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SUPPLEMENTARY INFORMATION

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The physiology and habitat of the last universal common ancestor

Madeline C. Weiss*, Filipa L. Sousa*, Natalia Mrnjavac, Sinje Neukirchen, Mayo Roettger, Shijulal Nelson-Sathi, William F. Martin‡

Institute of Molecular Evolution

Heinrich Heine University Düsseldorf

Universitätsstraße 1

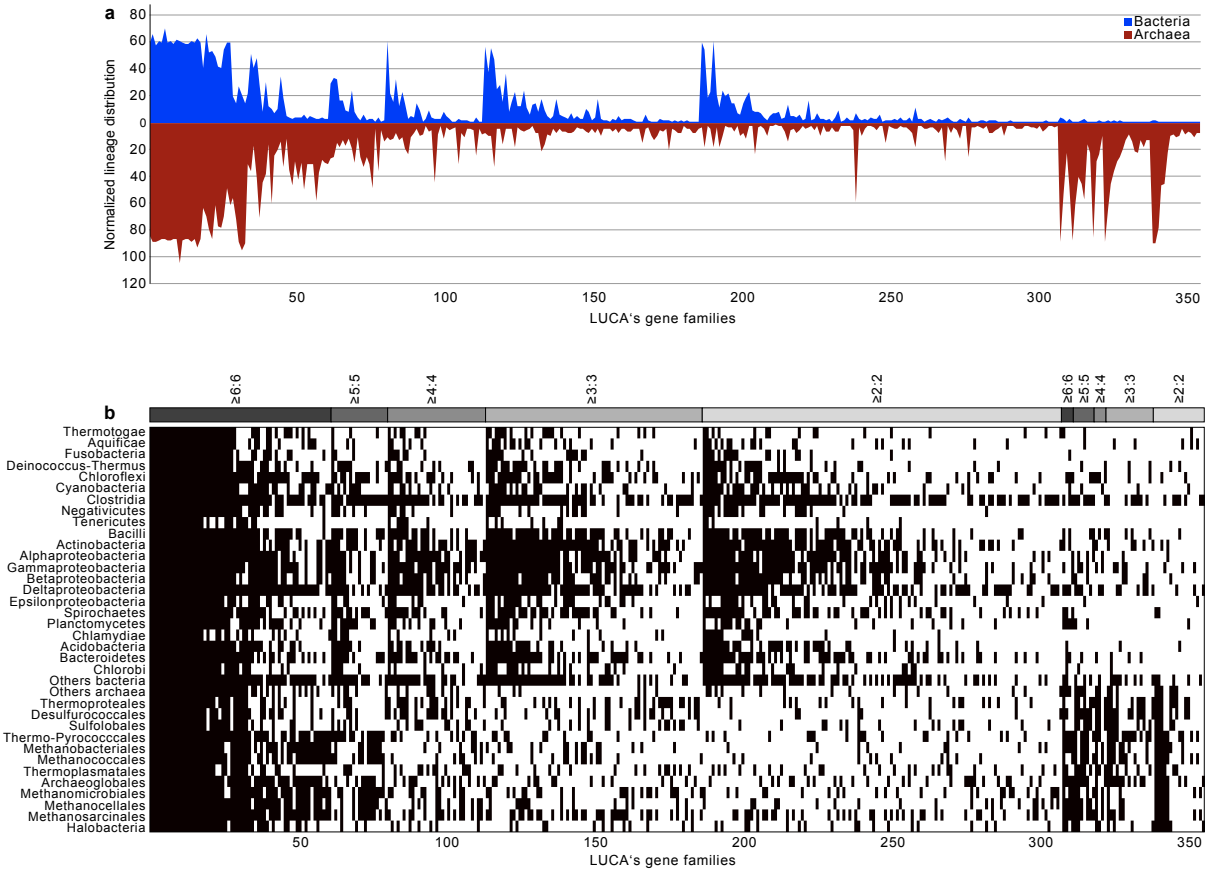
40225 Düsseldorf, Germany

* equal contribution

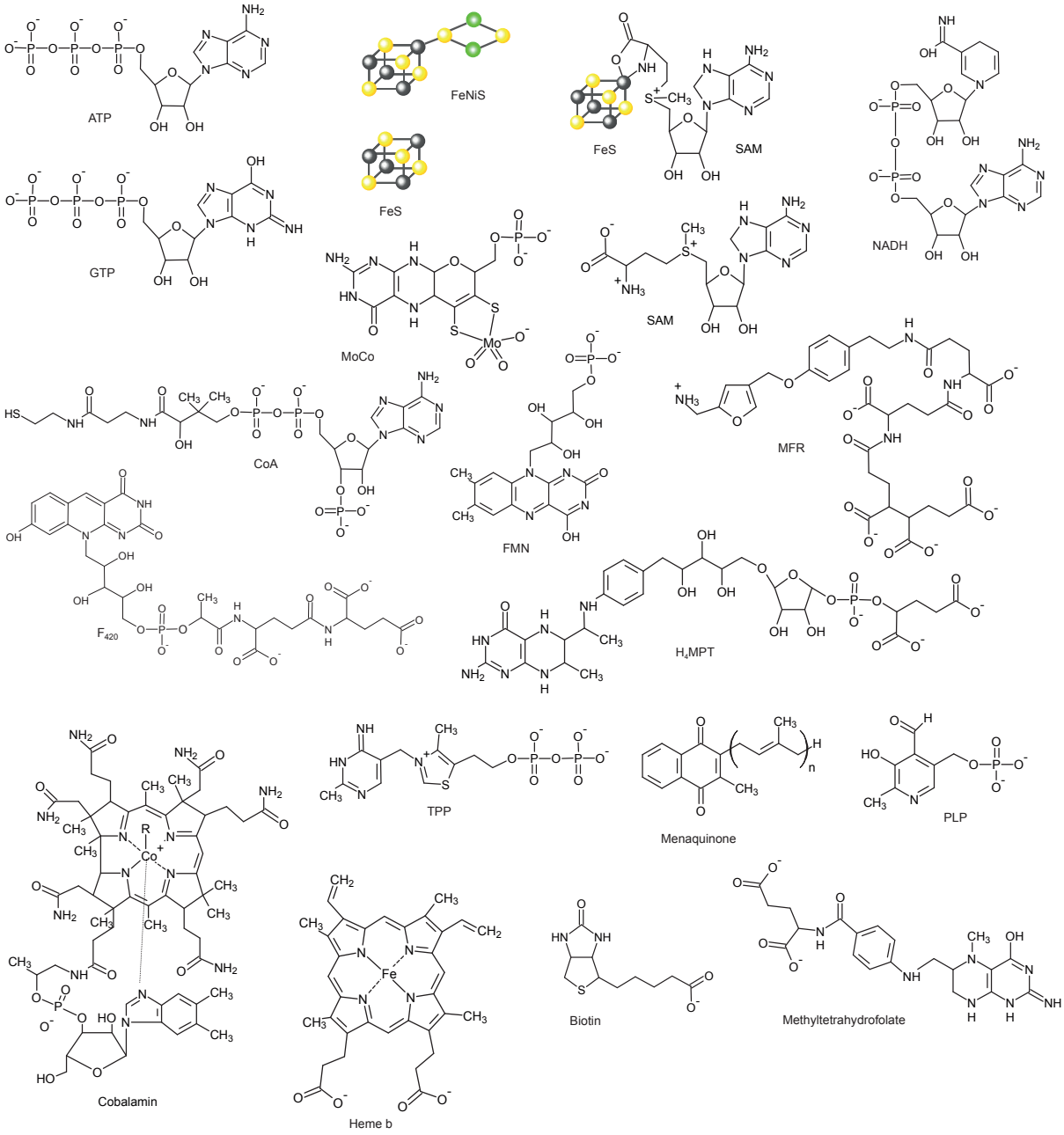
‡ bill@hhu.de

Supplementary Information

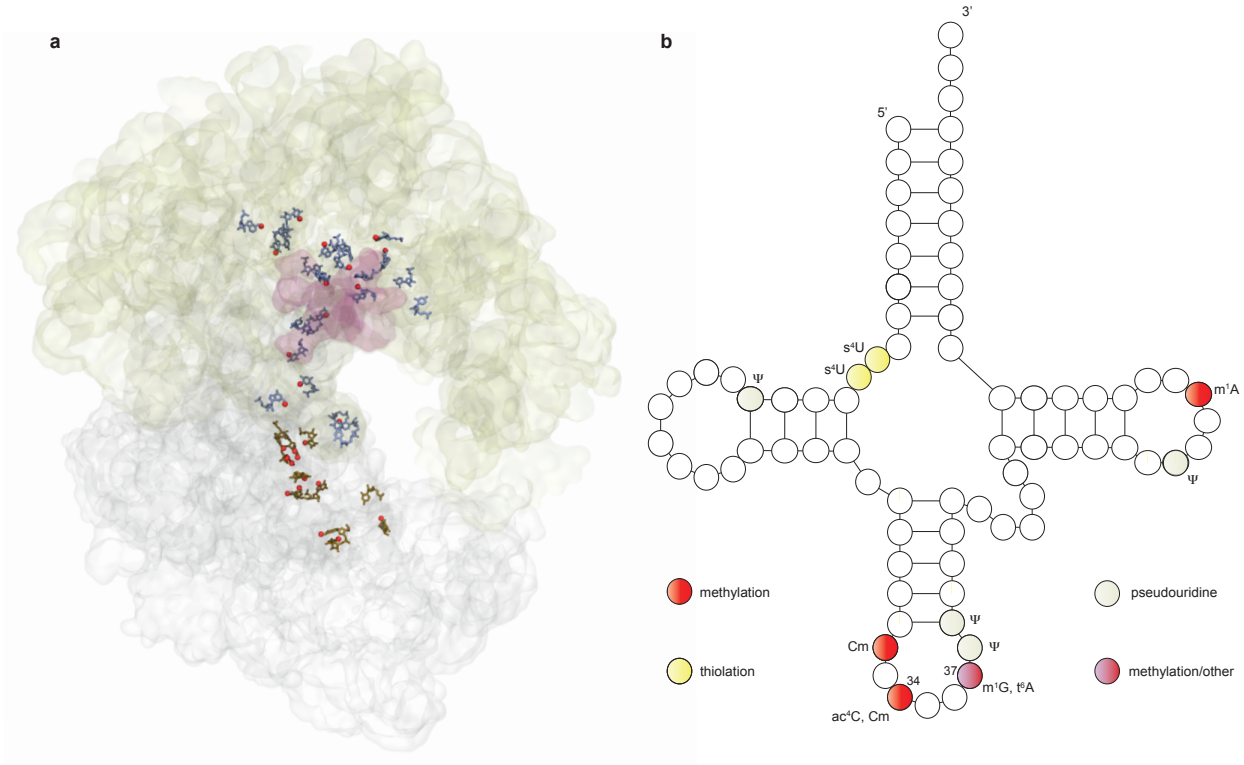
Supplementary Figures



Supplementary Fig. 1 – Archaeal and bacterial lineage distributions within LUCA's 355 gene families. a) Normalized lineage distribution for bacterial (blue) and archaeal (red) organisms present in each of the 355 LUCA's gene families (x-axis). Families (clusters) where one of the domains is overrepresented ≥20:1 (308 to 355) are sorted on the right side of panel a). The preponderance of archaeal versus bacterial sequences for these 48 proteins is likely due to the small archaeal sample (fewer detectable interdomain LGTs), though, none of these 48 are shown in Fig. 3. b) Taxonomic group representation (y-axis) of the organisms represented within LUCA's 355 gene families (x-axis) sorted by the number of bacterial and archaeal groups represented (top). Black ticks indicate gene presence in a given taxonomic group. The two identified groups of clusters were first sorted according to the number of bacterial and archaeal groups present (top) and then sorted by the total number of taxonomic groups present.



Supplementary Fig. 2 – Structures of the cofactors found in LUCA's protein set. SAM is shown both in free form and bound to an FeS cluster, which is the form encountered in radical SAM enzymes. Cobalamin was chosen as representative of corrin-based cofactors, menaquinone as representative of quinone cofactors, FMN as representative of flavins, MoCo as representative of molybdopterin-based cofactors, and heme b as representative of heme cofactors. NADPH is not shown as it differs from NADH in one phosphate group attached to the 2'-O of the ribose ring. Mononuclear metal centers (Fe and Cu) and the non-standard amino acid selenocysteine are not shown, nor are small protein electron carriers such as ferredoxin or rubredoxin. NTP is also listed as a cofactor, but not shown here as it stands for any of the nucleoside triphosphates in those cases when it's not known which one is bound by the enzyme, or when more than one nucleoside triphosphate can be used. Abbreviations: FeNiS – nickel-iron-sulfur cluster; FeS – iron-sulfur cluster; MoCo – molybdenum cofactor; SAM – S-adenosylmethionine; CoA – coenzyme A; MFR – methanofuran; H_4MPT – tetrahydromethanopterin; TPP - thiamine pyrophosphate; PLP - pyridoxal phosphate; NTP – nucleoside triphosphate.



Supplementary Fig. 3 – Modified nucleosides and the genetic code. a) Structure of the *E. coli* ribosome[1] (PDB ID: 4YBB), with the large and small subunits shown in green and silver, respectively. The peptidyl-transferase site is shaded pink[2,3]. The modified nucleosides of 23S rRNA are depicted in icy blue, while in 16S rRNA they are ochre. Modification of C2501 to 5-hydroxycytidine is not present in the structure. Methyl group carbons are shown as red balls. b) Cloverleaf secondary structure representation of tRNA showing only those posttranscriptional nucleoside modifications that are conserved among bacteria and archaea in both identity and position (see Methods). The presence of 5-methoxyuridine at position 34 in archaea has been disputed and is hence not shown[4]. Abbreviations as in Fig. 4.

Supplementary Tables

Supplementary Table 1. Cofactors in LUCA's proteins

Transition metal-based cofactors and redox electron carriers in LUCA's proteome

Functional category	Proteins tracing to LUCA	Cofactors* FeS	NAD(P)	Flavin†	MoCo‡	Fd§	Corrin	F_{420}	FeNiS	Heme
Information										
Ribosome biogenesis	19	-	-	-	-	-	-	-	-	-
Translation	12	-	-	-	-	-	-	-	-	-
RNA modification	8	1	1	-	-	-	-	-	-	-
DNA binding	15	1	-	-	-	-	-	-	-	-
Nucleic acid handling	17	-	-	-	-	-	-	-	-	-
Physiology										
Energy metabolism	2	-	-	-	-	-	-	-	-	-
Carbon assimilation	13	7	-	2	2	2	4	3	2	-
Nitrogen assimilation	7	4	1	1	3	4	-	-	-	-
Cofactor biosynthesis	19	4	3	1	1	-	-	-	-	-
Nucleotide metabolism	11	1	-	1	-	-	-	-	-	-
Amino acid metabolism	10	-	1	-	-	-	-	-	-	-
Redox chemistry	26	11	9	8	4	2	-	1	1	1
Protein modification	2	-	-	-	-	-	-	-	-	-
Lipid metabolism	10	-	2	1	-	-	-	-	-	-
Sugar-related	18	-	1	-	-	-	-	-	-	-
Cellular	31	1	-	-	-	-	-	-	-	-
Cell wall related	7	-	-	-	-	-	-	-	-	-
Transport	52	-	1	-	-	-	-	-	-	-
Others	7	-	-	-	-	-	-	-	-	-
Unknown & uncharacterized	61	2	1	1	-	-	1	-	-	-
Oxygen	8	1	2	1	-	-	-	-	-	2
Total	355	33	22	16	10	8	5	4	3	3

Other (mostly group transfer) cofactors in LUCA's proteome

Functional category	Proteins tracing to LUCA	Cofactors* ATP	SAM‖	CoA	GTP	H_4MPT	NTP	Sec¶	MFR
Information									
Ribosome biogenesis	19	1	-	3	4	-	1	-	-
Translation	12	9	-	-	2	-	-	-	-
RNA modification	8	1	4	-	-	-	-	-	-
DNA binding	15	-	1	-	-	-	-	-	-
Nucleic acid handling	17	7	-	-	1	-	-	1	-
Physiology									
Energy metabolism	2	-	-	-	-	-	-	-	-
Carbon assimilation	13	2	-	2	-	4	-	-	2
Nitrogen assimilation	7	6	-	-	-	-	-	-	-
Cofactor biosynthesis	19	3	4	-	-	-	-	-	-
Nucleotide metabolism	11	2	1	-	-	-	-	-	-
Amino acid metabolism	10	2	-	2	-	-	-	-	-
Redox chemistry	26	-	-	-	-	-	-	1	-
Protein modification	2	1	-	-	1	-	-	-	-
Lipid metabolism	10	-	-	5	-	-	-	-	-
Sugar-related	18	1	-	-	-	-	-	-	-
Cellular	31	7	1	1	3	-	-	-	-
Cell wall related	7	-	-	-	-	-	-	-	-
Transport	52	24	-	-	-	-	-	-	-
Others	7	2	1	-	-	-	-	1	-
Unknown & uncharacterized	61	1	3	1	2	-	2	-	-
Oxygen	8	-	-	-	-	-	-	-	-
Total	355	69	15	14	13	4	3	3	2

* Cofactors occurring only in one protein family or present exclusively in oxygen-related protein families are not shown (see Methods). Abbreviations: FeS – iron-sulfur cluster, SAM – S-adenosylmethionine, CoA – coenzyme A, MoCo – molybdenum cofactor, FeMoCo – iron-molybdenum cofactor, Fd – ferredoxin, H_4MPT – tetrahydromethanopterin, FeNiS – nickel-iron-sulfur cluster, Sec – selenocysteine, NTP – nucleoside triphosphate, MFR – methanofuran.

† FMN and FAD were counted as flavin. ‡ FeMoCo and tungsten-based pterins were counted as MoCo.

§ Ferredoxin, flavodoxin or methanophenazine were counted as Fd.

‖ Out of the 15 SAM dependent enzymes, 7 are radical SAM enzymes, 6 are non-radical, 1 uses decarboxylated SAM (dcSAM) as aminopropyl group donor, and 1 is uncharacterized.

¶ Selenium was listed in the table due to its potential catalytic role in selenoenzymes (see Methods).

Supplementary Table 2. Functional and taxonomic characterization of the 355 protein families potentially present in LUCA using a threshold of 25% global identity. (*provided as separate Excel file)

Supplementary Table 3. χ^2 test of independence

Lineage distribution	LUCA's 355 gene families (%)	11,093 protein families (%)
Archaeoglobales	0.39	0.25
Desulfurococcales	0.77	0.47
Halobacteria	2.17	1.74
Methanobacteriales	0.67	0.44
Methanocellales	0.35	0.23
Methanococcales	0.94	0.73
Methanomicrobiales	0.68	0.45
Methanosarcinales	1.15	0.77
Sulfolobales	0.73	0.95
Thermo-Pyrococcales	1.18	0.75
Thermoplasmatales	0.29	0.18
Thermoproteales	0.99	0.61
Other archaea	0.21	0.14
Acidobacteria	0.73	0.52
Actinobacteria	11.91	11.51
Alphaproteobacteria	10.38	9.86
Aquificae	0.58	0.40
Bacilli	9.53	13.99
Bacteroidetes	4.34	3.38
Betaproteobacteria	8.35	7.87
Chlamydiae	0.35	0.58
Chlorobi	0.84	0.55
Chloroflexi	1.31	0.98
Clostridia	7.62	6.30
Cyanobacteria	2.61	2.09
Deinococcus-Thermus	1.18	0.92
Deltaproteobacteria	4.45	3.23
Epsilonproteobacteria	1.56	2.12
Fusobacteria	0.31	0.21
Gammaproteobacteria	17.21	23.20
Negativicutes	0.43	0.28
Planctomycetes	0.47	0.33
Spirochaetes	1.63	1.42
Tenericutes	0.50	0.45
Thermotogae	1.14	0.73
Other bacteria	2.04	1.37
P-value	0.87	

Functional classification

COG distribution	LUCA's 355 gene families	11,093 clusters
Information	62	865
Metabolism	133	3127
Cellular	52	3793
Poorly characterised	58	1779
Not declared	50	1529
P-value	$<< 10^{-16}$	

χ^2 test of independence between the LUCA's 355 candidate gene families and the 11,093 protein families containing archaeal and bacterial homologues with regard to their lineage and to their functional (COG) distribution. For the χ^2 test, the absolute numbers of families, not their proportions were used.

Supplementary Table 4. Subset of LUCA's proteins (RNA modification, energy metabolism, C and N assimilation) and respective cofactors

Proteins	Cofactors
RNA modification	
tRNA U55 pseudouridine synthase TruB	-
tRNA C32,U32 (ribose-2'-O)-methylase TrmJ or a related methyltransferase	SAM
tRNA U38,U39,U40 pseudouridine synthase TruA	-
tRNA A37 threonylcarbamoyltransferase TsaD	ATP
RNA:NAD 2'-phosphotransferase, TPT1/KptA family	NAD
23S rRNA G2069 N7-methylase RlmK or C1962 C5-methylase RlmI	SAM
16S rRNA C967 or C1407 C5-methylase, RsmB/RsmF family	SAM
tRNA/tmRNA/rRNA uracil-C5-methylase, TrmA/RlmC/RlmD family	SAM, FeS
Energy metabolism	
Archaeal/vacuolar-type H^+-ATPase subunit I/STV1	-
BioD-like N-terminal domain of phosphotransacetylase	-
Carbon assimilation	
CO dehydrogenase/acetyl-CoA synthase delta subunit (corrinoid Fe-S protein)*	FeS [2x], corrinoid cofactor [2x], FeNiS
CO dehydrogenase/acetyl-CoA synthase gamma subunit (corrinoid Fe-S protein) [2x]*, †	FeS [2x], corrinoid cofactor [2x], FeNiS
CO or xanthine dehydrogenase, Mo-binding subunit	MoCo
Formylmethanofuran dehydrogenase subunit A*	MFR, H_4MPT, FeS, MoCo
Formylmethanofuran dehydrogenase subunit C*	MFR, H_4MPT, FeS, MoCo
Formylmethanofuran:tetrahydromethanopterin formyltransferase	MFR, H_4MPT
Coenzyme F_{420}-reducing hydrogenase, beta subunit [2x] †	FeS, F_{420}, flavin, Fd/methanophenazine
Methenyltetrahydromethanopterin cyclohydrolase	H_4MPT
Flavin-dependent oxidoreductase, luciferase family	F_{420}, H_4MPT
Acetyl-coenzyme A synthetase	(ATP, CoA)
Acyl-coenzyme A synthetase/AMP-(fatty) acid ligase	(ATP, CoA)
Nitrogen assimilation	
Nitrogenase molybdenum-iron protein, alpha and beta chains [2x] †	FeS, FeMoCo, ATP, Fd
Nitrogenase subunit NifH, an ATPase (fusion)	FeS, FeMoCo, Fd/flavodoxin, ATP
Nitrogenase subunit NifH, an ATPase	FeS, Fd/flavodoxin, ATP
Glutamine synthetase	ATP
Nitroreductase	flavin, NAD(P)
ADP-ribosylglycohydrolase	ATP

* In case of protein complexes, the cofactors of the entire complex are shown next to each of the subunits of the complex. Exceptionally, when the subunit couldn't be pinpointed to a specific protein complex with certainty, only the cofactors of that subunit are shown.

† When a subunit of a protein complex appears twice, it is scored as presence of an additional copy of that complex, with the associated cofactors.

Abbreviations used as in Supplementary Table 1.

Supplementary Table 5. Subset of LUCA's proteins (cofactor biosynthesis and redox chemistry) and respective cofactors

Proteins	Cofactors
Cofactor biosynthesis	
F_{420}-0:Gamma-glutamyl ligase (F_{420} biosynthesis)	-
Molybdenum cofactor biosynthesis enzyme	-
Molybdenum cofactor biosynthesis enzyme MoaA	SAM, FeS [2x]
Molybdopterin biosynthesis enzyme	MoCo
Dihydropteroate synthase	-
Glutamyl-tRNA reductase	NAD(P)
Mg-chelatase subunit ChlD[a]	ATP
Siroheme synthase (precorrin-2 oxidase/ferrochelatase domain)[a]	SAM, NAD(P)
Sirohydrochlorin ferrochelatase	FeS
Precorrin-6B methylase 1	SAM
Protoporphyrinogen oxidase (anaerobic)	flavin, menaquinone
2-iminoacetate synthase ThiH (thiamine biosynthesis)	SAM, FeS, NAD(P)
Sulfopyruvate decarboxylase, TPP-binding subunit (coenzyme M biosynthesis)[*]	TPP
Archaeal 2-phospho-L-lactate transferase CofD/UPF0052 family	-
Gamma-glutamyl:cysteine ligase YbdK, ATP-grasp superfamily	ATP
Glutathione synthase/RimK-type ligase, ATP-grasp superfamily	ATP
Predicted Fe-Mo cluster-binding protein, NifX family	-
4-hydroxybenzoate polyprenyltransferase	-
hypothetical protein (Uro-D domain)	-
Redox chemistry	
Sulfur relay (sulfurtransferase) protein, DsrC/TusE family	-
Rieske Fe-S protein	FeS
Archaeal flavoprotein	flavin
Aldehyde:ferredoxin oxidoreductase [2x]	FeS, MoCo, Fd
Fe-S oxidoreductase	FeS
Fe-S-cluster-containing dehydrogenase component	FeS
FMN-dependent dehydrogenase	flavin, NAD(P)
Formate hydrogenlyase subunit 6/NADH:UQ oxidoreductase 23 kD subunit (chain I)[*]	FeS
Electron transfer flavoprotein, alpha subunit [*]	flavin
Glycerol dehydrogenase or related enzyme, iron-containing ADH family	-
Predicted oxidoreductase (related to aryl-alcohol dehydrogenase)	NAD(P)
Predicted oxidoreductase of the aldo/keto reductase family	FeS, NAD(P)
NADH dehydrogenase, FAD-containing subunit[*]	flavin
Ni,Fe-hydrogenase I small subunit[*]	FeS, FeNiS, flavin, NAD(P)
Choline dehydrogenase or related flavoprotein	flavin, NAD(P)
Anaerobic SeCys-containing dehydrogenase	MoCo
Protein distantly related to bacterial ferritins	heme
Thioredoxin reductase	flavin, NAD(P)
MinD superfamily P-loop ATPase, contains an inserted ferredoxin domain	FeS
Cytochrome c biogenesis protein CcdA (sulfhydryl redox chemistry)	-
Nitroimidazol reductase NimA, FMN-containing flavoprotein, pyridoxamine 5'-P oxidase superfamily	flavin
Predicted dinucleotide-binding enzyme	F_{420}, NAD(P)
Tetrathionate reductase subunit A[*]	FeS, MoCo, Sec
Oxidoreductase domain-containing protein	NAD(P)
4Fe-4S ferredoxin	FeS

[*] In case of protein complexes, the cofactors of the entire complex are shown next to each of the subunits of the complex. Exceptionally, when the subunit couldn't be pinpointed to a specific protein complex with certainty, only the cofactors of that subunit are shown.
Abbreviations used as in Supplementary Table 1.

Supplementary Table 6. Archaea and bacteria basal branching lineages within the 355 phylogenetic trees of LUCA's gene families.

proxy	Archaea	no.	Bacteria	no.
$Tree_{Pure}$ [*]				
	Other archaea	18	Clostridia	30
	Methanomicrobiales	13	Deltaproteobacteria/Other bacteria[§]	18
	Methanosarcinales/Thermoplasmatales/ Thermoproteales/Thermo-Pyrococcales[§]	12	Actinobacteria	12
	Archaeoglbales	10	Chlorofelxi	8
	Methanococcales	9	Cyanobacteria/Gammaproteobacteria[§]	7
$Tree_{Mixed}$ [†]				
	Methanosarcinales/Archaeoglobales[§]	54	Clostridia	82
	Methanomicrobiales/Thermoplasmatales[§]	47	Other bacteria	72
	Methanobacteriales/Methanocellales[§]	46	Deltaproteobacteria	57
	Halobacteria	45	Actinobacteria	48
	Methanococcales	43	Bacilli	38
$Dist_{Root}$ [‡]				
	Methanosarcinales	48	Clostridia	87
	Thermo-Pyrococcales	47	Other bacteria	42
	Methanococcales	40	Deltaproteobacteria	32
	Thermoproteales	34	Chloroflexi	22
	Archaeoglobales	33	Gammaproteobacteria	21

[*] representatives from only one phylum/group in the basal branch.

[†] representatives from more than one phylum/group in the basal branch.

[‡] the number of trees in which a representative from the group indicated had the shortest distance to the root.

[§] groups represented equal number of times in the basal branch.

With respect to the phylogenetic position of the deepest branching lineages, the available taxon sample introduces a potential bias in the case of methanogens, which are overrepresented in the archaeal taxon sample (31.34% of archaeal genomes) but certainly not in the case of acetogens, which are not overrepresented in the sample (3.68% of bacterial genomes). In addition, Thaumarchaeota and other new archaeal phyla not belonging to the Eury- or Crenarchaeota are underrepresented in the present genome sample. This sampling bias reduces the number of archaea fulfilling both the monophyly and two phylum criteria.

Supplementary Table 7. SAM-dependent enzymes. (*provided as separate Excel file)

Supplementary Table 8. Functional and taxonomic characterization of one taxa misplaced protein families. (*provided as separate Excel file)

Supplementary Table 9. Functional and taxonomic characterization of one phyla misplaced protein families. (*provided as separate Excel file)

Supplementary Information References

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