DIVERGENCE OF AMP SYNTHETIC AND DEGRADATIVE MACHINERY, AND FUNCTIONAL IMPLICATIONS IN PSYCHROPHILES

By

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Abstract

A large group of organisms is well-suited to life at low temperatures (e.g., <20°C, termed psychrophiles) and is therefore able to colonize the majority of earth's biosphere. Interestingly, all observed kingdoms of psychrophiles have been shown to increase concentrations of ATP as temperatures decline; this phenomena has been described as an important compensatory mechanism to deal with decreased thermal energy. In an effort to understand this phenomena, both evolutionarily and metabolically, psychrophilic and mesophilic purine synthesis pathways were examined. Psychrophilic purine synthesis pathways tend to be enriched with *de novo* AMP synthetic enzymes, while mesophiles tend to be enriched with AMP degradative enzymes. The function of observed psychrophilic pathway structure was tested by engineering the mesophile *Escherichia coli* to reflect psychrophilic purine synthesis. Mutant *E. coli* was capable of growing up to 70% faster at low temperatures and became up to 10 fold more cold tolerant relative wild-type. These findings highlight an important evolutionary step in psychrophilic evolution.

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To members of the Shain lab past and present; past for providing a foundation for me to begin from and present for your help in the lab. Also to members of the Yakoby lab for friendly arguments and critical thinking. Specific thanks to Dr. Shain for many great opportunities, and advice along the way.

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Introduction

Life depends on gathering energy and expending its thermodynamic economy to prevent an organism from attaining maximum entropy and equilibration with the environment. Sufficient molecular kinetic energy is available at 37°C for biochemical reactions to occur, as evidenced by many extant species. However, at low temperatures biochemical reactions slow (predicted by the Q_{10} relationship, an approximation of the Arrhenius equations) and death occurs when thermal energy is insufficient to power biochemical reactions (i.e., insufficient energy to overcome the energy of activation barrier). Curiously, a large group of organisms is well-suited to life at low temperatures (e.g., <20°C, termed psychrophiles). Psychrophiles have been found to colonize all permanently cold environments from upper mountain regions to polar regions to deep sea (D'Amico et al, 2002). While bacteria are the predominate form of life found at low temperatures, having been observed living in environments as low as -20°C including permafrost and sea ice. Fungi, microalgae and some plants are also capable of thriving in such conditions. In terms of biological complexity, the glacial ice worm *Mesenchytraeus solifugus* stands in contrast. Of necessity, these organisms have evolved a number of mechanisms to allow them to cope with the various challenges that life in the cold presents.

Several adaptations have been observed; perhaps the most publicized are adaptations of the lipid bi-layer for maintenance of fluidity at low temperatures. The lipid bi-layer is sensitive to cold temperatures in that it tends to reach a gel phase where it loses

fluidity and function. To inhibit the onset of a gel phase (freezing), the psychrophile incorporates structural features into the bi-layer which serve to de-stabilize it. Chemical modifications, such as a higher content of unsaturated and polyunsaturated and methyl branched fatty acids and/or shorter chain length seeks to disrupt the membrane by steric mechanisms which cumulatively inhibit freezing (D'Amico et al 2006).

Other adaptations to prevent cytosolic freezing abound also. Many psychrophiles produce antifreeze proteins capable of binding ice crystals and inhibiting the formation of a crystal lattice. Cold shock proteins, analogous to heat shock chaperones, abound also. Yet regardless of how an organism deals with freezing, the problem of maintaining sufficient metabolic flux to perform essential biological tasks, especially in spite of predicted losses in reaction rates, is a fundamental problem for psychrophiles.

To cope with the problem of flux globally, psychrophiles' enzymes tend to exhibit substitutions slight enough to maintain architectural similarity with homologues across disparate taxa and temperature optima, yet sufficient to increase catalytic efficiency (k_{cat}/ *K*m; Siddiqui and Cavicchioli, 2006). On account of this, psychrophiles are able to maintain metabolic flux on par with their mesophilic and thermophilic counterprts (Georlette, 2003). Modifications in question typically include reductions of intra-molecular interactions (Bae and Philips, 2004), changes in the volume, and location and size of polar and apolar residues (Gianese et al, 2002; Farrell et al, 2004). Such changes result in a preference for maintaining flexibility at low temperatures at the expense of stability across a range of temperatures (Siddiqui and Cavicchioli, 2006).

An additional psychrophilic adaptation has also been recently observed: psychrophiles elevate ATP levels with decreasing temperature, even though their growth rates directly correlate with temperature (Napolitano and Shain, 2004; Napolitano and Shain, 2005; Morrison and Shain, 2008; Amato and Christner, 2009). In general, diminished temperatures result in reduced diffusion rates (due to increased viscosity of water) and Gibbs free energy is reduced. Increasing concentrations of a reactant at low temperatures rescue reductions in Gibbs free energy by minimizing the reactants' average distance to travel for reaction participation. For a metabolite as ubiquitous as ATP (ATP is involved in 492 known biochemical reactions), which drives many thermodynamically unfavorable reactions, an increase in its concentration may serve to drive a plethora of reactions which may not otherwise occur at low temperatures. to its dependent enzyme and abrogating temperature related Gibbs free energy reductions. Most likely, elevated levels of ATP represent a global response to decreased reaction rates at low temperatures. Increasing cellular ATP concentration is likely an integral component of psychrophilic evolution (Napolitano and Shain, 2004; Napolitano and Shain, 2005; Morrison and Shain, 2008)

Since less highly connected enzymes are correlated with rapid divergence, enzyme duplication correlates with flux (Vitkup et al, 2006), and the primary means of ATP synthesis comes from phosphorylation of AMP, I have approached questions of energy production in psychrophiles by examining network edges circumferential to AMP. As a result, ice worm genes recovered from the puring biosynthesis pathway are predicted

to enhance the size of their AMP pool. An examination of purine synthesis in psychrophiles and mesophiles of class gammaproteobacteria revealed psychrophiles to have an increased propensity to maintain a large AMP pool. Engineering the mesophilic bacterium *Escherichia coli* to reflect psychrophilic AMP edge structure resulted in up to 70% increased growth rate at low temperatures and 10 fold increase in cold tolerance over 8 days.

Materials and Methods

Computational Analyses of Divergence and Metabolic Networks

KEGG's KAAS feature automatically annotates sequenced genomes by searching for orthologs in reference genomes. For a genome to be annotated, it is BLAST searched in the forward and reverse directions against a reference set; hits with bit scores below 60 are discarded. Of the remainder, the ratios of the unknown gene's bit score of the current hit divided against the bit score of the highest hit, for forward and reverse hits are multiplied. Hits scoring >.95 are retained and assigned to the highest scoring ortholog groups (Moriya et al, 2007). The KAAS feature was employed in retrieving networks used to obtain in the following results.

Custom Perl and Matlab scripts were written to mine databases and analyze results, respectively. All prokarya and archae were initially retrieved from Kyoto Encyclopedia of Genes and Genomes (KEGG; n = 913 species), those without entries in the Prokaryotic Growth Temperature database (PGTDB) were excluded. Organisms apparently unable to manufacture AMP (similarity scores below acceptable threshold) were also excluded; 113 species within class γ-proteobacteria were used in the following analyses.

Network Scoring

The genome of each bacterium was scanned by a copy-number sensitive gene counter which counted AMP synthetic genes (enzyme commission numbers 6.3.4.4, 4.3.2.2, and 2.4.2.7), AMP degradative genes (enzyme commission numbers 3.5.4.6,

3.2.2.4, and 3.1.3.5) and degradative gene absences. For each copy of an AMP synthetic enzyme and missing AMP degradative pathway, an organism's score was incremented by one. The score was reduced by one for every copy of a primary AMP degradative gene.

Phylogeny

A custom made Perl script queried KEGG to obtain 16s rRNA sequences for all bacterial species of known temperature class. Sequences were aligned locally with Clustal 2.0.11 (Larkin et al, 2007) and Phylip (Felsenstein, 2005) was called to draw the tree using a neighbor joining algorithm. Representative species (typically one per genus) were selected, aligned and plotted as stated. Tree structure was checked against current literature (Brown and Volker, 2004). A Perl script was written to format and score the final tree.

Metabolic Divergence

KEGG orthology numbers K03500 K00163 K02115 K02114 K02112 corresponding to 16s rRNA, Pyruvate Dehydrogenase E1, F-type H+-transporting ATPase subunit gamma, F-type H+-transporting ATPase subunit epsilon, F-type H+-transporting ATPase subunit beta and enzyme commission numbers 6.3.4.4, 4.3.2.2, 2.4.2.7, 2.7.4.6, 3.2.2.4, 3.1.3.5, 2.7.2.3, 3.5.4.6, corresponding to Adenylosuccinate Synthetase, Adenylosuccinate Lyase, Adenylate Kinase, AMP nucleosidase, Phosphoglycerate Kinase and AMP Deaminase were used to query KEGG via a custom made Perl script. Nucleotide and amino acid sequences for the above genes and encoded proteins were returned for each organism and passed to Matlab for analysis. A custom Matlab script was

used to calculate pairwise identities using the Needleman and Wunsch similarity algorithm (the preferred method of calculating the ratio of non-synonymous to synonymous substitution ratio was unavailable to this analysis on account of wide divergence between species). Average identities and standard error of the mean among genes of a given temperature class (i.e., psychrophile or mesophile) was then calculated. Finally, copy number was counted and averaged by temperature class along with standard error of the mean.

Metabolic Compounds

Custom Perl scripts were written to mine all known biochemical reactions requiring AMP and ATP as a substrates.

Bacterial Strains

Psychrobacter cryohalolentis was purchased from the ATCC and recovered with growth in marine broth 2216 (Difco) at 15°C and plated on marine agar 2216 (Difco). DY330 was kindly provided by Nina Costantino and Donald Court; amnK (Morrison and Shain, 2008) was recovered from ~2 years of storage at 4°C by growth in liquid culture, plating and PCR verification of the strain. amnK was grown in 34 μg/mL chloramphenicol. DY330 and amnK were grown at 31°C unless stated otherwise.

***Psychrobacter cryohalolentis* Gene Cloning**

A 100 μL aliquot of a *Psychrobacter cryohalolentis* log phase culture was pelleted at 16k x *g* for 30 seconds in a microcentrifuge. Growth medium was aspirated with a pulled glass pipette and the pellet was resuspended with 200 μL dH_2O. The suspension

was boiled for 5 minutes, moved to ice and a 1:10 dilution was used as template in the following PCR reactions. Adenylosuccinate synthetase (AS) and adenylosuccinate lyase (AL) were amplified individually in reactions containing 39.75 µL dH20, 5 µL 10X PCR amplification buffer (100mM KCl, 100mM Tris-Cl pH 8.3, 15mM $MgCl_2$), 1 µL $MgCl_2$ 50mM, 1 µL dNTPs, 1 µL each of ATGGGTAAGAATGTCGTAGTT and AATTAAGCATCGTACGGATC or ATGAATCTACTTACCGCACTC and GCCAGTTAAATTTTAGCAATC, 1 µL *P. cryohalolentis* template and 0.25 µL Titanium Taq DNA polymerase (Clontech) for 3 minutes 94°C, 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds 72°C for 1 minute and 30 seconds and indefinite hold at 4°C. Products were electrophoresed on a .8% agarose gel. Following identification of a lone band of the correct size, AS and AL were cloned into pBAD TOPO TA expression vectors (Invitrogen) along with the manufacturer's provided positive control, LacZ, as per their direction. AS and AL pBAD constructs were sequenced (Genewiz) for verification of the correct insert in frame with the vector's start codon.

Preparation and Transformation of DY330 and amnK Cell Lines

DY330 and amnK were made chemically competent by inoculating 500 mL cultures with 1 mL of the respective overnight culture started from a single colony. Once OD_{600} reached ~.3, cultures were cooled in ice-water baths for 30 minutes. This early log material split into pre-chilled 250 mL centrifuge bottles and spun at 1800 rpm for 8 min at 0°C. Growth medium was decanted and each pellet pellet was gently resuspended in 2mL 100mM filter-sterilized $CaCl_2$. Competent cells were divided in 200uL aliquots and

either snap froze in liquid nitrogen or transformed immediately.

Competent DY330 and amnK lines were incubated on ice for 5 min with 50ng each of LacZ, AS and AL pBAD constructs (see 'Gene Cloning' above). DNA competent cell mixtures were heat shocked at 37°C for 2 min (as recommended by Nina Costantino, Donald Court lab, Nactional Cancer Institute, personal communication) and immediately transferred to ice. Room temperature LB was added to cooled transformation mixture and allowed to recover at 31° C without shaking. After two hours, Ampicillin was added to a final concentration of 50 µg/mL and transformants were grown at 31° C with shaking at 200 rpm. After 36 hours, cultures were plated. Strain genotypes were verified by amplification of endogenous genes, amplification of vector, its insert and sequencing of vector inserts.

Growth Rate

Single colonies of *P. cryohalolentis*, DY330, DY330:LacZ, DY330:AL, DY330:AS, amnK, amnK:Lacz, amnK:AL, and amnK:AS were picked and grown overnight under selection of the appropriate antibiotic; 50 µL of overnight culture was used to inoculate 4 mL LB cultures containing .02% L-Arabinose and 50 µg/mL ampicillin for transformants. After two hours of induction, cultures were moved to specified temperatures and allowed to equilibrate for an additional two hours; OD_{600} was read every 30 minutes to an hour for a minimum of two hours (minimum of three time points per culture). A custom Matlab program was written to calculate growth rates, compare and plot growth rates between cultures. Generation times were determined as the

quotient of log(2) and the slope of the line that minimized the squares' sum of vertical differences between the log of OD values and the line. Cell divisions per hour were calculated as the quotient of 60 minutes and generation time. The experiment was performed in independent triplicates; growth rates (cell divisions per hour) were averaged and standard error of the mean calculated for each data point.

Cold Tolerance

Specified cultures were grown overnight at 31°C with appropriate antibiotics. The following day, 10 μL of overnight culture was used to inoculate a 3 mL culture containing the appropriate antibiotic(s) and .02% L-Arabinose. At mid-log phase growth was stopped by cooling to 0°C. Either cell viability was determined and the cultures stored in an ice-water bath for 8 days, or were washed twice with PBS (Gibco), resuspended in two volumes PBS, viability determined and stored in an ice-water bath for 8 days. After 8 days cell viability was determined by triplicate plating (50ul of $1e^{-5}$ dilution for L-Arabinose stored cultures and 50ul of $2e^{-4}$ dilution for PBS stored cultures) on a selective plate. Counted colonies were used to determine the percent of viable colonies relative initial plating.

Library Screening

ClustalW was employed to align multiple sequences, whose highly conserved regions were used to design PCR primers. Library screening was performed essentially as previously described (Tartaglia and Shain, 2008), screening approximately 300,000 phage particles per screen. Probes with incorporated ^{32}[P]-αdCTP (Perkin-Elmer) were made

with standard reaction conditions. From ice worm cDNA template, forward and reverse primers GGNAMNTAYTAYCCNYT, GTNCCNADRTTNSWNGGRCA were used to amplify a universal phosphagen probe, ACNTGGMGNIIIYTNTGG and CATNGCCATDATDATRTTYTC amplifed a probe for adenylosuccinate lyase, and TGYCARGGNGGNAAYAAYGC, GGNCCDATNCCYTTYTTNGT amplified a probe for adenylosuccinate synthetase. Positive plaques were *in vivo* excised via Cre mediated Loxp recombination. Recombinant clones and PCR fragments were sequenced by Genewiz (South Plainfield, NJ).

AMP Deaminase

K188E Verification

M. solifugus AMP deaminase specific primers (ATGCGGACAGAAACACGTTCCA and TCCGAGTAGACGTTGTTTGCGA) were employed to amplify genomic and cDNA regions bearing residue 188. Purified ice worm genomic DNA and cDNA (Farrel et al, 2003) were used as templates. Standard reaction conditions were amplified at 94°C for 2 min 30 sec, 30 cycles of 94°C 30 sec, 64°C 40 sec, 72°C 1 min. Products were visualized on an agarose gel and sequenced (Genewiz, NJ).

Protein Modeling

AMPD crystal structure and primary structure sequence from *Arabidopsis thaliana* (PDB ID 2A3L chain A; Woo Han, 2006) was used to model ice worm AMPD (ExPASy server's Swiss Model). Simulated mutagenesis was performed by altering the

positions in question and re-modeling the mutant sequence. Model results were visualized with PyMol. The substrate binding plane was defined by four sterically constraining residues (K173, Y174, D444, D445) within 3.1A of the bound AMP analog coformycin 5'-phosphate.

Complementation Assay

Library Conversion

A 10mL LB culture was inoculated with a single *Escherichia coli* BM25.8 colony and grown overnight at 31°C with shaking at 190 rpm. The outgrown culture was diluted 1:10 in fresh LB and grown under the same conditions as above; when OD_{600} reached 1.2, 100 µL of 1M $MgCl_2$ was added (final concentration 10mM). *Mesenchytraeus solifugus* phage cDNA library was *in vivo* excised by mixing 200 µL BM25.8 culture with $2*10^6$ pfu of the library and incubation at 31°C with no shaking. After 1h, 500 µL of LB was added and agitated at 190 rpm for an additional hour. The titer (converted recombinant clones per micro liter) of the *Mesenchytraeus solifugus* plasmid cDNA (pTriplEx2) library was determined by plating with ampicillin selection. Subsequently, ~25,000 recombinant clones were plated on 200 150-mm LB-Amp plates (50 µg/mL ampicillin) and grown for ~12 hours at 37°C yielding $5*10^6$ independent recombinant clones. Clones were washed from each plate with 10mL LB, gently removed with a sterilized, bent pasteur pipette and retained in 250 mL ice water bathed centrifuge bottles. Resultant suspension was pelleted by centrifugation at 10,000 rpm for 20 minutes at 4°C, excess growth medium was decanted and pellets were frozen. The plasmid library was recovered

as needed with Promega's PureYield Plasmid Midiprep System.

Preparation of Electrocompetent XL1-Blue Cells

Cells were made competent according to Sambrook's electrocompetent procedure (Sambrook and Russel, 2001) and either used immediately or snap frozen in liquid nitrogen and stored at -80° C until needed.

Cold Tolerance Assay

XL1-Blue electrocompetent cells were thawed on ice and 1 µL of the *M. solifugus* pTriplEx2 library was added. After a 60 second incubation on ice, the mixture was transferred to ice-cold electroporation cuvettes. A Bio-Rad electroporator set with a field strength of 1.8 kV was used to electroporate cells with time constants <5msec. Immediately after electrocution, 1 mL of room-temperature LB was added to cuvettes and suspension was transferred to 17 x 120mm polypropylene tubes and allowed to recover at 37°C with shaking at 120 rpm. After one hour, ampicillin and Isopropyl β-D-1-thiogalactopyranoside (IPTG) were added to final concentrations of 50 µg/mL and 5mM respectively. After 2-4 hours of induction, 200 µL of transformants were plated per 150mm LB-Amp plate (50 µg/mL ampicillin) until transformed material was exhausted. Additionally, a control plasmid (containing a fragment of adenylosuccinate lyase) was transformed side by side. For each transformation, one plate was grown at 37°C to verify transformation. This process was repeated to accrue 50 plates at each temperature: 4°C, 8°C, and 15°C. Complementation to low-temperature growth was assessed by colony appearance before control.

Results

Purine synthesis network scoring

It has been established that disparate taxa elevate ATP levels while temperatures drop (Napolitano and Shain, 2004; Napolitano and Shain, 2005). Since AMP is the primary precursor of ATP, psychrophilic network architectural distinctions in AMP metabolism relative mesophiles were queried. To this end, a scoring system was devised that approximates an organism's propensity to maintain an AMP pool. For each copy of a gene encoding an AMP synthetic enzyme the score was incremented by one. The score was also incremented by one for missing degradative genes. For every copy of an AMP degradative enzyme, the score was decremented by one. Using this scoring scheme, psychrophiles are found to have a significantly higher score than mesophiles (Figure 1), highlighting a potential to maintain elevated AMP levels. Contributing to psychrophiles' elevated score is an average increase in copy numbers of synthetic genes combined with lower copy numbers of degradative genes and missing degradative enzymes (Figure 1).

Gammaproteobacteria phylogeny

In general, similar scores and temperature class group together (Figure 2); for example, see the clades containing *E. coli* or *P. ingrahamii*. Curiously, the clade containing *V. fischeri*, *A. salmonicida*, *V. parahaemolyticus*, and *P. profundum* are not neatly grouped by temperature class or score as other clades appear. Closer inspection reveals copy number of 5' nucleotidase correlates with divergence and temperature class. As species of this clade diverges, psychrophiles lose copies of 5' nucleotidase while their

mesophilic counterparts do not.

Divergence of energy associated genes in gammaproteobacteria

Excluding ATP Synthetase subunits, adenylosuccinate synthetase diverges at rates higher than other AMP dependent enzymes (Table 1, comparison of 'Mesophiles' versus 'Psychrophiles'). Additionally, adenylosuccinate synthetase is present, on average, in higher numbers in psychrophiles than in mesophiles (Table 1). The divergence of AMP nucleosidase could not be determined since no psychrophiles were found that maintained copies of this enzyme. Curiously, psychrophilic epsilon, gamma and delta subunits of the ATP synthetase diverge much more rapidly than mesophilic homologues.

Genetic manipulation of *E. coli*

An *E coli* strain (DY330) was genetically manipulated to mimic psychrophilic AMP synthetic and degradative pathways. First, recombineering was used to replace *E. coli's* amn gene with a cassette providing chloramphenicol resistance (Morrision Shain, 2008; here, derivatives of the amn knockout strain are designated amnK). The amn gene encodes AMP nucleosidase, *E. coli's* primary AMP degradative enzyme. Second, LacZ encoding β-galactosidase, and the purA and purB genes from the psychrophilic gammaproteobacterium *Psychrobacter cryohalolentis* were cloned into individual pBAD expression vectors. The purA gene encodes adenylosuccinate synthetase (AdsS), responsible for the first committed step of de novo AMP synthesis, and purB encodes adenylosuccinate lyase (AdsL), the final step in de novo AMP synthesis. In-frame clones were transformed into DY330 and amnK strains. The amn locus was amplified from each

strain, producing a 2.3kb fragment (representing amn) in DY330 backgrounds or a 1.7kb fragment in amnK backgrounds (representing the chloramphenicol cassette). Amplification of a cassette encoding chloramphenicol resistance yielded no products from DY330 background, but was amplified from amnK. Transformants were verified by amplification of pBAD vector plus insert: pBAD forward and the reverse insert of interest, and pBAD reverse and the forward insert of interest.

Growth rate analysis

Expression of LacZ or AdsL in the DY330 background lead to qualitatively identical growth rates at all assayed temperatures (Figure 3, top panel; note complete superimposition of data points across assayed temperature range). Whether expressing LacZ or AdsL, DY330 increased growth rates with temperature until maximal growth rates were achieved at 37° C and declined at 42° C. Exogenous AdsS expression in DY330 also increased growth rate with temperature until 37° C. Notably, at 8°, 10°, and 15° C, DY330 expressing AdsS increased its growth rate by 73%, 51% and 45% respectively over the same strain expressing LacZ. At temperatures approaching optimal, AdsS expression in DY330 slightly depressed growth rate relative the control (Figure 3, middle panel). Comparison of DY330 expressing AdsL or AdsS, yielded growth rate changes comparable to AdsS versus LacZ expression. AdsS expression conferred growth rates increased 50%, 65%, and 69% at 8°, 10° and 15° C over AdsL expression. At 37° C, AdsS expression decreased growth slightly (6%) from AdsL expression (Figure 3, bottom

panel; non overlapping error bars).

Expression of LacZ or AdsL in amnK resulted in qualitatively identical growth rates (all error bars overlap significantly and were removed), increasing across *E. coli'*s viable temperature window until 37° C; growth decreases at higher temperatures (Figure 4, top panel). Expression of AdsS in the amn null background apparently decreases its growth rate at 10° C (36% decrease) but not at 23° C (where growth rate is increased by 16%) or any other temperatures (Figure 4, middle panel; overlapping error bars). Comparing AdsS against AdsL expression in amnK yields qualitatively identical results: AdsS expression only changes amnK's growth rate at 23° C (increased by 15%) and 10° C (Figure 4, bottom panel; decreased by 34%).

Cold tolerance assay

After 8 days of cold storage (0° C) in LB .02% L-Arabinose, DY330 expressing LacZ and AdsL remained equally viable (Figure 5). In contrast, DY330 expressing AdsS treated in the same manner retained 35% viability, representing more than a 3-fold increase ($p = .01$) over the control and 2 fold increase over AdsL ($p = .04$). amnK constructs remained qualitatively identical (Figure 5).

Trehalose offers a cryoprotective effect at low temperature (Phadtare, 2004). In an effort to eliminate potentially similar contributions from L-Arabinose, cold tolerance was also measured after 8 days of storage in PBS. For both DY330 and amnK backgrounds, LacZ and AdsL expression resulted in similar viability after 8 days of cold storage. Notably, DY330 expressing AdsS remained more than five times as viable as LacZ or

AdsL ($p = .028$, $p = .03$ repsectively). amnK expressing AdsS remained nearly four times more viable as the same background expressing AdsL ($p = .033$), but not LacZ ($p = .065$; Figure 6).

Field work

Pika, Crown, and Kahiltna Glaciers, subglaciers of the Pika and Crown, and the Ramparts were observed for ice worms in the evening hours and under various weather conditions (Figure 7). While no specimens were found, an exhausted habitat was observed. In comparison to USGS maps, many glaciers had receded dramatically.

Ice worm AMP deaminase

AMP deaminase from all organisms examined contained a negative pocket (Figure 8 A) proximal to the substrate binding cleft which directly interacts with the substrate binding cleft by a loop of reduced flexibility owing to a proline residue. Ice worm specific substitution K188E occurs within this pocket (Figure 8 B). A predicted effect of this substitution is a reduction in active site size from 38 to 36 Å (Figure 9 A). The same substitution in *Arabidopsis thaliana* also reduces its active site to 36Å (Figure 9 B, C). The reicprocal substitution, E188K, in the ice worm grossly distorts active site architecture (Figure 9 D; Marotta et al, 2009).

Ice worm adenylosuccinate lyase

The sequence of a fragment of ice worm adenylosuccinate lyase (Figure 10) is shown in alignment with psychrophilic bacterium *Psychrobacter cryohalolentis,* leech *Helobdella robusta*, and high scoring BLAST (Altschul et al, 1997) hits lancelet

Branchiostoma floridae (bit score 376), horse *Equus Caballus* (bit score 372), humans *Homo sapiens* (bit score 364), mouse *Mus musculus* (bit score 363), zebrafish *Danio rerio* (bit score 358), and fly *Drosophila ananassae* (bit score 321). Despite aligning to corresponding active sites and lyase domains of adenylosuccinate lyase from other organisms (Pfam e-values of 3.3e-6), Pfam (Finn et al, 2008) found no significant similarity in ice worm adenylosuccinate lyase and any other known functional domains (insignificant Pfam-B hits for Lyase_1; e-values were 2.2 and .0027). Adenylosuccinate lyase C-terminus was also not found in the ice worm.

Within the fragment of ice worm adenylosuccinate lyase observed, known psychrophilic trends were followed. Position 245 is a solvent exposed position, and all mesophiles contain a hydrophilic residue that in most cases is charged (i.e., glutamate, aspartate). The ice worm and psychrophilic bacterium *P. cryohalolentis* have both substituted hydrophobic residues at this position (valine in the ice worm and alanine in *P. cryohalolentis*). Most organisms have a methionine at position 253 while the ice worm has glutamine. At position 265, ice worms have a threonine instead of the mesophilic's isoleucine, leucine or alanine.

Ice worm phosphagen kinase

BLAST analysis of ice worm phosphagen kinase fragments returned other phosphagen sequences. However, all hits had E-values greater than one.

Complementation assay

A total of 3 million ice worm cDNA clones were screened at 4°, 8°, and 15° C (~1

million clones per temperature) for complementation to low temperature growth in *E. coli*. Regardless of temperature, no transformed colonies appeared before control colonies.

Discussion

Gammaproteobacteria

Psychrophiles elevate ATP levels as temperatures decrease, even as growth rates increase with temperature. Since AMP synthesis represents the primary pathway to ATP, contributions of genes encoding edges of AMP were examined to identify a role in cold adaptation. A computational approach was taken to identify conserved distinctions in purine metabolism between psychrophiles and mesophiles within gammaproteobacteria. Functional consequences of these differences were tested by engineering *E. coli* (mesophile) purine metabolism to mimic psychrophilic pathway structure. Only two genetic manipulations were required and increased *E. coli*'s score from -1 to 1, a plausible score change over evolutionary time. Successively, these steps led to increased low temperature growth rate and increased cold tolerance relative by 10 fold over wild-type.

A hypothetical evolutionary pathway for the instance of a mesophile's adaptation to a psychrophile can be extrapolated based on data presented here. On account of the increased growth rate afforded by expression of a psychrophilic copy of AdsS at low temperatures, an early step will likely be the duplication of an AMP synthetic gene (i.e., AdsS) and/or introduction of a handful of key mutations in this enzyme. Previous analyses of metabolic network evolution have demonstrated that duplication/deletion events are common for high flux edges (Vitkup et al, 2006); higher copy numbers of AdsS in psychrophiles than mesophiles is also a trend shown here. Substitutions providing increased catalytic efficiency, even minimally, relative the ancestral form will

likely be favored. The combination of these individual events will result in increased growth rates at temperatures lower than optimal for the ancestral organism. The transition species will be able to copy its genome more often than the ancestor, allowing a greater number of mutations to occur over a given time period than for the ancestral species.

During this early period of adaptation, divergence of energy producing genes will occur. The known trends of psychrophilic enzyme evolution occur across a nearly limitless landscape and substitutions favoring flexibility will be met out. That no psychrophiles were found with a copy of amn suggests that its deletion is an early and integral component of psychrophilic evolution. The variability in number of AMP degradative genes found in comparison of psychrophiles and mesophiles is testament parsimony of silencing events and an early role in psychrophilic adaptation. Once these substitutions are made, the potential for the transition species is dramatically increased to explore the landscape of successful mutations and complete psychrophilic evolution.

Ice worm related

Phosphagens are a potential 'battery' in that they are phosphorylated guanidino compounds poised to phosphorylate ADP, increasing the ATP pool (Ellington, 2001). Since seven of the eight known phosphagens are represented in annelids and diffuse much more readily than ATP (phosphagens glycocyamine phosphate and creatine phosphate are able to diffuse nearly twice as fast as ATP; phosphagens are even capable of traversing the lipid bi-layer; Ellignton, 2001), they represent a potentially important energy storage means for ice worms. This perhaps even more so since ice worms are behaviorally

identical to their mesophilic counterparts yet complete their life cycle on ice. Lack of significant similarity of ice worm phosphagens with any phosphagens in the ncbi database may be indicative of seqeuncing errors or rapid divergence of these energy associated genes. Regardless, further investigation to these high-energy compounds has merit.

Of the ice worm genes examined (adenylosuccinate lyase and AMP deaminase), both followed known psychrophilic trends, displaying substitutions that reduce side chain volume and expose hydrophobic residues to solvent. AMP deaminase contained an ice worm specific substitution (K188E) which is predicted to alter active site architecture, diminishing it. More often than not, psychrophiles increase the size of the active site as a predicted mechanism to increase beneficial substrate enzyme collisions (D'Amico, 2006). A hypothetically decreased ability for a psychrophilic AMP degradative enzyme to bind substrate raises the possibility that ice worm AMP deaminase has adapted to function less well. In turn, this adaptation may play into the organism's elevated energy levels.

Despite screening the ice worm's transcriptome the equivalent of 150 times, no instances of complementation to low temperature growth were found. Keeping in mind the fact that simple manipulations in *E. coli* did not overtly change temperature preference or growth rate (amnK increases cold *tolerance* but not growth rate), a fruitful future for this experiment may be to screen for indicators of stress tolerance. Additionally, the expression of eukaryotic proteins in a prokaryote may lead to mis-folding problems for the foreign genes; this technical difficulty might be amended by repeating the experiment

in a tractable eukaryotic system.

Table 1. Average amino acid identities and copy number of specified genes for mesophiles (top) and psychrophiles (bottom).

Figure 1. Simplistic pathway scoring scheme suggests psychrophiles have a higher propensity than mesophiles to maintain a large AMP pool. Score is indicative of the sum of AMP Synthetic enzymes, missing degradative enzymes and decremented by degradative enzymes.

Figure 2. Representative 16s rRNA based phylogeny tree of class gammaproteobacteria. Mesophiles and psychrophiles are colored red and blue respectively; each species is followed by the number of AMP synthetic genes, AMP degradative genes, and its score.

Figure 3. Growth rate and percent change in growth at specified temperatures. Genetic background is *E. coli* DY330 expressing the indicated enzyme. In the bottom panel AdsL is used as the control. Data points are representative of independent triplicates and error bars indicate standard error of the mean; error bars were omitted in cases where their presence obfuscates data.

Figure 4. Growth rate and percent change in growth at specified temperatures. Genetic background is *E. coli* DY330^{amn} (Morrison and Shain 2008) expressing the indicated enzyme. In the bottom panel AdsL is used as the control. Data points are representative of independent triplicates and error bars indicate standard error of the mean; error bars were omitted in cases where their presence obfuscates data.

Figure 5. Cold tolerance measured by 8 days of incubation at 0° C in LB .02% L-Arabinose. Fraction of viable cells from *E coli* strains expressing the indicated protein. DY330:AdsS remains ~3.5 fold more viable than the control after cold storage (p = .0428); amnK:AdsS remained 2 fold more viable than DY330:LacZ (p = .046).

Figure 6. Cold tolerance measured by 8 days of incubation at 0° C in PBS. Fraction of viable cells from *E coli* strains expressing the indicated protein. Significant ($p < .05$) distinctions include DZ and DS, DZ and AS, DL and AS, AS and AL.

Figure 7. Locations in the vicinity of the Kahiltna Glacier, AK where ice worms searches were conducted.

Figure 8. Negatively charged pocket proximal to AMPD active site. *FAC1*, a representative structure of eukaryotic AMPD, contains a negatively charged cleft proximal to the substrate binding domain and active site (A). Ice worm specific K188E (B) is predicted to occur within this pocket.

Figure 9. Four sterically constraining residues (K173, Y174, D444 and D445) define the surface area of the AMPD substrate binding plane as ~38Å2 in A. thaliana (A), ~36Å2 in A. thaliana K188E (B), ~36Å2 in the ice worm E188 (C), and predicted loss of structure in ice worm E188K (D). Position 188 is visible to the right in each panel

Figure 10. Ice worm adenylosuccinate lyase multiple sequence alignment against high-scoring BLAST hits. Colormap is based on BLOSUM62 percent identity. Conservation is a measure of conserved physico-chemical properties. Quality is the likelihood of observing mutations for that column. Consensus is the consensus sequence.

Appendix 1

Compendium of all reactions known to require AMP as substrate.

ATP diphosphohydrolase (3.6.1.5)
ATP + 2 H2O ↔ AMP + 2 Orthophosphate

ATP diphosphohydrolase (diphosphate-forming); ATP pyrophosphohydrolase (3.6.1.8)
ATP + H2O ↔ AMP + Diphosphate

NAD+ phosphohydrolase (3.6.1.9, 3.6.1.22)
NAD+ + H2O ↔ AMP + Nicotinamide D-ribonucleotide

ADP phosphohydrolase (3.6.1.5)
ADP + H2O ↔ AMP + Orthophosphate

ATP:AMP phosphotransferase (2.7.4.3)
ATP + AMP ↔ 2 ADP

Uridine triphosphate:AMP phosphotransferase (2.7.4.10)
UTP + AMP ↔ UDP + ADP

FAD nucleotidohydrolase (3.6.1.9, 3.6.1.18)
FAD + H2O ↔ AMP + FMN

AMP aminohydrolase (3.5.4.6, 3.5.4.17)
AMP + H2O ↔ IMP + NH3

AMP phosphoribohydrolase (3.2.2.4)
AMP + H2O ↔ Adenine + D-Ribose 5-phosphate

Adenosine 5'-monophosphate phosphohydrolase (3.1.3.5)
AMP + H2O ↔ Adenosine + Orthophosphate

P1,P4-Bis(5'-adenosyl) tetraphosphate nucleotidohydrolase (3.6.1.17)
P1,P4-Bis(5'-adenosyl) tetraphosphate + H2O ↔ ATP + AMP

ATP:adenosine 5'-phosphotransferase (2.7.1.20, 2.7.1.74)
ATP + Adenosine ↔ ADP + AMP

(S)-Lactate adenylohydrolase (3.6.1.29)
P1,P2-Bis(5'-adenosyl) triphosphate + H2O ↔ ADP + AMP

P1,P3-Bis(5'-adenosyl) triphosphate adenylohydrolase (3.6.1.29)
P1,P3-Bis(5'-adenosyl) triphosphate + H2O ↔ ADP + AMP

Adenosine 3',5'-bisphosphate 3'(2')-phosphohydrolase (3.1.3.7)
Adenosine 3',5'-bisphosphate + H2O ↔ AMP + Orthophosphate

Deamino-NAD+:ammonia ligase (AMP-forming) (6.3.1.5)
ATP + Deamino-NAD+ + NH3 ↔ AMP + Diphosphate + NAD+

AMP:pyrophosphate phosphoribosyltransferase (2.4.2.7, 2.4.2.8)
AMP + Diphosphate ↔ Adenine + 5-Phospho-alpha-D-ribose 1-diphosphate

Adenosine 3',5'-phosphate 5'-nucleotidohydrolase (3.1.4.17)
3',5'-Cyclic AMP + H2O ↔ AMP

ATP:pyruvate,water phosphotransferase (2.7.9.2)
ATP + Pyruvate + H2O ↔ AMP + Phosphoenolpyruvate + Orthophosphate

ATP:pyruvate,orthophosphate phosphotransferase (2.7.9.1)
ATP + Pyruvate + Orthophosphate ↔ AMP + Phosphoenolpyruvate + Diphosphate

Acetate:CoA ligase (AMP-forming) (6.2.1.1)
ATP + Acetate + CoA ↔ AMP + Diphosphate + Acetyl-CoA

acetyl adenylate:CoA acetyltransferase (6.2.1.1)
Acetyl adenylate + CoA ↔ AMP + Acetyl-CoA

Deamido-NAD+:L-glutamine amido-ligase (AMP-forming) (6.3.5.1)
ATP + Deamino-NAD+ + L-Glutamine + H2O ↔ AMP + Diphosphate + NAD+ + L-Glutamate

5'-acetylphosphoadenosine acetylhydrolase (3.6.1.20)
Acetyl adenylate + H2O ↔ AMP + Acetate

nucleoside-triphosphate:AMP phosphotransferase (2.7.4.10)
Nucleoside triphosphate + AMP ↔ NDP + ADP

(Deoxyribonucleotide)n:5'-phospho-(deoxyribonucleotide)m ligase (AMP-forming) (6.5.1.1)
ATP + DNA(n) + 5'-Phospho-DNA(m) ↔ AMP + Diphosphate + DNA(n+m)

(Deoxyribonucleotide)n:5'-phospho-(deoxyribonucleotide)m ligase (AMP-forming, NMN-forming) (6.5.1.2)
NAD+ + DNA(n) + 5'-Phospho-DNA(m) ↔ AMP + Nicotinamide D-ribonucleotide + DNA(n+m)

Acid:CoA ligase (AMP-forming) (6.2.1.2)
ATP + Acid + CoA ↔ AMP + Diphosphate + Acyl-CoA

Long-chain carboxylate:CoA ligase (AMP-forming) (6.2.1.3)
ATP + Long-chain carboxylate + CoA ↔ AMP + Diphosphate + Acyl-CoA

ATP:GTP 3'-diphosphotransferase (2.7.6.5)
ATP + GTP ↔ AMP + Guanosine 3'-diphosphate 5'-triphosphate

polyribonucleotide ligase (cyclizing, AMP-forming) (6.5.1.3)
ATP + RNA ↔ AMP + Diphosphate + RNA(circular)

L-aspartate:ammonia ligase (AMP-forming) (6.3.1.1, 6.3.5.4)
ATP + L-Aspartate + NH3 ↔ AMP + Diphosphate + L-Asparagine

Adenylylsulfate sulfohydrolase (3.6.2.1)
Adenylyl sulfate + H2O ↔ AMP + Sulfate

5'-Acylphosphoadenosine acylhydrolase (3.6.1.20)
5'-Acylphosphoadenosine + H2O ↔ AMP + Carboxylate

L-aspartate:L-glutamine amido-ligase (AMP-forming) (6.3.5.4)
ATP + L-Aspartate + L-Glutamine + H2O ↔ AMP + Diphosphate + L-Asparagine + L-Glutamate

ATP:thiamine diphosphotransferase (2.7.6.2)
ATP + Thiamin ↔ AMP + Thiamin diphosphate

L-Phenylalanine racemase (ATP-hydrolysing) (5.1.1.11)
ATP + L-Phenylalanine + H2O ↔ AMP + Diphosphate + D-Phenylalanine

L-Tyrosine:L-arginine ligase (AMP-forming) (6.3.2.24)
ATP + L-Tyrosine + L-Arginine ↔ AMP + Diphosphate + L-Tyrosyl-L-arginine

AMP,sulfite:acceptor oxidoreductase (adenosine-5'-phosphosulfate-forming) (1.8.99.2)
Sulfite + Acceptor + AMP ↔ Adenylyl sulfate + Reduced acceptor

L-Lysine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + L-Lysine + beta-Alanine ↔ AMP + Diphosphate + beta-Alanyl-L-lysine

L-Arginine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + L-Arginine + beta-Alanine ↔ AMP + Diphosphate + beta-Alanyl-L-arginine

Propanoate:CoA ligase (AMP-forming) (6.2.1.1, 6.2.1.17)
ATP + Propanoate + CoA ↔ AMP + Diphosphate + Propanoyl-CoA

Propionyladenylate:CoA propionyltransferase (6.2.1.1, 6.2.1.17)
Propionyladenylate + CoA ↔ AMP + Propanoyl-CoA

ADP-glucose Glucose-1-phosphohydrolase (3.6.1.21)
ADP-glucose + H2O ↔ AMP + D-Glucose 1-phosphate

Anthranilate:CoA ligase (AMP-forming) (6.2.1.32)
ATP + Anthranilate + CoA ↔ AMP + Diphosphate + Anthranilyl-CoA

ATP:D-ribose-5-phosphate diphosphotransferase (2.7.6.1)
ATP + D-Ribose 5-phosphate ↔ AMP + 5-Phospho-alpha-D-ribose 1-diphosphate

ADP-ribose ribophosphohydrolase (3.6.1.13, 3.6.1.21)
ADP-ribose + H2O ↔ AMP + D-Ribose 5-phosphate

biotin:CoA ligase (AMP-forming) (6.2.1.11)
ATP + Biotin + CoA ↔ AMP + Diphosphate + Biotinyl-CoA

N6-(1,2-dicarboxyethyl)AMP AMP-lyase (fumarate-forming) (4.3.2.2)
N6-(1,2-Dicarboxyethyl)-AMP ↔ Fumarate + AMP

L-Histidine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + L-Histidine + beta-Alanine ↔ AMP + Diphosphate + Carnosine

Butanoate:CoA ligase (AMP-forming) (6.2.1.2)
ATP + Butanoic acid + CoA ↔ AMP + Diphosphate + Butanoyl-CoA

Xanthosine-5'-phosphate:ammonia ligase (AMP-forming) (6.3.4.1, 6.3.5.2)
ATP + Xanthosine 5'-phosphate + NH3 ↔ AMP + Diphosphate + GMP

Xanthosine-5'-phosphate:L-glutamine amido-ligase (AMP-forming) (6.3.5.2)
ATP + Xanthosine 5'-phosphate + L-Glutamine + H2O ↔ AMP + Diphosphate + GMP + L-Glutamate

Palmitate:CoA ligase (AMP-forming) (6.2.1.3)
ATP + Hexadecanoic acid + CoA ↔ AMP + Palmitoyl-CoA + Diphosphate

4-Hydroxybenzoate:CoA ligase (AMP-forming) (6.2.1.27)
ATP + 4-Hydroxybenzoate + CoA ↔ AMP + Diphosphate + 4-Hydroxybenzoyl-CoA

Acetoacetate:CoA ligase (AMP-forming) (6.2.1.16)
ATP + Acetoacetate + CoA ↔ AMP + Diphosphate + Acetoacetyl-CoA

Long-chain-fatty-acid:[acyl-carrier-protein] ligase (AMP-forming) (6.2.1.20)
ATP + Long-chain fatty acid + Acyl-carrier protein ↔ AMP + Diphosphate + Acyl-[acyl-carrier protein]

Acid:protein ligase (AMP-forming) (6.2.1.19)
ATP + Acid + Protein ↔ AMP + Diphosphate + Acyl-protein thioester

Benzoate:CoA ligase (AMP-forming) (6.2.1.25)
ATP + Benzoate + CoA ↔ AMP + Diphosphate + S-Benzoate coenzyme A

5'-Benzoylphosphoadenosine acylhydrolase (3.6.1.20)
5'-Benzoylphosphoadenosine + H2O ↔ AMP + Benzoate

aromatic aldehyde:NADP+ oxidoreductase (ATP-forming) (1.2.1.30)
Aromatic aldehyde + AMP + Diphosphate + NADP+ ↔ Aromatic acid + NADPH + ATP + H+

Oxalate:CoA ligase (AMP-forming) (6.2.1.8)
ATP + Oxalate + CoA ↔ AMP + Diphosphate + Oxalyl-CoA

ADP:thymidine 5'-phosphotransferase (2.7.1.118)
ADP + Thymidine ↔ AMP + dTMP

AMP:thymidine 5'-phosphotransferase (2.7.1.114)
AMP + Thymidine ↔ Adenosine + dTMP

Arachidonate:CoA ligase (AMP-forming) (6.2.1.15)
ATP + (5Z,8Z,11Z,14Z)-Icosatetraenoic acid + CoA ↔ AMP + Diphosphate + Arachidonyl-CoA

4-Coumarate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + 4-Coumarate + CoA ↔ AMP + Diphosphate + p-Coumaroyl-CoA

No name available (No EC number available)
Nicotinate + Glycine + ATP ↔ Nicotinurate + AMP + Diphosphate

No name available (3.6.1.21)
ADP-ribose + H2O ↔ alpha-D-Ribose 1-phosphate + AMP

Caffeate:CoA ligase (AMP-forming); 3,4-Dihydroxy-trans-cinnamate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + Caffeate + CoA ↔ AMP + Diphosphate + Caffeoyl-CoA

L-Citrulline:L-aspartate ligase (AMP-forming) (6.3.4.5)
ATP + L-Citrulline + L-Aspartate ↔ AMP + Diphosphate + N-(L-Arginino)succinate

L-Histidine:4-aminobutanoate ligase (AMP-forming) (6.3.2.11)
ATP + L-Histidine + 4-Aminobutanoate ↔ AMP + Diphosphate + Homocarnosine

Ferulate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + Ferulate + CoA ↔ AMP + Diphosphate + Feruloyl-CoA

Sinapate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + Sinapate + CoA ↔ AMP + Diphosphate + Sinapoyl-CoA

trans-Cinnamate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + trans-Cinnamate + CoA ↔ AMP + Diphosphate + Cinnamoyl-CoA

(R)-Pantoate:beta-alanine ligase (AMP-forming) (6.3.2.1)
ATP + (R)-Pantoate + beta-Alanine ↔ AMP + Diphosphate + Pantothenate

Phenylacetate:CoA ligase (6.2.1.30)
ATP + Phenylacetic acid + CoA ↔ AMP + Diphosphate + Phenylacetyl-CoA

ADPmannose sugarphosphohydrolase (3.6.1.21)
ADP-mannose + H2O ↔ AMP + D-Mannose 1-phosphate

4-Hydroxyphenylacetate:CoA ligase (AMP-forming) (6.2.1.-)
ATP + 4-Hydroxyphenylacetate + CoA ↔ AMP + Diphosphate + 4-Hydroxyphenylacetyl-CoA

4-Methylene-L-glutamate:ammonia ligase (AMP-forming) (6.3.1.7)
ATP + 4-Methylene-L-glutamate + NH3 ↔ AMP + Diphosphate + 4-Methylene-L-glutamine

D-Alanine:poly(ribitol phosphate) ligase (AMP-forming) (6.1.1.13)
ATP + D-Alanine + Poly(ribitol phosphate) ↔ AMP + Diphosphate + O-D-Alanyl-poly(ribitol phosphate)

Cholate:CoA ligase (AMP-forming) (6.2.1.7)
ATP + 3alpha,7alpha,12alpha-Trihydroxy-5beta-cholanate + CoA ↔ AMP + Diphosphate + Choloyl-CoA

L-Tyrosine:tRNA(Tyr) ligase (AMP-forming) (6.1.1.1)
ATP + L-Tyrosine + tRNA(Tyr) ↔ AMP + Diphosphate + L-Tyrosyl-tRNA(Tyr)

2-Furoate:CoA ligase (AMP-forming) (6.2.1.31)
ATP + 2-Furoate + CoA ↔ AMP + Diphosphate + 2-Furoyl-CoA

Deamino-NAD+ nucleotidohydrolase (3.6.1.9, 3.6.1.22)
Deamino-NAD+ + H2O ↔ AMP + Nicotinate D-ribonucleotide

Dephospho-CoA nucleotidohydrolase (3.6.1.9)
Dephospho-CoA + H2O ↔ Pantetheine 4'-phosphate + AMP

L-Alanine:tRNA(Ala) ligase (AMP-forming) (6.1.1.7)
ATP + L-Alanine + tRNA(Ala) ↔ AMP + Diphosphate + L-Alanyl-tRNA

6-Carboxyhyxanoate:CoA ligase (AMP-forming) (6.2.1.14)
ATP + 6-Carboxyhexanoate + CoA ↔ AMP + Diphosphate + 6-Carboxyhexanoyl-CoA

ATP:nucleoside-5'-phosphate pyrophosphotransferase (2.7.6.4)
ATP + Nucleoside 5'-phosphate ↔ AMP + 5'-Phosphonucleoside 3'-diphosphate

N(pai)-Methyl-L-histidine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + N(pi)-Methyl-L-histidine + beta-Alanine ↔ AMP + Diphosphate + beta-Alanyl-N(pi)-methyl-L-histidine

Adenylyl-[L-glutamate:ammonia ligase (ADP-forming)] adenylylhydrolase (3.1.4.15)
Adenylyl-[L-glutamate:ammonia ligase (ADP-forming)] + H2O ↔ AMP + [L-Glutamate:ammonia ligase (ADP-forming)]

ATP:2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine 6'-pyrophosphotransferase (2.7.6.3)
ATP + 2-Amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine ↔ AMP + 2-Amino-7,8-dihydro-4-hydroxy-6-(diphosphooxymethyl)pteridine

ADP-sugar sugarphosphohydrolase (3.6.1.21)
ADP-sugar + H2O ↔ AMP + alpha-D-Aldose 1-phosphate

ATP:selenide, water phosphotransferase (2.7.9.3)
ATP + Selenide + H2O ↔ AMP + Selenophosphate + Orthophosphate

Phytanate:CoA ligase (AMP-forming) (6.2.1.24)
ATP + Phytanate + CoA ↔ AMP + Diphosphate + Phytanoyl-CoA

L-Arginine:tRNA(Arg) ligase (AMP-forming) (6.1.1.19)
ATP + L-Arginine + tRNA(Arg) ↔ AMP + Diphosphate + L-Arginyl-tRNA(Arg)

L-Aspartate:tRNAAsp ligase (AMP-forming) (6.1.1.23)
ATP + L-Aspartate + tRNA(Asn) ↔ AMP + Diphosphate + L-Aspartyl-tRNA(Asn)

L-Asparagine:tRNA(Asn) ligase (AMP-forming) (6.1.1.22)
ATP + L-Asparagine + tRNA(Asn) ↔ AMP + Diphosphate + L-Asparaginyl-tRNA(Asn)

L-Cysteine:tRNA(Cys) ligase (AMP-forming) (6.1.1.16)
ATP + L-Cysteine + tRNA(Cys) ↔ AMP + Diphosphate + L-Cysteinyl-tRNA(Cys)

L-Glutamate:tRNA(Gln) ligase (AMP-forming) (6.1.1.24)
ATP + L-Glutamate + tRNA(Gln) ↔ AMP + Diphosphate + L-Glutamyl-tRNA(Gln)

L-Glutamine:tRNA(Gln) ligase (AMP-forming) (6.1.1.18)
ATP + L-Glutamine + tRNA(Gln) ↔ AMP + Diphosphate + Glutaminyl-tRNA

Glycine:tRNA(Gly) ligase (AMP-forming) (6.1.1.14)
ATP + Glycine + tRNA(Gly) ↔ AMP + Diphosphate + Glycyl-tRNA(Gly)

L-Histidine:tRNA(His) ligase (AMP-forming) (6.1.1.21)
ATP + L-Histidine + tRNA(His) ↔ AMP + Diphosphate + L-Histidyl-tRNA(His)

L-Isoleucine:tRNA(Ile) ligase (AMP-forming) (6.1.1.5)
ATP + L-Isoleucine + tRNA(Ile) ↔ AMP + Diphosphate + L-Isoleucyl-tRNA(Ile)

L-Leucine:tRNA(Leu) ligase (AMP-forming) (6.1.1.4)
ATP + L-Leucine + tRNA(Leu) ↔ AMP + Diphosphate + L-Leucyl-tRNA

L-Lysine:tRNALys ligase (AMP-forming) (6.1.1.6)
ATP + L-Lysine + tRNA(Lys) ↔ AMP + Diphosphate + L-Lysyl-tRNA

L-Methionine:tRNAMet ligase (AMP-forming) (6.1.1.10)
ATP + L-Methionine + tRNA(Met) ↔ AMP + Diphosphate + L-Methionyl-tRNA

L-Phenylalanine:tRNA(Ala) ligase (AMP-forming) (6.1.1.20)
ATP + L-Phenylalanine + tRNA(Phe) ↔ AMP + Diphosphate + L-Phenylalanyl-tRNA(Phe)

L-Proline:tRNA(Pro) ligase (AMP-forming) (6.1.1.15)
ATP + L-Proline + tRNA(Pro) ↔ AMP + Diphosphate + L-Prolyl-tRNA(Pro)

L-Serine:tRNA(Ser) ligase (AMP-forming) (6.1.1.11)
ATP + L-Serine + tRNA(Ser) ↔ AMP + Diphosphate + L-Seryl-tRNA(Ser)

L-Threonine:tRNA(Thr) ligase (AMP-forming) (6.1.1.3)
ATP + L-Threonine + tRNA(Thr) ↔ AMP + Diphosphate + L-Threonyl-tRNA(Thr)

L-Tryptophan -tRNA(Trp) ligase (AMP-forming) (6.1.1.2)
ATP + L-Tryptophan + tRNA(Trp) ↔ AMP + Diphosphate + L-Tryptophanyl-tRNA(Trp)

L-Valine:tRNAVal ligase (AMP-forming) (6.1.1.9)
ATP + L-Valine + tRNA(Val) ↔ AMP + Diphosphate + L-Valyl-tRNA(Val)

biotin:CoA ligase (AMP-forming); biotinyl-5'-AMP:CoA biotinyltransferase (6.2.1.11)
Biotinyl-5'-AMP + CoA ↔ AMP + Biotinyl-CoA

Ubiquitin:protein-lysine N -ligase (AMP-forming) (6.3.2.19)
ATP + Ubiquitin + Protein lysine ↔ AMP + Diphosphate + Protein N-ubiquityllysine

4-Chlorobenzoate:CoA ligase (6.2.1.33)
ATP + 4-Chlorobenzoate + CoA ↔ AMP + Diphosphate + 4-Chlorobenzoyl-CoA

Chenodeoxycholate:CoA ligase (AMP-forming) (6.2.1.7)
ATP + Chenodeoxycholate + CoA ↔ AMP + Diphosphate + Chenodeoxycholoyl-CoA

O-Succinylbenzoate:CoA ligase (AMP-forming) (6.2.1.26)
ATP + 2-Succinylbenzoate + CoA ↔ AMP + Diphosphate + 2-Succinylbenzoyl-CoA

Photinus-luciferin:oxygen 4-oxidoreductase(decarboxylating,ATP-hydrolysing) (1.13.12.7)
Photinus luciferin + Oxygen + ATP ↔ Oxidized Photinus luciferin + hn + AMP + Diphosphate + CO2

2-Isopentenyl-diphosphate:AMP delta2-isopentenyltransferase (2.5.1.27)
Dimethylallyl diphosphate + AMP ↔ Diphosphate + N6-(delta2-Isopentenyl)-adenosine 5'-monophosphate

alpha(omega)-Dicarboxylic acid:CoA ligase (AMP-forming) (6.2.1.23)
ATP + alpha,omega-Dicarboxylic acid + CoA ↔ AMP + Diphosphate + omega-Carboxyacyl-CoA

(R)-4'-Phosphopantothenate:L-cysteine ligase (6.3.2.5)
ATP + D-4'-Phosphopantothenate + L-Cysteine ↔ AMP + Diphosphate + (R)-4'-Phosphopantothenoyl-L-cysteine

RNA-3'-phosphate:RNA ligase (cyclizing, AMP-forming) (6.5.1.4)
ATP + RNA 3'-terminal-phosphate ↔ AMP + Diphosphate + RNA terminal-2',3'-cyclic-phosphate

Acetate:citrate-(pro-3S)-lyase(thiol-form) ligase (AMP-forming) (6.2.1.22)
ATP + Acetate + Citrate (pro-3S)-lyase (thiol form) ↔ AMP + Diphosphate + Citrate (pro-3S)-lyase (acetyl form)

3alpha,7alpha-Dihydroxy-5beta-cholestanate:CoA ligase (AMP-forming) (6.2.1.7, 6.2.1.28)
ATP + 3alpha,7alpha-Dihydroxy-5beta-cholestanate + CoA ↔ AMP + Diphosphate + 3alpha,7alpha-Dihydroxy-5beta-cholestanoyl-CoA

Biotin:apo-[acetyl-CoA:carbondioxide ligase (ADP-forming)] ligase (AMP-forming) (6.3.4.15)
ATP + Biotin + Apo-[acetyl-CoA:carbon-dioxide ligase (ADP-forming)] ↔ AMP + Diphosphate + [Acetyl-CoA:carbon-dioxide ligase (ADP-forming)]

Biotin:apo[methylmalonyl-CoA:pyruvate carboxyltransferase] ligase (AMP-forming) (6.3.4.9)
ATP + Biotin + Apo-[methylmalonyl-CoA:pyruvate carboxytransferase] ↔ AMP + Diphosphate + [Methylmalonyl-CoA:pyruvate carboxytransferase]

3alpha,7alpha,12alpha-Trihydroxy-5beta-cholestanate:CoA ligase (AMP-forming) (6.2.1.7)
ATP + 3alpha,7alpha,12alpha-Trihydroxy-5beta-cholestanoate + CoA ↔ AMP + Diphosphate + 3alpha,7alpha,12alpha-Trihydroxy-5beta-cholestanoyl-CoA

Biotin:apo-[propionyl-CoA:carbon-dioxide ligase (ADP-forming)] (6.3.4.10)
ATP + Biotin + Apo-[propionyl-CoA:carbon-dioxide ligase (ADP-forming)] ↔ AMP + Diphosphate + [Propionyl-CoA:carbon-dioxide ligase (ADP-forming)]

Biotin:apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)] ligase (AMP-forming) (6.3.4.11)
ATP + Biotin + Apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)] ↔ AMP + Diphosphate + [3-Methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)]

Selenomethionine:tRNAMet ligase (AMP-forming) (6.1.1.10)
ATP + Selenomethionine + tRNA(Met) ↔ AMP + Diphosphate + Selenomethionyl-tRNA(Met)

L-2-Aminoadipate-6-semialdehyde:NAD(P)+ 6-oxidoreductase (1.2.1.31)
L-2-Aminoadipate adenylate + Holo-Lys2 ↔ alpha-Aminoadipoyl-S-acyl enzyme + AMP

L-2-aminohexanedioate:L-cysteine:L-valine ligase (AMP-forming, valine-inverting) (6.3.2.26)
L-2-Aminoadipate + L-Valine + L-Cysteine + 3 ATP + H2O ↔ delta-(L-2-Aminoadipyl)-L-cysteinyl-D-valine + 3 AMP + 3 Diphosphate

AMP,sulfite:(acceptor) oxidoreductase (1.8.99.2)
2 Ferricytochrome c + Selenite + AMP ↔ 2 Ferrocytochrome c + Adenylylselenate

Biotinyl-5'-AMP:apo-[carboxylase] ligase (AMP-forming) (6.3.4.9, 6.3.4.10, 6.3.4.11, 6.3.4.15)
Biotinyl-5'-AMP + Apo-[carboxylase] ↔ AMP + Holo-[carboxylase]

Calmodulin:ubiquitin ligase (AMP-forming) (6.3.2.21)
n ATP + Calmodulin + n Ubiquitin ↔ n AMP + n Diphosphate + (Ubiquitin)n-calmodulin

2,4-dichlorobenzoate:CoA ligase (6.2.1.-)
CoA + ATP + 2,4-Dichlorobenzoate ↔ Diphosphate + 2,4-Dichlorobenzoyl-CoA + AMP

Benzoyl acetate: CoA ligase (6.2.1.-)
Benzoyl acetate + CoA + ATP ↔ Diphosphate + Benzoyl acetyl-CoA + AMP

L-N2-(2-carboxyethyl)arginine cyclo-ligase (AMP-forming) (6.3.3.4)
L-N2-(2-Carboxyethyl)arginine + ATP ↔ Deoxyguanidinoproclavaminic acid + AMP + Diphosphate

No name available (No EC number available)
3-(2-Hydroxyphenyl)propanoate + ATP + CoA + FAD + NAD+ ↔ Salicylate + AMP + Diphosphate + FADH2 + Acetyl-CoA + NADH + H+

L-Aspartate:tRNA(Asp) ligase (AMP-forming) (6.1.1.12, 6.1.1.23)
tRNA(Asp) + L-Aspartate + ATP ↔ L-Aspartyl-tRNA(Asp) + Diphosphate + AMP

L-Glutamate:tRNA(Glu) ligase (AMP-forming) (6.1.1.17, 6.1.1.24)
tRNA(Glu) + L-Glutamate + ATP ↔ L-Glutamyl-tRNA(Glu) + Diphosphate + AMP

No name available (6.2.1.-)
Cyclohexane-1-carboxylate + CoA + ATP ↔ Cyclohexane-1-carboxyl-CoA + AMP + Diphosphate

Phenylphosphate synthase (2.7.9.-)
Phenol + ATP + H2O ↔ Phenolic phosphate + AMP + Orthophosphate

AMP,sulfite:glutathione-disulfide oxidoreductase (adenosine-5'-phosphosulfate-forming) (1.8.4.9)
AMP + Sulfite + Glutathione disulfide ↔ Adenylyl sulfate + 2 Glutathione

acetone:carbon-dioxide ligase (AMP-forming) (6.4.1.6)
Acetone + CO2 + ATP + 2 H2O ↔ Acetoacetate + AMP + 2 Orthophosphate

ADP:D-glucose 6-phosphotransferase (2.7.1.147)
ADP + D-Glucose ↔ AMP + D-Glucose 6-phosphate

ADP:D-fructose-6-phosphate 1-phosphotransferase (2.7.1.146)
ADP + D-Fructose 6-phosphate ↔ AMP + D-Fructose 1,6-bisphosphate

perillic acid:CoA ligase (AMP-forming) (6.2.1.-)

Perillic acid + CoA + ATP ↔ Perillyl-CoA + H2O + AMP + Diphosphate

cis-2-Methyl-5-isopropylhexa-2,5-dienoate-CoA ligase (6.2.1.-)
cis-2-Methyl-5-isopropylhexa-2,5-dienoic acid + ATP + CoA ↔ cis-2-Methyl-5-isopropylhexa-2,5-dienoyl-CoA + AMP + Diphosphate

trans-2-Methyl-5-isopropylhexa-2,5-dienoate-CoA ligase (6.2.1.-)
trans-2-Methyl-5-isopropylhexa-2,5-dienoic acid + ATP + CoA ↔ trans-2-Methyl-5-isopropylhexa-2,5-dienoyl-CoA + AMP + Diphosphate

(3R)-3-isopropenyl-6-oxoheptanoate:CoA ligase (AMP-forming) (6.2.1.-)
(3R)-3-Isopropenyl-6-oxoheptanoate + CoA + ATP ↔ (3R)-3-Isopropenyl-6-oxoheptanoyl-CoA + H2O + AMP + Diphosphate

4-coumarate:CoA ligase (AMP-forming) (6.2.1.12)
5-Hydroxyferulate + CoA + ATP ↔ 5-Hydroxyferuloyl-CoA + AMP + Diphosphate

No name available (6.2.1.-)
Pseudoecgonine + ATP + CoA ↔ Pseudoecgonyl-CoA + AMP

No name available (No EC number available)
L-Proline + ATP ↔ L-Prolyl-[pcp] + AMP + Diphosphate

No name available (No EC number available)
L-Tyrosine + ATP ↔ L-Tyrosyl-[pcp] + AMP + Diphosphate

No name available (No EC number available)
3-Amino-4,7-dihydroxy-8-methylcoumarin + 3-Dimethylallyl-4-hydroxybenzoate + ATP ↔ Novobiocic acid + AMP + Diphosphate

No name available (No EC number available)
3-Methylpyrrole-2,4-dicarboxylic acid + 3-Amino-4,7-dihydroxy-8-methylcoumarin + ATP ↔ Coumeroic acid + AMP + Diphosphate

No name available (No EC number available)
Coumeroic acid + 3-Amino-4,7-dihydroxy-8-methylcoumarin + ATP ↔ Coumermic acid + AMP + Diphosphate

No name available (6.2.1.-)
Adipate + CoA + ATP ↔ Adipyl-CoA + AMP + Diphosphate

AMP,sulfite:thioredoxin-disulfide oxidoreductase(adenosine-5'-phosphosulfate-forming) (1.8.4.10)
AMP + Sulfite + Thioredoxin disulfide ↔ Adenylyl sulfate + Thioredoxin

long-chain-fatty-acid:[acyl-carrier-protein] ligase (AMP-forming) (6.2.1.20)
ATP + Acid + Acyl-carrier protein ↔ AMP + Diphosphate + Acyl-[acyl-carrier protein]

N-Methylanthranilate:CoA ligase (AMP-forming) (6.2.1.32)
ATP + N-Methylanthranilate + CoA ↔ AMP + Diphosphate + N-Methylanthraniloyl-CoA

FAD AMP-lyase (cyclic-FMN-forming) (4.6.1.15)
FAD ↔ AMP + Riboflavin cyclic-4',5'-phosphate

poly(ribonucleotide):poly(ribonucleotide) ligase (AMP-forming) (6.5.1.3)
ATP + RNA(n) + RNA(m) ↔ AMP + Diphosphate + RNA(n+m)

L-lysine:tRNAPyl ligase (AMP-forming) (6.1.1.25)
ATP + L-Lysine + tRNA(Pyl) ↔ AMP + Diphosphate + L-Lysyl-tRNA(Pyl)

No name available (2.7.7.63)
Lipoyl-AMP + Apoprotein ↔ Protein N6-(lipoyl)lysine + AMP

No name available (6.2.1.-)
ATP + 8-[(1R,2R)-3-Oxo-2-{(Z)-pent-2-enyl}cyclopentyl]octanoate + CoA ↔ AMP + Diphosphate + OPC8-CoA

citronellyl-CoA ligase (6.2.1.-)
Citronellate + CoA + ATP ↔ Citronellyl-CoA + AMP + Diphosphate

4-fluorobenzoate:CoA ligase (6.2.1.33)
4-Fluorobenzoate + CoA + ATP ↔ 4-Fluorobenzoyl-CoA + AMP + Diphosphate

No name available (6.1.1.11)
ATP + L-Serine + tRNA(Sec) ↔ AMP + Diphosphate + L-Seryl-tRNA(Sec)

6-thioxanthine 5'-monophosphate:L-glutamine amido-ligase (AMP-forming) (6.3.5.2)
6-Thioxanthine 5'-monophosphate + ATP + L-Glutamine + H2O ↔ 6-Thioguanosine monophosphate + AMP + Diphosphate + L-Glutamate

AMP,sulfite:acceptor oxidoreductase (adenosine-5'-phosphosulfate-forming) (1.8.99.2)
AMP + Sulfite + FAD ↔ Adenylyl sulfate + FADH2

No name available (6.1.1.-)
L-Serine + tRNA(Cys) + ATP ↔ O-Phosphoseryl-tRNA(Cys) + Diphosphate + AMP

L-pyrrolysine:tRNA(Pyl) ligase (AMP-forming) (6.1.1.26)
ATP + L-Pyrrolysine + tRNA(Pyl) ↔ AMP + Diphosphate + L-Pyrrolysyl-tRNA(Pyl)

[enzyme]-S-sulfanylcysteine:tRNA uridine sulfurtransferase (ATP-hydrolysing) (No EC number available)
tRNA uridine + [Enzyme]-S-sulfanylcysteine + ATP + Reduced acceptor ↔ tRNA containing 2-thiouridine + [Enzyme]-cysteine + AMP + Diphosphate + Acceptor

ATP diphosphohydrolase (3.6.1.5)
ATP + 2 H2O ↔ AMP + 2 Orthophosphate

ATP diphosphohydrolase (diphosphate-forming); ATP pyrophosphohydrolase (3.6.1.8)
ATP + H2O ↔ AMP + Diphosphate

NAD+ phosphohydrolase (3.6.1.9, 3.6.1.22)
NAD+ + H2O ↔ AMP + Nicotinamide D-ribonucleotide

ADP phosphohydrolase (3.6.1.5)
ADP + H2O ↔ AMP + Orthophosphate

ATP:AMP phosphotransferase (2.7.4.3)
ATP + AMP ↔ 2 ADP

Uridine triphosphate:AMP phosphotransferase (2.7.4.10)
UTP + AMP ↔ UDP + ADP

FAD nucleotidohydrolase (3.6.1.9, 3.6.1.18)
FAD + H2O ↔ AMP + FMN

AMP aminohydrolase (3.5.4.6, 3.5.4.17)
AMP + H2O ↔ IMP + NH3

AMP phosphoribohydrolase (3.2.2.4)
AMP + H2O ↔ Adenine + D-Ribose 5-phosphate

Adenosine 5'-monophosphate phosphohydrolase (3.1.3.5)
AMP + H2O ↔ Adenosine + Orthophosphate

P1,P4-Bis(5'-adenosyl) tetraphosphate nucleotidohydrolase (3.6.1.17)
P1,P4-Bis(5'-adenosyl) tetraphosphate + H2O ↔ ATP + AMP

ATP:adenosine 5'-phosphotransferase (2.7.1.20, 2.7.1.74)
ATP + Adenosine ↔ ADP + AMP

(S)-Lactate adenylohydrolase (3.6.1.29)
P1,P2-Bis(5'-adenosyl) triphosphate + H2O ↔ ADP + AMP

P1,P3-Bis(5'-adenosyl) triphosphate adenylohydrolase (3.6.1.29)
P1,P3-Bis(5'-adenosyl) triphosphate + H2O ↔ ADP + AMP

Adenosine 3',5'-bisphosphate 3'(2')-phosphohydrolase (3.1.3.7)
Adenosine 3',5'-bisphosphate + H2O ↔ AMP + Orthophosphate

Deamino-NAD+:ammonia ligase (AMP-forming) (6.3.1.5)
ATP + Deamino-NAD+ + NH3 ↔ AMP + Diphosphate + NAD+

AMP:pyrophosphate phosphoribosyltransferase (2.4.2.7, 2.4.2.8)
AMP + Diphosphate ↔ Adenine + 5-Phospho-alpha-D-ribose 1-diphosphate

Adenosine 3',5'-phosphate 5'-nucleotidohydrolase (3.1.4.17)
3',5'-Cyclic AMP + H2O ↔ AMP

ATP:pyruvate,water phosphotransferase (2.7.9.2)
ATP + Pyruvate + H2O ↔ AMP + Phosphoenolpyruvate + Orthophosphate

ATP:pyruvate,orthophosphate phosphotransferase (2.7.9.1)
ATP + Pyruvate + Orthophosphate ↔ AMP + Phosphoenolpyruvate + Diphosphate

Acetate:CoA ligase (AMP-forming) (6.2.1.1)
ATP + Acetate + CoA ↔ AMP + Diphosphate + Acetyl-CoA

acetyl adenylate:CoA acetyltransferase (6.2.1.1)
Acetyl adenylate + CoA ↔ AMP + Acetyl-CoA

Deamido-NAD+:L-glutamine amido-ligase (AMP-forming) (6.3.5.1)
ATP + Deamino-NAD+ + L-Glutamine + H2O ↔ AMP + Diphosphate + NAD+ + L-Glutamate

5'-acetylphosphoadenosine acetylhydrolase (3.6.1.20)
Acetyl adenylate + H2O ↔ AMP + Acetate

nucleoside-triphosphate:AMP phosphotransferase (2.7.4.10)
Nucleoside triphosphate + AMP ↔ NDP + ADP

(Deoxyribonucleotide)n:5'-phospho-(deoxyribonucleotide)m ligase (AMP-forming) (6.5.1.1)
ATP + DNA(n) + 5'-Phospho-DNA(m) ↔ AMP + Diphosphate + DNA(n+m)

(Deoxyribonucleotide)n:5'-phospho-(deoxyribonucleotide)m ligase (AMP-forming, NMN-forming) (6.5.1.2)
NAD+ + DNA(n) + 5'-Phospho-DNA(m) ↔ AMP + Nicotinamide D-ribonucleotide + DNA(n+m)

Acid:CoA ligase (AMP-forming) (6.2.1.2)
ATP + Acid + CoA ↔ AMP + Diphosphate + Acyl-CoA

Long-chain carboxylate:CoA ligase (AMP-forming) (6.2.1.3)
ATP + Long-chain carboxylate + CoA ↔ AMP + Diphosphate + Acyl-CoA

ATP:GTP 3'-diphosphotransferase (2.7.6.5)
ATP + GTP ↔ AMP + Guanosine 3'-diphosphate 5'-triphosphate

polyribonucleotide ligase (cyclizing, AMP-forming) (6.5.1.3)
ATP + RNA ↔ AMP + Diphosphate + RNA(circular)

L-aspartate:ammonia ligase (AMP-forming) (6.3.1.1, 6.3.5.4)
ATP + L-Aspartate + NH3 ↔ AMP + Diphosphate + L-Asparagine

Adenylylsulfate sulfohydrolase (3.6.2.1)
Adenylyl sulfate + H2O ↔ AMP + Sulfate

5'-Acylphosphoadenosine acylhydrolase (3.6.1.20)
5'-Acylphosphoadenosine + H2O ↔ AMP + Carboxylate

L-aspartate:L-glutamine amido-ligase (AMP-forming) (6.3.5.4)
ATP + L-Aspartate + L-Glutamine + H2O ↔ AMP + Diphosphate + L-Asparagine + L-Glutamate

ATP:thiamine diphosphotransferase (2.7.6.2)
ATP + Thiamin ↔ AMP + Thiamin diphosphate

L-Phenylalanine racemase (ATP-hydrolysing) (5.1.1.11)
ATP + L-Phenylalanine + H2O ↔ AMP + Diphosphate + D-Phenylalanine

L-Tyrosine:L-arginine ligase (AMP-forming) (6.3.2.24)
ATP + L-Tyrosine + L-Arginine ↔ AMP + Diphosphate + L-Tyrosyl-L-arginine

AMP,sulfite:acceptor oxidoreductase (adenosine-5'-phosphosulfate-forming) (1.8.99.2)
Sulfite + Acceptor + AMP ↔ Adenylyl sulfate + Reduced acceptor

L-Lysine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + L-Lysine + beta-Alanine ↔ AMP + Diphosphate + beta-Alanyl-L-lysine

L-Arginine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + L-Arginine + beta-Alanine ↔ AMP + Diphosphate + beta-Alanyl-L-arginine

Propanoate:CoA ligase (AMP-forming) (6.2.1.1, 6.2.1.17)
ATP + Propanoate + CoA ↔ AMP + Diphosphate + Propanoyl-CoA

Propionyladenylate:CoA propionyltransferase (6.2.1.1, 6.2.1.17)
Propionyladenylate + CoA ↔ AMP + Propanoyl-CoA

ADP-glucose Glucose-1-phosphohydrolase (3.6.1.21)
ADP-glucose + H2O ↔ AMP + D-Glucose 1-phosphate

Anthranilate:CoA ligase (AMP-forming) (6.2.1.32)
ATP + Anthranilate + CoA ↔ AMP + Diphosphate + Anthranilyl-CoA

ATP:D-ribose-5-phosphate diphosphotransferase (2.7.6.1)
ATP + D-Ribose 5-phosphate ↔ AMP + 5-Phospho-alpha-D-ribose 1-diphosphate

ADP-ribose ribophosphohydrolase (3.6.1.13, 3.6.1.21)
ADP-ribose + H2O ↔ AMP + D-Ribose 5-phosphate

biotin:CoA ligase (AMP-forming) (6.2.1.11)
ATP + Biotin + CoA ↔ AMP + Diphosphate + Biotinyl-CoA

N6-(1,2-dicarboxyethyl)AMP AMP-lyase (fumarate-forming) (4.3.2.2)
N6-(1,2-Dicarboxyethyl)-AMP ↔ Fumarate + AMP

L-Histidine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + L-Histidine + beta-Alanine ↔ AMP + Diphosphate + Carnosine

Butanoate:CoA ligase (AMP-forming) (6.2.1.2)
ATP + Butanoic acid + CoA ↔ AMP + Diphosphate + Butanoyl-CoA

Xanthosine-5'-phosphate:ammonia ligase (AMP-forming) (6.3.4.1, 6.3.5.2)
ATP + Xanthosine 5'-phosphate + NH3 ↔ AMP + Diphosphate + GMP

Xanthosine-5'-phosphate:L-glutamine amido-ligase (AMP-forming) (6.3.5.2)
ATP + Xanthosine 5'-phosphate + L-Glutamine + H2O ↔ AMP + Diphosphate + GMP + L-Glutamate

Palmitate:CoA ligase (AMP-forming) (6.2.1.3)
ATP + Hexadecanoic acid + CoA ↔ AMP + Palmitoyl-CoA + Diphosphate

4-Hydroxybenzoate:CoA ligase (AMP-forming) (6.2.1.27)
ATP + 4-Hydroxybenzoate + CoA ↔ AMP + Diphosphate + 4-Hydroxybenzoyl-CoA

Acetoacetate:CoA ligase (AMP-forming) (6.2.1.16)
ATP + Acetoacetate + CoA ↔ AMP + Diphosphate + Acetoacetyl-CoA

Long-chain-fatty-acid:[acyl-carrier-protein] ligase (AMP-forming) (6.2.1.20)
ATP + Long-chain fatty acid + Acyl-carrier protein ↔ AMP + Diphosphate + Acyl-[acyl-carrier protein]

Acid:protein ligase (AMP-forming) (6.2.1.19)
ATP + Acid + Protein ↔ AMP + Diphosphate + Acyl-protein thioester

Benzoate:CoA ligase (AMP-forming) (6.2.1.25)
ATP + Benzoate + CoA ↔ AMP + Diphosphate + S-Benzoate coenzyme A

5'-Benzoylphosphoadenosine acylhydrolase (3.6.1.20)
5'-Benzoylphosphoadenosine + H2O ↔ AMP + Benzoate

aromatic aldehyde:NADP+ oxidoreductase (ATP-forming) (1.2.1.30)
Aromatic aldehyde + AMP + Diphosphate + NADP+ ↔ Aromatic acid + NADPH + ATP + H+

Oxalate:CoA ligase (AMP-forming) (6.2.1.8)
ATP + Oxalate + CoA ↔ AMP + Diphosphate + Oxalyl-CoA

ADP:thymidine 5'-phosphotransferase (2.7.1.118)
ADP + Thymidine ↔ AMP + dTMP

AMP:thymidine 5'-phosphotransferase (2.7.1.114)
AMP + Thymidine ↔ Adenosine + dTMP

Arachidonate:CoA ligase (AMP-forming) (6.2.1.15)
ATP + (5Z,8Z,11Z,14Z)-Icosatetraenoic acid + CoA ↔ AMP + Diphosphate + Arachidonyl-CoA

4-Coumarate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + 4-Coumarate + CoA ↔ AMP + Diphosphate + p-Coumaroyl-CoA

No name available (No EC number available)
Nicotinate + Glycine + ATP ↔ Nicotinurate + AMP + Diphosphate

No name available (3.6.1.21)
ADP-ribose + H2O ↔ alpha-D-Ribose 1-phosphate + AMP

Caffeate:CoA ligase (AMP-forming); 3,4-Dihydroxy-trans-cinnamate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + Caffeate + CoA ↔ AMP + Diphosphate + Caffeoyl-CoA

L-Citrulline:L-aspartate ligase (AMP-forming) (6.3.4.5)
ATP + L-Citrulline + L-Aspartate ↔ AMP + Diphosphate + N-(L-Arginino)succinate

L-Histidine:4-aminobutanoate ligase (AMP-forming) (6.3.2.11)
ATP + L-Histidine + 4-Aminobutanoate ↔ AMP + Diphosphate + Homocarnosine

Ferulate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + Ferulate + CoA ↔ AMP + Diphosphate + Feruloyl-CoA

Sinapate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + Sinapate + CoA ↔ AMP + Diphosphate + Sinapoyl-CoA

trans-Cinnamate:CoA ligase (AMP-forming) (6.2.1.12)
ATP + trans-Cinnamate + CoA ↔ AMP + Diphosphate + Cinnamoyl-CoA

(R)-Pantoate:beta-alanine ligase (AMP-forming) (6.3.2.1)
ATP + (R)-Pantoate + beta-Alanine ↔ AMP + Diphosphate + Pantothenate

Phenylacetate:CoA ligase (6.2.1.30)
ATP + Phenylacetic acid + CoA ↔ AMP + Diphosphate + Phenylacetyl-CoA

ADPmannose sugarphosphohydrolase (3.6.1.21)
ADP-mannose + H2O ↔ AMP + D-Mannose 1-phosphate

4-Hydroxyphenylacetate:CoA ligase (AMP-forming) (6.2.1.-)
ATP + 4-Hydroxyphenylacetate + CoA ↔ AMP + Diphosphate + 4-Hydroxyphenylacetyl-CoA

4-Methylene-L-glutamate:ammonia ligase (AMP-forming) (6.3.1.7)
ATP + 4-Methylene-L-glutamate + NH3 ↔ AMP + Diphosphate + 4-Methylene-L-glutamine

D-Alanine:poly(ribitol phosphate) ligase (AMP-forming) (6.1.1.13)
ATP + D-Alanine + Poly(ribitol phosphate) ↔ AMP + Diphosphate + O-D-Alanyl-poly(ribitol phosphate)

Cholate:CoA ligase (AMP-forming) (6.2.1.7)
ATP + 3alpha,7alpha,12alpha-Trihydroxy-5beta-cholanate + CoA ↔ AMP + Diphosphate + Choloyl-CoA

L-Tyrosine:tRNA(Tyr) ligase (AMP-forming) (6.1.1.1)
ATP + L-Tyrosine + tRNA(Tyr) ↔ AMP + Diphosphate + L-Tyrosyl-tRNA(Tyr)

2-Furoate:CoA ligase (AMP-forming) (6.2.1.31)
ATP + 2-Furoate + CoA ↔ AMP + Diphosphate + 2-Furoyl-CoA

Deamino-NAD+ nucleotidohydrolase (3.6.1.9, 3.6.1.22)
Deamino-NAD+ + H2O ↔ AMP + Nicotinate D-ribonucleotide

Dephospho-CoA nucleotidohydrolase (3.6.1.9)
Dephospho-CoA + H2O ↔ Pantetheine 4'-phosphate + AMP

L-Alanine:tRNA(Ala) ligase (AMP-forming) (6.1.1.7)
ATP + L-Alanine + tRNA(Ala) ↔ AMP + Diphosphate + L-Alanyl-tRNA

6-Carboxyhyxanoate:CoA ligase (AMP-forming) (6.2.1.14)
ATP + 6-Carboxyhexanoate + CoA ↔ AMP + Diphosphate + 6-Carboxyhexanoyl-CoA

ATP:nucleoside-5'-phosphate pyrophosphotransferase (2.7.6.4)
ATP + Nucleoside 5'-phosphate ↔ AMP + 5'-Phosphonucleoside 3'-diphosphate

N(pai)-Methyl-L-histidine:beta-alanine ligase (AMP-forming) (6.3.2.11)
ATP + N(pi)-Methyl-L-histidine + beta-Alanine ↔ AMP + Diphosphate + beta-Alanyl-N(pi)-methyl-L-histidine

Adenylyl-[L-glutamate:ammonia ligase (ADP-forming)] adenylylhydrolase (3.1.4.15)
Adenylyl-[L-glutamate:ammonia ligase (ADP-forming)] + H2O ↔ AMP + [L-Glutamate:ammonia ligase (ADP-forming)]

ATP:2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine 6'-pyrophosphotransferase (2.7.6.3)
ATP + 2-Amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine ↔ AMP + 2-Amino-7,8-dihydro-4-hydroxy-6-(diphosphooxymethyl)pteridine

ADP-sugar sugarphosphohydrolase (3.6.1.21)
ADP-sugar + H2O ↔ AMP + alpha-D-Aldose 1-phosphate

ATP:selenide, water phosphotransferase (2.7.9.3)
ATP + Selenide + H2O ↔ AMP + Selenophosphate + Orthophosphate

Phytanate:CoA ligase (AMP-forming) (6.2.1.24)
ATP + Phytanate + CoA ↔ AMP + Diphosphate + Phytanoyl-CoA

L-Arginine:tRNA(Arg) ligase (AMP-forming) (6.1.1.19)
ATP + L-Arginine + tRNA(Arg) ↔ AMP + Diphosphate + L-Arginyl-tRNA(Arg)

L-Aspartate:tRNAAsp ligase (AMP-forming) (6.1.1.23)
ATP + L-Aspartate + tRNA(Asn) ↔ AMP + Diphosphate + L-Aspartyl-tRNA(Asn)

L-Asparagine:tRNA(Asn) ligase (AMP-forming) (6.1.1.22)
ATP + L-Asparagine + tRNA(Asn) ↔ AMP + Diphosphate + L-Asparaginyl-tRNA(Asn)

L-Cysteine:tRNA(Cys) ligase (AMP-forming) (6.1.1.16)
ATP + L-Cysteine + tRNA(Cys) ↔ AMP + Diphosphate + L-Cysteinyl-tRNA(Cys)

L-Glutamate:tRNA(Gln) ligase (AMP-forming) (6.1.1.24)
ATP + L-Glutamate + tRNA(Gln) ↔ AMP + Diphosphate + L-Glutamyl-tRNA(Gln)

L-Glutamine:tRNA(Gln) ligase (AMP-forming) (6.1.1.18)
ATP + L-Glutamine + tRNA(Gln) ↔ AMP + Diphosphate + Glutaminyl-tRNA

Glycine:tRNA(Gly) ligase (AMP-forming) (6.1.1.14)
ATP + Glycine + tRNA(Gly) ↔ AMP + Diphosphate + Glycyl-tRNA(Gly)

L-Histidine:tRNA(His) ligase (AMP-forming) (6.1.1.21)
ATP + L-Histidine + tRNA(His) ↔ AMP + Diphosphate + L-Histidyl-tRNA(His)

L-Isoleucine:tRNA(Ile) ligase (AMP-forming) (6.1.1.5)

ATP + L-Isoleucine + tRNA(Ile) ↔ AMP + Diphosphate + L-Isoleucyl-tRNA(Ile)

L-Leucine:tRNA(Leu) ligase (AMP-forming) (6.1.1.4)
ATP + L-Leucine + tRNA(Leu) ↔ AMP + Diphosphate + L-Leucyl-tRNA

L-Lysine:tRNALys ligase (AMP-forming) (6.1.1.6)
ATP + L-Lysine + tRNA(Lys) ↔ AMP + Diphosphate + L-Lysyl-tRNA

L-Methionine:tRNAMet ligase (AMP-forming) (6.1.1.10)
ATP + L-Methionine + tRNA(Met) ↔ AMP + Diphosphate + L-Methionyl-tRNA

L-Phenylalanine:tRNA(Ala) ligase (AMP-forming) (6.1.1.20)
ATP + L-Phenylalanine + tRNA(Phe) ↔ AMP + Diphosphate + L-Phenylalanyl-tRNA(Phe)

L-Proline:tRNA(Pro) ligase (AMP-forming) (6.1.1.15)
ATP + L-Proline + tRNA(Pro) ↔ AMP + Diphosphate + L-Prolyl-tRNA(Pro)

L-Serine:tRNA(Ser) ligase (AMP-forming) (6.1.1.11)
ATP + L-Serine + tRNA(Ser) ↔ AMP + Diphosphate + L-Seryl-tRNA(Ser)

L-Threonine:tRNA(Thr) ligase (AMP-forming) (6.1.1.3)
ATP + L-Threonine + tRNA(Thr) ↔ AMP + Diphosphate + L-Threonyl-tRNA(Thr)

L-Tryptophan -tRNA(Trp) ligase (AMP-forming) (6.1.1.2)
ATP + L-Tryptophan + tRNA(Trp) ↔ AMP + Diphosphate + L-Tryptophanyl-tRNA(Trp)

L-Valine:tRNAVal ligase (AMP-forming) (6.1.1.9)
ATP + L-Valine + tRNA(Val) ↔ AMP + Diphosphate + L-Valyl-tRNA(Val)

biotin:CoA ligase (AMP-forming); biotinyl-5'-AMP:CoA biotinyltransferase (6.2.1.11)
Biotinyl-5'-AMP + CoA ↔ AMP + Biotinyl-CoA

Ubiquitin:protein-lysine N -ligase (AMP-forming) (6.3.2.19)
ATP + Ubiquitin + Protein lysine ↔ AMP + Diphosphate + Protein N-ubiquityllysine

4-Chlorobenzoate:CoA ligase (6.2.1.33)
ATP + 4-Chlorobenzoate + CoA ↔ AMP + Diphosphate + 4-Chlorobenzoyl-CoA

Chenodeoxycholate:CoA ligase (AMP-forming) (6.2.1.7)
ATP + Chenodeoxycholate + CoA ↔ AMP + Diphosphate + Chenodeoxycholoyl-CoA

O-Succinylbenzoate:CoA ligase (AMP-forming) (6.2.1.26)
ATP + 2-Succinylbenzoate + CoA ↔ AMP + Diphosphate + 2-Succinylbenzoyl-CoA

Photinus-luciferin:oxygen 4-oxidoreductase(decarboxylating,ATP-hydrolysing) (1.13.12.7)
Photinus luciferin + Oxygen + ATP ↔ Oxidized Photinus luciferin + hn + AMP + Diphosphate + CO2

2-Isopentenyl-diphosphate:AMP delta2-isopentenyltransferase (2.5.1.27)
Dimethylallyl diphosphate + AMP ↔ Diphosphate + N6-(delta2-Isopentenyl)-adenosine 5'-monophosphate

alpha(omega)-Dicarboxylic acid:CoA ligase (AMP-forming) (6.2.1.23)
ATP + alpha,omega-Dicarboxylic acid + CoA ↔ AMP + Diphosphate + omega-Carboxyacyl-CoA

(R)-4'-Phosphopantothenate:L-cysteine ligase (6.3.2.5)
ATP + D-4'-Phosphopantothenate + L-Cysteine ↔ AMP + Diphosphate + (R)-4'-Phosphopantothenoyl-L-cysteine

RNA-3'-phosphate:RNA ligase (cyclizing, AMP-forming) (6.5.1.4)

ATP + RNA 3'-terminal-phosphate ↔ AMP + Diphosphate + RNA terminal-2',3'-cyclic-phosphate

Acetate:citrate-(pro-3S)-lyase(thiol-form) ligase (AMP-forming) (6.2.1.22)
ATP + Acetate + Citrate (pro-3S)-lyase (thiol form) ↔ AMP + Diphosphate + Citrate (pro-3S)-lyase (acetyl form)

3alpha,7alpha-Dihydroxy-5beta-cholestanate:CoA ligase (AMP-forming) (6.2.1.7, 6.2.1.28)
ATP + 3alpha,7alpha-Dihydroxy-5beta-cholestanate + CoA ↔ AMP + Diphosphate + 3alpha,7alpha-Dihydroxy-5beta-cholestanoyl-CoA

Biotin:apo-[acetyl-CoA:carbondioxide ligase (ADP-forming)] ligase (AMP-forming) (6.3.4.15)
ATP + Biotin + Apo-[acetyl-CoA:carbon-dioxide ligase (ADP-forming)] ↔ AMP + Diphosphate + [Acetyl-CoA:carbon-dioxide ligase (ADP-forming)]

Biotin:apo[methylmalonyl-CoA:pyruvate carboxyltransferase] ligase (AMP-forming) (6.3.4.9)
ATP + Biotin + Apo-[methylmalonyl-CoA:pyruvate carboxytransferase] ↔ AMP + Diphosphate + [Methylmalonyl-CoA:pyruvate carboxytransferase]

3alpha,7alpha,12alpha-Trihydroxy-5beta-cholestanate:CoA ligase (AMP-forming) (6.2.1.7)
ATP + 3alpha,7alpha,12alpha-Trihydroxy-5beta-cholestanoate + CoA ↔ AMP + Diphosphate + 3alpha,7alpha,12alpha-Trihydroxy-5beta-cholestanoyl-CoA

Biotin:apo-[propionyl-CoA:carbon-dioxide ligase (ADP-forming)] (6.3.4.10)
ATP + Biotin + Apo-[propionyl-CoA:carbon-dioxide ligase (ADP-forming)] ↔ AMP + Diphosphate + [Propionyl-CoA:carbon-dioxide ligase (ADP-forming)]

Biotin:apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)] ligase (AMP-forming) (6.3.4.11)
ATP + Biotin + Apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)] ↔ AMP + Diphosphate + [3-Methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)]

Selenomethionine:tRNAMet ligase (AMP-forming) (6.1.1.10)
ATP + Selenomethionine + tRNA(Met) ↔ AMP + Diphosphate + Selenomethionyl-tRNA(Met)

L-2-Aminoadipate-6-semialdehyde:NAD(P)+ 6-oxidoreductase (1.2.1.31)
L-2-Aminoadipate adenylate + Holo-Lys2 ↔ alpha-Aminoadipoyl-S-acyl enzyme + AMP

L-2-aminohexanedioate:L-cysteine:L-valine ligase (AMP-forming, valine-inverting) (6.3.2.26)
L-2-Aminoadipate + L-Valine + L-Cysteine + 3 ATP + H2O ↔ delta-(L-2-Aminoadipyl)-L-cysteinyl-D-valine + 3 AMP + 3 Diphosphate

AMP,sulfite:(acceptor) oxidoreductase (1.8.99.2)
2 Ferricytochrome c + Selenite + AMP ↔ 2 Ferrocytochrome c + Adenylylselenate

Biotinyl-5'-AMP:apo-[carboxylase] ligase (AMP-forming) (6.3.4.9, 6.3.4.10, 6.3.4.11, 6.3.4.15)
Biotinyl-5'-AMP + Apo-[carboxylase] ↔ AMP + Holo-[carboxylase]

Calmodulin:ubiquitin ligase (AMP-forming) (6.3.2.21)
n ATP + Calmodulin + n Ubiquitin ↔ n AMP + n Diphosphate + (Ubiquitin)n-calmodulin

2,4-dichlorobenzoate:CoA ligase (6.2.1.-)
CoA + ATP + 2,4-Dichlorobenzoate ↔ Diphosphate + 2,4-Dichlorobenzoyl-CoA + AMP

Benzoyl acetate: CoA ligase (6.2.1.-)
Benzoyl acetate + CoA + ATP ↔ Diphosphate + Benzoyl acetyl-CoA + AMP

L-N2-(2-carboxyethyl)arginine cyclo-ligase (AMP-forming) (6.3.3.4)
L-N2-(2-Carboxyethyl)arginine + ATP ↔ Deoxyguanidinoproclavaminic acid + AMP + Diphosphate

No name available (No EC number available)
3-(2-Hydroxyphenyl)propanoate + ATP + CoA + FAD + NAD+ ↔ Salicylate + AMP + Diphosphate + FADH2 + Acetyl-CoA + NADH + H+

L-Aspartate:tRNA(Asp) ligase (AMP-forming) (6.1.1.12, 6.1.1.23)
tRNA(Asp) + L-Aspartate + ATP ↔ L-Aspartyl-tRNA(Asp) + Diphosphate + AMP

L-Glutamate:tRNA(Glu) ligase (AMP-forming) (6.1.1.17, 6.1.1.24)
tRNA(Glu) + L-Glutamate + ATP ↔ L-Glutamyl-tRNA(Glu) + Diphosphate + AMP

No name available (6.2.1.-)
Cyclohexane-1-carboxylate + CoA + ATP ↔ Cyclohexane-1-carboxyl-CoA + AMP + Diphosphate

Phenylphosphate synthase (2.7.9.-)
Phenol + ATP + H2O ↔ Phenolic phosphate + AMP + Orthophosphate

AMP,sulfite:glutathione-disulfide oxidoreductase (adenosine-5'-phosphosulfate-forming) (1.8.4.9)
AMP + Sulfite + Glutathione disulfide ↔ Adenylyl sulfate + 2 Glutathione

acetone:carbon-dioxide ligase (AMP-forming) (6.4.1.6)
Acetone + CO2 + ATP + 2 H2O ↔ Acetoacetate + AMP + 2 Orthophosphate

ADP:D-glucose 6-phosphotransferase (2.7.1.147)
ADP + D-Glucose ↔ AMP + D-Glucose 6-phosphate

ADP:D-fructose-6-phosphate 1-phosphotransferase (2.7.1.146)
ADP + D-Fructose 6-phosphate ↔ AMP + D-Fructose 1,6-bisphosphate

perillic acid:CoA ligase (AMP-forming) (6.2.1.-)
Perillic acid + CoA + ATP ↔ Perillyl-CoA + H2O + AMP + Diphosphate

cis-2-Methyl-5-isopropylhexa-2,5-dienoate-CoA ligase (6.2.1.-)
cis-2-Methyl-5-isopropylhexa-2,5-dienoic acid + ATP + CoA ↔ cis-2-Methyl-5-isopropylhexa-2,5-dienoyl-CoA + AMP + Diphosphate

trans-2-Methyl-5-isopropylhexa-2,5-dienoate-CoA ligase (6.2.1.-)
trans-2-Methyl-5-isopropylhexa-2,5-dienoic acid + ATP + CoA ↔ trans-2-Methyl-5-isopropylhexa-2,5-dienoyl-CoA + AMP + Diphosphate

(3R)-3-isopropenyl-6-oxoheptanoate:CoA ligase (AMP-forming) (6.2.1.-)
(3R)-3-Isopropenyl-6-oxoheptanoate + CoA + ATP ↔ (3R)-3-Isopropenyl-6-oxoheptanoyl-CoA + H2O + AMP + Diphosphate

4-coumarate:CoA ligase (AMP-forming) (6.2.1.12)
5-Hydroxyferulate + CoA + ATP ↔ 5-Hydroxyferuloyl-CoA + AMP + Diphosphate

No name available (6.2.1.-)
Pseudoecgonine + ATP + CoA ↔ Pseudoecgonyl-CoA + AMP

No name available (No EC number available)
L-Proline + ATP ↔ L-Prolyl-[pcp] + AMP + Diphosphate

No name available (No EC number available)
L-Tyrosine + ATP ↔ L-Tyrosyl-[pcp] + AMP + Diphosphate

No name available (No EC number available)
3-Amino-4,7-dihydroxy-8-methylcoumarin + 3-Dimethylallyl-4-hydroxybenzoate + ATP ↔ Novobiocic acid + AMP +

Diphosphate

No name available (No EC number available)
3-Methylpyrrole-2,4-dicarboxylic acid + 3-Amino-4,7-dihydroxy-8-methylcoumarin + ATP ↔ Coumeroic acid + AMP + Diphosphate

No name available (No EC number available)
Coumeroic acid + 3-Amino-4,7-dihydroxy-8-methylcoumarin + ATP ↔ Coumermic acid + AMP + Diphosphate

No name available (6.2.1.-)
Adipate + CoA + ATP ↔ Adipyl-CoA + AMP + Diphosphate

AMP,sulfite:thioredoxin-disulfide oxidoreductase(adenosine-5'-phosphosulfate-forming) (1.8.4.10)
AMP + Sulfite + Thioredoxin disulfide ↔ Adenylyl sulfate + Thioredoxin

long-chain-fatty-acid:[acyl-carrier-protein] ligase (AMP-forming) (6.2.1.20)
ATP + Acid + Acyl-carrier protein ↔ AMP + Diphosphate + Acyl-[acyl-carrier protein]

N-Methylanthranilate:CoA ligase (AMP-forming) (6.2.1.32)
ATP + N-Methylanthranilate + CoA ↔ AMP + Diphosphate + N-Methylanthraniloyl-CoA

FAD AMP-lyase (cyclic-FMN-forming) (4.6.1.15)
FAD ↔ AMP + Riboflavin cyclic-4',5'-phosphate

poly(ribonucleotide):poly(ribonucleotide) ligase (AMP-forming) (6.5.1.3)
ATP + RNA(n) + RNA(m) ↔ AMP + Diphosphate + RNA(n+m)

L-lysine:tRNAPyl ligase (AMP-forming) (6.1.1.25)
ATP + L-Lysine + tRNA(Pyl) ↔ AMP + Diphosphate + L-Lysyl-tRNA(Pyl)

No name available (2.7.7.63)
Lipoyl-AMP + Apoprotein ↔ Protein N6-(lipoyl)lysine + AMP

No name available (6.2.1.-)
ATP + 8-[(1R,2R)-3-Oxo-2-{(Z)-pent-2-enyl}cyclopentyl]octanoate + CoA ↔ AMP + Diphosphate + OPC8-CoA

citronellyl-CoA ligase (6.2.1.-)
Citronellate + CoA + ATP ↔ Citronellyl-CoA + AMP + Diphosphate

4-fluorobenzoate:CoA ligase (6.2.1.33)
4-Fluorobenzoate + CoA + ATP ↔ 4-Fluorobenzoyl-CoA + AMP + Diphosphate

No name available (6.1.1.11)
ATP + L-Serine + tRNA(Sec) ↔ AMP + Diphosphate + L-Seryl-tRNA(Sec)

6-thioxanthine 5'-monophosphate:L-glutamine amido-ligase (AMP-forming) (6.3.5.2)
6-Thioxanthine 5'-monophosphate + ATP + L-Glutamine + H2O ↔ 6-Thioguanosine monophosphate + AMP + Diphosphate + L-Glutamate

AMP,sulfite:acceptor oxidoreductase (adenosine-5'-phosphosulfate-forming) (1.8.99.2)
AMP + Sulfite + FAD ↔ Adenylyl sulfate + FADH2

No name available (6.1.1.-)
L-Serine + tRNA(Cys) + ATP ↔ O-Phosphoseryl-tRNA(Cys) + Diphosphate + AMP

L-pyrrolysine:tRNA(Pyl) ligase (AMP-forming) (6.1.1.26)
ATP + L-Pyrrolysine + tRNA(Pyl) ↔ AMP + Diphosphate + L-Pyrrolysyl-tRNA(Pyl)

[enzyme]-S-sulfanylcysteine:tRNA uridine sulfurtransferase (ATP-hydrolysing) (No EC number available)
tRNA uridine + [Enzyme]-S-sulfanylcysteine + ATP + Reduced acceptor ↔ tRNA containing 2-thiouridine + [Enzyme]-cysteine + AMP + Diphosphate + Acceptor

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