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Proteases in bacterial pathogenesis

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Abstract

Bacterial pathogens rely on proteolysis for protein quality control under adverse conditions experienced in the host, as well as for the timely degradation of central virulence regulators. We have focused on the contribution of the conserved Lon, Clp, HtrA and FtsH proteases to pathogenesis and have highlighted common biological processes for which their activities are important for virulence.

Keywords: Virulence regulators; Pathogenesis; Lon, Clp, HtrA and FtsH proteases; Type III secretion; Quorum sensing

1. Introduction

Bacterial pathogens carry an array of factors that contribute to virulence either directly in host interactions or indirectly as, for example, permitting replication in the host environment. Similar to the external milieu, the host offers a highly adverse environment with microbial attacks such as free radicals in the macrophages, iron limitation and altered temperature. In this review, we focus on the contribution of the conserved proteases Lon, Clp, HtrA and FtsH to pathogenesis. Commonly, they contribute to infection in at least two ways: First, they are part of the protein quality control machinery required for the turnover of unfolded proteins generated in the adverse host environment. Secondly, growing evidence supports a conserved role in specific and controlled proteolysis of regulatory proteins in response to temporal, spatial or environmental stimuli. Through selected examples, we highlight some of the mechanisms by which these proteases contribute to pathogenesis and reveal that, particularly in Gram-positive bacteria, Clp complexes are central in controlling virulence factor production, while in Gram-negative bacteria conserved proteases in general contribute to pathogenesis by affecting biological processes across the bacterial envelope. Additional research is required, however, to reveal the multitude of mechanisms by which regulated proteolysis influences pathogenesis.

2. Lon

2.1. Distribution and characteristics

The Lon protease is a cytoplasmic serine protease that is composed of hexameric rings of single peptide chains carrying the peptidase domain, an AAA^+ domain (ATPases associated with various cellular activities) and a domain displaying chaperone activity [62]. Lon is widely distributed in different kingdoms including the archaea, but is lacking in some members of the Bacillales and in the Lactobacillales of the Firmicutes including important pathogens such as *Streptococcus pneumoniae, Listeria monocytogenes* and *Staphylococcus aureus*. In *Escherichia coli*, the degradation of non-native proteins is predominantly carried out by Lon [61] and the substrate specificity was recently characterized revealing that Lon recognizes specific sequences rich in aromatic residues that are accessible in the unfolded polypeptide chain, but hidden in most native protein structures [21]. Although detailed mechanistic studies are still missing for other species, it is tempting to speculate that similar substrate specificity may also apply.

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2.2. Lon in type III secretion

Salmonella enterica serovar Typhimurium is a Gram-negative pathogen that causes gastroenteritis in humans, and systemic diseases similar to typhoid fever in mice. It colonizes the small intestine and invades normally non-phagocytic epithelial cells to gain access to the underlying tissue. *S.* typhimurium mutants lacking Lon revealed a surprising phenotype with massive induction of apoptosis in macrophages, enhanced invasion of epithelial cells and increased production of proteins expressed by the *Salmonella* pathogenicity island 1, SPI1 [5,57]. Important in these processes is the type III secretion system (TTSS) encoded by SPI1 [66]. The SPI1 TTSS is composed of multiple proteins that form a needle-like structure through which effector proteins are injected into the host cell cytosol, where they rearrange actin cytoskeleton leading to uptake of the bacteria. Expression of the TTSS proteins is controlled by a complex cascade of transcriptional regulators in which the activator of TTSS genes, HilA (hyper-invasion-locus-A) is regulated by the AraC/XylS family activators, HilC and HilD [52]. Detailed studies revealed that both HilC and HilD are targets of Lon proteolysis and in the absence of *lon,* the accumulation of the transcriptional activators leads to stimulation of SPI1 gene expression [54].

From the enhanced expression of SPI1 genes observed in the absence of Lon one might predict that *lon* mutant cells are more virulent than wild type cells in an animal model. However, this did not turn out to be the case. In fact, *S.* typhimurium cells lacking Lon increased the 50% lethal dose in mice between 10^3 and 10^6 fold depending on the inoculation and were unable to survive in murine macrophages [55]. In addition, mutant cells were highly susceptible to oxidative stress and acid, thus suggesting that during infection the most important contribution of the protease is to resist oxygen-dependent killing associated with the respiratory burst and the low phagosomal pH [55].

Also in other pathogens, type III secretion components are targets of Lon degradation. In the plant pathogen *Pseudomonas syringae,* a TTSS is expressed from the hypersensitive response and pathogenicity island, *hrp* and again, the absence of Lon resulted in constitutive expression of the TTSS genes and accumulation of effector proteins as a consequence of the stabilization of the positive transcriptional regulator HrpR [8,37]. Intriguingly, in the presence of Lon, HrpR is normally degraded with a half-life of 3–6 min, whereas under conditions inducing the *hpr* genes such as in minimal medium the half-life is increased to >35 min, [8]. The mechanism by which proteolysis responds to environmental change is unknown, but it may depend on whether HrpR is occupied by binding to DNA or other proteins.

In *Yersinia pestis*, the etiological agent of plague, Lon is important for virulence, but in this case expression of a plasmid-encoded TTSS is repressed in the absence of Lon as a consequence of accumulation of the small nucleoid-associated protein, YmoA [27]. YmoA is a transcriptional repressor of TTSS genes and it is rapidly degraded at 37 °C in wild type *Y. pestis* cells, but remains stable in cells lacking both the Lon and the Clp protease. Interestingly, the stability of YmoA is temperature-dependent with increased stability at lower temperature [27]. Thus, enhanced degradation of YmoA at 37 °C, the temperature of the host, relieves the transcriptional repression exerted by YmoA and induces TTSS gene expression. Lon is also involved in temperature-dependent regulation of the transcriptional activator RovA, in *Yersinia pseudotuberculosis*. RovA is a DNA binding protein which coordinates expression of multiple metabolic stress and virulence genes and contributes to colonization and host-associated stress adaptation. As shown in Fig. 1, RovA functions as a protein thermometer that at 37 °C has reduced DNA binding due to a conformational change and when released from the DNA its susceptibility to Lon-mediated degradation is greatly enhanced [22]. Since the temperature-dependent conformational change of RovA is reversible, Lon is required to irreversibly remove RovA from the cytoplasm and thereby prevent recurring binding to DNA [22].

2.3. Lon in quorum sensing

In some bacterial pathogens, the expression of virulence factors is tightly regulated in response to the density of the bacterial population. For this purpose, quorum sensing systems are used to keep track of the microbial cell count. *Pseudomonas aeruginosa* is a Gram-negative, opportunistic pathogen that infects immunocompromised patients and is known to colonize the lungs of cystic fibrosis patients. Virulence of *P. aeruginosa* relies on the production of a wide range of virulence factors largely regulated by two acyl-homoserine lactone (HSL)-mediated quorum sensing systems, LasR/LasI and RhlR/RhlI. Recently, Lon was implicated in the activity of both systems as the half-lives of the HSL-synthetases LasI and RhlI were greatly prolonged in a *lon* mutant, suggesting that Lon controls the production of homoserine lactones [56]. In the related organism, *Pseudomonas putida*, quorum sensing

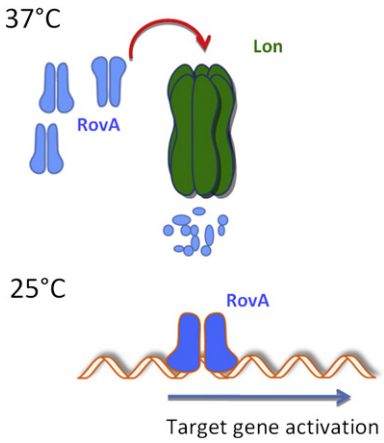

Fig. 1. Lon in thermal sensing in *Yersinia*. At low temperature (25 °C), the transcriptional activator, RovA (blue) binds to target sequences such as in the promoter of the *inv* gene encoding invasin. At higher temperature (37 °C), the protein undergoes thermal de-stabilization, releases DNA and is degraded by Lon (green).