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Mini Review

Clp chaperones and proteases are central in stress survival, virulence and antibiotic resistance of *Staphylococcus aureus*

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ABSTRACT

Intracellular proteolysis carried out by energy-dependent proteases is one of the most conserved biological processes. In all cells proteolysis maintains and shapes the cellular proteome by ridding the cell of damaged proteins and by regulating abundance of functional proteins such as regulatory proteins. The ATP-dependent ClpP protease is highly conserved among eubacteria and in the chloroplasts and mitochondria of eukaryotic cells. In the serious human pathogen, *Staphylococcus aureus* inactivation of *clpP* rendered the bacterium avirulent emphasizing the central role of proteolysis in virulence. The contribution of the Clp proteins to virulence is likely to occur at multiple levels. First of all, both Clp ATPases and the Clp protease are central players in stress responses required to cope with the adverse conditions met in the host. The ClpP protease has a dual role herein, as it both eliminates stress-damaged proteins as well as ensures the timely degradation of major stress regulators such as Spx, LexA and CtsR. Additionally, as we will summarize in this review, Clp proteases and Clp chaperones impact on such central processes as virulence gene expression, cell wall metabolism, survival in stationary phase, and cell division. These observations together with recent findings that Clp proteins contribute to adaptation to antibiotics highlights the importance of this interesting proteolytic machinery both for understanding pathogenicity of the organism and for treating staphylococcal infections.

Introduction

Staphylococcus aureus is a serious human pathogen that can give rise to a variety of infections ranging from harmless wound infections to life-threatening conditions like bacteremia, osteomyelitis and heart valve infections (Lowy, 1998). Antibiotic resistance is an increasing problem with the spread of methicillin resistant strains (MRSAs) both in the hospitals and in the community (Otto, 2012). Yet, for the majority of time *S. aureus* is colonizing harmlessly warm-blooded animals and humans and for the latter approximately 30% are permanently colonized by the organism (DeLeo et al., 2010). In the balance between harmless symbiosis and devastating infection, *S. aureus* tightly controls production of virulence and colonization factors. At the same time it relies on advanced stress response systems that will allow survival and adaptation to changing environmental habitats. One of the molecular machineries that in *S. aureus* occupy roles in both virulence and environmental adaptation is the Clp proteolytic system. Clp proteases are found well conserved in most bacterial species and they are composed of a core proteolytic chamber flanked by one of several possible ATPases that determine substrate specificity. Importantly, these ATPases also have chaperone activity that in combination with ClpP enable entry into the secluded, proteolytic chamber but in the absence of ClpP may function as independent molecular chaperones (Savijoki et al., 2006; Frees et al., 2007, 2013). When originally examined in *S. aureus*, several studies indicated that the proteolytic subunit, ClpP and the ClpATPase, ClpX are essential for virulence as inactivation completely abolished abscess formation in a mouse model and eliminated expression of one of the major staphylococcal hemolysins, α-hemolysin (Mei et al., 1997; Frees et al., 2003). Also, intracellular replication in bovine mammary cells was eliminated for mutants lacking either *clpP*, *clpX* or *clpB* and was significantly reduced for *clpC* mutant cells (Frees et al., 2004). While the mechanisms behind the defects in virulence remain unknown, they must be related to two key biological functionalities of the Clp complex namely in degradation of short-lived regulatory proteins (Elsholz et al., 2010a) or in protein quality control (Frees et al., 2004). Recently, a large number of ClpP substrates in *S. aureus* were identified by using catalytically inactive ClpP or ClpC variants ("clpP^{TRAP}") that will retain but not degrade substrates translocated into the proteolytic chamber (Fig. 1, Feng et al., 2013; Graham et al., 2013). This study revealed that in *S. aureus* the Clp targets encompass a number of central regulatory proteins (CtsR, Spx, HrcA, PerR, CodY) as well as proteins

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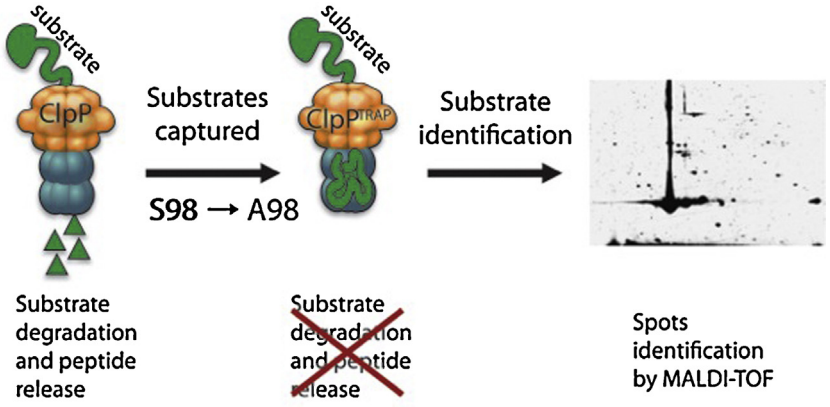

Fig. 1. Large scale identification of substrates of the ClpP protease by using a proteolytically inactive ClpP-variant. To directly identify substrates of the ClpP protease, the active site was mutated, and the proteolytic inactive ClpP chamber now functions as a "ClpP^{TRAP}" that will retain but not degrade substrates translocated into the proteolytic chamber. Captured substrates were co-purified along with the His-tagged ClpP complex and identified by mass spectrometry.

with central physiological functions such as RecA, FtsZ, NrdE, Pmp, GlmS and DnaK. Thus, with dual functionality in degrading both non-native proteins generated during stress as well as specific key regulatory proteins, the Clp proteolytic system appears to be a cornerstone in processes of importance to survival and pathogenicity, and as such, may not be an important target for design of new interventions.

Structure

The Clp proteolytic chamber is a barrel shaped structure that is conserved among bacteria and is composed of two rings of heptameric ClpP. Access to this secluded proteolytic chamber is restricted by pores that are too narrow to allow entry of folded proteins. In order to be degraded, substrates must first interact with the Clp ATPase component that powers unfolding and subsequent translocation of the substrate into the ClpP proteolytic chamber (Frees et al., 2007). In *S. aureus*, the ClpC and ClpX ATPases can perform this function. Two additional Clp ATPases are encoded by the organism, namely ClpL and ClpB, but they lack the conserved tripeptide consensus sequence (IG(F/L)) required for interaction with ClpP (Martin et al., 2008). During proteolysis in *S. aureus* this structure undergoes dramatic conformational changes. In the proteolytic active state ClpP adopts an extended conformation with the catalytic triad (His-123, Asp-172, Ser-98) aligned in a proper geometry for proteolytic activity. In contrast, in the closed conformation the side-chains of the catalytic triad are flipped hampering proteolysis (Zhang et al., 2011; Gersch et al., 2012). Following degradation the products are likely to be released through pores in the side of the barrel (Geiger et al., 2011). In some cases proteolysis is regulated through the spatial and/or temporal use of adaptor proteins, which are directly involved in the recognition and delivery of specific substrate proteins to the proteases (Dougan et al., 2002; Battesti and Gottesman, 2013). For example ClpXP can degrade substrates independently of adaptors but the presence of the adaptor-like protein YjbH greatly enhances the proteolytic activity (Engman et al., 2012; Lies and Maurizi, 2008).

Notably, ClpP-like proteins are also common among *S. aureus* phages indicating that proteolytic control has a central function in phage biology (Boyle-Vavra et al., 2011). An additional Clp proteolytic complex baring resemblance to the eukaryotic 26S proteasome is formed by the products of the *clpY* and *clpQ* genes (Frees et al., 2005b). Despite the name, the proteolytic subunits ClpQ and ClpP are not related, and as no distinct phenotype has been linked to the function of the ClpYQ it will not be discussed further here.

Stress tolerance

During infection bacterial pathogens are likely to encounter dramatic environmental changes and may experience shift in temperature, oxidative stress, antimicrobial peptides and other conditions aimed at inactivating the invading microorganism. Such stress exposures may lead to protein unfolding, and removal of unfolded and non-native proteins is necessary for cellular functionality and growth (Truscott et al., 2011). In *S. aureus* inactivation of *clpP*, *clpC*, *clpB* and to a lesser extent *clpL* abolished or reduced growth at 45 °C (Frees et al., 2003, 2004, 2012). Since ClpB and ClpL are not functional ClpP partners these results suggested that ClpCP is degrading non-native proteins in *S. aureus*, a finding recently confirmed by trapping protein substrates at high and ambient temperature, respectively (Feng, in preparation). The contribution of ClpB and ClpL to stress survival is likely as chaperones either to prevent protein unfolding or promote disaggregation (Glover and Lindquist, 1998). This notion was supported by the finding that both ClpB and ClpL are required for thermoinduced thermotolerance where pre-exposure to intermediate high temperature improves survival at high temperatures (Frees et al., 2004). Surprisingly, inactivation of *clpX* allowed growth at an even higher temperature than the wild type cells. Although the basis for this finding is currently unknown it shows that Clp proteins contribute to virulence through stress-independent (via ClpXP) and stress-dependent (ClpCP and ClpB) pathways (Frees et al., 2003, 2004).

Also in *Bacillus subtilis*, non-native proteins are degraded by the ClpCP complex and it takes place in a process also requiring the adaptor protein, MecA. MecA is necessary not only for substrate recognition but also for the oligomerization of ClpC into a hexamer and binding to ClpP (Kirstein et al., 2006; Schlothauer et al., 2003). A MecA homologue is also found in *S. aureus* where it is designated teicoplanin resistance factor A (TrfA, see below, Renzoni et al., 2009). TrfA is a target of the Clp protease (Feng et al., 2013) and a mutant lacking the corresponding gene is temperature sensitive indicating that TrfA may assist ClpCP mediated proteolysis of non-native proteins also in *S. aureus* (Feng et al., in preparation). McsB is another adaptor-like protein also found in *B. subtilis* that was trapped as Clp target (Elsholz et al., 2010b; Wozniak et al., 2012; Feng et al., 2013). Inactivation of *S. aureus McsB* impairs growth at high temperature as well as in the presence of heavy metals, osmotic pressure, oxidative stress and at low pH (Wozniak et al., 2012). In this case the heat sensitivity may be due to lack of ClpC expression as McsB in *B. subtilis* is needed for the degradation of the negative heat shock regulator, CtsR that controls *clpC* expression (Elsholz et al., 2011a, see below).