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Effects of Antibiotics and a Proto-oncogene Homolog on Destruction of Protein Translocator SecY*

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Abstract

Protein secretion occurs via translocation by the evolutionarily-conserved Sec complex. LacZ hybrid proteins have long been used to study translocation in *Escherichia coli*. Some LacZ hybrids were thought to block secretion by physically jamming the Sec complex leading to cell death. We found that jammed Sec complexes caused the degradation of essential translocator components by the protease FtsH. Increasing the amounts or the stability of the membrane protein YccA, a known inhibitor of FtsH, counteracted this destruction. Antibiotics that inhibit translation elongation also jammed the translocator and caused degradation of translocator components, which likely contributes to their effectiveness. Intriguingly, YccA is a functional homologue of the proto-oncogene product Bax Inhibitor-1, which may share a similar mechanism of action in regulating apoptosis upon prolonged secretion stress.

Protein translocation is a fundamental process essential for the delivery of most extra-cytoplasmic proteins to their final destination. This process is mediated by an evolutionarily-conserved heterotrimeric membrane protein complex called the Sec61 complex (Sec61αβγ) in mammals and the Sec complex (SecY,E and G) in prokaryotes (1). In *E. coli*, two pathways target proteins to the Sec complex (2); the post-translational Sec pathway, which targets most outer membrane (OM) and periplasmic proteins (3), and the co-translational pathway, used primarily by inner membrane (IM) proteins, where the ribosome-nascent chain complex is targeted to the Sec complex by the signal recognition particle (SRP) (4). In both cases, proteins are initially directed to the SecY translocator via an amino-terminal signal sequence, which may or may not be cleaved upon translocation. The nature of this signal sequence determines which targeting pathway is used (2).

Genetic analysis of protein secretion is facilitated by *lacZ* (specifies beta-galactosidase) gene fusions (5,6). When the signal sequence of the OM protein LamB is fused to LacZ, the resulting hybrid protein is targeted for the post-translational translocation pathway (5). On induction with maltose, large amounts of hybrid protein are made and rapid folding of LacZ sequences in the cytoplasm causes a lethal jamming of the Sec complex (Fig. 1A), as evidenced by the accumulation in the cytoplasm of the precursor forms of wild-type secreted proteins (7). Under non-inducing conditions (in the absence of maltose) where low amounts of hybrid protein are made, lethal jamming does not occur, but because the hybrid protein is inefficiently secreted, some LacZ remains in the cytoplasm where it is active and strains

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carrying this fusion exhibit a Lac⁺ phenotype. When the signal sequence of this hybrid is changed to increase its hydrophobicity, the resulting H*LamB-LacZ hybrid is directed instead to the alternative co-translational SRP pathway (4). Since full-length LacZ is never exposed to the cytoplasm when secretion occurs co-translationally, the H*LamB-LacZ hybrid is translocated efficiently to the periplasm and jamming does not occur (Fig. 1B). In the oxidizing environment of the periplasm LacZ misfolds. Thus, under non-inducing conditions, strains carrying this fusion are Lac⁻. Under inducing conditions, toxic hybrid protein aggregates accumulate in the periplasm so even though no Sec complex jamming is observed, these strains are as maltose-sensitive as strains producing LamB-LacZ (4).

The Cpx two-component system regulates gene expression in response to misfolded proteins in the cell envelope. Dominant mutations, called *cpxA*^{*}, activate this system and suppress the inducer-sensitivity caused by periplasmic LacZ aggregates (8). Suppression of this toxicity was due solely to the induction of the periplasmic protease, DegP, by the Cpx system. Indeed, increasing production of DegP by cloning the structural gene on a multi-copy plasmid suppresses toxicity caused by H*LamB-LacZ (4). The protease degrades the toxic, periplasmic LacZ aggregates. Activation of the Cpx system also relieves the inducer-sensitive phenotype caused by LamB-LacZ (7). This was surprising because this hybrid protein exerts its toxic effects in the cytoplasm by jamming the Sec complex. Indeed, overproduction of DegP is necessary, but not sufficient to relieve the toxicity caused by LamB-LacZ (4). Activation of the Cpx system using *cpxA*^{*} alleles results in the efficient translocation of LamB-LacZ to the periplasm where it can then be degraded by DegP (7). This reduced LacZ activity in uninduced cells (Fig. 2). Apparently a Cpx-inducible gene product(s), other than DegP, facilitates translocation of LamB-LacZ.

To search for this Cpx-inducible factor(s) we utilized the uninduced Lac phenotype of LamB-LacZ strains. Under these conditions, artificially increasing the production of the relevant factor in fusion strains that are *cpxA*⁺ should increase the efficiency of LamB-LacZ secretion and thus decrease LacZ activity. Alternatively, inactivating the Cpx-regulated gene responsible for the efficient secretion of LamB-LacZ in a strain carrying the *cpxA*^{*} allele should increase LacZ activity. We began by testing genes regulated by Cpx (9).

The *yccA* gene is regulated in a Cpx-dependent fashion (9,10), and we have confirmed this by RT-PCR (Fig. S1) (11). Overexpression of *yccA* from an IPTG-inducible promoter with a construct integrated at the lambda attachment site in a *cpxA*⁺ fusion strain reduced LacZ activity comparable to that seen in *cpxA*^{*} fusion strains (Fig. 2) (11). Conversely, deletion of *yccA* in the *cpxA*^{*} fusion strain caused a modest increase in LacZ activity. The fact that *yccA*⁻ is not completely epistatic to *cpxA*^{*} indicates there are other Cpx-regulated factors that function to enhance translocation. Here, we focused on YccA, since its increased production is sufficient to relieve jamming.

When SecY is not in a complex with SecE and SecG, it is degraded by FtsH, an essential, ATP-dependent, membrane-bound protease (12). In a search for mutants in which SecY was stabilized, a mutant form of an IM protein, YccA, which lacks eight amino acids in the amino-terminus, was identified (13). This mutant protein, YccA11, binds to FtsH but unlike wild-type YccA, it is resistant to FtsH-mediated degradation (13). We replaced the chromosomal copy of *yccA* with *yccA11* in a *cpxA*⁺ *lamB-lacZ* fusion strain and found YccA11 was even more effective than over-expressed wild-type YccA at reducing LacZ activity (Fig. 2). Thus YccA, and even more so YccA11, can apparently stimulate secretion of the LamB-LacZ hybrid protein under non-inducing conditions.

It had long been thought that the toxicity caused by high-level production of LamB-LacZ was due to a physical jamming of the Sec complex (7,8). Our results suggested an

alternative hypothesis; perhaps the cell responds to “jamming” by promoting the energy-dependent degradation of SecY by FtsH. Translocator destruction would also cause the accumulation of the precursor forms of secreted proteins, and result ultimately in cell death. Induction caused the degradation of SecY in strains carrying LamB-LacZ (Fig. 3A) but not in strains carrying the LamB Δ 60-LacZ fusion in which the signal sequence is deleted (Fig. 3B) (11). Moreover, this degradation was prevented by the *cpxA*^{*} mutation (Fig. 3A). As noted above (7), this mutation prevents jamming, as evidenced by the lack of accumulation of the precursor form of MalE in induced *cpxA*^{*} *lamB-lacZ* fusion strains (Fig. 3C). Overproduction of YccA alone was sufficient to prevent jamming in the *lamB-lacZ cpxA*⁺ strain (Fig. 3C) and to prevent SecY destruction (Fig. 3B). The mutant YccA11 protein also stabilizes SecY (Fig. 3B). Thus, the toxicity associated with high-level production of LamB-LacZ is not entirely due to a physical jamming, but rather is due, at least in part, to proteolytic destruction of SecY.

YccA inhibits the protease FtsH (13). If this is the protease that degrades jammed SecY then mutations that inactivate FtsH should result in hybrid protein secretion into the periplasm even better than the *cpxA*^{*} mutations do. Although the *ftsH* gene is essential, we could test this prediction using a strain that carries *sfhC21* (14). This allele suppresses the lethality of *ftsH* null but by itself does not dramatically alter the LacZ phenotype (Fig. S2). Removing FtsH strongly reduced LacZ activity (Fig. 2) reflecting enhanced secretion of LamB-LacZ to the periplasm.

SRP-mediated co-translational secretion couples the processes of translation and translocation (Fig. 1) (2). Antibiotics that block translation elongation should produce proteins that are effectively fused to a ribosome by an unhydrolyzed, unreleased tRNA (15). We found that antibiotics that block translation elongation, such as chloramphenicol (Cm) and tetracycline (Tc), caused proteolytic destruction of SecY while antibiotics that affect other stages of gene expression, such as translation initiation (kasugamycin) or transcription (rifamycin) did not (Fig. 4) (11). Furthermore, destruction of SecY upon treatment with Cm was FtsH-dependent (Fig. 4C). We have also investigated the fate of SecE and SecG. SecE was also degraded in the presence of Cm, although more slowly than SecY, whereas SecG was not (Fig. 4C, D). Finally, in agreement with the notion that SRP-dependent substrates define the target for FtsH-mediated translocon destruction upon treatment with antibiotics that inhibit translation elongation, high-level production of PhoA, a protein that is secreted in SRP-independent fashion, increased the stability of SecY upon treatment with Cm (Fig. S3).

Our results reveal a new activity for several old antibiotics. Because the secretion of many proteins occurs in a co-translational fashion, agents that inhibit translation elongation will result in “jammed” translocators, and this will lead to proteolytic destruction of these translocators. The suicidal nature of SecY destruction reflects the fact that SecY is required to insert newly synthesized SecY in the membrane (16). This chicken and the egg-like problem may help explain why antibiotics that inhibit translation elongation exhibit bactericidal effects much sooner in some bacteria than in others (17,18). Perhaps the most susceptible bacteria have lower amounts of functional SecY.

We propose that *E. coli*, and perhaps all cells, have mechanisms that respond to SecY (or Sec61) translocators that are struggling with difficult substrates by degrading the stressed translocator. Clearly such degradative activities can have dire consequences, and thus cells also have mechanisms to limit this suicidal activity. In *E. coli*, this limiting activity is controlled, at least in part, by the Cpx envelope stress response and YccA.

YccA is homologous to human Bax Inhibitor-1 (BI-1), an anti-apoptotic protein that restrains the activity of the tumor-suppressor, Bax (19). BI-1 is so highly conserved that YccA from *E. coli* protects yeast cells against ectopically expressed human Bax (20). BI-1 is a proto-oncogene and is over-expressed in various types of cancer. Although Bax function is not fully understood, it contributes to cellular apoptosis caused by prolonged stress in the protein secretion pathway in eukaryotes (21), a pathway that involves the endoplasmic reticulum (ER) and is clearly related to the secretion stress caused by LacZ hybrids in bacteria. Moreover, Lisbona *et al.* (22) recently reported that BI-1 is a negative regulator of ER stress. Despite its obvious importance, the mechanism of action of BI-1 remains unknown, in part due to the inherent difficulty of studying the effects of Bax and its inhibitors in eukaryotic cells (20,23). Owing to the highly-conserved function of BI-1 and YccA, studies of the bacterial counterpart may shed light on the mechanistic role of this important cell death regulator in human cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References and notes

1. Rapoport TA. Nature 2007;450:663. [PubMed: 18046402]
2. Hegde RS, Bernstein HD. Trends Biochem Sci 2006;31:563. [PubMed: 16919958]
3. Veenendaal AK, van der Does C, Driessen AJ. Biochim Biophys Acta 2004;1694:81. [PubMed: 15546659]
4. Bowers CW, Lau F, Silhavy TJ. J Bacteriol 2003;185:5697. [PubMed: 13129940]
5. Silhavy TJ, Shuman HA, Beckwith J, Schwartz M. Proc Natl Acad Sci U S A 1977;74:5411. [PubMed: 414221]
6. Bassford P, Silhavy TJ, Beckwith J. J Bacteriol 1979;139:19. [PubMed: 110778]
7. Cosma CL, Danese PN, Carlson JH, Silhavy TJ, Snyder WB. Mol Microbiol 1995;18:491. [PubMed: 8748033]
8. Snyder WB, Davis LJ, Danese PN, Cosma CL, Silhavy TJ. J Bacteriol 1995;177:4216. [PubMed: 7635808]
9. Price NL, Raivio TL. J Bacteriol. December 19;2008
10. Yamamoto K, Ishihama A. Biosci Biotechnol Biochem 2006;70:1688. [PubMed: 16861804]
11. Materials and methods are available as supporting material on *Science* Online
12. Kihara A, Akiyama Y, Ito K. Proc Natl Acad Sci U S A 1995;92:4532. [PubMed: 7753838]
13. Kihara A, Akiyama Y, Ito K. J Mol Biol 1998;279:175. [PubMed: 9636708]
14. Ogura T, et al. Mol Microbiol 1999;31:833. [PubMed: 10048027]
15. Walsh, C. Antibiotics: Actions, Origins, Resistance. ASM Press; Washington, DC: 2003.
16. Akiyama Y, Ito K. J Biol Chem 1989;264:437. [PubMed: 2535843]
17. Feldman WE, Manning NS. Antimicrob Agents Chemother 1983;23:551. [PubMed: 6344785]
18. Rahal JJ JR, Simberkoff MS. Antimicrob Agents Chemother 1979;16:13. [PubMed: 38742]
19. Xu Q, Reed JC. Mol Cell 1998;1:337. [PubMed: 9660918]
20. Chae HJ, et al. Gene 2003;323:101. [PubMed: 14659883]
22. Smith MI, Deshmukh M. Cell Death Differ 2007;14:1011. [PubMed: 17218955]
22. Lisbona F, et al. Mol Cell 2009;33:679. [PubMed: 19328063]
23. Huckelhoven R. Apoptosis 2004;9:299. [PubMed: 15258461]
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Fig. 1. Protein translocation in *E. coli*

(A) Jamming of SecY occurs during post-translational translocation of the LamB-LacZ hybrid, resulting in accumulation of precursor proteins such as pMalE (in red) with their signal sequence (in green) still attached. (B) When the hybrid is targeted for co-translational translocation (H*LamB-LacZ), it is secreted into the periplasm. Properties of the LacZ fusion strains are summarized in the text box. (C) Co-translational translocation of a wild-type SRP substrate (purple). (D) During co-translational translocation, antibiotics that inhibit peptide chain elongation jam the translocator. Orange circles; translating ribosome, OM; outer membrane, IM; inner membrane, IMP; IM protein, P; periplasm.

Fig. 2. YccA affects translocation of LamB-LacZ

LacZ activity of *lamB-lacZ* strains assayed in the absence of maltose induction to determine the efficiency of translocation. Lane 1, Pop3186 (*cpxA⁺ yccA⁺ ftsH⁺*); 2, WBS226 (*cpxA^{*} yccA⁺ ftsH⁺*); 3, JVS724 (*cpxA⁺ yccA⁺ λatt pYccA ftsH⁺*); 4, JVS621 (*cpxA^{*} ΔyccA ftsH⁺*); 5, JVS716 (*cpxA⁺ yccA11 ftsH⁺*); 6, JVS679 (*cpxA⁺ yccA⁺ sfhC21 ΔftsH*). See Table S1 for complete genotypes. Activities are expressed as a per cent of wild type (WT). Error bars indicate standard deviation (n=3).

Fig. 3. Increased YccA stabilizes SecY and enables translocation of LamB-LacZ

(A) Immunoblot of SecY from strains carrying either wild-type *lamB* or the *lamB-lacZ* fusion and with either wild-type *cpxA* or the constitutive *cpxA^{*}* allele incubated in the presence (+) or absence (-) of maltose for 4 hours. (B) Immunoblot of SecY from strains incubated in the presence of maltose for 6 hours, of *cpxA⁺* strains carrying the *lamB-lacZ* fusion with (wt; Pop3186) and without (Δ ; BZR60) its signal sequence and expressing either endogenous levels of YccA (+), wild type *yccA* on a multi-copy plasmid (pYccA) or the protease-resistant form of YccA (*yccA11*) from the chromosomal gene. (C) Immunoblot of MalE from cultures incubated in the presence of maltose for 1.5 hours. The empty vector (pTrc99a) and the *yccA* over-expression plasmid (pYccA) were induced with 2 μ M IPTG 4 hours prior to and during induction with maltose.

Fig. 4. Antibiotic inhibition of translation elongation results in SecY degradation

(A) Immunoblot of SecY from cultures untreated ($t=0$) or incubated for the indicated times with 30 mg/L chloramphenicol (Cm), 10 mg/L tetracycline (Tc) or 100 mg/L rifampicin (Rif). (B) Immunoblot of SecY from cultures untreated (no) or incubated for the indicated times with 30 mg/L Cm, 100 mg/L Rif or 200mg/L kasugamycin (Ksg). (C) Normalized levels of SecY, SecE and SecG in an *ftsH*⁺ strain (Pop3186, stippled bars) and an *ftsH* null strain (JVS679, closed bars) as determined by immunoblotting in cultures incubated for 30 min. with Cm or Ksg. Values of untreated cultures have been set to 100%; each value is the mean of duplicate cultures and the range is indicated. (D) The levels of SecY (squares), SecE (triangles) and SecG (circles) were determined by immunoblotting in cultures incubated for the indicated times with Cm (solid lines) or Rif (dashed lines).