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Energy Dependent Proteolysis in Archaea

Hira Amjad[1*], Aftab Ahmad[2], Nouman Rasool[1]

[1]*Department of Chemistry, University of Management and Technology, Lahore, Pakistan.*

[2]*Department of Bio-Chemistry/US Center for Advanced Studies in Agriculture and Food Security (USPCAS-AFS), University of Agriculture, Faisalabad, Pakistan.*

Abstract

Archaea, the third domain of life, are genotypically and phenotypically diverse collection of microorganisms which have a mutual evolutionary ancestor. There are four kingdoms of Archeae recognized so far i.e. Crenarchaeota, Euryarchaeota, Nanoarchaeota, Korarchaeota. Proteolysis plays a critical role in regulating proteins, maintaining cell growth and rapidly removing them when they are useless thus avoiding accumulation and chaos inside the cell. Protein turnover in Archaea is regulated by both energy dependent and energy independent proteolysis. Energy dependent proteolysis also known as ATP-dependent proteolysis adjusts mitochondrial biogenesis and eliminates misfolded proteins. The main players of energy dependent proteolysis are Proteasome, Lon protease, ClpAP protease, FtsH like protease. The ATP-independent protease can either metabolize proteins or regulates protein quality to restock amino acid pool but its major flaw is that it cannot remove misfolded or unwanted proteins from the biological system as compared to energy dependent proteolysis. Energy dependent proteolysis is a more efficient way to conserve energy while inactivating/degrading unwanted protein in Archaea. Energy dependent proteolysis is time taking and accurate mechanism to get rid of or to translocate protein in Archaea. The recycling of obsolete protein and protein quality control is mainly managed by energy dependent proteolysis in Archaea.

Keywords: Archaea, Proteolysis, Protein turnover, Protease

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***Corresponding Author,* e-mail**: hira.amjad.ali@gmail.com

1. Introduction

The innate ability of proteins and peptides to undergo degradation in the cell is often termed as cellular proteolysis. The cell proteolysis is essential for most biological functions that allows removal of mis-folded and dysfunctional proteins or leader sequences controlling cellular localization, control of transcription factor and removal of precursor processing. All these processes are crucial for development, differentiation and maintenance of cell cycle. (King *et al.,* 1996; Gottemann, 1999; Sauer *et al.,* 2004; Oikonomo-poulou *et al.,* 2006; Lopez-Otin *et al.,* 2008; Turk *et al.,* 2012). Proteolysis is amino acid sequence specific and the three dimensional structure of proteins results in a high degree of selectivity for the action of proteases on proteins. (Barrett *et al.,* 2003; Miranda *et al.,* 2008). Hence evidently, proteolysis is important for balanced growth of cells and enables the cell to respond against external stress and planned signal development.

Proteolysis may be processive or non processive (Yura *et al.,* 2000). Both processive and non-processive proteolysis are included in the regulation of cellular processes. Non- processive proteolytic commonly known as energy independent proteolysis usually results proteins maturation and their regulation for specific sites (Rudner *et al.,* 1999). This may provide specific signal input and end up with high specificity but the problem with misfolded proteins never ends. Processive proteolysis or energy dependent proteolysis is of special interest for its list of application and advantages. Energy dependent proteolysis made its mechanism in

different ways like time management to develop precise process, tool for protein translocation and recycling of obsolete proteins (Alberts and Miake-lye. 1992; Sauer and Baker, 2011).

Although proteolysis has been widely studied in bacteria and eukaryotes but it is now being studied in Archaea, the third domain of life. (Anderson *et al.,* 1880; Sullivan *et al.,* 2003). Archaea are a diverse collection of micro-organisms and was first discovered in 1980 with optimal growth temperature 80^{o}C and above (Stetter.2013). The four kingdoms of Archaea till known are Crenarchaeota, Euryarchaeota, Nanoarchaeota and Korarchaeota. All of them have their own specific characteristics, but still they share some common properties along with their evolutionary ancestor. They mostly grow in a catastrophic environment consisting extremes in temperature, pH, salt contents, pressure and available resources ratio as compared to other living organisms (Rothchild *et al.,* 2001). Thus Archaea are a rich source of protease that are useful in microbiology, enzymology, biotechnology and fuel industry (Egorova and Antranikian. 2005; Reeve and Schleper. 2011).

The energy dependent proteolysis in Archaea is mostly accomplished by four major groups, i.e. Proteasome, Lon protease, ClpAP protease and FtsH like (Lupas *et al.,* 1997; Gottemann 1999; Ward *et al.,* 2002).

AAA+ ATPase complex

The energy dependent proteolysis often has an AAA+ ATPase complex to perform its task. They usually perform protein quality control and used in regulatory circuits in all circuits (Hanson *et al.,* 2005). The AAA+ ATPase complex is usually composed of six functional units Walker A, Walker B motifs, sensor 1, sensor 2 residues, Arginase finger and pore loop (Ezrberger *et al.,* 2006). ATPase domain with its highly conserved residues i.e. lysine in Walker A, aspartate in Walker B, asparagine in Sensor 1, arginine in Sensor 2 and Arginine finger help in ATP hydrolysis and thus in ATP dependent proteolysis. The two highly conserved arginine residues contribute to less degree in ATPase activity but are involved in activation of the protease domain. The Sensor 2 (arginine residue) is involved in inter domain communication which is of prime importance (Ogura *et al.,* 2004). The AAA+ ring is recognizes specific native substrate while pore loop is responsible for translocation and unfolding of protein thus leading it to proteolytic chamber. The protein substrate recognition involves binding of peptide segments in axial pore of AAA+ ring while the conformation changes in ring due to ATP binding and hydrolysis translocate it and unfold it for its entrance in the proteolytic chamber (Baker and Sauer, 2006). The substrate sequences that facilitate binding to pore are degradation tags or degrons as they recognize them and functions in four ways by binding to an auxiliary site on the protease, binding to an adaptor protein binding protease and intervening reactions that allow protease recognition. (Inobe and Matouschek, 2008; Tatsuta and Langer, 2009; Sauer and Baker, 2011).

Proteasomes

Proteasomes are present in almost every member of kingdom Archaea. Archaeal proteasomes have a special mechanism of compartments for specific degradation of unwanted proteins. Proteasomes are self-compartmentalizing energy dependent proteolytic complex, first stated by Dahlmann and co-workers as muticatalytic proteinase, later named as proteasomes (Dahlmann *et al.,* 1985). The simplest type of proteasome in Archaea was found in *Thermoplasma acidophilum* (Arrigo *et al.,* 1988). The proteasome has been characterized in number of Archaea. *Thermoplasma acidophilum* was first Archaea where proteasome was characterized while the first actinobacteria was *Rhodococcus erythropolis*. Whereas it was latter characterized in *P.furiosus, M.jannaschii, M.thermophila, P.horikoshii , H.marismortui, A.fulgidus, S.sulfotaricus T.kodakaraensis, P.abysii, P.horikoshii,T.volcanium, A.pernix, P.aerophilum, S.tokodaii, S.acidocaldarius, H.volcanii* and *Nanoarchaeum equitans* among Archaea (Maupin-Furlow *et al.,* 2006; Forouzan *et al.,* 2012).

Proteasome consists of catalytic 20S core which combines with AAA-type unfoldase such as proteasome activating nucleotidase for effective proteolysis (Lupas *et al.,* 1997). The 20S core or 20S proteasome is a barrel-shaped particle of nearly 600-700kDa that contain four stacked heptameric protein rings (Zwickl *et al.,* 2000). The two inner rings termed as β-type subunits enclosing along the length passage which are surrounded

by α-type subunits that are likely of interface AAA-type unfoldase (Lupas *et al.,* 1997) .The basic function is performed by β subunit which may be active or inactive with Gly-Thr-Thr motif where the bond between Gly and Thr is hydrolysed to expose its N-terminal threonine active site which is further responsible for peptide bond hydrolysis (Seemuller *et al.,* 1995). The unfolded proteins enters β ring for degradation but α ring make sure that only unfolded protein enters in the chamber. The α and β rings accumulated from α and β subunits, respectively, provide a number of catalytic sites like β-ring have 14 catalytic sites of 3 types that line the catalytic chamber (Baumeister *et al.,* 1998). The three types of catalytic sites give three main activities: cleavage after hydrophobic side chain i.e. chymotrypsin like, cleavage after basic side chain i.e. trypsin like, cleavage after acidic side chain i.e. post glutamyl peptidase, commonly known as caspase like activity (Kisselev *et al.,* 2002).

The most common comment about the mechanism is proteasome activating nucleoside (PAN) combines with 20S proteasome and the other end is combined with unfolded protein with it and use ATP molecule to make PAN and unfolded protein complex that results the entrance of unfolded protein through α ring and in turn β ring for proteolytic cleavage (Maupin-Furlow *et al.,*

2004). This ATPase complex actually performs different functions simultaneously as they combine with 20S proteasome, selectively bind the substrate, cause the gated substrate entry channel in 20S to open the unfold globular or partially folded protein and facilitate translocation of unfolded substrate (Zwickl *et al.,* 2000).

Thus the structure and function of this ATPase complex is very important in understanding the mechanism of proteasome. The ATPase complex is composed of four structural domains and a C-terminal tail motif:

i. N-terminal coiled (CC) domain.

ii. Oligonucleotide binding (OB) domain.

iii. C-terminal helical domain.

iv. AAA ATPase domain.

v. C-terminal HbYX motif.

i. N-terminal coiled (CC) domain

The CC domain comprises of α-helical dimers. At CC domain base there lie oligonucleotide binding domain where proline residue is highly conserved. Thus the N-terminal domain of proteasomal ATPase acts like a chaperone in the cell avoiding the accumulation of misfolded proteins. It is assumed that as translocation ends at CC domain, proteins unfold at the mouth of ATPase ring and thus avoid accumulation of unfolded domain with other cytosolic proteins (Benaroudj and Goldberg. 2000; Djuranovic *et al.,* 2009).

ii. Oligonucleotide binding (OB) domain

It has surface loops containing five-stranded β sandwiched in folded form; while these loops may bind to the protein. This protein along with the CC domain is critical points for the regulation as they are essential for initial substrate binding, its handling and its entrance to the central pore (Zhang *et al.,* 2009).

iii. C-terminal helical domain

It usually consists of α-helical structure formed by the assembly of α-subunits and combine to form gate of proteasome and hence allow the regulation of substrate entry. This helical further fills the cleft in β-stranded sandwich of oligonucleotide binding domain. Actually there is an ADP hinge between the OB domain and this helical domain combines them (Smith *et al.,* 2011).

iv. AAA ATPase domain

Protein degradation and protein modeling in Archaea are facilitated by AAA ATPase domain. This ATPase uses ATP to catalyze substrate unfolding and its translocation. The structure of proteasomal ATPase is hexameric where it could bind only with two ATPs and two ADPs at a time. This leads to an ATPase hydrolysis cycle (Liu *et al.,* 2006; Kim *et al.,* 2013). The most convenient and known model about its hydrolysis suggest that the presence of three different binding nucleotide sites present in the hexameric ATPase ring. The three nucleotide binding sites can be mention as two high affinity, two low affinity, and two non-binding subunits. Hence the two ATP-bound states is the active state and regulate 20S binding and gating process very efficiently (Smith *et al.,* 2011).

The unfolding of substrate requires ATP hydrolysis but the ATP cost and time, mainly depends upon the stability and tertiary structure of the substrate. The unfolding and translocation of substrate are simultaneous process where the translocation of substrate through its narrow pore loop results the unfolding of that substrate (Peth *et al.,* 2013).

v. C-terminal HbYX motif

The substrate entrance in proteolytic chamber is prevented by the N-termini of α-subunits. Thus N-termini form the gate to prevent useful protein's degradation. The C-termini of PAN in proteasome have the self-sufficient activity to induce the gate opening of 20S (Groll *et al.,* 1997). The PAN activity is recognized by the HbYX motif while the peptides links to C-termini of PAN can bind to 20S and stimulates gate opening for the unwanted protein resulting their degradation. This must be noted that this PAN binds with ATPase complex to initiate its function (Groll *et al.,* 2000; Smith *et al.,* 2007).

The HbYX motif combines with pocket located between α-subunits and thus inducing rotation in N-terminal tail of α-subunits. This rotation displaces a reverse-turn loop destabilizing the closed gate conformation and stabilizing the open one (Rabl *et al.,* 2008). Task of the HbYX motif was based on the presence of a small or hydrophobic residue in third last and a Phe or Tyr residue in penultimate position (Yu *et al.,* 2010).

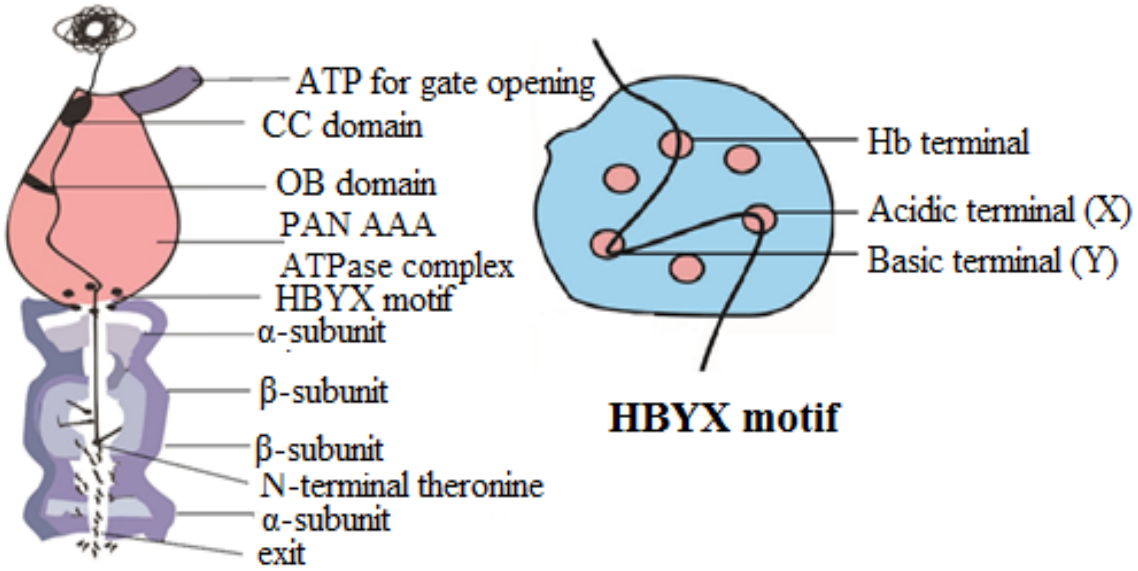

Fig: 1- Basic structure of proteasome. (A) Showing whole components including PAN AAA ATPase complex, α-subunit and β-subunit. (B) Showing HbYX motif at junction of PAN complex and α-subunit.

Basic mechanism of α- subunit:

The basic mechanism account for the entrance and approach of obsolete protein for cleavage at β-subunit through α-subunit. The proteasome interacting proteins takes the unwanted protein towards the α-subunit where the PAN combines with the 20S using ATP forming ATPase complex. This PAN ATPase combines with C-terminal HbYX domain that results in pocket modification in α-subunit rendering changes in conformation of N-terminal of α-subunits. This change in conformation lead to gate opening for the entrance of substrate while the maintenance of open gate conformation is performed by highly conserved residues Tyr8, Asp9, Pro19, Tyr26 of α-subunit (numbered according to *T. acidophilum*) (Forster *et al.,* 2003). The waiting HbYX motif has its conserved arginine and tyrosine side chains. Here the carboxyl group of terminal arginine forms a salt bridge with amino group of lysine in 20S α-subunit more specifically in intersubunit pocket, while this arginine residue does not react specifically with any other residue. Meanwhile tyrosine form specific interactions with a number of residues within intersubunit pocket using its hydroxyl group on the aromatic ring forming hydrogen bond with the carbonyl group of glycine near reverse turn loop of α-subunit. Finally the first residue of HbYX motif, i.e. hydrophobic leucin forms bond with the hydrophobic residues like leucin and valine in inter-subunit pocket (Yu *et al.,* 2010). These interaction results in rendering open gate conformation along with reverse-turn loop stabilization. The substrate passes through the PAN ATPase complex through the pore loop that result in unfolding and translocation of that substrate and finally approach the proteolytic chamber for its degradation (Smith *et al.,* 2011) (Kim *et al.,* 2014).

Basic mechanism of β-subunit:

The mechanism of β-subunit is still vague. The substrate after entering the proteolytic site facing the catalytic residues has to undergo the proteolytic cleavage. It has three catalytic sites that three different types of activities i.e. chymotrypsin like, trypsin like and caspase like, but the most prominent observed is chymotrypsin like where the hydroxyl group of N-terminal threonine residues attack peptide bonds to undergo proteolytic cleavage (Smith *et al.,* 2011). The studies on thermoplasma proteasome revealed that side chain hydroxyl of Thr1 is active catalytic nucleophile but the activation of this hydroxyl group is made by either the free amino acid or by the amine group of lysine. The lysine along with glutamic acid residue acts as a charge relay to activate Thr1. Another most conserved residue is Gly1 with N-terminal Thr1 which is necessary for its efficient working (Kisselev *et al.,* 2002). The confirmed mechanism for peptide bond hydrolysis is not known, but as the mechanism is similar to serine protease so it can be predicted as follows

The hydroxyl group of N-terminal Thr1 attacks the scissile bond, i.e. carbonyl group of peptide bond of substrate resulting in tetrahedral intermediate formation that breakdown into acyl-enzyme and the cleaved peptide product. Nucleophilic attack of this acyl-enzyme yields another peptide product and hence all the peptide bonds cleave to give peptide product. The rate of this cleavage depends on the chain length of polypeptide. But it should be noted that the cleavage does not occur with random proteins rather preferred amino acids are recognized and undergoes the process. They may be proline at P, Leucin at P1 or amino acids that promotes β-turns at P1 (Emmerich *et al.,* 2000; Maupin-Furlow *et al.,* 2004).

Inhibition of proteasome

The inhibitors for proteasome may consist of a wide range from natural to artificial, from hydrophobic to hydrophilic and so on. The proteasome activity can be cease at different levels and different positions. The inhibition may occur at α-subunit by alternating the arginine group as well as tyrosine which are crucial for the open gate conformation (Rabl *et al.,* 2008). If any of the residues is replaced by any other residue the function will remain unfinished. Similarly the 2 ATP-bound state is very important if the conformation or site for the ATPase complex changes and the required conformation i.e. two ATP and an ADP is not provided then the maintenance of open gate conformation does not achieve (Peth *et al.,* 2013). While the changing in HbYX motif also influence the entrance and approach substrate to proteolytic site. Further if the hydroxyl group of N-terminal Thr1 does not form bond with carbonyl group of peptide this will stop the proteolytic process. The most common inhibitor is lactacystin, clasto-lactacystin beta-lactone, vinyl sulfones, and

epoxyketones (dick *et al.,* 1996; Bogyo *et al.,* 1997). The sulphonyl and carbonyl group in them combines with the threonine N-terminal ceasing its catalytic activity.

Lon protease

Lon protease is conserved in eukaryotes, prokaryotes and archaea. The ATP dependent Lon protease belongs to AAA superfamily. Lon protease progress in ATP dependent and ATP independent manner. The ATP dependent process of Lon protease involve recognition and degradation of folded protein while the recognition, translocation and degradation of folded proteins can only be performed through ATP hydrolysis process. Hence it specifically degrades proteins rendering to protein quality and cellular physiology. Lon protease is responsible for about 50% turnover of abnormal protein in *E.coli*. The first Lon protease was first discovered in 1980th in *E.coli* while latter in Archaea, it was discovered in *Thermococcus kodakaraensis, Thermoplasma acidophilum, Natrialba magadii, Thermococcus onnurineus, Methanococcus jannaschii, Pyrococcus abyssi, Pyrococcus horikoshii Archaeoglobus fulgidus, Haloferax volcanii. Thermotoga maritime and Thermus thermophiles* (Fukui *et al.,* 2002; Gimenez *et al.,* 2015).

Archaeal Lon protease resembles serine type protease but differs in typical serine type such as chymotrypsin which has a catalytic triad while it has a serine-lysine dyad (Rotanova *et al.,* 2004). Archaeal Lon protease is about 600 kDa and consists of mostly three regions N-terminal domain, ATPase domain and C-terminal domain (An *et al.,* 2010). The Lon protease is mostly associated with membrane where the dock of transmembrane domain inserted in ATPase region that anchors the protein into cytoplasmic side of membrane (Rotanova *et al.,* 2004).

The three domains are attach to one another in a specific manner that follows the bond formation of N-terminal with ATPase domain which further combines with C-terminal through covalent bond. Here N-terminal is substrate or protein recognizing domain, ATPase domain unfold the protein and translocate these polypeptides into thread like structure more specifically walker A and B motif helps in binding and hydrolyzing while the C-terminal domain is proteolytic domain having a conserved Ser-Lys catalytic dyad (Venkatesh *et al.,* 2012; Gur *et al.,* 2013).

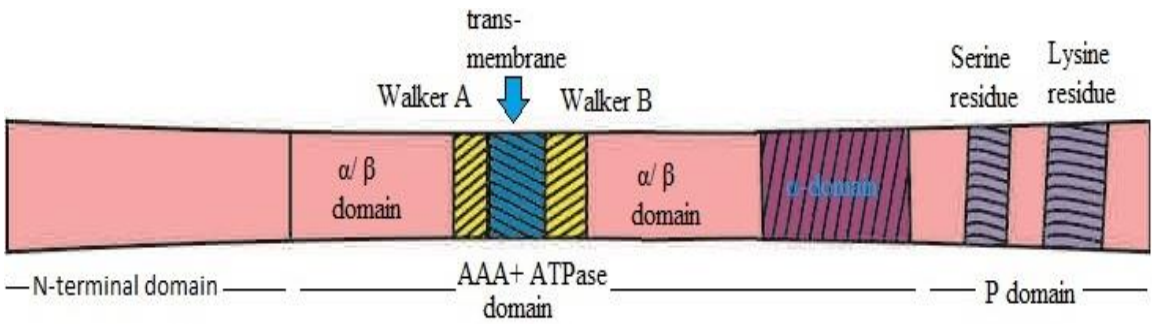

Fig: 2- Showing different component of LON protease in Archaea.

This catalytic dyad is actually responsible for the degradation of proteins and proteolysis in absence of this dyad no proteolysis occur even in presences of all the three domains. The communication between ATPase domain and proteolytic sites is very important; any mutation with ATPase domain can also cease the proteolytic activity. So it also depends upon intersubunit interactions. This specific arrangement results in formation of an oligomer more specifically hexameric ring structure. This configuration creates a catalytic cavity inside the ring which performs proteolysis (Ogura *et al.,* 2004).

AAA ATPase domain

The hexameric ring structure is formed by combination of different subunits. The first putative membrane-anchoring region leads to the AAA ATPase domain extends to α- subdomain and α/β subdomain present in

walker A. These domains further surround the ADP molecule. The ADP molecule are further submerged in two types of monomers, one tightly bound ADP monomer (T monomer) and other loosely bound ADP monomer (L monomer) while both are readily created by hydrolysis of ATP While the three hetro-dimers are placed parallel in alternate manner (Vineyard *et al.,* 2006; Cha *et al.,* 2010). The two trans-membranes are present between Walker A and Walker B that are responsible for anchoring and binding of substrate. This AAA ATPase domain further leads to P domain or proteolytic domain which has the proteolytic sites in it (Augustin *et al.,* 2009)

P domain

C-terminal domain is also called P-terminal domain as it is the proteolytic domain. The C-terminal of α-domain from the AAA ATPase region combines immediately with the N-terminal of P-domain. The P-domain consists of six α-domain and ten β-strands which forms ring shape hexamer with 6 symmetric folds. The N-terminal of β-strand 1 and its antiparallel β-strand 2 form β-hairpin loop while the β-strand 3 and β-strand 4 are separated by the α-domain 1 thus the whole 16 subunits are combined into a loop to form folds. The hexameric ring along with these subunits is characterized by hydrophilic interactions with some hydrophobic grooves in it. The α- domain mostly forms hydrogen bonding. The proximal surface of hexameric ring is positively charged due to presence of positively charged residues while the distal surface is negatively charged with negatively charged and polar residues. The proximal surface due to its charge may act as gating processor for substrate entrance in P domain. The entrance of substrate mimics like a lock and key model where the charged region modifies shape in proximity of substrate (Botos *et al.,* 2004).

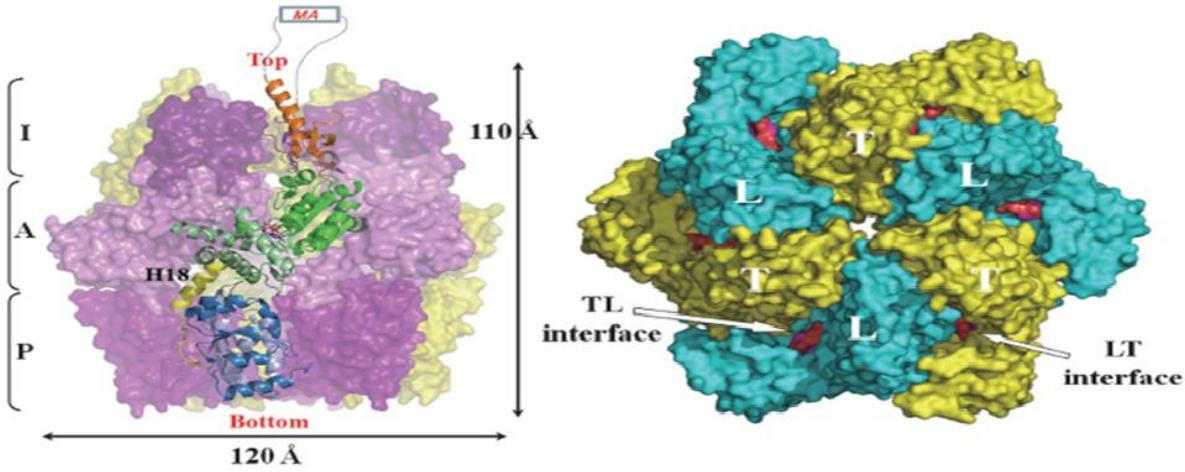

Fig: 3- Structure of Lon Protease. (A) Showing ribbon model of Lon Protease in Archaea. (B) Showing space model of T and L monomer while the red hinges are ADP molecules. (Cha *et al*, 2010)

Basic mechanism of Lon protease

The basic mechanism of Lon protease is determined in different bacteria and Archaea. The process initiates with the folded or unfolded protein recognition by the N-terminal domain which is further bind and translocate to trans-membrane in ATPase domain. While this anchorage leads protein to enter the substrate into protease domain or proteolytic domain (Li *et al.,* 2005; Gur *et al.,* 2013). The binding of substrate with AAA ATPase domain results in the change in the conformation of the α-domain which further changes the conformation of subunits in P domain which results in the chamber broadening for substrate entrance. It may occur that before entering to proteolytic chamber the polypeptide chain break down into small different fragment which further cleave in proteolytic chamber by the catalytic dyad present there. It must be noted that the most active of dyad for proteolysis is lysine residue. The serine may initiate the mechanism but the absence of lysine or its replacement with any other basic residue results in ending of its function (Cha *et al.,* 2010).

The Lon protease degrades substrate consisting of ~50-30 amino acids. It mostly cleaves the hydrophobic residues at position -1. The cleavage pattern follows usually leucine at position 1, serine at -1 and glutamine

at P2 and P5 (Nishii *et al.,* 2002). The interesting fact is serine and glutamine are both polar residues besides leucine which is non-polar and hydrophobic in nature. This give arise to fact that it cleaves uncharged amino acids while hydrophobic are more prominent one. This affinity may be due to conformational changes in AAA ATPase domain and P domain that cleave Ser and Gln specifically. It may cleave readily leucine, alanine, valine and sometimes phenylalanine due to their uncharged hydrophobic nature (Nishii *et al.,* 2005; Liao *et al.,* 2010).

Inhibitors of Lon protease

The nature of Lon protease is serine type so the inhibitors are also the serine inhibitors such phenylmethyl sulfonyl fluoride (PMSF). The most common inhibitor used is peptide boronate which preferentially targets proteolytic Ser hence end the proteolytic activity but the interesting fact is that it use ATP for its adduct formation which make it easier to avoid by the hydrolysis of ATP. Another inhibitor for Lon protease is coumarin derivatives which attack Ser nucleophile to end its proteolytic activity and they do not need any ATP for their functioning but due to their low molecular weight they can be reversible by the chemical reaction (Frase *et al.,* 2007; Bayot *et al.,* 2008).

ClpP protease

ClpP protease is a cylindrical, ATP dependent, periplasmic serine protease which is highly conserved in Bacteria and Eukaryotes and its presence in Archaea has now been discovered. It is also responsible for proteolysis but the main assisting entity is AAA+ chaperons that bind target and translocate substrate to undergo degradation by ClpP protease. It was first identified in *E.coli* while in Archaea it is recently identified during less than a decade. In Archaea it is first identified in *Methanothermobacter thermautotrophicus* (COG0542) and then found in *Methanocaldococcus jannaschii, Pyrobolus fumarii, Pyrobaculum aerophilum, Pyrobaculum arsenaticum, Pyrobaculum calidifontis, Thermococcus gammatolerans, Thermococcus kodakarensis, Pyrobaculum neutrophilum, Thermococcus onnurineus, Thermofilum pendens, Thermococcus sibiricus, Methanococcus maripaludis, Methanococcus voltae, Methanotorris igneus, Archaeoglobus fulgidus* (Chandu and Nandi. 2004)

The basic structure of ClpP protease for all domains is same but there is always some specific features for each domain. For Archaea, the system is less specific while the structure consists of only AAA domain and Clp domain which have the proteolytic chamber. The ClpP protease is composed of a cylindrical like structure which surrounds a large chamber having proteolytic sites. The ClpP protease form complex with AAA ATPase complex that binds, unfolds, denature substrates and translocate them to axial pores into proteolytic chamber for proteolysis. ClpP degrades substrate to 7-8 residues (Wang *et al.,* 1998; Bolhuis *et al.,*, 1999).

ClpP protease has cylindrical shaped tetradecamer of about 300kDa. It consists of six monomers with α/β repeating units that result in folds by hydrophobic interaction. Monomers can be divided into handle region that facilitate ring interaction and a head domain. The handle region is present between two ClpP heptameric rings where charge-charge interaction between residues stabilizes them (Yu *et al.,* 2007). There is an axial pore large enough to accept unfolded polypeptide chains leading to proteolytic cavity having fourteen serine protease active sites (Szyk *et al.,* 2006) (Alexopoulos *et al.,* 2012). Proteolytic cavity consists of a catalytic triad which is serine, histadine and aspartic acid. There are mainly two types of components which are responsible for ClpP activity, ClpA and ClpX ATPase chaperon (Gribun *et al.,* 2005). Without small substrate can enter the cavity and degrade but the long polypeptide as well as folded proteins cannot be degraded. In that situation the activators like acyl depeptidase can help ClpP to cleave the folded protein (Singh *et al.,* 2000; Li *et al.,* 2010). The translocational process is directional in ClpP where the carboxy terminus proceeds first while the substrate entrance to proteolytic chamber is also C to N- terminal.

The proteolysis initiate with the recognition and binding of substrate using AAA ATPase complex along with ClpA which further forms an axial pore with ClpP (Effantin *et al.,* 2010). This axial pore is responsible for the entrance of recognized substrate and is divided into two entities. The first part consists of 7-8 hydrophobic residues that make the pore lining while the second part consists of axial protrusion that bulge out the apical

surface of ClpP for substrate capturing. Mostly the hydrophobic, non-polar, charged and branched substrate enters for the cleavage and this specificity is maintained by the axial pore on ClpP and the assisting AAA ATPase domain (Baker and Sauer 2012). It is to be noted that the presence of N-terminal residues on ClpP are responsible for the degradation of folded proteins while in their absence it ceases but the efficiency for the unfolded protein to be degraded also increases. The active residues for cleavage are serine and histadine. As the substrate enters the proteolytic chamber, the active residues hydrolyse them through their carboxy terminal where the Ser residue is the most active to cleave the peptide and then the His (Bewley *et al.*, 2009).

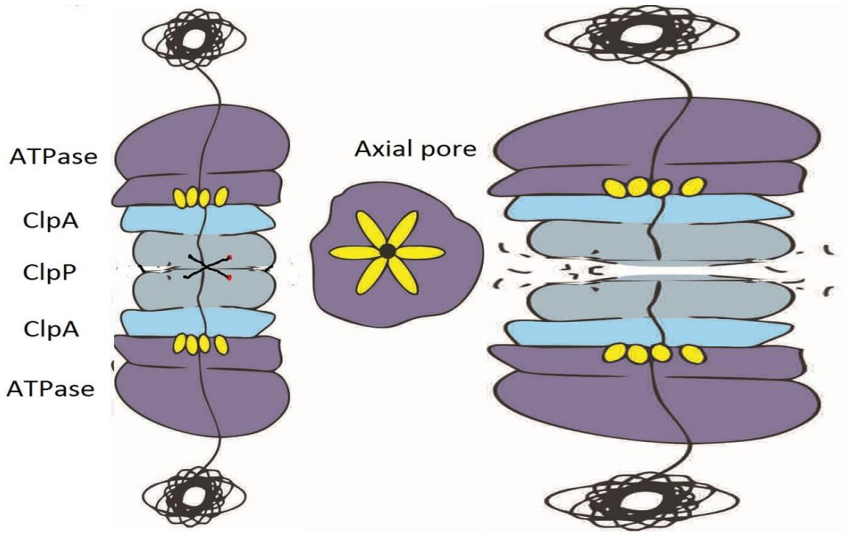

Fig: 4- Structure of ClpP protease. (A) Showing different components of protease along with Axial pore. (B) Showing the proteolysis and exit of amino acid residues.

There are three suppositions given for the substrate exit from the ClpP, according to them the entrance of substrate occur from the axial pore but after proteolytic action, the fragment may escape from the other side of chamber through the ClpA placed on the other side. The theronine concept about the exit is the peptide exit from the same entrance axial pore through passive diffusion after releasing of the chaperon from it. The third concept is unique one, according to it the peptide release through the side pore that momentarily formed between the two heptameric ClpP rings (Yu *et al.*, 2007).

Inhibitors of ClpP

The ClpP are caseinolytic protease. The most common inhibitors are acyldepsipeptides as well as β-lactones. They most commonly combines with the AAA ATPase complex competing the ClpP or may undergo conformational changes by binding with N-terminal of ClpP subunit thus open gate conformation is reversed, stopping any substrate to enter in the proteolytic chamber for degradation (Li *et al.*, 2010). β-lactones more specifically combine with carbonyl group of active site near the hydrophobic pocket thus making it impossible for active site to proceed proteolytic activity (Gersch *et al.*, 2013).

FtsH ATPase/RPt

Putative ATPase found in a variety of Archaea has similarity to ATP-dependent FtsH protease and Rpt subunits of 26S proteasome. The COG1223 forms clusters with protein and have AAA proteins motif including binding with ATP and Mg^{+2}. But the fact is that the Zn^{+2}-dependent proteolytic site as well as N-terminal trans-membrane region like that of FtsH is absent. So this Rpt/FtsH like ATPase is cytoplasmic does not directly hydrolysis peptide bonds, may act as an ATP-dependent unfoldase in protein degradation (Maupin-Furlow *et al.*, 2000).

Concluding remarks

Energy dependent proteolysis plays a critical role in protein turnover. This involves a quality control mechanism using protein regulation in a cell by destroying short lived or damaged proteins at a very precise time. Sometimes the rate of removal of a specific protein must be higher than the rate of cell division which can be made possible by proteolysis to continue cell cycle (Rasool *et al.,* 2013). Thus, it plays a critical role in cell cycle while it is in its growth phase or stationary phase. The energy used in this kind of proteolysis may be used to avoid any unfavorable steps like the binding of a competitive substrate or alteration of substrate interaction site or change in the conformation of protease that may alter the process precision. The energy required in energy dependent proteolysis is expended to increase the suitability for protease and potential substrate. This leads to complete degradation of protein once the process is initiated. The energy is also used to check the process and manage time for its precision. The energy dependent proteolysis is considered to be energy economical process as it has been seen that the energy required per peptide bond formation is 3 ATP molecules but for Lon protease in *E.coli* the cleavage required only 2 ATP molecules which in turn conserve 1 ATP per peptide and 3% of total energy cost (Gottesman *et al.,* 1992). It is also responsible for the degradation of nascent polypeptides whose synthesis is sometimes interrupted resulting in the faulty translation and formation of mis-folded protein that may accumulate and disturbs the balanced cell cycle. The energy dependent proteolysis provide tool for the translocation of protein that are destined to degradation. Thus it recycles obsolete and mis-folded protein and maintains balanced growth of cell and respond against the external stresses.

References

1. Alberts B, Miake-Lye R. Unscrambling the puzzle of biological machines: the importance of the details. Cell. 1992; 68(3):415-20.
2. Alexopoulos JA, Guarné A, Ortega J. ClpP: a structurally dynamic protease regulated by AAA+ proteins. Journal of structural biology. 2012; 179(2):202-10.
3. An YJ, Lee CR, Supangat S, Lee HS, Lee JH, Kang SG, Cha SS. Crystallization and preliminary X-ray crystallographic analysis of Lon from *Thermococcus onnurineus* NA1. Acta Crystallographica Section F: Structural Biology and Crystallization Communications. 2010; 66(1):54-6.
4. Anderson EC. On the Partial Metabolism, by the Liver, of Leucin and Tyrosin into Urea. Medico-chirurgical transactions. 1880; 63:245.
5. Arrigo AP, Tanaka K, Goldberg AL, Welch WJ. Identity of the 19S'prosome'particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature. 1988; 331:192–194.
6. Augustin S, Gerdes F, Lee S, Tsai FT, Langer T, Tatsuta T. An intersubunit signaling network coordinates ATP hydrolysis by m-AAA proteases. Molecular cell. 2009; 35(5):574-85.
7. Baker TA, Sauer RT. ATP-dependent proteases of bacteria: recognition logic and operating principles. Trends in biochemical sciences. 2006; 31(12):647-53.
8. Baker TA, Sauer RT. ClpXP, an ATP-powered unfolding and protein-degradation machine. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2012; 1823(1):15-28.
9. Barrett AJ, Woessner JF, Rawlings ND, editors. Handbook of proteolytic enzymes. Elsevier; 2013.
10. Bayot A, Basse N, Lee I, Gareil M, Pirotte B, Bulteau AL, Friguet B, Reboud-Ravaux M. Towards the control of intracellular protein turnover: mitochondrial Lon protease inhibitors versus proteasome inhibitors. Biochimie. 2008; 90(2):260-9.
11. Benaroudj N, Goldberg AL. PAN, the proteasome-activating nucleotidase from archaebacteria, is a protein-unfolding molecular chaperone. Nature cell biology. 2000; 2(11):833-9.
12. Bewley MC, Graziano V, Griffin K, Flanagan JM. Turned on for degradation: ATPase-independent degradation by ClpP. Journal of structural biology. 2009; 165(2):118-25.
13. Bogyo M, Gaczynska M, Ploegh HL. Proteasome inhibitors and antigen presentation. Peptide Science. 1997; 43(4):269-80.
14. Bolhuis A, Matzen A, Hyyryläinen HL, Kontinen VP, Meima R, Chapuis J, Venema G, Bron S, Freudl R, van Dijl JM. Signal Peptide Peptidase-and ClpP-like Proteins of *Bacillus subtilis* Required

for Efficient Translocation and Processing of Secretory Proteins. Journal of Biological Chemistry. 1999; 274(35):24585-92.
15. Botos I, Melnikov EE, Cherry S, Tropea JE, Khalatova AG, Rasulova F, Dauter Z, Maurizi MR, Rotanova TV, Wlodawer A, Gustchina A. The catalytic domain of *Escherichia coli* Lon protease has a unique fold and a Ser-Lys dyad in the active site. Journal of Biological Chemistry. 2004; 279(9):8140-8.
16. Cha SS, An YJ, Lee CR, Lee HS, Kim YG, Kim SJ, Kwon KK, De Donatis GM, Lee JH, Maurizi MR, Kang SG. Crystal structure of Lon protease: molecular architecture of gated entry to a sequestered degradation chamber. The EMBO journal. 2010; 29(20):3520-30.
17. Chandu D, Nandi D. Comparative genomics and functional roles of the ATP-dependent proteases Lon and Clp during cytosolic protein degradation. Research in microbiology. 2004; 155(9):710-9.
18. Dahlmann B, Kuehn L, Rutschmann MA, Reinauer H. Purification and characterization of a multicatalytic high-molecular-mass proteinase from rat skeletal muscle. Biochemical Journal. 1985; 228(1):161-70.
19. Dick LR, Cruikshank AA, Grenier L, Melandri FD, Nunes SL, Stein RL. Mechanistic Studies on the Inactivation of the Proteasome by Lactacystin A central role for CLASTO-LACTACYSTIN β-LACTONE. Journal of Biological Chemistry. 1996; 271(13):7273-6.
20. Djuranovic S, Hartmann MD, Habeck M, Ursinus A, Zwickl P, Martin J, Lupas AN, Zeth K. Structure and activity of the N-terminal substrate recognition domains in proteasomal ATPases. Molecular cell. 2009; 34(5):580-90.
21. Effantin G, Maurizi MR, Steven AC. Binding of the ClpA unfoldase opens the axial gate of ClpP peptidase. Journal of Biological Chemistry. 2010; 285(19):14834-40.
22. Egorova K, Antranikian G. Industrial relevance of thermophilic Archaea. Current opinion in microbiology. 2005; 8(6):649-55.
23. Emmerich NP, Nussbaum AK, Stevanovic S, Priemer M, Toes RE, Rammensee HG, Schild H. The human 26 S and 20 S proteasomes generate overlapping but different sets of peptide fragments from a model protein substrate. Journal of Biological Chemistry. 2000; 275(28):21140-8.
24. Erzberger JP, Berger JM. Evolutionary relationships and structural mechanisms of AAA+ proteins. Annual Review of Biophysics and Biomolecular Structure. 2006; 35:93-114.
25. Forouzan D, Ammelburg M, Hobel CF, Ströh LJ, Sessler N, Martin J, Lupas AN. The archaeal proteasome is regulated by a network of AAA ATPases. Journal of Biological Chemistry. 2012; 287(46):39254-62.
26. Förster A, Whitby FG, Hill CP. The pore of activated 20S proteasomes has an ordered 7-fold symmetric conformation. The EMBO Journal. 2003; 22(17):4356-64.
27. Frase H, Lee I. Peptidyl boronates inhibit *Salmonella enterica* and *serovar Typhimurium* Lon protease by a competitive ATP-dependent mechanism. Biochemistry. 2007; 46(22):6647-57.
28. Fukui T, Eguchi T, Atomi H, Imanaka T. A membrane-bound archaeal Lon protease displays ATP-independent proteolytic activity towards unfolded proteins and ATP-dependent activity for folded proteins. Journal of bacteriology. 2002; 184(13):3689-98.
29. Gersch M, Gut F, Korotkov VS, Lehmann J, Böttcher T, Rusch M, Hedberg C, Waldmann H, Klebe G, Sieber SA. The mechanism of caseinolytic protease (ClpP) inhibition. Angewandte Chemie International Edition. 2013; 52(10):3009-14.
30. Giménez MI, Cerletti M, De Castro RE. Archaeal membrane-associated proteases: insights on *Haloferax volcanii* and other haloarchaea. Frontiers in microbiology. 2015; 6(39):1-9.
31. Gottesman S, Maurizi MR. Regulation by proteolysis: energy-dependent proteases and their targets. Microbiological reviews. 1992; 56(4):592-621.
32. Gottesman S. Proteolysis in Bacterial Regulatory Circuits 1. Annual review of cell and developmental biology. 2003; 19(1):565-87.
33. Gottesman S. Regulation by proteolysis: developmental switches. Current opinion in microbiology. 1999; 2(2):142-7.
34. Gribun A, Kimber MS, Ching R, Sprangers R, Fiebig KM, Houry WA. The ClpP double ring tetradecameric protease exhibits plastic ring-ring interactions, and the N termini of its subunits form

flexible loops that are essential for ClpXP and ClpAP complex formation. Journal of Biological Chemistry. 2005; 280(16):16185-96.
35. Groll M, Bajorek M, Köhler A, Moroder L, Rubin DM, Huber R, Glickman MH, Finley D. A gated channel into the proteasome core particle. Nature Structural & Molecular Biology. 2000; 7(11):1062-7.
36. Groll M, Ditzel L, Löwe J, Stock D, Bochtler M, Bartunik HD, Huber R. Structure of 20S proteasome from yeast at 2.4Å resolution. Nature. 1997; 386: 463–471.
37. Gur E. The Lon AAA+ protease. InRegulated Proteolysis in Microorganisms. Springer Netherlands. 2013 (35-51).
38. Hanson PI, Whiteheart SW. AAA+ proteins: have engine, will work. Nature Reviews Molecular Cell Biology. 2005; 6(7):519-29.
39. Inobe T, Matouschek A. Protein targeting to ATP-dependent proteases. Current opinion in structural biology. 2008; 18(1):43-51.
40. Kim YC, Li X, Thompson D, DeMartino GN. ATP binding by proteasomal ATPases regulates cellular assembly and substrate-induced functions of the 26 S proteasome. Journal of Biological Chemistry. 2013; 288(5):3334-45.
41. Kim YC, Smith DM. The 26S Proteasomal ATPases: Structure, Function, Regulation, and Potential for Cancer Therapies. InResistance to Proteasome Inhibitors in Cancer. Springer International Publishing. 2014 (347-364).
42. King RW, Deshaies RJ, Peters JM, Kirschner MW. How proteolysis drives the cell cycle. Science. 1996; 274(5293):1652-59.
43. Kisselev AF, Kaganovich D, Goldberg AL. Binding of Hydrophobic Peptides to Several Non-catalytic Sites Promotes Peptide Hydrolysis by All Active Sites of 20 S Proteasomes, evidence for peptide-induced channel opening in the α-rings. Journal of Biological Chemistry. 2002; 277(25):22260-70.
44. Li DH, Chung YS, Gloyd M, Joseph E, Ghirlando R, Wright GD, Cheng YQ, Maurizi MR, Guarné A, Ortega J. Acyldepsipeptide antibiotics induce the formation of a structured axial channel in ClpP: a model for the ClpX/ClpA-bound state of ClpP. Chemistry & biology. 2010; 17(9):959-69.
45. Li M, Rasulova F, Melnikov EE, Rotanova TV, Gustchina A, Maurizi MR, Wlodawer A. Crystal structure of the N-terminal domain of *Escherichia coli* Lon protease. Protein science. 2005; 14(11):2895-900.
46. Liao JH, Lin YC, Hsu J, Lee AY, Chen TA, Hsu CH, Chir JL, Hua KF, Wu TH, Hong LJ, Yen PW. Binding and Cleavage of *E. coli* HUβ by the *Escherichia coli* Lon Protease. Biophysical journal. 2010; 98(1):129-37.
47. Liu CW, Li X, Thompson D, Wooding K, Chang TL, Tang Z, Yu H, Thomas PJ, DeMartino GN. ATP binding and ATP hydrolysis play distinct roles in the function of 26S proteasome. Molecular cell. 2006; 24(1):39-50.
48. López-Otín C, Bond JS. Proteases: multifunctional enzymes in life and disease. Journal of Biological Chemistry. 2008; 283(45):30433-7.
49. Lupas A, Flanagan JM, Tamura T, Baumeister W. Self-compartmentalizing proteases. Trends in biochemical sciences. 1997; 22(10):399-404.
50. Maupin-Furlow JA, Gil MA, Karadzic IM, Kirkland PA, Reuter CJ. Proteasomes: perspectives from the Archaea. Frontiers Bioscience. 2004; 9(1743):1758.
51. Maupin-Furlow JA, Humbard MA, Kirkland PA, Li W, Reuter CJ, Wright AJ, Zhou G. Proteasomes from structure to function: perspectives from Archaea. Current topics in developmental biology. 2006; 75:125-69.
52. Maupin-Furlow JA, Wilson HL, Kaczowka SJ, Ou MS. Proteasomes in the archaea: from structure to function. Frontiers Bioscience. 2000; 5(9):D837-865.
53. Miranda LP, Holder JR, Shi L, Bennett B, Aral J, Gegg CV, Wright M, Walker K, Doellgast G, Rogers R, Li H. Identification of potent, selective, and metabolically stable peptide antagonists to the calcitonin gene-related peptide (CGRP) receptor. Journal of medicinal chemistry. 2008; 51(24):7889-97.

54. Nishii W, Maruyama T, Matsuoka R, Muramatsu T, Takahashi K. The unique sites in SulA protein preferentially cleaved by ATP-dependent Lon protease from *Escherichia coli*. European Journal of Biochemistry. 2002; 269(2):451-7.
55. Nishii W, Suzuki T, Nakada M, Kim YT, Muramatsu T, Takahashi K. Cleavage mechanism of ATP-dependent Lon protease toward ribosomal S2 protein. FEBS letters. 2005; 579(30):6846-50.
56. Ogura T, Whiteheart SW, Wilkinson AJ. Conserved arginine residues implicated in ATP hydrolysis, nucleotide-sensing, and inter-subunit interactions in AAA and AAA+ ATPases. Journal of structural biology. 2004; 146(1):106-12.
57. Oikonomopoulou K, Hansen KK, Saifeddine M, Vergnolle N, Tea I, Diamandis EP, Hollenberg MD. Proteinase-mediated cell signalling: targeting proteinase-activated receptors (PARs) by kallikreins and more. Biological chemistry. 2006; 387(6):677-85.
58. Peth A, Nathan JA, Goldberg AL. The ATP costs and time required to degrade ubiquitinated proteins by the 26 S proteasome. Journal of Biological Chemistry. 2013; 288(40):29215-22.
59. Rabl J, Smith DM, Yu Y, Chang SC, Goldberg AL, Cheng Y. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. Molecular cell. 2008; 30(3):360-8.
60. Rasool N, Rashid N, Bashir Q, Siddiqui MA. Proteolytic inventory of *Thermococcus kodakaraensis*. African Journal of Microbiology Research. 2013; 7(25):3139-50.
61. Reeve JN, Schleper C. Archaea: very diverse, often different but never bad?. Current opinion in microbiology. 2011; 14(3):271-3.
62. Rotanova TV, Melnikov EE, Khalatova AG, Makhovskaya OV, Botos I, Wlodawer A, Gustchina A. Classification of ATP-dependent proteases Lon and comparison of the active sites of their proteolytic domains. European Journal of Biochemistry. 2004; 271(23-24):4865-71.
63. Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature. 2001; 409(6823):1092-101.
64. Rudner DZ, Fawcett P, Losick R. A family of membrane-embedded metalloproteases involved in regulated proteolysis of membrane-associated transcription factors. Proceedings of the National Academy of Sciences. 1999; 96(26):14765-70.
65. Sauer RT, Baker TA. AAA+ proteases: ATP-fueled machines of protein destruction. Annual review of biochemistry. 2011; 80:587-612.
66. Sauer RT, Bolon DN, Burton BM, Burton RE, Flynn JM, Grant RA, Hersch GL, Joshi SA, Kenniston JA, Levchenko I, Neher SB. Sculpting the proteome with AAA+ proteases and disassembly machines. Cell. 2004; 119(1):9-18.
67. Seemuller E, Lupas A, Stock D, Lowe J. Proteasome from *Thermoplasma acidophilum*: a threonine protease. Science. 1995; 268(5210):579-82.
68. Singh SK, Grimaud R, Hoskins JR, Wickner S, Maurizi MR. Unfolding and internalization of proteins by the ATP-dependent proteases ClpXP and ClpAP. Proceedings of the National Academy of Sciences. 2000; 97(16):8898-903.
69. Smith DM, Chang SC, Park S, Finley D, Cheng Y, Goldberg AL. Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's α ring opens the gate for substrate entry. Molecular cell. 2007; 27(5):731-44.
70. Smith DM, Fraga H, Reis C, Kafri G, Goldberg AL. ATP binds to proteasomal ATPases in pairs with distinct functional effects, implying an ordered reaction cycle. Cell. 2011; 144(4):526-38.
71. Stetter KO. A brief history of the discovery of hyperthermophilic life. Biochemical Society Transactions. 2013; 41(1):416-20.
72. Sullivan JA, Shirasu K, Deng XW. The diverse roles of ubiquitin and the 26S proteasome in the life of plants. Nature Reviews Genetics. 2003; 4(12):948-58.
73. Tatsuta T, Langer T. AAA proteases in mitochondria: diverse functions of membrane-bound proteolytic machines. Research in microbiology. 2009; 160(9):711-7.
74. Turk B, Turk D, Turk V. Protease signalling: the cutting edge. The EMBO journal. 2012; 31(7):1630-43.
75. Venkatesh S, Lee J, Singh K, Lee I, Suzuki CK. Multitasking in the mitochondrion by the ATP-dependent Lon protease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2012; 1823(1):56-66.

76. Vineyard D, Patterson-Ward J, Lee I. Single-turnover kinetic experiments confirm the existence of high-and low-affinity ATPase sites in *Escherichia coli* Lon protease. Biochemistry. 2006; 45(14):4602-10.
77. Wang J, Hartling JA, Flanagan JM. Crystal Structure Determination of *Escherichia coli* ClpP Starting from an EM-Derived Mask. Journal of structural biology. 1998; 124(2):151-63.
78. Ward DE, Shockley KR, Chang LS, Levy RD, Michel JK, Conners SB, Kelly RM. Proteolysis in hyperthermophilic microorganisms. Archaea. 2002; 1(1):63-74.
79. Yu AY, Houry WA. ClpP: a distinctive family of cylindrical energy-dependent serine proteases. FEBS letters. 2007; 581(19):3749-57.
80. Yu Y, Smith DM, Kim HM, Rodriguez V, Goldberg AL, Cheng Y. Interactions of PAN's C-termini with archaeal 20S proteasome and implications for the eukaryotic proteasome–ATPase interactions. The EMBO journal. 2010; 29(3):692-702.
81. Yura TA, Kanemori MA, Morita MT. The heat shock response: regulation and function. Bacterial stress responses. ASM Press, Washington, DC. 2000:3-18.
82. Zhang F, Wu Z, Zhang P, Tian G, Finley D, Shi Y. Mechanism of substrate unfolding and translocation by the regulatory particle of the proteasome from *Methanocaldococcus jannaschii*. Molecular cell. 2009; 34(4):485-96.
83. Zwickl P, Baumeister W, Steven A. Dis-assembly lines: the proteasome and related ATPase-assisted proteases. Current opinion in structural biology. 2000; 10(2):242-50.