Investigating the origins of B_{12} biosynthesis in the most ancient roots of the tree of life

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Abstract

Vitamin B_{12}, also known as B_{12} or cobalamin, is a vital nutrient required across all branches of life, but the ability to synthesize this complex molecule *de novo* is limited to only a few archaea and bacteria. *De novo* synthesis begins with glutamate and utilizes over 30 gene products to produce an active cobalamin [1]. Previous studies suggest that of the available bacterial genomes, only half utilizing cobalamin can synthesize it [2]. The other half either take up complete cobalamin from the environment via an ABC transporter, or scavenge incomplete corrinoids (partial cobalamin molecules) as precursors to synthesize active cobalamin [3, 4].

The evolutionary histories and identities of multiple genes within these B_{12} pathways are unknown, leaving gaping holes in our understanding of the only source of biosynthesis of the vitamin so essential to human survival. Genes of particular interest to this investigator are those responsible for producing reductases that act upon the central cobalt atom of B_{12}. Three reductases with unknown gene identities are located within the B_{12} biosynthetic pathway and it is the aim of this research to identify those genes responsible.

Background

Vitamin B_{12}

- Most complex cofactor
- Essential micronutrient required by life
- Only made by certain bacteria and archaea.
- Structure contains modified tetrapyrrole ring bearing resemblance to heme, chlorophyll, siroheme and coenzyme F_{430}.
- Each organism's B_{12} pathway unique

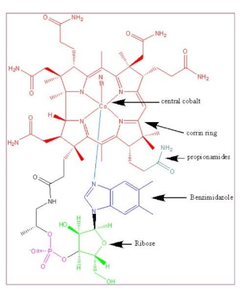

Fig. 1. Vitamin B_{12} (cobalamin). The central cobalt atom is the key to the cofactor's biological activity and versatility.

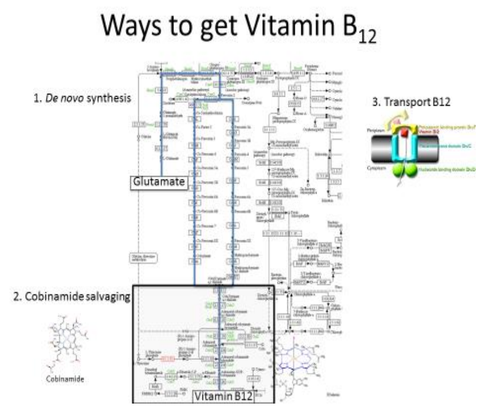

Fig. 2. Bacterial vitamin B_{12} biosynthetic pathway. Organisms can utilize one of three major ways to get vitamin B_{12}. 1. De novo synthesis, 2. Cobinamide salvaging, 3. Transport of external B_{12}.

B_{12} synthesis in Thermotogae phylum as starting model

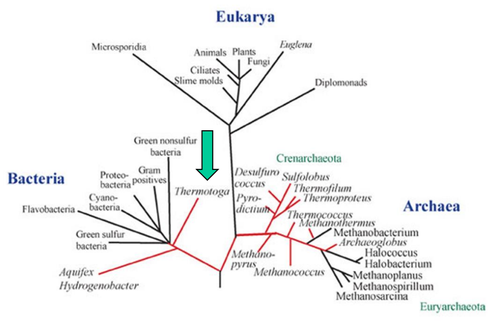

Fig. 3. Placement of Thermotogae at the base of the tree of life. The Thermotogae (green arrow) is a deep branching phylum that possess all three types of B_{12} genes.

- We have previously shown two members of this phylum capable of the complete de novo synthesis of B_{12}. [5]

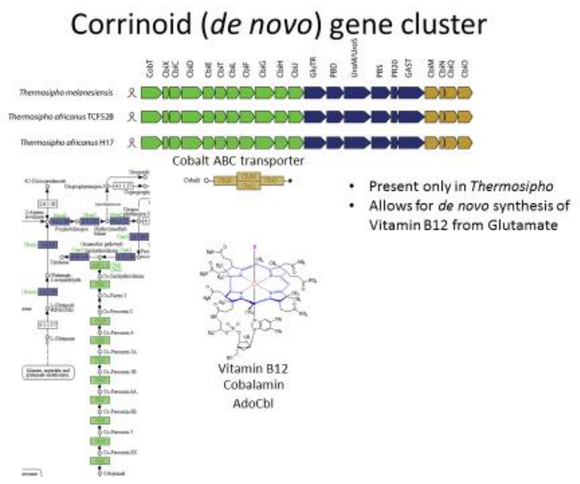

Fig. 4. B_{12} related gene clusters in *Thermosipho.* Present as an operon in Thermosipho species. From presence/absence analysis 20 continuous genes (unique to *Thermosipho*) involved in vitamin B_{12} synthesis were revealed.

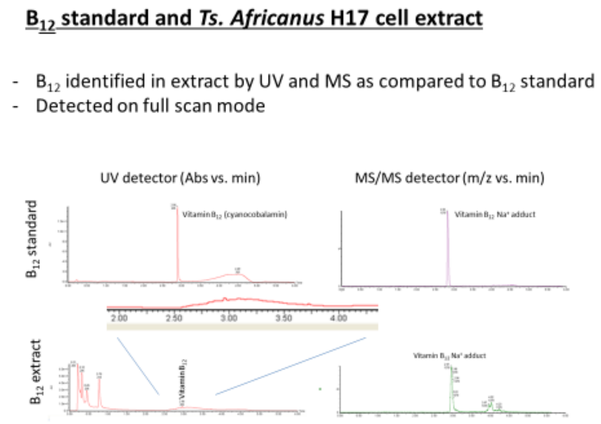

Fig. 5. B_{12} production experimentally verified in *Thermosipho*, Cell extracts of *Ts. Africanus H17* and *TCF* tested for B_{12} production by UPLC/MS (shown above) and *Lactobacillus* assay. This was verified by qRT-PCR analysis of gene expression

Identifying reductases in the B_{12} pathway

- Parts of the B_{12} pathways poorly understood
  - Specifically reductases that act upon the central cobalt atom of the B_{12}
- Reduction of the cobalt is the driving force behind B_{12}
- Few reductases have been identified
  - *Salmonella enterica* and *Escherichia coli* [6]
  - *Pseudomonas denitrificans* [7, 8]

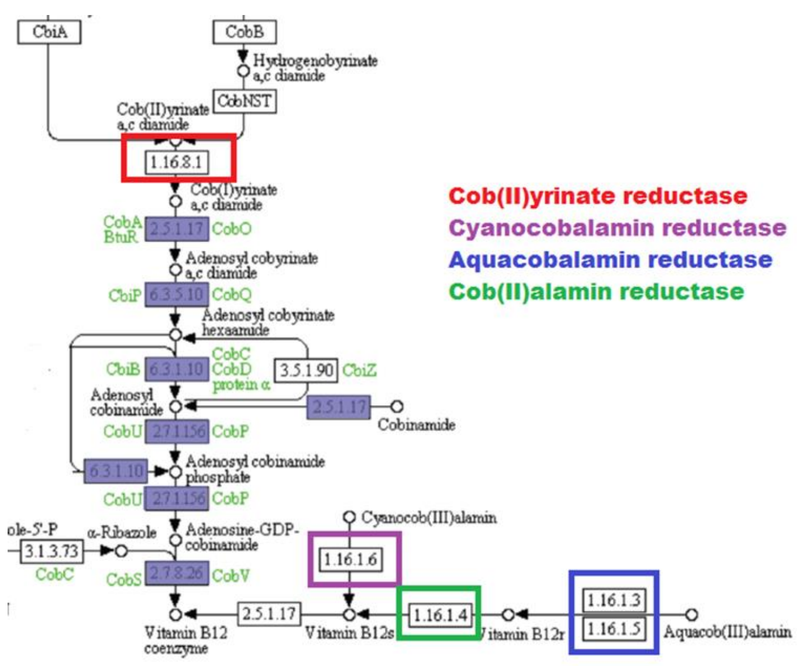

Fig. 6. B_{12} biosynthesis and recycling in *Thermosipho melanesiensis* (KEGG). [8] The missing reductases are displayed within colored boxes. Red is Cob(II)yrinate reductase (1.16.8.1), Purple is cyanocobalamin reductase (1.16.1.4), Blue is aquacobalamin reductase (1.16.1.3 and 1.16.1).

- We aim to identify the B_{12} reductases
- Three candidates have been identified :
  - Tmel_ 0728, Tmel_0733 and Tmel_1756.
- Candidates share domains with known reductases (Pfam database [10])
- Candidates were identified by BLAST searches against B_{12} proteins in
  - *Brucella melitensis, Rhodobacter capsulatus* and *Pseudomonas denitrificans*

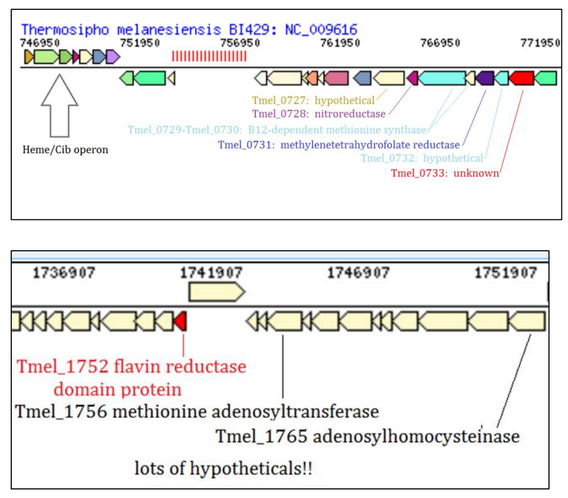

Fig. 6. Gene neighborhoods of reductase candidates. Genes of interest (top: Tmel_0728 and 0733, bottom: Tmel_1752) are located within the vicinity of genes related to B_{12} biosynthesis or B_{12} dependent reactions.

Reductase Protein Candidate	Domain/ Family Match	# Amino acids	Mol. Mass	% α % β
Tmel_0728	Nitroreductase	182	21 kDa	79.7 58.2
Tmel_0733	4Fe-4S Binding Domain	434	48 kDa	60.6 68.9
Tmel_1752	Flavin Reductase	152	17 kDa	77.0 67.1

Table 1. Gene candidate sequence analysis as done by Pfam and CFSSP. [9, 10]

- These three candidates are currently being expressed for purification and characterization
- Candidates will be compared to known reductases from *Salmonella enterica, Escherichia coli* and *Pseudomonas denitrificans*

Conclusion

The main goal of this work is to identify the genes responsible for the cobalt reductases that act within the B_{12} pathways of the Thermotogae phylum. Three reductase candidates have been identified within *Thermosipho* and are currently being expressed and characterized for further experiments.

References

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