RamA, which controls expression of the MDR efflux pump AcrAB-TolC, is regulated by the Lon protease

Vito Ricci, Jessica M. A. Blair and Laura J. V. Piddock*

Antimicrobials Research Group, School of Immunity and Infection and Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, UK

*Corresponding author. Antimicrobials Research Group, School of Immunity and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK. Tel: +44-121-414-6966; Fax: +44-121-414-6819; E-mail: l.j.v.piddock@bham.ac.uk

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Objectives: RamA regulates the AcrAB-TolC multidrug efflux system. Using Salmonella Typhimurium, we investigated the stability of RamA and its impact on antibiotic resistance.

Methods: To detect RamA, we introduced ramA::3XFLAG::aph into plasmid pACYC184 and transformed this into Salmonella Typhimurium SL1344/ramA::cat and lon::aph mutants. An N-terminus-deleted mutant [pACYC184/ramA(Δ2-21)::3XFLAG::aph] in which the first 20 amino acids of RamA were deleted was also constructed. To determine the abundance and half-life of FLAG-tagged RamA, we induced RamA with chlorpromazine (50 mg/L) and carried out western blotting using anti-FLAG antibody. Susceptibility to antibiotics and phenotypic characterization of the lon mutant was also carried out.

Results: We show that on removal of chlorpromazine, a known inducer of ramA, the abundance of RamA decreased to pre-induced levels. However, in cells lacking functional Lon, we found that the RamA protein was not degraded. We also demonstrated that the 21 amino acid residues of the RamA N-terminus are required for recognition by the Lon protease. Antimicrobial susceptibility and phenotypic tests showed that the lon mutant was more susceptible to fluoroquinolone antibiotics, was filamentous when observed by microscopy and grew poorly, but showed no difference in motility or the ability to form a biofilm. There was also no difference in the ability of the lon mutant to invade human intestinal cells (INT-407).

Conclusions: In summary, we show that the ATP-dependent Lon protease plays an important role in regulating the expression of RamA and therefore multidrug resistance via AcrAB-TolC in Salmonella Typhimurium.

Keywords: Salmonella, transcription factors, proteolysis

Introduction

RamA is an AraC/XylS transcriptional activator that regulates the expression of the genes encoding the AcrAB-ToLC resistance-nodulation-division multidrug efflux system in Salmonella enterica serovar Typhimurium and other Enterobacteriaceae, including Klebsiella and Enterobacter spp. In Salmonella Typhimurium, the expression of ramA is repressed by RamR, a member of the TetR family of transcriptional repressors, which usually contain an N-terminal DNA-binding domain and a C-terminal ligand-binding regulatory domain. The function of RamR can be ablated by N-terminal DNA-binding domain and a C-terminal ligand-binding domain. Unlike many transcription factors, MarA, SoxS and RamA are not expressed constitutively but are synthesized de novo in response to their respective inducers. In the case of SoxS, SoxR is directly activated by redox-cycling agents and in turn activates the expression of soxS. With MarA, MarR represses the transcription of marA, but in the presence of inducers such as salicylate, the expression of marA is increased. The exact mechanism by which RamR activity is controlled is as yet still unclear. On removal of the inducer, the first step in re-setting the system is to halt the synthesis of the transcription factor, which is typically via one of four mechanisms: binding of a ligand, covalent modification, partner protein or altering the level of the transcription factor. Following the cessation of transcription, the mechanism by which MarA and SoxS are removed is a reduction
in the level of the protein by an active process of proteolysis involving the Lon protease.\textsuperscript{22}

Lon is an ATP-dependent protease belonging to the AAA\textsuperscript{+} (ATPases associated with a variety of cellular activities) super-family of enzymes.\textsuperscript{23} Lon associates into hexameric rings in Gram-negative bacteria and is a homo-oligomer of sub-units each composed of an N-terminal domain, an ATP-binding domain, a substrate sensor and discriminatory domain, and a proteolytically active C-terminal domain. Lon performs a wide range of different cellular functions, and studies with several species have shown that Lon is involved in unfolding misfolded proteins, as well as in their degradation.\textsuperscript{23} Lon has also been extensively studied in \textit{E. coli} and known Lon targets include RcsA, a transcriptional activator for capsule synthesis\textsuperscript{24} RamA and its impact on antibiotic resistance.\textsuperscript{29} 

\textit{P. aeruginosa} are also hypersusceptible to ciprofloxacin, filamented\textsuperscript{30} and fluoroquinolone antibiotics.\textsuperscript{29} Lon mutants of 

do sensitive to UV light and DNA damage\textsuperscript{28} and hypersusceptible to antibiotics.\textsuperscript{22,27} Using Typhimurium SL1344 was used 

- Controls
- \textit{Salmonella}

Using Typhimurium, we investigated the stability of Lon protease of \textit{P. aeruginosa} has also been shown to be a negative regulator of quorum sensing.\textsuperscript{32} The Lon protease of \textit{Salmonella Typhimurium} negatively controls \textit{Salmonella} pathogenicity island (SPI)-1 expression through degradation of the HilC and HilD transcriptional regulators.\textsuperscript{33,34} and Lon, along with CpaP, is also involved in controlling haem biosynthesis by degrading HemA.\textsuperscript{23,35,36}

We hypothesized that the RamRA system is reset after induction by an active process following the removal of chloromazine. Using \textit{Salmonella Typhimurium}, we investigated the stability of RamA and its impact on antibiotic resistance.

**Materials and methods**

**Bacterial strains and growth conditions**

The strains and plasmids used in this study are shown in Table 1. The widely used representative strain \textit{Salmonella Typhimurium} SL1344 was used throughout. Growth was routinely performed in \textit{Luria–Bertani} (LB) broth unless indicated otherwise.

<table>
<thead>
<tr>
<th>Table 1. Strains and plasmids used in this study</th>
</tr>
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<tbody>
<tr>
<td>Strain/genotype</td>
</tr>
<tr>
<td>SL1344 wild-type</td>
</tr>
<tr>
<td>SL1344::ramA::cat</td>
</tr>
<tr>
<td>SL1344lon::aph</td>
</tr>
<tr>
<td>SL1344lon::aph::ramA::cat</td>
</tr>
<tr>
<td>SL1344(pACYC-ramA::3XFLAG-aph)</td>
</tr>
<tr>
<td>SL1344ramA::cat (pACYC-ramA::3XFLAG-aph)</td>
</tr>
<tr>
<td>PLasmid</td>
</tr>
<tr>
<td>pSUB11</td>
</tr>
<tr>
<td>pACYC184</td>
</tr>
</tbody>
</table>

**Construction of gene-deleted mutants**

To establish whether Lon was responsible for the degradation of RamA in \textit{Salmonella Typhimurium}, we constructed a lon and ramA double mutant in \textit{Salmonella Typhimurium} (SL1344) using the method described by Datsenko and Wanner.\textsuperscript{37} To distinguish between the two, we made the lon mutant using a kanamycin selectable marker, and the ramA mutant using a chloramphenicol marker. For the lon mutant, primers were designed to amplify the aph cassette from the \textit{pKDI} plasmid which contained 40 bases at the 5\textsuperscript{’} ends that were complementary to the end of the \textit{lon} gene (forward primer 5\textsuperscript{’}-ACCCTGCAAGCTGGTCCAGAACCAGTGCCCGCAATATGGCTG TAGGGCTGGAGCTGCTTC-3\textsuperscript{’} and reverse primer 5\textsuperscript{’}-TACCCCGGCAGCCACAAGGGAAATAGCCATGGTCCAT-3\textsuperscript{’}). For the ramA mutant, primers were designed to amplify the cat cassette from the \textit{pKD} plasmid which contained 40 bases at the 5\textsuperscript{’} ends that were complementary to the end of the ramA gene (forward primer 5\textsuperscript{’}-GCTCAAGGATTATGCTGGATGATAGTGTAGGCTGGAGCTGCTTC-3\textsuperscript{’} and reverse primer 5\textsuperscript{’}-TTTAAGAAAGGCTGGCCAGGTAACGTGGGCTTAGGGAAAAATGCTGGTCCAT-3\textsuperscript{’}). SL1344 containing the \textit{pKD} plasmid was transformed with the PCR fragments, and DNA sequencing was carried out to confirm the deletions. To make the SL1344lon::aph::ramA::cat double mutant, we performed P22 transduction.

**Western blotting**

Bacterial samples required for western blotting were harvested by centrifugation, and cell pellets were resuspended in 50 mM Tris/\textit{HCl} (pH 8.0). Protein extracts were prepared by sonication on ice with an \textit{MSE} Soniprep 150 (Sanyo, UK) for four pulses of 30 s with a 30 s pause between each pulse. A Bradford assay was carried out to quantify the protein concentration and 10 \mu g of protein was run on 4\%–12\% NuPAGE\textsuperscript{G} Bis-Tris mini gels with NuPAGE\textsuperscript{B} MES SDS running buffer (Life Technologies, UK). Protein was transferred to nitrocellulose transfer membranes (Whatman, UK), and analysed using the ECL detection system (GE Healthcare UK).

**FLAG-tagged RamA plasmid construction**

To make the plasmid construct, a DNA fragment carrying the ramA::3XFLAG-aph flanked by HindIII and NruI restriction sites was amplified from a previously made chromosomal ramA::3XFLAG fusion using the following primers: forward primer 5\textsuperscript{’}-GCAATAAGGCTGGCCAGGTAACGTGGGCTTAGGGAAAAATGCTGGTCCAT-3\textsuperscript{’} and reverse primer 5\textsuperscript{’}-GCCTGGAGCTGCTTC-3\textsuperscript{’}. For the SL1344lon::aph::ramA::cat::3XFLAG-aph was subsequently transformed into the SL1344lon::aph::cat and SL1344lon::aph::ramA::cat mutants.

In order to establish whether the N-terminal sequence of RamA is involved in Lon protease recognition, we constructed a deletion mutant in which the first 20 amino acids of RamA were deleted following the initiation codon. To achieve this we designed a pair of primers (5\textsuperscript{’}-GCTAAGCTTACCCCGGAAGAACCTCGTCAAGAAAGCAGGAAAAATGCTGGTCCAT-3\textsuperscript{’} and 5\textsuperscript{’}-GCCTGGAGCTGCTTC-3\textsuperscript{’}). For the SL1344lon::aph::ramA::cat::3XFLAG-aph that amplified the ramA::3XFLAG-aph flanked by HindIII and NruI restriction sites in which codons 2 to 21 were deleted. Following amplification, purification and double-restriction digest with HindIII and NruI, the amplimer was cloned into pACYC184.\textsuperscript{38} pACYC184::ramA::3XFLAG-aph was subsequently transformed into the SL1344lon::aph::cat mutat.
**Determination of RamA abundance following removal of inducer**

To establish the abundance of the RamA::3XFLAG protein following the removal of the inducer, we grew a culture of SL1344::ramA::cat (pACYC::ramA::3XFLAG) to mid-logarithmic growth phase, and induced it with chlorpromazine (50 mg/L) for 30 min. Aliquots of the induced culture were taken every 10 min. Following induction, the remaining culture was split into two and the cells were collected by centrifugation, washed and resuspended in LB medium containing or lacking chlorpromazine (50 mg/L). These cultures were re-incubated at 37°C for a further 30 min with aliquots removed every 10 min. All aliquots taken were subjected to sonication and then SDS-PAGE. For detection of RamA::3XFLAG protein, western blotting was performed as previously described.

**RamA half-life experiments**

To investigate the absence of Lon on the expression and stability of RamA::3XFLAG, we performed half-life experiments in which we grew SL1344::ramA::cat (pACYC184::ramA::3XFLAG) and SL1344lon::aph/ramA::cat (pACYC184::ramA::3XFLAG) in the presence of chlorpromazine, an inducer of RamA. To investigate the effect of the N-terminal 20 amino acid deletion on the expression of RamA::3XFLAG, we grew the SL1344::ramA::cat (pACYC184::ramA::3XFLAG) mutant in the presence of chlorpromazine. Mutants were grown in 300 mL of LB broth, containing 50 mg/L kanamycin and/or chloramphenicol, at 37°C with shaking at 180 rpm until an optical density (OD; measured at 600 nm) of 0.5 was attained. Chlorpromazine at a concentration of 50 mg/L was added to the cultures, which were then reincubated at 37°C for a further 60 min. Following treatment with spectinomycin (100 mg/L) to halt protein synthesis, aliquots (25 mL) were transferred every minute for 10 min to centrifuge tubes standing on ice. Each sample was then centrifuged and protein was extracted by sonication. SDS-PAGE was performed followed by western blotting as previously described.

**Determination of susceptibility to fluoroquinolones**

The MIC of each fluoroquinolone tested was determined by the standardized agar doubling-dilution method as described previously by the BSAC (http://www.bsac.org.uk).39

**Growth kinetics**

The rate of growth in LB broth and minimal medium (Teknova, USA) of SL1344 and SL1344lon::aph was determined over 24 h at 37°C using a FLUOSter OPTIMA (BMG Labtech, UK) plate reader as previously described.12

**Motility assays**

The ability of SL1344lon::aph to migrate through (swimming) or across (swarming) semi-solid agar was determined by making agar plates based on MOPS minimal medium (Teknova) supplemented with 0.25% and 0.5% (w/v) agar. Plates were inoculated by stabbing them with a sterile loop and the cells were incubated at 30°C over 5 days, and zones of migration through the agar were measured daily for each strain. Data were obtained in four separate experiments, each containing two technical replicates. All data were analysed with a Student’s t-test; P values of <0.05 were taken as significant.

**Crystal Violet biofilm assay**

Overnight cultures of strains were diluted in fresh, antibiotic-free LB broth without salt to an OD of 0.1 at 600 nm. Ninety-six-well polystyrene microtitre trays (Sterilin) were inoculated with 200 μL of this suspension and incubated at 30°C for 48 h with gentle agitation. After incubation, the liquid was removed from all the wells and the wells were washed with sterile distilled water to remove any unbound cells. Biofilms were stained by adding 200 μL of 1% Crystal Violet to the appropriate wells for 15 min. Crystal Violet was removed and each well was washed with sterile distilled water to remove any unbound dye. The stained biofilm was solubilized by adding 200 μL of 70% ethanol and the OD was measured at 600 nm using a FLUOSter OPTIMA (BMG Labtech). All biofilm assays were carried out three times with two biological and four technical replicates per repeat. A Student’s t-test was used to compare the statistical significance of the results between the Lon mutant and SL1344 (wild-type).

**Measurement of SPI gene expression**

SL1344 and SL1344lon::aph containing a chromosomal green fluorescent protein (GFP) reporter fused to the promoter of the prgH gene were grown overnight in LB broth at 37°C with shaking. A 4% inoculum was added to 10 mL of minimal media and incubated at 37°C with shaking until mid-log phase (an OD<sub>600</sub> of 0.6). Cells were harvested from 500 μL of culture by centrifugation and resuspended in 1 mL of PBS. An aliquot of 100 μL of each cell suspension was added to a 96-well plate and bacteria were analysed by flow cytometry using an Accuri C6 cytometer (BD Biosciences, USA). Three biological replicates were carried out and 10000 data points were collected for each sample.

**Adherence and invasion of bacteria to human intestinal cells (INT-407) growing in tissue culture**

Assays were performed as previously described.10

**Microscopic morphology**

Microscope slides were inoculated with a loopful of bacteria taken from mid-logarithmic growth cultures of SL1344 and SL1344lon::aph grown in LB medium. Cells were heat fixed, Gram-stained and examined with a light microscope at x100 magnification.

**Results**

**Chlorpromazine induces expression of RamA**

To date, few inducers of RamA have been reported; however, Nikaido et al.1,41 demonstrated an increased expression of ramA in response to the bacterial metabolite indole. Work carried out by Bailey et al.3 using comparative reverse-transcription PCR, and more recently by Lawler et al.42 with transcriptional GFP reporter fusions, showed that chlorpromazine, a phenothiazine, also induced the expression of ramA. For the purposes of our present experiments, we wanted to establish whether the increase in transcription of ramA by chlorpromazine translated into increased levels of RamA protein, and in order to do this we carried out western blotting in the absence and presence of chlorpromazine (50 mg/L). Previous studies have had difficulties in detecting chromosomally encoded proteins such as MarA by western blotting.22,43 To overcome this and produce detectable quantities of protein, we used a multicopy plasmid (pACYC184; Table 1) that harboured a ramA::3XFLAG fusion and blotted with an anti-FLAG antibody. In the absence of chlorpromazine, detectable amounts of RamA were produced, presumably due to the use of a multicopy plasmid. In the presence of chlorpromazine, 3-fold more RamA was detected; this showed that induction with chlorpromazine increased RamA production (Figure 1).
Abundance of RamA after removal of inducer is degraded by an active process

To ascertain how the RamRA system is reset and whether this is by an active or a passive process, we determined the abundance of RamA by western blot following the removal of chlorpromazine. As shown in Figure 2, the relative abundance of RamA was increased following induction and remained constant throughout the initial induced period of 30 min until the cells were harvested and transferred to LB containing or lacking chlorpromazine (50 mg/L). In the LB medium containing chlorpromazine, the amount of RamA remained constant, as was observed in the initial induced culture; however, after 10 min in LB medium with no chlorpromazine, the amount of RamA fell rapidly.

RamA is unstable with a short half-life

Our data (Figure 2) indicate that, in the absence of inducer, the abundance of RamA diminished, suggesting that RamA has a short half-life. As the previous experiment was carried out over a longer time period, and so that we could determine the half-life of RamA more accurately, we induced RamA synthesis using chlorpromazine and determined the abundance of RamA in multiple samples taken every 2 min. Following western blotting, our data revealed that the abundance of RamA rapidly decreased and indicated that the half-life of RamA is ~2 min (Figure 3a).

RamA is more stable in a lon mutant

Having shown that RamA is unstable and has a short half-life, we proceeded to investigate what was responsible for the instability of the RamA. ATP-dependent proteases are known to play an important role in gene regulation by degrading regulatory proteins such as transcription factors and, as previously reported by Griffith et al., the E. coli Lon protease is responsible for the rapid turnover of the transcriptional activators SoxS and MarA following induction by their respective inducers. To investigate whether the Lon protease in Salmonella Typhimurium carries out a similar role and degrades RamA, we constructed a lon deletion mutant in Salmonella Typhimurium (SL1344) and carried out half-life experiments. Our data revealed that, over the 10 min period tested and following induction, the abundance of the RamA protein remained high in the lon-deleted mutant (Figure 3b) and the half-life increased to >10 min. These data show that RamA is more stable and more abundant in the absence of lon.

The N-terminus of RamA is required for Lon protease recognition

Proteases degrade specific proteins in environments occupied by a variety of different proteins, so in order to target the appropriate protein, proteases are able to recognize specific substrates. Protease recognition signals have been found to reside at the N- and C-termini of proteins, which may reflect the accessibility of these ends. Previous work carried out in E. coli with the transcriptional activators SoxS and MarA identified that the N-terminus of these proteins play a primary role in Lon protease recognition. To determine whether the N-terminus of RamA is required for Lon-mediated degradation, we constructed an N-terminus-deleted RamA plasmid construct and determined the effect of this deletion on the half-life of RamA. We found that the N-terminal deletion increased the half-life of RamA from 2 min to >10 min (Figure 3c), suggesting that the N-terminus of RamA is important for proteolytic degradation by Lon protease.

The lon-disrupted mutant is not multidrug resistant

Previous work with other bacterial species has shown that lon mutants exhibit certain phenotypic characteristics. Therefore, to see whether the same was true for Salmonella, we determined...
the phenotype of the lon-disrupted mutant. In agreement with previous work on *P. aeruginosa* and *E. coli*, the *lon* mutant was 2- to 4-fold more susceptible to the fluoroquinolone antibacterials ciprofloxacin, norfloxacin and nalidixic acid compared with the wild-type (Table 2). Compared with the wild-type parental strain, SL1344, the SL1344 *lon::aph* mutant also grew poorly in both MOPS minimal medium and LB medium, but showed no difference in swarming or swimming motility or the ability to form a biofilm (although SL1344 forms biofilms poorly due to mutations in *mlrA* and *adrA*) (data not shown). There was also no difference in the ability of the SL1344 *lon::aph* to invade human intestinal cells (INT-407). Despite this, a greater percentage of SL1344 *lon::aph* mutants expressed SPI-1 than did the parental strain (Table 2).

### Discussion

RamA is the primary regulator of expression of AcrAB-TolC in most Enterobacteriaceae and understanding how the components of efflux pumps and the factors that regulate them are controlled will provide essential biological information. Furthermore, knowledge about such mechanisms is essential in the search for new antibacterial compounds.

Transcription factors are regulated in a variety of ways, which ultimately control their activity or their expression. One mechanism is the modification of the DNA-binding affinity of the transcription factor by small ligands, whose concentrations can vary in response to nutrient availability or stress. One example of this is the reduction in the DNA-binding affinity of the Lac repressor by the small molecule allolactose, which is an inducer that binds to the Lac repressor, stopping repression and allowing the transcription of lacZ and related genes.

Another mechanism is the modulation in activity of some transcription factors by covalent modification. A good example of this mechanism is that of NarL, which binds to its target DNA only when phosphorylated by its cognate sensor kinases NarX and NarQ.

A third mechanism is where the concentration of a transcription factor controls its activity, either by regulating the expression of the transcription factor or by proteolysis. One example is the transcription of *soxS* that is controlled by SoxR, which is directly activated by redox-cycling agents and in turn activates the expression of *soxS*.

The fourth mechanism by which transcription factors are regulated is sequestration by a regulatory protein to which the

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**Figure 3.** Determination of the half-life of RamA::3XFLAG and RamA(Δ2-21)::3XFLAG. Western blots and densitometry plots demonstrating the abundance of RamA::3XFLAG following removal of inducer in (a) SL1344 *ramA::aph* and (b) SL1344 *lon::aph/ramA::cat* over a 10 min time period. (c) Abundance of RamA(Δ2-21)::3XFLAG following removal of the inducer in SL1344 *ramA::cat* over a 10 min time period.

**Table 2.** Phenotype of *Salmonella Typhimurium lon::aph*

<table>
<thead>
<tr>
<th>Strain</th>
<th>CPZ (mg/L)</th>
<th>CIP (mg/L)</th>
<th>NOR (mg/L)</th>
<th>NAL (mg/L)</th>
<th>MIC (mg/L)</th>
<th>Growth in LB and MOPS minimal medium, generation time (min)</th>
<th>Motility, colony diameter (mm)</th>
<th>Percentage of population expressing SPI-1</th>
<th>Filament formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL1344</td>
<td>512</td>
<td>0.03</td>
<td>0.25</td>
<td>4</td>
<td>47.5±5</td>
<td>100.2±8.9</td>
<td>35±4</td>
<td>13±3</td>
<td>14.12±4.2</td>
</tr>
<tr>
<td>SL1344 <em>lon::aph</em></td>
<td>256</td>
<td>0.007</td>
<td>0.06</td>
<td>1</td>
<td>92.5±9.5</td>
<td>340.3±12.2</td>
<td>36±5</td>
<td>12±4</td>
<td>70.52±3.7</td>
</tr>
</tbody>
</table>

CPZ, chlorpromazine; CIP, ciprofloxacin; NOR, norfloxacin; NAL, nalidixic acid.
transcription factor binds. An example is that of Mlc, which represses several glucose-related genes, including the phosphotransferase system (PTS) genes ptsH and ptsG. Induction of these genes by glucose occurs as a response to the flux of glucose through the PTS and involves the sequestration of Mlc to membranes containing dephosphorylated PtsG. Data arising from this study strongly indicate that the level of RamA is regulated by the Lon protease. Ultimate proof of the involvement of Lon protease in the degradation of RamA could be obtained by performing proteolytic assays.

The proteolysis of transcription factors, in a constitutive or regulated manner, plays a key role in controlling many regulatory networks and studies of the ATP-dependent Lon protease in different bacteria have shown its involvement in many biological processes. To date, the Lon protease has been shown to regulate two systems in Salmonella: the regulation of SPI-1 gene expression and the regulation of haem biosynthesis. We have now shown that the Lon protease in Salmonella also regulates the expression of RamA, and that it is required for the levels of RamA to be reset to basal level in the absence of induction.

The Lon protease is known to play an important role in protein quality control by degrading misfolded proteins; however, Lon also unfolds and degrades stably folded proteins that have accessible recognition tags. Studies on UmuD, SoxS and MarA have identified an N-terminal degradation tag as being essential for Lon proteolysis. In this study, we demonstrated that the N-terminal region of RamA consisting of the peptide sequence MTISAQVIDTIVQTMDDLNQ is important for Lon recognition and subsequent degradation of RamA.

The phenotype of the Salmonella Lon mutant also suggests that the Lon protease participates in some other pathways, as described for other bacterial species. For instance, the lack of effect of the inactivation of the lon gene upon biofilm formation in certain bacterial species; however, it has been shown that FlhD and FlhC in Salmonella Typhimurium are degraded by the CipX protease and not the Lon protease. The lack of an effect on motility may also explain the lack of effect of the inactivation of the lon gene upon biofilm formation as motility is known to be required for the initial stages of biofilm formation in certain bacterial species; however, it should be noted that Salmonella Typhimurium SL1344 is poor at forming biofilms due to a mutation in the mtrA and adrA genes, which are involved in cellulose production. Takaya et al. may reflect the use of different Salmonella strains and hence genotype and phenotype.

In summary, this study has revealed that there is a higher order of regulation of RamA and thus of the AcrAB-ToIC MDR efflux system. The involvement of the Lon protease in this process is further evidence that the expression of acrAB and toIC is under multilevel control and that there could be many ways to prevent the overproduction of AcrAB-ToIC and therefore prevent enhanced efflux and multidrug resistance.

Acknowledgements

We thank Steve Busby for helpful discussions and reading this manuscript, and Dr Andrew Bailey for technical assistance.

Funding

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Transparency declarations

None to declare.

References


2. van der Staa T, Mevius DJ. Lon negatively regulates SPI-1 expression as disruption of the lon gene in Salmonella Typhimurium markedly stimulated the invasion of human intestinal cells (INT-407), with a 10-fold increase in the invasion level of the lon mutant compared with that of the parental strain. Despite observing an increase in SPI-1 expression, we did not see an increased invasion of human intestinal cells (INT-407). One reason for this may be that SPI-1 is not the only determinant of invasion and inactivation of lon could alter invasion in other ways. The differences between our study and those of Takaya et al. may reflect the use of different Salmonella strains and hence genotype and phenotype.


9. Zheng J, Cui S, Meng J. Effect of transcriptional activators RamA and SoxS on expression of multidrug efflux pumps AcrAB and AcrEF in...
Lon regulates expression of RamA


