329 M 1.21 YP_802583 YP_802586 YP_003543219 M M 1.22 YP_802542 YP_802586 YP_003543219 M M 1.23 YP_802513 YP_802563 YP_003038762 YP_003543219 M 1.24 YP_802577 YP_802533 </td <td>L7/L12</td> <td>YP_802560</td> <td>YP_802598</td> <td>YP_003038704</td> <td>YP_003543226</td> <td></td>	L7/L12	YP_802560	YP_802598	YP_003038704	YP_003543226	
L10 VP_802561 VP_802503 VP_802563 VP_802563 M L13 VP_802563 VP_802601 VP_000386702 VP_00354325 M L14 VP_802537 VP_802804 VP_000387700 VP_003543370 M L15 VP_802537 VP_802860 VP_00038728 VP_00038732 M L16 VP_802531 VP_802812 VP_00038732 VP_00038732 M L17 VP_802531 VP_802851 VP_00038734 VP_00038735 M L13 VP_80237 VP_802851 VP_00038734 VP_000543249 M L14 VP_80230 VP_80280 VP_00038734 VP_000543249 M L21 VP_80230 VP_802864 VP_00038762 VP_00543249 M L23 VP_802803 VP_90038762 VP_00543201 M M L24 VP_802804 VP_00308762 VP_00543313 M M L24 VP_802804 VP_00308762 VP_00543204 M M L23 VP_802513 VP_802864 VP_00338762 VP_00354336 M	L9		YP_802912			
L11 YP_802563 YP_802640 YP_8023643 YP_802344 YP_802344 YP_802364 YP_802363 YP_8023373 M L14 YP_802364 YP_802864 YP_90038728 YP_900343370 M L15 YP_802366 YP_802861 YP_90038728 YP_900343375 M L16 YP_802311 YP_802872 YP_90038726 YP_90038732 M L18 YP_802331 YP_802872 YP_90038733 YP_90038732 M L19 YP_802487 YP_802870 YP_90038734 YP_900343290 M L20 YP_802848 YP_90038716 YP_900343266 YP_90034326 L23 YP_802870 YP_900343762 YP_90034330 M L24 YP_802810 YP_900338786 YP_90034330 M L25 YP_802810 YP_900338786 YP_90034320 M L34 YP_802810 YP_90034373 YP_90034320 M L34 YP_802810 YP_900343873 YP_90034320 M L34	L10	YP_802561	YP_802599	YP_003038703	YP_003543226	Μ
L13 VP_802844 VP_00308663 VP_00354245 L14 VP_802537 VP_802869 VP_00038720 VP_00353370 M L15 VP_802536 VP_802881 VP_00308728 VP_00358376 M L16 VP_802539 VP_802862 VP_00358736 YP_00358736 M L17 VP_802539 VP_802872 VP_00358733 YP_00358735 M L19 VP_802547 VP_802861 VP_00358733 YP_003543287 M L20 VP_802548 VP_802860 VP_003543249 M M L21 VP_802548 VP_802860 VP_00354326 VP_00354326 M L23 VP_802549 VP_802876 VP_003543201 M M L24 VP_802876 VP_00354326 VP_00354321 M M L25 VP_802876 VP_00354326 VP_00354329 M M L24 VP_802876 VP_00354326 VP_00354324 M M L34 VP_802867 VP_00354326 VP_00354326 M M L35 VP_80247	L11	YP_802563	YP_802601	YP_003038702	YP_003543228	Μ
L14 YP_802544 YP_802878 YP_00303720 YP_0035377 M L15 YP_802366 YP_802869 YP_00303728 YP_00353377 M L16 YP_802546 YP_802811 YP_00303728 YP_00353375 M L17 YP_802331 YP_802810 YP_00303728 YP_00353375 M L18 YP_802531 YP_802810 YP_00303743 YP_003543249 M L20 YP_802847 YP_80283 YP_003038716 YP_003543249 M L21 YP_80280 YP_003038716 YP_003543249 M L23 YP_80283 YP_003038762 YP_003543249 M L24 YP_802810 YP_003038762 YP_003543219 M L27 YP_802513 YP_802840 YP_003543249 M L38 YP_802847 YP_00338786 YP_003543249 M L34 YP_802840 YP_00338793 YP_003543249 M L34 YP_802847 YP_00338793 YP_003543248 M	L13		YP_802804	YP_003038663	YP_003543245	
L15 YP_802537 YP_802860 YP_003038728 YP_00354377 L16 YP_802516 YP_802881 YP_003543385 YP_003543372 M L17 YP_802539 YP_802862 YP_003543385 YP_003543375 M L19 YP_802810 YP_00308726 YP_003543287 M L10 YP_802477 YP_802813 YP_003543287 M L11 YP_802487 YP_802886 YP_003543249 M L24 YP_802886 YP_003543201 T L25 YP_802877 YP_802886 YP_003543201 L28 YP_802513 YP_802810 YP_00338762 YP_003543210 L28 YP_802513 YP_802847 YP_00354326 M L31 YP_8028170 YP_00354326 M M L32 YP_802813 YP_802816 YP_003543249 M L34 YP_802870 YP_003543240 M M L35 YP_802871 YP_003543249 M M L34 YP_802871 YP_00354326 YP M L35 <	L14	YP_802544	YP_802878	YP_003038720	YP_003543370	Μ
L16 YP_802546 YP_802881 YP_00363718 YP_003543268 M L17 YP_802531 YP_802872 YP_00338726 YP_00354375 M L18 YP_802511 YP_802872 YP_00358726 YP_003543287 M L10 YP_802611 YP_00358726 YP_003543287 M L21 YP_802511 YP_00358724 YP_003543249 M L22 YP_802580 YP_00354326 YP_003543312 Y L23 YP_802581 YP_003543210 Y <td>L15</td> <td>YP_802537</td> <td>YP_802869</td> <td>YP_003038728</td> <td>YP_003543377</td> <td></td>	L15	YP_802537	YP_802869	YP_003038728	YP_003543377	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	L16	YP_802546	YP_802881	YP_003038718	YP_003543368	Μ
L18 YP_802870 YP_802870 YP_00338726 YP_003543275 M L19 YP_802810 YP_00338734 YP_003543275 M L20 YP_802510 YP_90338734 YP_003543326 M L21 YP_802530 YP_802800 YP_00338716 YP_003543312 L23 YP_802578 YP_00338762 YP_003543201 L24 YP_802579 YP_802808 YP_003543219 L25 YP_802529 YP_802801 YP_00338762 YP_003543219 L26 YP_802513 YP_802801 YP_00338766 YP_0035433219 L30 YP_802801 YP_802801 YP_00338766 YP_003543350 M L31 YP_802423 YP_802801 YP_00338397 YP_003543349 M L32 YP_802420 YP_802801 YP M M L33 YP_802521 YP_802536 YP_00338773 YP_003543348 M L36 YP_802536 YP_903038779 YP_003543348 M M S2 YP_802547 YP_802804 YP_00338773 YP_003543376 M	L17	YP_802531	YP_802862	YP_003543385	YP_003038732	
L19 VP_802810 VP_802837 VP L20 VP_802487 VP_802561 VP_003038744 VP_003543287 M L21 VP_802530 VP_802800 VP_003543312 VP L22 VP_802588 VP_802806 VP_003543312 VP L23 VP_802868 VP_003038762 VP_003543313 M L24 VP_802513 VP_802624 VP_003038762 VP_003543313 M L28 VP_802529 VP_802624 VP_003038762 VP_003543316 M L29 VP_802543 VP_80264 VP_003038762 VP_003543356 M L30 VP_802543 VP_80264 VP_003038762 VP_003543349 M L32 VP_802423 VP_80253 VP_003543349 M M L34 VP_802556 VP_80267 VP_003543349 M M L34 VP_802556 VP_80267 VP_003543348 M M L34 VP_802556 VP_80267 VP_003038717 VP_003543380 M S2 VP_802417 VP_802575 VP_00303873	L18	YP_802539	YP_802872	YP_003038726	YP_003543375	Μ
L20 YP_802487 YP_802501 YP_802503 YP_8	L19		YP_802810	YP_003038743	YP_003543287	Μ
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	L20	YP_802487	YP_802651	YP_003038794	YP_003543249	Μ
L22 YP_802548 YP_802883 YP_003038716 YP_003543366 L23 YP_802866 YP_003543201 YP_003543201 L24 YP_802877 YP_003543313 M L27 YP_802529 YP_802801 YP_00338762 YP_003543219 L29 YP_802800 YP_003543219 M L30 YP_802870 YP_003543349 M L31 YP_802423 YP_802633 YP_003036697 YP_003543248 L33 YP_802536 YP_802650 YP_003038697 YP_003543248 L34 YP_802536 YP_003038697 YP_003543248 M L35 YP_802650 YP_003038697 YP_003543248 M L36 YP_802536 YP_900368779 YP_003543248 M SSU S YP_802547 YP_802867 M S3 YP_802547 YP_802864 YP_003038779 YP_003543243 M S5 YP_802541 YP_802844 YP_00303877 YP_003543336 M S5 YP_802541 YP_802844 YP_003038773 YP_003543334 M	L21	YP_802530	YP_802800		YP_003543312	
L23 YP_80286 L24 YP_802513 YP_802658 YP_003543201 L25 YP_802513 YP_802624 YP_003038762 YP_003543219 L28 YP_802513 YP_802624 YP_00338762 YP_003543219 L29 YP_80280 YP_003543219 YP_003543256 M L30 YP_802870 YP_003543356 M L31 YP_802423 YP_802777 YP_003543249 M L32 YP_802512 YP_802563 YP_003038793 YP_003543248 M L34 YP_802560 YP_003038793 YP_003543248 M L35 YP_802547 YP_802567 YP_003038719 YP_003543184 M S2 YP_802447 YP_802755 YP_003038717 YP_003543343 M S3 YP_802477 YP_802821 YP_003038717 YP_003543343 M S4 YP_802541 YP_900308777 YP_003543344 M S5 YP_802541 YP_900308777 YP_003543344 M S6 YP_802541 YP_900308777 YP_003543344 M	L22	YP_802548	YP_802883	YP_003038716	YP_003543366	
L24 YP_802877 L25 YP_802529 YP_802608 YP_003038762 YP_003543210 L27 YP_802529 YP_802801 YP_003038762 YP_003543219 L28 YP_802803 YP_802800 YP_802802 YP_003543356 M L30 YP_802492 YP_802777 YP_003543356 M L31 YP_802492 YP_802277 YP_003543349 M L33 YP_802512 YP_802563 YP_003038697 YP_003543248 M L35 YP_802564 YP_003038793 YP_003543248 M M L36 YP_802536 YP_802867 YP_003038719 YP_003543380 M SSU S YP_802417 YP_802862 YP_003038719 YP_003543243 M S3 YP_802417 YP_802861 YP_003038717 YP_003543343 M S4 YP_802533 YP_802864 YP_00303873 YP_003543343 M S5 YP_802533 YP_802864 YP_00303873 YP_003543344 M S8 YP_802533 YP_802865 YP_00303	L23		YP_802886			
L25VP_80258VP_003543201L27VP_802513VP_802501VP_00308762VP_003543313ML28VP_802513VP_80264VP_003087662VP_003543219L29VP_80280VP_003543356ML31VP_802433VP_802918VP_003543356ML32VP_802512VP_802570VP_003543329ML33VP_802512VP_802563VP_00308697VP_003543220L34VP_802584MML35VP_802560VP_00308793VP_00354324L36VP_802536VP_802867VP_003088711VP_003543380SSUSVP_802417VP_802711VP_003088717VP_003543184MS3VP_802547VP_802711VP_003088717VP_003543367MS4VP_802533VP_802864VP_003038776VP_003543376MS5VP_802533VP_802864VP_003038777VP_003543376MS4VP_802556VP_802847VP_00308777VP_003543376MS6VP_802541VP_802847VP_00308727VP_003543373MS8VP_802541VP_802864VP_00308774VP_003543373MS9VP_802553VP_802867VP_00308774VP_003543374MS10VP_802553VP_802865VP_00308774VP_003543374MS11VP_802554VP_802865VP_00308772VP_003543375MS12VP_802557VP_802867VP_00308772VP_003543357M </td <td>L24</td> <td></td> <td>YP_802877</td> <td></td> <td></td> <td></td>	L24		YP_802877			
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	L25		YP_802658		YP_003543201	
L28 YP_802513 YP_802624 YP_003038786 YP_003543219 L29 YP_80280 YP_80280 YP_802412 YP_80280 L31 YP_802423 YP_802918 YP_003543356 M L32 YP_802492 YP_802777 YP_003543349 M L33 YP_802512 YP_802584 M M L34 YP_802586 YP_00308793 YP_003543248 M L35 YP_802536 YP_00308779 YP_003543248 M SSU SU SU SU YP_802447 YP_802755 YP_00308779 YP_003543243 M S3 YP_80247 YP_802852 YP_003038717 YP_003543367 M S3 YP_802533 YP_802864 YP_003038777 YP_003543367 M S4 YP_802871 YP_003038787 YP_003543376 M S5 YP_802854 YP_003038787 YP_003543376 M S6 YP_802892 YP_003038787 YP_003543376 M S7 YP_802453 YP_802893 YP_003038707 YP_003543376 M <td>L27</td> <td>YP_802529</td> <td>YP_802801</td> <td>YP_003038762</td> <td>YP_003543313</td> <td>Μ</td>	L27	YP_802529	YP_802801	YP_003038762	YP_003543313	Μ
L29YP_80280L30YP_802870L31YP_802423YP_802870L32YP_802492YP_802777YP_003543349ML33YP_802492YP_802633YP_802512YP_802633YP_003038697L34YP_802584ML35YP_802650YP_003038793YP_802536YP_802867YP_003038714SSUSSUSSUSSUSSUS1YP_802417YP_802755YP_802533YP_802753YP_802547YP_802864YP_802533YP_802864YP_802533YP_802864YP_802533YP_802864YP_802533YP_802864YP_802533YP_802864YP_802533YP_802871S6YP_802533YP_802556YP_903038787S7YP_802533S9YP_802874YP_802533YP_802874YP_802533YP_802874YP_802534YP_802874S9YP_802534YP_802865YP_003038707S9YP_802874YP_802535YP_802889YP_802534YP_802864YP_802535YP_8028707YP_802534YP_802871S1YP_802534YP_802865YP_003038711YP_802535YP_802864YP_802535YP_802865YP_802865YP_00334373S1YP_802535YP_802866YP_0033371S1YP_802535YP_802866YP_00338773S13	L28	YP_802513	YP_802624	YP_003038786	YP_003543219	
L30 YP_802870 L31 YP_802423 YP_802918 $YP_003543356$ ML32 YP_802492 YP_802777 $YP_003543349$ ML33 YP_802512 $YP_8025623$ $YP_003038697$ $YP_003543220$ L34 YP_802584 ML35 YP_802560 $YP_003038793$ $YP_003543248$ L36 YP_802536 YP_802867 $YP_003038713$ $YP_003543340$ SSUS YP_802757 $YP_003038779$ $YP_003543243$ MS2 YP_802417 YP_802757 $YP_003038717$ $YP_003543243$ MS3 YP_802533 YP_802824 $YP_003038717$ $YP_003543243$ MS4 YP_802533 YP_802871 $YP_003038717$ $YP_003543367$ MS4 YP_802533 YP_802871 $YP_003038727$ $YP_003543376$ MS5 YP_802533 YP_802841 $YP_003038777$ $YP_003543376$ MS8 YP_802556 YP_802892 $YP_00303877$ $YP_003543374$ MS8 YP_802551 YP_802803 $YP_003038707$ $YP_003543344$ MS11 YP_802533 YP_802865 $YP_003038707$ $YP_003543354$ MS11 YP_802535 YP_802865 $YP_003038707$ $YP_003543354$ MS13 YP_802535 YP_802865 $YP_003038707$ $YP_003543357$ MS14 YP_802542 YP_802865 $YP_003038707$ $YP_003543357$ MS15 YP_802545 YP_802866 YP_00303	L29		YP_802880			
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	L31	YP_802423	YP_802918		YP_003543356	М
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$\begin{array}{c c c c c c c c c c c c c c c c c c c $	L33	YP_802512	YP_802623	YP_003038697	YP_003543220	
L35YP_802650YP_003038793YP_003543248L36YP_802536YP_802867YP_003038711YP_003543380SSU </td <td>L34</td> <td></td> <td>YP_802584</td> <td></td> <td></td> <td>Μ</td>	L34		YP_802584			Μ
L36 YP_802536 YP_802867 YP_003038811 YP_003543380 SSU SSU SSU SSU S1 YP_802417 YP_802755 YP_003038779 YP_003543184 M S3 YP_802547 YP_802711 YP_003038711 YP_003543367 M S3 YP_802533 YP_802864 YP_003038717 YP_003543383 M S5 YP_802538 YP_802864 YP_003038727 YP_003543383 M S5 YP_802556 YP_802892 YP_003038708 YP_003543358 M S8 YP_802556 YP_802892 YP_003038708 YP_003543358 M S8 YP_802541 YP_802874 YP_003038708 YP_003543358 M S9 YP_802443 YP_802874 YP_003038724 YP_003543373 M S9 YP_802541 YP_802893 YP_00308664 YP_003543344 S10 YP_802553 YP_80289 YP_003038711 YP_003543360 M S11 YP_802557 YP_80289 YP_003038711 YP_003543382 M S12 YP_802557 YP_80289 YP_003038707 YP_003543381 M S12 YP_802557 YP_802893 YP_003038707 YP_003543381 M S13 YP_802535 YP_802865 YP_003038707 YP_003543381 M S14 YP_802535 YP_802893 YP_003038707 YP_003543381 M S14 YP_802542 YP_802875 YP_00308729 YP_003543381 M S14 YP_802542 YP_802875 YP_00308723 YP_003543381 M S14 YP_802542 YP_802875 YP_00308723 YP_003543351 M S15 YP_802466 YP_80279 YP_003543351 M S16 YP_802545 YP_802875 YP_00308723 YP_003543354 M S17 YP_802545 YP_802807 YP_00308723 YP_003543354 M S16 YP_802545 YP_802875 YP_00308719 YP_003543355 M S17 YP_802245 YP_802875 YP_00308719 YP_003543355 M S18 YP_802545 YP_802879 YP_00308719 YP_003543355 M S19 YP_802549 YP_802807 YP_00308719 YP_003543356 M S19 YP_802549 YP_802807 YP_00308719 YP_003543356 M S10 YP_802545 YP_802879 YP_00308719 YP_003543365 M S10 YP_802545 YP_802879 YP_00308719 YP_003543365 M S20 YP_802664 YP_802871 YP_802843 YP_00338719 YP_003543365 M S20 YP_802543 YP_802864 YP_00308715 YP_003543345 YP_003543321	L35		YP_802650	YP_003038793	YP_003543248	
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31 11_3032417 11_30324373 11_3030379 11_00343134 M 52 YP_802417 YP_802711 YP_003038781 YP_003543367 M S3 YP_802533 YP_802882 YP_003038717 YP_003543367 M S4 YP_802533 YP_802864 YP_003038727 YP_003543376 M S5 YP_80256 YP_802914 YP_003038787 YP_003543314 M S7 YP_802566 YP_802892 YP_003038724 YP_003543373 M S8 YP_802541 YP_802892 YP_003038724 YP_003543373 M S9 YP_802443 YP_802803 YP_003038724 YP_003543360 M S11 YP_802535 YP_802893 YP_003038730 YP_003543352 M S12 YP_802557 YP_802865 YP_003038729 YP_003543372 M S13 YP_802542 YP_802866 YP_003038723 YP_003543372 M S14 YP_802542 YP_802867 YP_003038723 YP_003543372 M S14 YP_802545 YP_802867 YP_003038723	S3U S1	VD 802447	VD 802755	VD 003038770	VD 0035/318/	м
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33 11_002047 11_002082 11_00008717 11_00004300 11_00000000 S4 YP_802533 YP_802864 YP_00038803 YP_0003543383 M S5 YP_802538 YP_802871 YP_003038727 YP_0003543376 S S6 YP_802914 YP_003038727 YP_003543376 M S7 YP_802566 YP_802892 YP_003038708 YP_003543373 M S8 YP_802541 YP_802874 YP_003038724 YP_003543360 M S9 YP_802533 YP_802803 YP_003038711 YP_003543360 M S11 YP_802557 YP_802865 YP_003038707 YP_003543360 M S12 YP_802557 YP_802866 YP_003038729 YP_003543381 M S14 YP_802542 YP_802866 YP_003038723 YP_003543354 M S14 YP_802542 YP_802866 YP_003038723 YP_003543354 M S14 YP_802542 YP_802807 YP_003038742 YP_003543369 M S15 YP_802545 YP_802807 YP_003038719 YP_003543	S2 S3	VD 802547	VD 802882	VD_003038717	VP_003543367	M
34 Π_{2} Π	SJ S4	VD 802533	VP 802864	VD_003038803	VP_003543383	M
So 11_002371 11_0033717 11_0033717 11_0034307 S6 YP_802914 YP_003038787 YP_003543314 S7 YP_802556 YP_802892 YP_003038708 YP_003543373 M S8 YP_802541 YP_802874 YP_003038724 YP_003543373 M S9 YP_802443 YP_802803 YP_003038711 YP_003543360 M S10 YP_802553 YP_802865 YP_003038707 YP_003543382 M S11 YP_802557 YP_802866 YP_003038729 YP_003543381 M S14 YP_802542 YP_802875 YP_003038723 YP_003543341 M S14 YP_802542 YP_802875 YP_003038723 YP_003543345 M S15 YP_802546 YP_802879 YP_003038742 YP_003543345 M S15 YP_802545 YP_802879 YP_003038719 YP_003543345 S S16 YP_802545 YP_802844 YP_003038718 YP_003543345 S S S17 YP_802545 YP_802884 YP_003038715 YP_003543315 S	S5	YP 802538	YP 802804	YP_003038727	YP 003543376	141
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S10 YP_802553 YP_802889 YP_003038711 YP_00354324 S11 YP_802534 YP_802889 YP_003038730 YP_003543382 M S12 YP_802557 YP_802893 YP_003038707 YP_003543357 M S13 YP_802535 YP_802866 YP_003038729 YP_003543372 M S14 YP_802542 YP_802875 YP_003038723 YP_003543372 M S15 YP_802466 YP_802790 YP_003543354 M S16 YP_802551 YP_802807 YP_003038719 YP_003543369 S17 YP_802545 YP_802879 YP_003038718 YP_003543315 S18 YP_802549 YP_802844 YP_003038715 YP_003543365 M S20 YP_802644 YP_003038715 YP_003543343 M S21 YP_802610 YP_003543321 YP_003543321	50	YP 802443	YP 802803	YP_003038664	VP 003543244	101
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S16 YP_802521 YP_802807 YP_003038742 YP_003543345 S17 YP_802545 YP_802879 YP_003038719 YP_003543369 S18 YP_802913 YP_003038788 YP_003543315 S19 YP_802549 YP_802844 YP_003038715 YP_003543365 M S20 YP_802664 YP_003543343 YP_003543343 YP_003543343 S21 YP_802610 YP_003543321 YP_003543321	S15	YP 802466	YP 802790		YP 003543354	
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S20 YP_802664 YP_003543343 S21 YP_802610 YP_003543321	S19	YP 802549	YP 802884	YP 003038715	YP 003543365	М
S21 YP_802610 YP_003543321	S20		YP_802664	_	YP_003543343	
	S21		YP_802610		YP_003543321	



Fig. 3. Inference of the set of MRPs present in the last common ancestor of Unikonts and its subsequent evolution in Metazoa, Fungi and Amoebozoa. For details see legend to Fig. 2.

The loss of genes is a frequently observed phenomenon in endosymbiotic events (Moya et al., 2008), but the mitochondrial endosymbiosis appears to be special in having involved both losses and gains. Concerning the mitoribosome, 19 eukaryoticspecific proteins (Ek-MRPs, 9 in the LSU and 10 in the SSU) are present in at least one representative of a Unikont and a Bikont and thus must have appeared before LECA. These proteins were therefore added to the complement of initial Bt-MRPs (Table 1), meaning that the LECA mitoribosome already contained at least 72 proteins (42 in the LSU and 30 in the SSU), 18 proteins more than the original alphaproteobacterial ribosome. These 72 MRPs include 53 Bt-MRPs (26 of which may have been already translocated to the nuclear genome) and 19 Ek-MRPs (Fig. 2). Taken together, these results imply an already largely modified mitoribosome in the LECA and a fairly mature process in genome reduction after endosymbiosis, pointing to a consistent time frame between the endosymbiosis and the diversification of present-day eukaryotic lineages.

3.2. Evolution of the mitoribosome during eukaryotic diversification (after LECA)

The mitoribosomal proteomes of the ancestors of Unikonts and Bikonts are remarkably similar (Fig. 2), with only one gain (L56) and one loss (S1) in the lineage leading to Unikonts. This may suggest that the split between the lineages leading to these two ancestors occurred very rapidly or that mitoribosomes entered in a stationary phase in these two lineages after the acquisition of 19 Ek-MRPs. The loss and gain involving two proteins in the ancestor of Unikonts appear to constitute two important synapomorphies for this group. Conversely, none of the Ek-MRPs characterized in the two proteomes of Kinetoplastids are exclusively present in Bikonts (Table 1), preventing for the time being the identification of synapomorphies for this major eukaryotic supergroup.

Except in the case of Ppe1 (see above), we could not find any homologue of MRPs in any genome of amitochondriates (Table 1), indicating that the loss of mitochondria is virtually always accompanied by the loss of all MRPs, even those encoded in the nucleus of the host. This implies multiple independent gene losses from the host genome, because at least 19 Ek-MRPs and 26 Bt-MRPs were already encoded in the nucleus of LECA. This phenomenon would have repeated multiple times during the evolution of eukaryotes because the transition to an amitochondriate state occurred independently in various unrelated lineages. Our observation indicates that no MRPs were recruited for other functions, differently from other proteins of mitochondrial origin that were retained in amitochondriate



Fig. 4. Inference of the set of MRPs present in the last common ancestor of Bikonts and its subsequent evolution in Archaeplastida, Alveolata, Heterokonta and Excavata. For details see legend to Fig. 2. The missing MRPs that might have been replaced by chloroplastic homologues are indicated in green. Chl indicates that the corresponding chloroplastic protein is encoded in the chloroplast genome.

eukaryotes (Clark and Roger, 1995; Germot et al., 1996; Soltys and Gupta, 1994; Sutak et al., 2004; Tachezy et al., 2001; Tovar et al., 2003).

By assuming the most parsimonious scenarios to take into account MRP losses and gains, we then reconstructed the evolution of the mitoribosome in the six present-day eukaryotic supergroups for which complete genome sequences are currently available (Figs. 3 and 4, respectively). This was inferred according to a reference eukaryotic tree rooted between Unikonts and Bikonts. However, because no gains or losses of MRP are exclusive of Bikonts, assuming alternative rootings of the eukaryotic tree (e.g., within bikonts (Roger and Simpson, 2009)) does not change our inference.

3.2.1. Opisthokonta

We inferred 73 MRPs in the ancestor of Opisthokonta (44 LSU and 29 SSU), including two gains (L40, L44) that appear to

be specific synapomorphies of Opisthokonta, thanks to the proteomic characterizations of mitoribosomes from representatives of Metazoa and Fungi (Table 1 and Fig. 3). The inclusion in our sampling of the choanoflagellate Monosiga brevicollis changes sensibly the picture of the evolution of the mitochondrial ribosome in Opisthokonta that may be gathered by analyzing Metazoa and Fungi only (Fig. 3). In fact, M. brevicollis has retained 11 Bt-MRP coding genes (L2, L5, L14, L16, S3, S4, S8, S12, S13, S14, S19) in its mitochondrial genome while these have moved to the nuclear genome in all other metazoans and fungi analyzed except for S3 in some fungi (Table 1), including Allomyces macrogynus, a deeply branching chytrid (Bullerwell and Gray, 2004) (not shown). The sistership of Metazoa and Choanoflagellata implies that the transfer of these genes to the nuclear genome of Metazoa and Fungi occurred at least twice independently (Fig. 3) and is thus more recent than it was inferred from the analysis of mammals and fungionly (Smits et al., 2007).



Fig. 5. Bayesian phylogenetic tree of mitochondrial ribosomal protein S8. Numbers at nodes represent posterior probabilities (PP). For clarity, only PP greater than 0.5 are shown. The scale bar represents the average number of substitutions per site that occurred during the evolution of this protein. Note that the mitochondrial sequences from *Leishmania* and *Trichomonas* do not cluster with the other mitochondrial homologues. However, based on typical sequence signatures and additional phylogenetic analyses, they are to be considered as genuine mithochondrial versions, they placement at the base of bacteria being likely due to an artefact caused by their fast evolutionary rate.

Interestingly, these genes are still encoded in the mitochondrial genomes of free-living unicellular representatives of other major eukaryotic lineages (e.g., *Phytophtora*, *Thalassiosira*, green and red algae) (Table 2). A tempting hypothesis is that the

transfer to the host nucleus of these MRPs might be linked to the passage to a multicellular lifestyle in the ancestor of Metazoans and that of Fungi. However, this does not appear to be the case for Archaeplastida.



Fig. 6. Inference of the set of ribosomal proteins that was present in the last archaeal common ancestor and its subsequent evolution along the archaeal phyla: Crenarchaeota and Euryarchaeota (Woese et al., 1990), and the two additional proposed phyla: Korarchaeota (Barns et al., 1996) and Thaumarchaeota (Brochier-Armanet et al., 2008). Universal proteins are shown in black, whereas proteins found only in archaea and eukaryotes are in brown. Finally, proteins that are found only in archaea are shown in red. Protein acquisitions are indicated by dark green arrows, whereas losses are highlighted by dark red arrows. The proteins inferred to have been present in the last archaeal common ancestor are those MRPs shared by at least one present-day archaeon and one present-day eukaryote. These losses have been inferred by positing a sistership of Archaea and Eukaryotes (3-domains hypothesis). Under the alternative hypothesis that Eukaryotes derive from within the Euryarchaeota, the five losses at the base of Euryarchaeota would become five gains in the lineage leading to Crenarchaeota and Eukaryotes.

Compared to Metazoa and Fungi, the impressive number of 18 MRPs (of which eight Bt-MRPs) appears to have been lost in the lineage leading to *M. brevicollis* (Fig. 3). In order to confirm these gene losses and to verify whether these have been compensated by the recruitment of novel proteins it will be essential to have sequence data from a second choanoflagellate and/or to characterize the mitoribosomal proteome of a choanoflagellate representative.

After the divergence of *Monosiga* the mitochondrial ribosomal proteome of Metazoa appears to have undergone five losses and 11 gains. In the absence of proteomic characterizations other than mammals, it cannot be possible to infer the eventual gain of proteins in other metazoan lineages. Nevertheless, only few losses appear to have occurred during metazoan diversification. In contrast, our increased taxonomic sampling allows to infer a more dynamic evolution of the mitochondrial ribosome in Fungi than previously reported by Smits et al. (Smits et al., 2007), with many losses and gains in both Basidiomycetes and Ascomycetes (Fig. 3). For example, we found a previously undetected S34 homologue in one fungal genome of our taxonomic sampling (*Ustilago maydis*). Moreover, an S34 homologue is present also in two other basidiomycetes not considered in our taxonomic sampling (*Laccaria bicolor* and *Coprinopsis cinerea*) (not shown). This means that the ancestor of Fungi likely harboured S34, which was thus lost many times independently during the evolution of this group (Fig. 3).

Four of the proteins characterized in the mitoribosomal proteome of *S. cerevisiae* appear to be specific novel recruitments in the branch of Saccharomycotina, since these have homologues in other members of this lineage (not shown) but in no other fungal genome. We also inferred five losses in the branch leading to *Schizosaccharomyces pombe*. It would be extremely interesting to characterize the mitochondrial

ribosomal proteome of additional representatives of Fungi, and in particular other lineages such as Basidiomycetes, Zygomycetes or Chytridiomycetes, in order to verify whether the recruitment of new MRPs is a widespread phenomenon in Fungi.

3.2.2. Amoebozoa

We found a previously unreported homologue of L34 in the genome of Dictyostelium discoideum, while we could not find any homologues of S6 and S16, contrary to previous reports (Smits et al., 2007). As in the case of Monosiga, an impressive number of 14 losses, 11 of which involving Bt-MRPs, appears to have occurred in D. discoideum (Fig. 3). It will be essential to obtain additional genomes from representatives of Amoemitochondriate bozoa (especially from relatives of Entamoeba), as well as proteomic characterizations to verify whether these losses are a general feature of this phylum and have been compensated by the inclusion of new proteins.

3.2.3. Archaeplastida

We inferred 66 MRPs in the ancestor of Archaeplastida (38 LSU and 28 SSU). Then, subsequent evolution of mitochondrial ribosomes appears to have been very dynamic (Fig. 4). Experimental studies on land plants have shown that mitochondrial genes are regularly transferred to the nucleus (Adams et al., 2000; Bergthorsson et al., 2003; Fallahi et al., 2005), and there are examples of shared usage of ribosomal proteins between mitochondrial, chloroplastic and the cytosolic ribosomes (e.g., S13 and L21 (Adams et al., 2002; Gallois et al., 2001)).

S23 was previously identified in Metazoans and Fungi only and therefore considered as a specific acquisition of Opisthokonta (Smits et al., 2007). However, we found previously undetected homologues of S23 in the two land plants genomes (Table 1b). Its presence in land plants pushes the origin of this protein back to LECA and increases the number of independent losses in all other major lineages (Figs. 2 and 4). S8 homologues were previously reported from the two land plants genomes (Smits et al., 2007). However, phylogenetic analysis shows that these are in fact cytoplasmic ribosomal homologues (Fig. 5) and it was experimentally shown that the cytoplasmic S8 copy works also in the mitochondrion (Adams et al., 2002). Intriguingly, a bona fide mitoribosomal S8 homologue is present in the mitochondrial genomes of Ostreococus tauri and the red algae Cyanidioschyzon merolae, meaning that this protein was lost (and/or replaced by its cytosolic counterpart) at least twice independently, i.e. in Chlamydomonas reinhardtii and land plants. Finally, phylogenetic analysis indicates that the S5 and S13 homologues reported by Smits et al. (Smits et al., 2007) in C. reinhardtii are in fact chloroplastic ribosomal copies (Supplementary Figs. 1 and 2).

We found only one difference between the mitochondrial ribosomal proteome of *Oryza sativa* and that of *Arabidopsis thaliana*. In fact, S13 has been lost in *A. thaliana*, consistent with a recent duplication of the gene coding for the chloroplastic copy of S13 that was recruited to work in the

mitochondrion (Adams et al., 2002). On the contrary, we inferred a high number of losses in green and red algae (up to 15 in *C. reinhardtii* and *C. merolae* (Fig. 4)), with few overlaps, indicating that most of these losses occurred independently in these algal lineages. It would be interesting to investigate these lineages by a proteomic approach to see if some of these losses have been compensated by the recruitment of MRPs of chloroplastic origin encoded in the nucleus, when these are present (indicated in green in Fig. 3), or even of MRPs that are still encoded in the chloroplast genome (indicated by chl in Fig. 3) since this would require transport from one organelle to the other.

3.2.4. Heterokonta-Alveolata

Phylogenetic analysis indicates that Heterokonta and Alveolata share a common ancestor that possessed a chloroplast issued from a secondary endosymbiosis with a red alga (see (Simpson and Roger, 2004) and (Archibald, 2009) and references therein). We inferred for the first time the mitoribosome of this ancestor, which contained 59 MRPs (35 LSU and 24 SSU). Subsequent evolution of the mitoribosome appears to have been very different in Heterokonts and Alveolates. We inferred the loss of one Bt-MRP and of three Ek-MRPs in the branch leading to Phytophtora ramorum and Thalassiosira pseudonana (Fig. 4). No further losses occurred in P. ramorum, whereas six nuclear encoded MRPs (L35, L36, L43, S6, Ppe1) appear to have been specifically lost in T. pseudonana (Fig. 4). By contrast, more losses occurred during evolution of Alveolata (Fig. 4). We highlighted five previously unidentified MRPs (L6, L10, L25, L54, S10, (Smits et al., 2007)) in the three apicomplexan genomes analyzed (Table 1). These apicomplexan homologues are often very divergent (not shown) and we probably detected them thanks to a better HMM model based on our wide taxonomic sampling. Importantly, two (L6 and L25) have no homologues in humans (Table 1) and may thus represent potential targets for antimalarial treatment. We found also previously unreported homologues of L35, L41, and Ppe1 in the two genomes from Ciliates (Table 1). This allows us to infer the presence of L35 in the ancestor of Bikonts and the presence of Ppe1 in the ancestor of Alveolata (Fig. 4).

3.2.5. Excavata

We inferred 62 MRPs in the ancestor of Excavata (38 LSU and 24 SSU). With respect to previous analyses (Smits et al., 2007), we included a free-living representative (*Naegleria* gruberi). Moreover, we took advantage of the recent proteomic characterization of the mitoribosome from a second kinetoplastid representative (*Trypanosoma brucei*) (Zikova et al., 2008). This allowed us to identify previously unreported MRP homologues (Table 1 in red with an X) and better reconstruction of the evolution of the mitoribosome in this important eukaryotic supergroup, as well as the events that have taken place during the emergence of kinetoplastid parasites. In fact, we infer that 14 losses have occurred specifically in the branch leading to the two parasitic kinetoplastids (Fig. 4); some of these losses may have been compensated by the specific appearance of a very large number of new MRPs (Fig. 4), as shown by the proteomic characterization of *Leishmania* and *Trypanosoma*. Indeed only one of the 95 novel MRPs that have been characterized in the two kinetoplastids is present in *N. gruberi* (Table 1), which indicates that these are genuine recent acquisitions in the ancestor of these parasites (Fig. 4). It will be crucial to obtain the mitoribosome proteome of another member of Excavata to verify if massive acquisitions of novel MRPs are a peculiarity of kinetoplastids or of the whole phylum.

3.3. Evolutionary considerations

Ribosomes are frequently considered as "frozen" cellular machines, because of their key function. However, our phylogenomic investigation shows that this does not apply to mitochondrial ribosomes, which have undergone a very dynamic history, both before and after LECA. It has been speculated that the mitochondrial endosymbiosis coincided with the origin of Eukaryotes, where the ancestor of mitochondria would have been engulfed by an archaeon, the association starting a burst of evolution giving rise to eukaryotes (Martin and Müller, 1998). However, the mitoribosomal complex has gained at least 19 novel MRPs in the time frame going from the endosymbiosis of the mitochondrial ancestor up to LECA. Finally under a scenario of eukaryotic origins coinciding with the engulfment of an alphaproteobacterium by an archaeon, it might have been expected that the increase in size of the mitochondrial ribosome would have been accomplished through recruitment of proteins already present in the archaeal host instead of new ones. It is perhaps more likely that the enrichment of the mitoribosome with novel proteins occurred in a host that was rather a protoeukaryote (either derived from an archaeon or representing a separate lineage), with a genome containing already a large fraction of eukaryotic-specific genes. Consistently, comparison with different endosymbionts shows that the loss/translocation of ribosomal proteins is a late event in the reductive process, indicating that the endosymbiosis at the origin of the mitochondrion did not coincide with LECA but occurred some time before in an ancestor. Finally, it has been put forward that the three-domains structure of universal phylogenetic trees is the fruit of a reconstruction artefact caused by an acceleration of evolutionary rates in eukaryotes, whereas the true tree should show eukaryotes as deriving from within archaea (Cox et al., 2008; Foster et al., 2009; Lake, 1988; Rivera and Lake, 1992, 2004; Tourasse and Gouy, 1999). However, despite the very fast evolutionary rate of ribosomal mitochondrial proteins, their bacterial origin could always be easily recovered by phylogenetic analysis. This suggests either that the hypothesis of an artefact affecting universal trees does not hold, or that another type of artefact other than accelerated evolutionary rates is at work.

It should be noted that the loss of only one Bt-MRP occurred before LECA, indicating that, initially, the ancestral alphaproteobacterial ribosome experienced accretion of novel proteins uniquely, whose role was therefore not to compensate for the loss of Bt-MRPs. Such increase in size of the mitoribosome is puzzling, as it has been neither observed in the ribosomes of other bacterial endosymbionts nor in other organelles such as plastids. Interestingly, the addition of novel proteins appears to have continued during the diversification of present-day eukaryotic lineages, and is still ongoing. Only one loss and one gain of MRPs could be inferred after the split between Unikonts and Bikonts, but a large number of such events occurred subsequently, during the diversification of present-day eukaryotic lineages, and this process is still ongoing, as exemplified by great differences between mitoribosome of closely related organisms. Interestingly, these gains and losses have occurred repeatedly and independently in different lineages. It is worth noting that both Bt-MRPs and Ek-MRPs have been equally affected by this dynamic of gene losses, suggesting that there is no general trend in protein dispensability.

In contrast to the chaotic history of mitoribosomes, their cytosolic ribosomal counterparts have remained largely stable during eukaryotic evolution (data not shown). Therefore, there is definitely some unique and still unclear factor driving the instability in composition of mitochondrial ribosomes. Intriguingly, the archaeal ribosome also appears to have experienced a dynamic history. It has in fact experienced multiple independent losses during the history of this domain (Fig. 6), in clear contrast with its bacterial counterpart, whose composition is much more stable (Lecompte et al., 2002). However, there is other evidence of streamlining in additional informational archaeal systems (see for example (Gribaldo and Brochier, 2006) and (de Crecy-Lagard et al., 2010)). This indicates that the archaeal ancestor was more complex (at least for some cellular systems) than its present-day descendants. Interestingly, and differently from mitoribosomes, losses have involved uniquely those MRPs shared between Archaea and Eukarya (in brown in Fig. 6) but not universal MRPs (in black in Fig. 6). It will be interesting to investigate further the reasons for such instability of the archaeal ribosome.

4. Conclusions

The combination of genomic and proteomic data in phylogenomic analyses promises to become a major tool of the post-genomic era to dissect early evolutionary events. Too little is known about the composition of the majority of cellular systems, especially in representatives of major eukaryotic phyla other than mammals or yeasts. Proteomics of mitoribosomes from protist lineages should be relatively easy to accomplish and would provide essential insights for the reconstruction of a complete picture of early and more recent events in its evolution, which may shed light on LECA and therefore eukaryotic origins.

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Appendix. Supplementary data

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