Overexpression of the *hslVU* operon suppresses SOS-mediated inhibition of cell division in *Escherichia coli*

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Abstract A multicopy clone was isolated which conferred resistance to the SOS inducer nitrofurantoin in an *Escherichia coli lon* mutant. Plasmid pHL1 was found to contain a 7–8 kbp *Hind*III DNA insert from a region of the chromosome at 88.5 minutes. Further characterisation of pHL1 revealed that resistance to nitrofurantoin was due to the overexpression of the *hslV-hslU* operon which encodes an ATP-dependent protease complex in *E. coli*. The overexpression of *hslVU* also conferred resistance to ultraviolet irradiation in the *lon* mutant. It is proposed that when overproduced, the HslV-HslU protease complex can degrade SulA which is an endogenous inhibitor of the essential cell division protein FtsZ. The ability of HslVU to degrade SulA in vivo suggests that Lon and HslVU may share a range of substrates. Furthermore, the suppression of *lon* could be used as a simple genetic test of proteolytic activity of cloned HslVU.
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Key words: HslV; HslU; Cell division; SulA; Lon

1. Introduction

In *Escherichia coli*, DNA damage induces the expression of several genes in a process referred to as the SOS response [1]. One of the SOS-inducible genes, *sfiA*, encodes a specific inhibitor of cell division which acts to delay division until DNA repair has taken place [2,3]. The *sfiA*-encoded SulA protein is rapidly degraded by the heat shock protease La (Lon), and hence mild mutagenic agents such as ultraviolet (UV) irradiation or nitrofurantoin are readily tolerated by *lon*+ strains of *E. coli* but are lethal to *lon*⁻ mutants due to irreversible inhibition of division which results from the action of a more stable SulA [4,5]. The target of the SulA inhibitor is FtsZ [6–8], an essential cell division protein [9,10] which is also a GTPase [11–13]. Genetic [14–16] as well as in vitro studies [17] have shown that SulA and FtsZ interact to form a complex, a process thought to require GTP [17]. In this paper, the possible involvement of other proteins, e.g. division proteins, in the interaction between FtsZ and SulA was investigated genetically by seeking multicopy clones that conferred resistance to nitrofurantoin in a *lon* mutant from a random plasmid library. One such clone, designated pHL1, was obtained. Further analysis of pHL1 revealed that it contained the heat shock operon *hslV-hslU* [18] within a larger DNA fragment which also included the essential cell division gene *ftsN* [19]. However, the resistance to nitrofurantoin is shown to be mediated by the HslV-HslU heat shock protease complex and not by FtsN. It is therefore likely that when overpro-

duced the HslV-HslU protease complex can degrade the division inhibitor SulA.

2. Materials and methods

2.1. Bacterial strains

All strain were derivatives of *E. coli* K-12. Strain SG20252 (obtained from M. Berlyn) is a *lon* mutant carrying the *lon-100* mutation which is cotransducible with the *zba-3000::*Tn*10* (Tet^r). W3110L(*lon-100*) was constructed by P1 transduction (P1 (SG20252)×W3110). Most of the Tet^r transductants were also sensitive to nitrofurantoin (2 μg ml^{-1}) on LB agar plates. One such transductant was designated W3110L. Strain DH5α (laboratory stock) was used for cloning following standard procedures [20].

2.2. Construction of plasmids

Fig. 1 shows cloned DNA inserts in the plasmids which were constructed in this study. Plasmid pHL1 was isolated as a multicopy suppresser of strain W3110L in the presence of nitrofurantoin (NF; 2 μg ml^{-1}). pHL2 was constructed by excising a ∼2.1 kbp *Eco*RI fragment from pHL1 followed by religation of the vector backbone. The ∼7.5 kbp *Hind*III insert in pHL1 was sub-cloned into pUC19 to give pHL4. pHL4 was restricted with *Sph*I and the backbone religated to yield pHL5. pHL4ΔEV was constructed by restriction of pHL4 with *Eco*RV followed with religation of the plasmid backbone.

The *hslVU* operon was amplified from plasmid pHL1 using primers W3559 (−157 5'-GCACCCTCAA<u>AAGCTT</u>GAAGATGGC-3' −133; base numbers are in reference to the start codon of *hslV*, and underlined is the recognition sequence for *Hind*III) and W3562 (+25 5'-CAATGAT<u>GAATTC</u>GATTGAACGCG-3' +2; numbers are in reference to the stop codon of *hslU*, and underlined is the recognition sequence for *Eco*RI). The *hslV* gene was amplified using primers W3559 and W3561 (+43 5'-CGCTGAC<u>GAATTC</u>GCGTGGGG-3' +23; base numbers are in reference to the stop codon of *hslV*, and underlined is the recognition sequence for *Eco*RI), and *hslU* was amplified using primers W3560 (−129 5'-TTAGAAAACACT<u>AAGCTT</u>AGCGCCCG-3' −104; base numbers are in reference to the start codon of *hslU*, and underlined is the recognition sequence for *Hind*III) and W3562. In each case, therefore, the *hslVU* operon, *hslV* or *hslU* genes were cloned as *Hind*III-*Eco*RI inserts into pUC19 such that these genes could be transcribed from the *lac* promoter in the vector. Plasmid pJF118VU was constructed by sub-cloning the *hslVU* operon as a *Hind*III-*Eco*RI DNA fragment from pUC19VU into the expression vector pJF118HE [21].

3. Results

3.1. Isolation of plasmid pHL1

In order to isolate multicopy suppressers of the sensitivity of a *lon* mutant to SOS-mediated inhibition of cell division, strain W3110L was transformed with two plasmid libraries (random clones of *Hind*III- or *Bam*HI-digested genomic DNA isolated from the prototroph W3110 and cloned into pBR325) which were a generous gift from M. Masters. Competent cells of strain W3110L were transformed with DNA from either library and transformants were selected on LB plates containing ampicillin (amp; 100 μg ml^{-1}) and NF (2 μg ml^{-1}). Plates were incubated at 37°C overnight. Five col-

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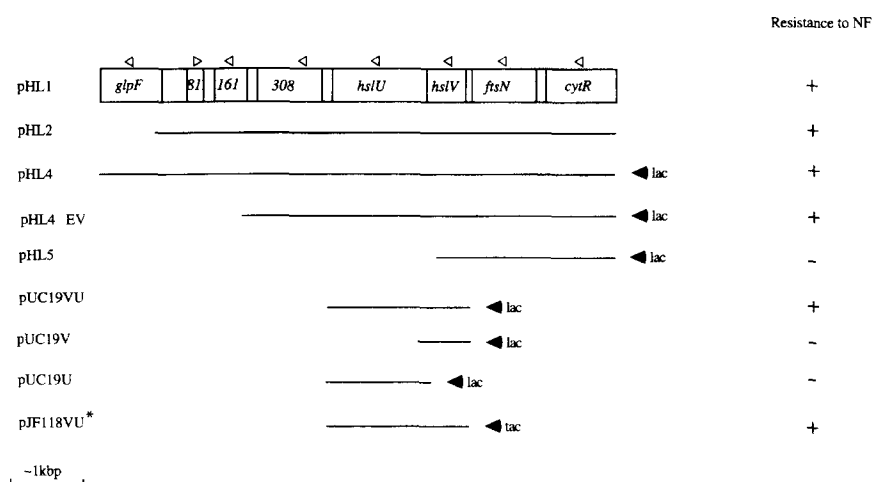E-mail: medhat@holyrood.ed.ac.uk

Resistance to NF

Fig. 1. Plasmid constructs and their ability to confer resistance to nitrofurantoin (NF) in strain W3110L (lon). Overnight cultures of plasmid-carrying strain W3110L were diluted and equal volumes were plated out on LB amp and LB NF agar plates such that 200–300 colonies were obtained on LB amp plates. Plating efficiency was calculated as the ratio between the number of colonies obtained on LB NF plate divided by the number of colonies obtained on the corresponding LB amp plate. + indicates plating efficiency of 1 (NF-resistant) and − indicates plating efficiency of less than 5×10^{-3} (NF-sensitive). Strain W3110L carrying either pUC19 or pBR325 was NF-sensitive (data not shown). * Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the medium to a final concentration of 1 mM.

onies were obtained using the HindIII library whereas only one colony was obtained using the BamHI library. To eliminate the possibility that NF resistance was the result of a chromosomal mutation, plasmid DNA was prepared from all six isolates and re-transformed into strain W3110L. In each case, the transformation mix was divided into two equal volumes; one volume was plated on LB agar containing ampicillin only whereas the other half was plated on LB containing both ampicillin and nitrofurantoin. In all cases, 200–500 transformants were obtained on LB amp plates but only in one case were transformants also obtained on LB plates containing both amp and NF. This clone was designated pHL1.

3.2. Overexpression of hslVU confers resistance to nitrofurantoin

A custom-made oligonucleotide was used to sequence the 5' end of the HindIII insert in plasmid pHL1. Comparison of the sequence to DNA sequences in databases revealed 100% identity with glpF which encodes glycerol facilitator protein [22]. Amongst the other open reading frames contained within the HindIII insert are ftsN which is an essential cell division gene [19] and the hslV-hslU operon encoding two heat shock proteins recently shown to form an ATP-dependent protease complex [23–29]. Several plasmids were constructed in order to determine which of the cloned gene(s) in pHL1 were responsible for the resistance to nitrofurantoin. Fig. 1 shows a diagram of the inserts and open reading frames in these plasmids and the plating efficiencies of strain W3110L carrying the various plasmids. Fig. 1 demonstrates that resistance to nitrofurantoin is due to the overexpression of the hslVU operon (plasmids pHL1, pHL4, pHL4ΔEV, pUC19VU and pJF118VU). Overexpression of only hslV (plasmid pUC19V) or only hslU (plasmid pUC19U) did not confer resistance to nitrofurantoin, neither did the overexpression of only ftsN. Plasmid pJF118VU was also found to suppress the sensitivity of strain W3110L to ultraviolet irradiation (data not shown). Furthermore, microscopic examination revealed that the over-

expression of hslVU suppressed the nitrofurantoin-induced inhibition of cell division (Fig. 2).

4. Discussion

The SulA protein of E. coli, encoded by the SOS-inducible gene sfiA, is an inhibitor of cell division. The target for SulA is the essential cell division protein FtsZ which is a GTPase that plays a critical role in the initiation of cell division [11–13,30]. The SOS response can be induced by treatment with UV or mild mutagens such as nitrofurantoin [1]. The ability of E. coli to survive SOS-mediated division inhibition depends on the rapid degradation of the SulA protein by the ATP-dependent heat shock protease La (Lon) which is encoded by the lon gene. In the absence of La (Lon), such as in the case of a lon mutant, SOS-mediated division inhibition becomes irreversible due to formation of a stable FtsZ-SulA complex, suggesting that SulA is the in vivo substrate for the La (Lon) protease [5].

The hslVU operon of E. coli encodes two heat shock proteins, HslV and HslU, which form an ATP-dependent protease complex in vitro where HslU is the ATPase component of the complex and HslV is a peptidase with homology to the 20S subunit of the eukaryotic proteasome [23–29]. In vivo, the overexpression of hslVU results in the general down-regulation of the heat shock response as well as overall increase in proteolysis of misfolded puromycylpolypeptides and hence resistance to puromycin [28]. In addition, inactivation of the chromosomal copy of hslU results in partial suppression of a dnaA46(Ts) mutation further suggesting the involvement of HslVU in the degradation of misfolded peptides [31]. However, until now no substrate of the HslVU protease has been identified in vivo, and the assay of activity of HslVU has relied mainly on in vitro proteolysis of synthetic peptides or casein by purified protease [23–25,27]. The findings presented here suggest that once overproduced the HslVU protease complex can degrade the SOS-inducible division inhibitor
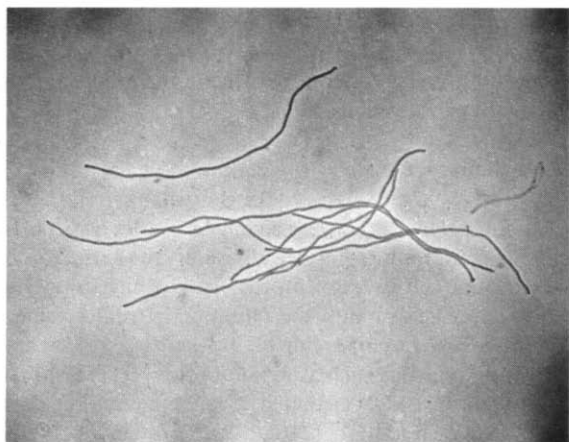
A

B

Fig. 2. Micrographs of cells of strain W3110L (*lon*) carrying either pJF118VU (A) or pJF118HE (B) grown in LB broth (containing 1 mM IPTG) for 3 h after the addition of nitrofurantoin (final concentration 2 µg ml^{-1}).

SulA in vivo. This activity requires the combined over-expression of *hslV* and *hslU* confirming that complex formation is required for proteolytic function in vivo [28] and further suggests that the degradation of SulA by HslVU complex is an inefficient process. In addition, since *lon*$^-$ *hslVU*$^+$ strains are sensitive to SOS-mediated inhibition of cell division it is unlikely that SulA is the primary substrate of HslVU although a role for HslVU in the turnover of SulA during normal growth cannot be excluded, especially since Lon-independent, ATP-dependent degradation of SulA in vivo has already been demonstrated [32]. Whilst *lon* and *hslVU* are independently dispensable in the cell it would be of interest to determine whether a *lon hslVU* double mutant is viable.

The identification of SulA as an in vivo substrate of HslVU allows for a simple and rapid genetic test for the activity of cloned HslVU. Mutations which affect the function of either proteins or their ability to form a complex could be easily isolated using this simple test.

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