Table 5. Enzymatic activities associated with the P-loop hydrolase fold (c.37) in the purine and pyrimidine metabolism subnetworks and candidates of origin that are relevant to the RNA world.

Enzymatic activity	Reaction scheme[a]	N_d/N_g[b]	D[c]	Functional link[d]	RNA world-relevant[e]
Purine metabolism subnetwork					
EC 2.7.1.25	Adenylylsulfate =>3′-Phosphoadenylyl sulfate (R00509)	126/104	1.56 (1-5)	+	+
EC 2.7.1.74	AMP <=> Adenosine (R02321)	4/4	1	+	+
EC 2.7.1.76	Deoxyadenosine => dAMP (R02089)	2/2	1	+	–
EC 2.7.1.113	dGMP <=> Deoxyguanosine (R01967)	29/29	1	+	–
EC 2.7.4.3	ADP <=> AMP dADP <=> dAMP (R00127, R01547)	240/239	1.65 (1-2)	+	+
EC 2.7.4.8	GMP <=> GDP dGDP <=> dGMP (R00332)	191/193	1.06 1-4)	+	+
EC 2.7.7.4	Adenylyl sulfate => Sulfate (R00529)	95/197	1.95 (1-5)	+	–
EC 2.7.7.7	dATP or dGTP => DNA (R00379)	616/1866	1.06 (1-4)	+	–
EC 3.1.4.17	cAMP => AMP (R03259)	0/66	1.67 (1-4)	–	?
EC 3.1.5.1	dGTP => Deoxyguanosine (R01856)	0/3	1	–	–
EC 3.6.1.3	ATP =>ADP (R00086)	162/101	1.89 (1-6)	+	+
EC 3.6.4.1	ATP =>ADP (R00086)	13/13	1.46 (1-2)	+	+
EC 4.6.1.1	ATP => cAMP (R00089)	2/176	1.46 (1-5)	–	?
EC 6.3.4.4[f]	IMP => Adenylosuccinate (R01135)	194/194	1	+	+
Pyrimidine metabolism subnetwork					
EC 2.7.1.21	Deoxyuridine => dUMP (R01567)	102/101	1.09 (1-2)	+	–
EC 2.7.1.48	Uridine => UMP Cytidine => CMP (R00964, R00513)	–	–	+	+
EC 2.7.1.74	Deoxycytidine => dCMP (R02321)	4/4	1	+	–
EC 2.7.4.9	dTDP <=> dTMP dUDP <=> dUMP (R02094, R02098)	205/212	1.02 (1-2)	+	–
EC 2.7.4.10	UDP => UTP (R00333, R00157)	3/3	1.67 (1-2)	+	+
EC 2.7.4.14	CDP <=> CMP UDP <=> UMP dCDP <=> dCMP (R00158, R00512, R01665)	186/188	1.04 (1-3)	+	+
EC 2.7.7.7	dTTP or dCTP => DNA (R00379)	616/1866	1.94 (1-4)	+	–
EC 3.5.4.12	dCMP => dUMP (R01663)	3/88	1.05 (1-2)	–	–
EC 6.3.4.2[f]	UTP => CTP (R00571)	214/215	1.99 (1-2)	+	+

[a] The reaction scheme describes fundamental compounds involved in either reversible (<=>) or directional (=>) metabolic reactions (described by KEGG database entries in parentheses). Compounds and their KEGG entry numbers in parentheses include: Adenosine (C00212); Adenylosuccinate (C03794); Adenylylsulfate (C00224); ADP, Adenosine 5′-diphosphate (C00008); AMP, Adenosine 5′-monophosphate (C00020); ATP, Adenosine 5′-triphosphate (C00002); cAMP, 3′,5′-cyclic AMP (C00575); CDP, Cytidine 5′-diphosphate (C00112); CMP, Cytidine 5′-monophosphate (C00055); CTP, Cytidine 5′-triphosphate (C00063); Cytidine (C00475); dAMP, 2′-Deoxyadenosine 5′-monophosphate (C00360); dATP, 2′-Deoxyadenosine 5′-triphosphate (C00206); dCDP, 2′-Deoxycytidine 5′-diphosphate (C00705); dCMP, 2′-Deoxycytidine 5′-monophosphate (C00239); dCTP, 2′-Deoxycytidine 5′-triphosphate (C00358); 2′-Deoxyadenosine (C00559); 2′-Deoxycytidine (C00881); 2′-Deoxyguanosine (C00330); 2′-Deoxyuridine (C00526); dGDP, 2′-Deoxycytidine 5′-diphosphate (C00361); dGMP, 2′-Deoxyguanosine 5′-monophosphate (C00362); dGTP, 2′-Deoxyguanosine 5′-triphosphate (C00286); DNA (C00039); dTDP, Deoxythymidine 5′-diphosphate (C00363); dTMP, Deoxythymidine 5′-monophosphate (C00364); dTTP, Deoxythymidine 5′-triphosphate (C00459); dUDP, 2′-Deoxyuridine 5′-diphosphate (C01346); dUMP, 2′-Deoxyuridine 5′-monophosphate (C00365); GDP, Guanosine 5′-diphosphate (C00035); GMP, Guanosine 5′-monophosphate (C00144); IMP, Inosine 5′-monophosphate (C00130); 3′ Phosphoadenylyl sulfate (C00053); Sulfate (C00059); UDP, Uridine 5′-diphosphate (C00015); UMP, Uridine 5′-monophosphate (C00105); Uridine (C00299); UTP, Uridine 5′-triphosphate (C00075).

[b] Number of c.37 domains (N_d) assigned using a HMM library/number of gene sequences analyzed (N_g). Detailed fold superfamily assignments of genes associated with enzymatic activities can be visualized as pull-down menus in MANET.

[c] Average number of domains (D) identified in protein sequences. A range of values is provided in parentheses.

[d] Direct association of EC activity with c.37 fold. Only four out of 23 EC entries were associated indirectly with the c.37 fold. This includes EC 4.6.1.1 (genes LIC13201 and LA4008 of *Leptospira interrogans*) and EC 3.5.4.12 (gene SPBC2G2.13c of *Schyzosaccharomyces pombe*, gene orf19.7243 of *Candida albicans*, and gene CMQ291C of *Cyanidioschyzon merolae*). Indirect associations of EC 3.1.4.17 and EC 3.1.5.1 derive from crystallographic studies. Functions associated with all these EC entries are probably the result of relatively recent recruitment processes since indirect associations were rare (identified in few organisms).

[e] Enzymes that catalyze DNA precursors known to be missing in minimal genomes suggest an ancient ancestor of life with an RNA-based genome[41] and are here considered not relevant for an RNA world origin of metabolism. The relevance of signal molecules such as cAMP for the RNA world cannot be established, and this is marked with a question mark.

[f] Ligases forming C-N bonds (EC 6.3.4) that play significant roles in nucleotide salvage pathways usually promote at least two transfer reactions, a phosphoryl group transfer followed by a nucleophilic displacement in a transition state, for example of the 6-phosphoryl group of 6-phosphoryl-IMP by the α-amino group of L-aspartate in the EC 6.3.4.4 reaction[42]. This complicated catalysis may suggest they derive by recruitment from ancient phosphotransferases (EC. 2.7).