Ferroplasma acidarmanus: A new Archeal AAA+ ATPase with nuclease function

A. James, K. Arthur, C. Paulsingh, R. Naraine and S. Kotelnikova
Department of Microbiology, St. George’s University, Grenada

Introduction

- Extremely acidophilic cell wall-lacking Archaean Ferroplasma acidarmanus Fer1 is a facultative anaerobe. In presence of O₂, it oxidizes Fe²⁺ chemolithoautotrophically [1], while anaerobically, it can couple chemooanerotrophic growth on yeast extract to the reduction of Fe³⁺ [2].
- The microbial community at the Iron Mountain acid-generating site (CA, USA) is 85% dominated by Archaea of the genus Ferroplasma [1], which is one of most resistant organisms known to copper and nickel [2,12].
- Temperature optima is 35-42°C; pH optima is 1.0-1.7, capable of growing at pH 0 [2], pH of cytosol was shown to be around 5.6 [11], when ATP is not stable therefore ADP is the best candidate used for hydrolysis and as a source of phosphate group for cell signaling in Ferroplasma.
- AAA or AAA+ is an abbreviation for ATPases Associated with diverse cellular Activities. Proteins share a common conserved sequence of approximately 230 amino acids. AAA+ superfamily is evolutionary large functionally diverse family of ring-shaped p-loop NTPTases, which exert their activity through the energy-dependent remodeling or translocation of macromolecules. These proteins are involved in a range of processes, including DNA replication, protein degradation, membrane fusion, microtubule and peroxisome biogenesis, signal transduction and the regulation of gene expression.

- **Aim:** Utilize comparative genomics analysis to identify function for a hypothetical gene (OID: 638394430) from archaean *F. acidarmanus fer1*.
- **Hypothesis:** The protein is signalling AAA+-ase with nuclease function.

Methods

- The gene OID 638394430 was randomly assigned as a Fall 2012 Genetic BIOL 320 class project.
- Over a period of 8 weeks the gene was comparatively analyzed by identifying orthologous sequences with known structural and functional domains.
- This was achieved using a variety of bioinformatics software from IGM-ACT including Kyoto Encyclopedia of Genes and Genomes (KEGG), Protein Data Base (PDB), BLASTn and BLASTp at NCBI.gov, COG, Prosite, pFam, InterProScan (EBI, EMBL.gov), Psort, Phobius, Signal P, TMHMM, Gen3D, Superfamily and MetaCyc.
- NCBI Protein Blast, and gene neighborhood maps were used for analysis of surrounding genes. We ran separate searches for each individual domain composing this complex protein while revealing a “magic” story behind this hypothetical motif.

Figure 1: Conserved Domain sites present in Gene 638394430 and its gene neighborhood

Results and Discussion

- The gene (1377 bp) DNA 15184-16560(-), codes for 458 amino acids, with isoelectric point 6.8174. The computer predicted location of the open reading frame contained no Shine-Dalgarno sequence but an AT rich possible alternative binding site upstream of start codon.
- No transmembrane helices were identified. Psort and SOSUI predicted cytoplasmic localization while TMHMM and Phobius indicated an extracellular localization. Most ATPases are known to be located on the interior of the cellular membrane [3], however presence of signaling peptide indicates that protein may be secreted. HSP78 AAA+-chaperon in mitochondria showed cellular localization on both sides of the mitochondrial membrane [3].
- We found that the gene OID 638394430 was subjected to a horizontal gene transfer (Fig 2A) based on the difference of GC content (30 mol%). The genome of *F. acidarmanus* (36.7 mol%) was subjected to multiple horizontal gene transfer events [4]. This is not unusual for microorganisms to intensify horizontal gene transfer and DNA recombination DNA repair under stress conditions [5, 7]. The top genes orthologs were found in *Methanohalophilus petrolearius* and *Acidiloprofundum boonei* [10]. Thermococcus gammatolerans and Methanocaldococcus jannaschi (Fig 2A).
- The protein had homology with COG 1672 ATPase (AAA+ superfamily). InterproScan allowed to identify at least four separate domains (Fig 1). The domains comprised of possible P-Loop ATPase hydrolyse, winged helix-turn-helix transcriptional repressor, DUF234 ADP-driven DNA nuclease (resolvase) and a signal transduction response regulator, which is activated due to phosphorylation of Aspatic residue (Fig 1).
- We identified Pfam homologs for DUF234 as DNA nucleases and resolvases from transposons, herpes virus and bacteriophages [6] (Fig 1). Dimer typical for DNA endonucleases is seen on Fig 2b and dimerization interphase in domain 2 of the motif. The response regulator and repressor may be part two-component system guiding both de-repression of a gene and the recombination DNA repair.
- *F. acidarmanus* is known to resist acidity and high concentrations of heavy metals [1,2,12]. We identified two ABC-type metal/transporters domain from the end of the gene (Fig 1) and orthologs of RND family of periplasmic ABC efflux pumps, metal resistance from an extremophile *Acidiloprofundum* and *Methanocaldococcus�annaschi* (Fig 2A).
- The functions of the identified domains within this complex gene suggest that the gene may be involved in mediating stress response by facilitating DNA recombination similar to phase variation [5,8], mating type switching in yeasts [9] at high concentrations of reduced metals at high temperature or acidity. To ascertain the response of the gene to the intracellular concentration of metals (Cu²⁺, Ni²⁺, As³⁺) and contribution of the gene to recombination or ABC influx pump regulation, the physiology of knockout mutants or recombinant expression might be tested in the future research.

Conclusions

- Given the results presented above, this gene indeed is multi-domain signalling AAA+ ATPase with nuclease function. It may be a part of two-component signal transduction pathway and stress induced DNA recombination leading to a switch in gene expression.

References


Figure 2: A) Phylogenetic divergence for Ferroplasma acidarmanus Fer1 gene 638394430 using maximum likelihood and approximate likelihood-ratio test, B) Three dimensional Alignment of gene 638394430 onto top PDB hit of Chain A, The Walker-type ATPase Paby3046 of Pyrococcus abyssi (PDB: 2GRH, A)