Brief Communication

Cell Chemical Biology

Apratoxin Kills Cells by Direct Blockade of the Sec61 Protein Translocation Channel

Highlights

- Mutations in Sec61α outline the apratoxin A binding site
- Apratoxin A competes with cotransin for Sec61α binding
- Apratoxin A inhibits translocation of a broad spectrum of Sec61 substrates
- Apratoxin A prevents pre-gating TMD-Sec61 interactions

Authors

Anja O. Paatero, Juho Kellosalo, Bryan M. Dunyak, ..., William H. Gerwick, Jack Taunton, Ville O. Paavilainen

Correspondence

ville.paavilainen@helsinki.fi

In Brief

Paatero et al. discovered that apratoxin directly targets the Sec61 protein translocation channel to prevent cotranslational biogenesis of secreted and membrane proteins. By acting on the Sec61 lateral gate, apratoxin blocks ER translocation of a broad spectrum of Sec61 substrates.

Paatero et al., 2016, Cell Chemical Biology *23*, 561–566
May 19, 2016 © 2016 Elsevier Ltd.
http://dx.doi.org/10.1016/j.chembiol.2016.04.008

CellPress

Please cite this article as: Paatero et al., Apratoxin Kills Cells by Direct Blockade of the Sec61 Protein Translocation Channel, Cell Chemical Biology (2016), http://dx.doi.org/10.1016/j.chembiol.2016.04.008

Cell Chemical Biology
Brief Communication

Apratoxin Kills Cells by Direct Blockade of the Sec61 Protein Translocation Channel

Anja O. Paatero,[1] Juho Kellosalo,[1] Bryan M. Dunyak,[3] Jehad Almaliti,[2] Jason E. Gestwicki,[3] William H. Gerwick,[2] Jack Taunton,[4] and Ville O. Paavilainen[1,*]
[1]Institute of Biotechnology, University of Helsinki, Viikinkaari 1, Biocenter 3, Helsinki 00014, Finland
[2]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093, USA
[3]Department of Pharmaceutical Chemistry, Institute for Neurodegenerative Disease, University of California at San Francisco, San Francisco, CA 94038, USA
[4]Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA 94158, USA
*Correspondence: ville.paavilainen@helsinki.fi
http://dx.doi.org/10.1016/j.chembiol.2016.04.008

SUMMARY

Apratoxin A is a cytotoxic natural product that prevents the biogenesis of secretory and membrane proteins. Biochemically, apratoxin A inhibits cotranslational translocation into the ER, but its cellular target and mechanism of action have remained controversial. Here, we demonstrate that apratoxin A prevents protein translocation by directly targeting Sec61α, the central subunit of the protein translocation channel. Mutagenesis and competitive photocrosslinking studies indicate that apratoxin A binds to the Sec61 lateral gate in a manner that differs from cotransin, a substrate-selective Sec61 inhibitor. In contrast to cotransin, apratoxin A does not exhibit a substrate-selective inhibitory mechanism, but blocks ER translocation of all tested Sec61 clients with similar potency. Our results suggest that multiple structurally unrelated natural products have evolved to target overlapping but non-identical binding sites on Sec61, thereby producing distinct biological outcomes.

INTRODUCTION

Modulating protein homeostasis with small molecules has emerged as a promising avenue to treat human disease (Balch et al., 2008). As such, identifying novel agents with the ability to remodel the disease proteome through diverse mechanisms is of key importance. Apratoxins comprise a class of marine natural products with potent cytotoxic effects toward many cancer cell lines. These hybrid peptide/polyketide macrocycles have demonstrated efficacy in preliminary xenograft models of colon adenocarcinoma (Chen et al., 2011; Tidgewell et al., 2010). However, understanding their mechanism of action has been hampered by the inability to unequivocally identify their cellular target, despite extensive effort.

Recently, apratoxin A ("apratoxin" hereafter; Figure 1A) was reported to inhibit the cotranslational translocation of secretory and membrane proteins into the ER (Liu et al., 2009). This multi-step process requires the signal recognition particle (SRP), the SRP receptor, and the Sec61 complex; it is facilitated by numerous accessory factors, including ER lumenal chaperones and the ER membrane proteins TRAM, TRAP complex, Sec62, and Sec63. Each of these proteins could potentially mediate the effects of apratoxin. However, another recent study found that apratoxin binding to Hsp70 paralogs promotes the degradation of a subset of Hsp90 clients by enhancing their interactions with Hsp70/Hsc70 (Shen et al., 2009). Interestingly, the ER luminal form of Hsp70, binding immunoglobulin protein (BiP), binds directly to Sec61 and Sec63. Extensive biochemical and genetic evidence has implicated BiP in protein translocation into the ER (Alder et al., 2005; Hamman et al., 1998; Schäuble et al., 2012; Tyedmers et al., 2000). Hence, apratoxin modulation of BiP activity could potentially reconcile the two apparently contradictory studies described above. In this work, we sought to resolve these discrepancies and identify the direct, physiologically relevant target of apratoxin.

RESULTS

Resistance Mutations Reveal Sec61α as the Target of Apratoxin

We first sought to test whether apratoxin can influence the activity of purified Hsp70 isoforms or their co-activator complexes. We tested the effect of apratoxin on J-domain-induced ATPase activity of human Hsc70 and BiP (Figure 1B). To our surprise, addition of apratoxin to concentrations as high as 100 μM did not reduce the ATPase rate of either Hsp70 isoform (Figure 1C). To examine the potential effects on the chaperone function of Hsp70, we tested the capacity of apratoxin to inhibit J-protein-mediated refolding of chemically denatured luciferase. Also in this experiment, we failed to observe any effect on Hsc70- or BiP-catalyzed luciferase refolding at apratoxin concentrations up to 1 μM (Figures 1D and 1E). These data suggested that apratoxin-mediated downregulation of newly synthesized proteins is likely caused by modulation of a target other than Hsc70 or BiP.

We next turned to a genetic selection approach to identify the target of apratoxin (Wacker et al., 2012). We exposed HCT-116

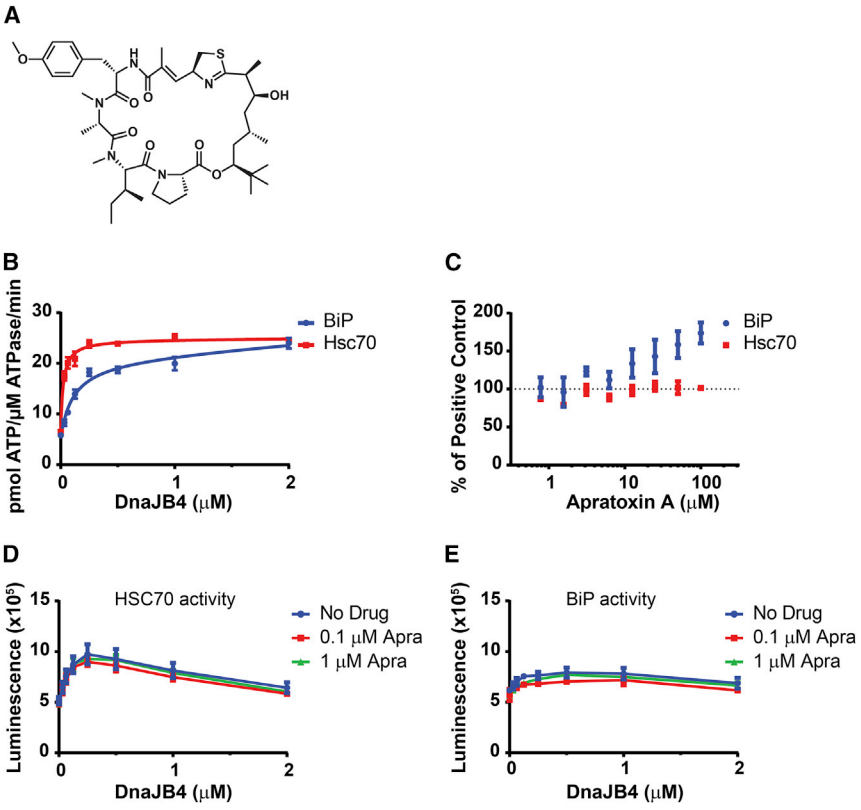

Figure 1. Apratoxin Does Not Target Hsp70 In Vitro

(A) Structure of apratoxin A.

(B) The human J protein DnaJB4 stimulates ATP turnover by the Hsp70 molecular chaperones, Hsc70 (*HSPA8*) or BiP (*HSPA5*), as determined by the colorimetric malachite green assay.

(C) Apratoxin A does not inhibit the J-stimulated ATPase activity of Hsc70 or BiP. Hsc70 or BiP (5 μM) were stimulated by subsaturating concentrations of J protein (0.1 μM DnaJB4) to optimize sensitivity to inhibitors.

(D) Incubation of apratoxin with DnaJB4 plus Hsc70 has a negligible effect on the J-mediated refolding of chemically denatured firefly luciferase by Hsp70 molecular chaperones.

(E) As in (D), except BiP was used instead of Hsc70. The data represent the mean ± SEM, n = 3.

colon cancer cells to 10–30 nM apratoxin (IC_{50} ~ 0.2 nM, Figure 2A) for 9 days. During this time most of the cells died, but six colonies grew and were isolated. Cell lines derived from these colonies proved highly resistant (up to 600-fold desensitization) to apratoxin (Figure 2A), whereas sensitivity to three other cytotoxic drugs was not significantly altered (Figure S1). Sequencing the coding regions of *HSPA8* (encodes Hsc70), *HSPA5* (BiP), and *S61A1* (Sec61α) revealed that all apratoxin-resistant clones contained heterozygous mutations in *S61A1*, with no mutations identified in *HSPA8* or *HSPA5*. Single-nucleotide transitions in *S61A1* resulted in two amino acid alterations, Thr86Met (T86M) and Tyr131His (Y131H). In a human Sec61α homology model, these mutations are located on transmembrane segment 2 (TM-2) in the vicinity of the plug domain (T86M) and at the lumenal tip of TM-3 (Y131H) also near the plug (Figure 2B). Interestingly, mutations near this region of Sec61α have been shown to cause resistance to two structurally unrelated Sec61 inhibitors, cotransin and decatransin (Figure S2A), suggesting that all three compounds may have overlapping binding sites on Sec61α (Junne et al., 2015; MacKinnon et al., 2014). This prompted us to test whether mutations previously shown to confer resistance to cotransin (MacKinnon et al., 2014) would also impart resistance to apratoxin, and vice versa. Indeed we found varying degrees of cross-resistance (Figures S2B and S2C). Notably, the apratoxin-resistant mutants identified in this study (135- to 600-fold shift in potency) are much less resistant to CT8 (1.5- to 10-fold shift) (Figure S2B). Hence, while apratoxin and CT8 likely share overlapping binding sites, each inhibitor forms distinct interactions with Sec61.

To ensure that the identified mutations are sufficient to confer resistance to apratoxin, we generated stable HEK293 cell lines that express wild-type or mutant Sec61α constructs. Cells expressing wild-type Sec61α were sensitive to apratoxin (IC_{50} ~ 13 nM, Figure 2C), whereas cells expressing Sec61α mutants were highly desensitized (IC_{50} 1,000 nM for T86M and 250 nM for Y131H, Figure 2C). These results indicate that the Sec61α mutants assemble into functional translocons and confer significant levels of apratoxin resistance, despite the presence of endogenous wild-type Sec61α. The proximity of the identified mutations to sites that were previously implicated in cotransin binding (Figure S2A) raised the possibility that apratoxin and cotransin may bind Sec61α competitively. To test this, we used a previously described cotransin photoaffinity probe CT7, an isosteric and equipotent analog of the highly substrate-selective cotransin CT8 (MacKinnon et al., 2007). Photo-crosslinking of CT7 to Sec61α in ER microsomes was blocked by addition of excess apratoxin (Figure 2D). Importantly, apratoxin competes for CT7 binding to Sec61α at least as efficiently as the potent cotransin analog CT9. Collectively, our data suggest that apratoxin and cotransins bind in a mutually exclusive manner to a site near the lumenal plug of Sec61.

Apratoxin Prevents Formation of an Early Pre-gating Intermediate

To dissect the biochemical mechanism by which apratoxin inhibits co-translational translocation, we used a reconstituted mammalian translation system supplemented with ER microsomes (Sharma et al., 2010). As reported earlier (Liu et al., 2009), apratoxin had no effect on translation. However, apratoxin potently blocked cotranslational translocation of the secreted proteins preprolactin, BiP, and human hepatocyte growth factor (HGF) (Figure 3A). In addition, apratoxin inhibited cotranslational membrane insertion of type I (interleukin-7 receptor [IL7R]), type II (tumor necrosis factor α [TNFα]), and polytopic (mucolipin-1 [MCOLN]) membrane proteins (Figures 3B and 3C). Apratoxin

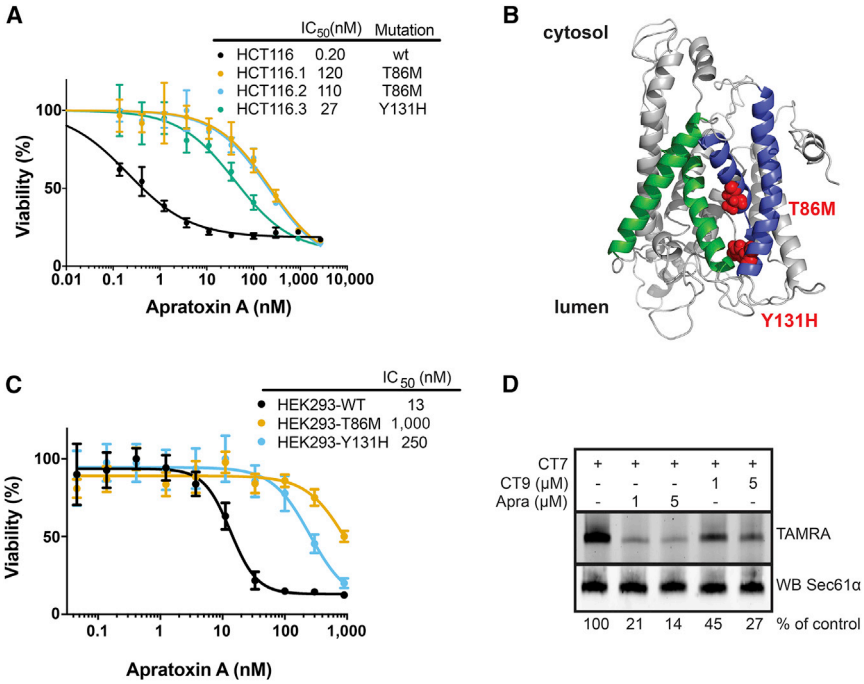

Figure 2. Apratoxin Targets Lumenal End of Sec61α

(A) Parental HCT-116 cells and resistant clones were treated with increasing concentrations of apratoxin for 72 h, and viability was assessed by the Alamar Blue assay (mean ± SD, n = 4).

(B) Homology model of human Sec61α showing the location of apratoxin resistance mutations (red). Lateral gate helices are colored blue (TM2/3) and green (TM7/8).

(C) HEK293-FRT cells stably expressing wild-type (WT) or mutant Sec61α were assayed as in (A).

(D) CT7 photo-crosslinking with ER microsomes. Microsomes were incubated with 100 nM CT7 in the presence or absence of excess CT9 or apratoxin, photolyzed, and the photo-crosslinked CT7/Sec61α adduct was detected by click chemistry with TAMRA-azide, followed by in-gel fluorescence imaging.

See also Figures S1 and S2.

inhibited all of these proteins with similar potency (IC_{50} 10–100 nM), suggesting that unlike CT8, apratoxin does not discriminate between different Sec61 clients.

We next sought to identify the apratoxin-sensitive stage of the translocation process. For this purpose, we assembled ribosome-nascent chain complexes (RNCs) programmed with mRNA encoding the first 126 residues of human TNFα (either FLAG- or hemagglutinin [HA]-tagged) and lacking a stop codon (126-mers). After translating until the end of the mRNA the ribosome stalls, creating a synchronized population of Sec61-targeted RNCs. Following detergent solubilization, the nascent chains were immunopurified with anti-FLAG beads and analyzed for the presence of Sec61α. Absence of Sec61α in the sample programmed with HA-TNFα indicates that Sec61α specifically co-purifies with stalled TNFα RNCs (Figure 4A). Moreover, the amount of co-purified Sec61α was not affected by apratoxin, demonstrating that it does not affect SRP-dependent targeting to the Sec61 complex (Figure 4A).

Prior to membrane insertion, the sole transmembrane domain (TMD) of TNFα specifically docks to the cytosolic face of the Sec61 lateral gate, an interaction that is stabilized by cotransin binding (MacKinnon et al., 2014). To test whether apratoxin has a similar stabilizing effect, we prepared ER-targeted TNFα 96-mers in the presence of saturating concentrations of either apratoxin or CT8 and assessed TNFα-Sec61 interactions by cysteine crosslinking. As expected, CT8 allowed crosslinking between a cysteine in the TNFα TMD and a cysteine in Sec61α (Figures 4B and S3), previously identified as Cys13. In the presence of apratoxin, this crosslink was conspicuously absent, suggesting that apratoxin prevents the TNFα TMD from adopting the docked configuration at the cytosolic tip of the Sec61 lateral gate.

Since cotransin arrests the TNFα TMD helix in a defined orientation in the Sec61α cytosolic vestibule (MacKinnon et al., 2014), we sought to test whether apratoxin stabilizes the TMD in the same location, but in a slightly altered conformation. For this purpose, we tested a series of TNFα 96-mer constructs with a single cysteine engineered at different positions. As previously reported (MacKinnon et al., 2014), Sec61α crosslinks were observed with a peak intensity at around TNFα position 50, indicating a preferred orientation of the CT8-stabilized TNFα TMD helix (Figures 4C and 4D). By contrast, apratoxin prevented the formation of significant TMD crosslinks to Sec61α, regardless of where the cysteine was introduced (Figures 4C and 4D). Importantly, both CT8 and apratoxin promoted TMD crosslinking to the single cytosolic cysteine of Sec61β (Figures 4C and 4D), consistent with successful targeting of the TNFα nascent chain to the cytosolic face of Sec61 (Figure 4A). The fact that TMD crosslinks to Sec61α are observed in the presence of CT8, but not apratoxin, suggests that apratoxin blocks translocation at an earlier stage than CT8, before the TMD inserts into the cytosolic vestibule of Sec61α. Nevertheless, it remains possible that apratoxin does not entirely prevent the TMD from docking to Sec61α (Figure 4E), but might instead stabilize it within the cytosolic vestibule in a distinct conformation that precludes chemical crosslinking at any position along the helix.

DISCUSSION

Thus far, most studies on the potent cytotoxic natural product apratoxin have focused on its cellular effects. Its ability to inhibit growth of many cancer cell lines has been suggested to result from inhibition of secretory protein biogenesis and the degradation of Hsp90 client proteins (Liu et al., 2009; Shen et al., 2009). However, lack of knowledge of apratoxin's molecular target precludes deep insight into its anti-cancer effects and in vivo toxicity (Chen et al., 2014). Using a combination of cellular and biochemical approaches, we have revealed Sec61 as the cellular target of apratoxin and have dissected the biochemical mechanism by which it inhibits cotranslational translocation into the ER. Our data reveal that apratoxin interacts with Sec61 through a site

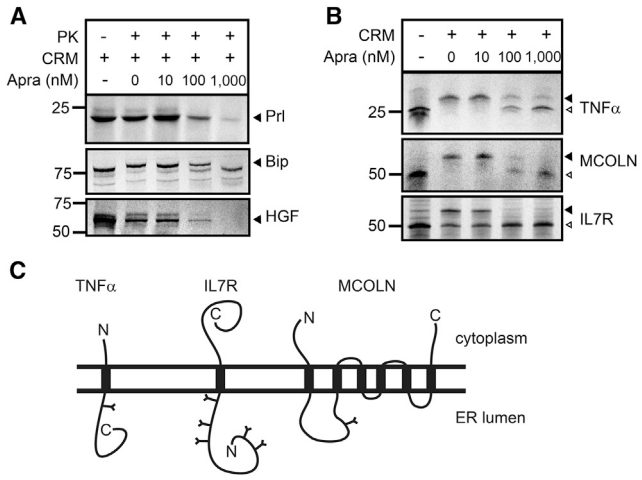

Figure 3. Apratoxin Is a Broad-Spectrum Sec61 Inhibitor

(A) In vitro translocation (IVT) assays for secreted proteins analyzed by protease protection. Full-length bovine prolactin (Prl), hamster binding immunoglobulin protein (Bip), and human hepatocyte growth factor (HGF) were translated in the presence of canine rough microsomes, ^{35}S-methionine, and increasing concentrations of apratoxin. ER translocation was assessed by treatment with proteinase K (PK). It should be noted that BiP is largely protease resistant and upon PK treatment forms shorter fragments. The protease-protected species are indicated by a closed triangle.

(B) IVT of integral membrane proteins analyzed without PK treatment. Apratoxin inhibits membrane insertion of human tumor necrosis factor α (TNFα), human mucolipin 1 (MCOLN), and human interleukin-7 receptor (IL7R). Correctly integrated glycosylated proteins and non-translocated unglycosylated species are indicated (closed and open triangles, respectively). The TNFα construct used contains an engineered glycosylation site (I134N) in its C-terminal ectodomain. CRM, canine rough microsomes; PK, proteinase K.

(C) Schematic of membrane topologies of the integral membrane proteins assayed in (B). The putative glycosylation sites of the model proteins are indicated.

that at least partially overlaps with two well-characterized Sec61 inhibitors, cotransin and decatransin (Junne et al., 2015; MacKinnon et al., 2014). As many natural product binding hotspots also mediate important physiological interactions (Villar et al., 2014), it is conceivable that the Sec61 lumenal cavity may also be targeted by physiological factors that influence Sec61-mediated secretory protein biogenesis. This work provides mechanistic insight into differences between substrate-selective (cotransin) and substrate-nonselective (apratoxin) Sec61 inhibitors in influencing secretory protein biogenesis. By interfering with membrane integration at a stage preceding nascent chain docking onto the Sec61 lateral gate (MacKinnon et al., 2014; Plath et al., 1998), apratoxin prevents an early integration step that is likely shared by all Sec61 substrates. Conversely, cotransin perturbs a later transition along the integration pathway that only a limited number of Sec61 substrates may depend on. Further structural studies are needed to ultimately resolve how inhibitors with varying degrees of substrate selectivity engage the Sec61 channel.

The fact that Sec61 modulators inhibit protein translocation through distinct mechanisms suggests they may have evolved to modulate target-cell physiology for distinct purposes. Indiscriminate translocation blockers (apratoxin, decatransin) inhibit proliferation of a wide variety of target cells whereas substrate-selective modulators (HUN-7293/cotransin) may facilitate parasitic or symbiotic interactions between the producing fungus and a target organism. Interestingly, several additional small-molecule inhibitors of co-translational translocation (Eeyarestatin I, mycolactone, CADA) have recently been described (Cross et al., 2009; Hall et al., 2014; Vermeire et al., 2014). Hence, it will be interesting to determine whether these other inhibitors also directly target Sec61 or act on other important translocon components.

SIGNIFICANCE

Prior to this work, apratoxins had been shown to exhibit potent cytotoxicity against many cancer cell lines, likely due to their ability to prevent protein translocation into the ER. However, inability to unequivocally identify the cellular target of apratoxin has prevented insight into its mechanism of action. We made the discovery that apratoxin directly targets Sec61α, the central subunit of the Sec61 protein translocation channel. By acting on the lumenal end of the Sec61 lateral gate, apratoxin blocks biogenesis of a broad range of Sec61 clients. Surprisingly, the interaction site of apratoxin overlaps with those of two unrelated Sec61 inhibitors. Our work defines a natural product binding hotspot on Sec61α that different natural products have evolved to target in order to modulate target-cell physiology for distinct purposes. In the future, it may be possible to discover new lead molecules that interact with the Sec61 lumenal hotspot to facilitate substrate-selective modulation of Sec61 substrate protein biogenesis.

EXPERIMENTAL PROCEDURES

Reagents

Apratoxin was produced and purified as described by Gutiérrez et al. (2008). See Supplemental Experimental Procedures for other reagents.

Cell-Free Translation/Translocation Assays

Cell-free transcription, translation, translocation, and proteinase K protection assays were performed as previously described (Sharma et al., 2010). In brief, DNA templates were transcribed and used immediately in the subsequent translation/translocation reactions, which were assembled at 0°C in the presence of apratoxin or CTs, ^{35}S-methionine (PerkinElmer), and canine pancreatic microsomes (CRMs). Translation was carried out at 32°C for 30–60 min. In addition, FLAG-tagged, stalled intermediates were immunoprecipitated. See Supplemental Experimental Procedures for details.

Bis-Maleimide Crosslinking

Bis-maleimide crosslinking was performed with bis-maleimidohexane (Pierce) as previously described (MacKinnon et al., 2014), with the following modifications: CRMs were obtained from Promega, membranes were sedimented for 10 min at 49,000 rpm at 4°C in an S100-AT3 rotor (Thermo Scientific), and the crosslinking reactions were trichloroacetic acid-precipitated prior to SDS-PAGE. CT8-treated reactions were immunoprecipitated for confirming protein identities.

Photoaffinity Labeling

CRMs containing 100 nM Sec61 were treated with either 1 or 5 μM CT9, apratoxin, or DMSO for 30 min at 0°C, followed by incubation with 100 nM CT7 for 10 min. Samples were then photolyzed, and crosslinked proteins were detected by click chemistry, SDS-PAGE, and in-gel fluorescent scanning as previously described (MacKinnon et al., 2007; MacKinnon and Taunton, 2009).

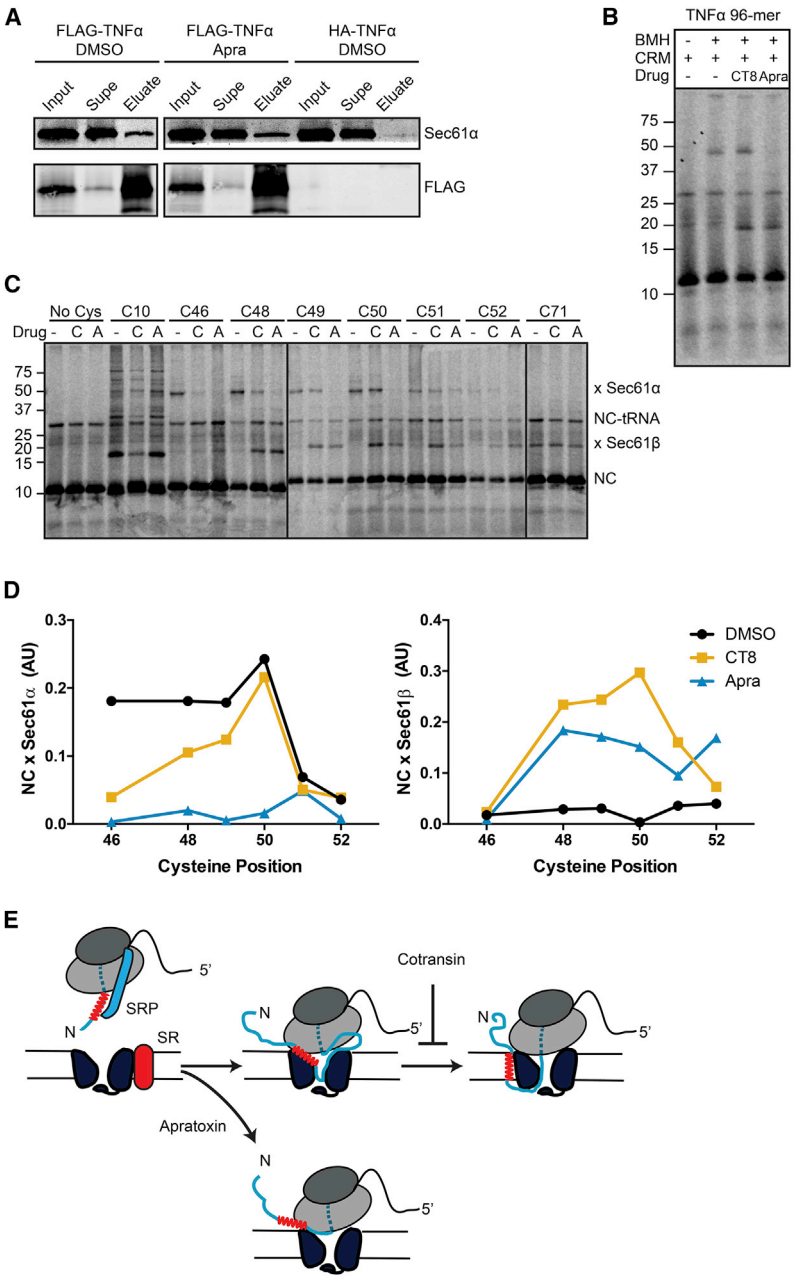

Figure 4. Apratoxin Prevents Formation of an Early Pre-gating Intermediate

(A) TNFα 126-mers containing an N-terminal FLAG- or HA-tag were translated in the presence or absence of 1 μM apratoxin and CRMs, solubilized with digitonin, and immunoprecipitated with anti-FLAG affinity resin. Immunoprecipitation eluates were analyzed by immunoblotting for FLAG-TNFα and Sec61α.

(B) Autoradiograph showing microsome-targeted TNFα C30A 96-mers assembled in the presence or absence of CT8 or apratoxin (1 μM), isolated, and treated with bis-maleimidohexane (BMH) as indicated. Bands corresponding to the nascent chain (NC) and the NC crosslinked to Sec61α and Sec61β are indicated (for immunoprecipitation confirmation, see Figure S3). Residual NC-tRNA is also indicated.

(C) BMH crosslinking reactions of TNFα 96-mers containing a single cysteine at indicated positions in the TMD. The intensities of NC crosslinked to Sec61α were quantified from the gels shown.

(D) Nascent chain crosslinking intensities for Sec61α or Sec61β were quantified from phosphorimaging data in (C) and plotted as a function of cysteine position.

(E) Model for apratoxin-mediated inhibition of translocation. Apratoxin does not affect RNC targeting to Sec61, but instead prevents the TMD from docking onto the Sec61 lateral gate.

See also Figure S3.

Cell-Culture and Cell-Viability Assays

Cell-viability assays were performed by plating 2,500 cells in black 96-well plates with clear, flat bottoms, and treating with CT8 or apratoxin the following day. After 72 h, cell viability was analyzed using the Alamar Blue assay (Life Technologies) according to the manufacturer's instructions. See Supplemental Experimental Procedures for details.

Selection of Resistant Clones, RNA Purification, RT-PCR, and Sequencing

For derivation of resistant cell lines, HCT-116 cells were incubated with 10–30 nM apratoxin for 9 days, after which cell colonies were isolated by ring cloning and cultured in drug-free media. Total RNA was isolated from HCT116 cells using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Total cDNA was synthesized using anchored oligo(dT) primers and Superscript III reverse transcriptase (Invitrogen), and different cDNAs were amplified with Phusion polymerase (Thermo Fisher Scientific) and sequenced bidirectionally by Sanger sequencing.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.chembiol.2016.04.008.

AUTHOR CONTRIBUTIONS

A.O.P., J.T., B.M.D., J.E.G., and V.O.P. designed the experiments. A.O.P., J.K., B.M.D., J.A., and V.O.P. conducted the experiments. A.O.P., B.M.D., J.A., J.E.G., W.H.G., J.T., and V.O.P. analyzed and interpreted the data. A.O.P., J.T., and V.O.P. drafted or revised the article.

ACKNOWLEDGMENTS

This work was supported by the Academy of Finland (grant 289737), Sigrid Juselius Foundation and Biocentrum Helsinki (V.O.P.), the US NIH (NS059690 to J.E.G., CA100851 to W.H.G.), and Howard Hughes Medical Institute (J.T.). We thank Dr. Ramanujan Hegde (MRC Laboratory of Molecular Biology, Cambridge, UK) for providing translocation reagents. Dr. Marja Lohela is acknowledged for critical comments on the manuscript.

Received: October 9, 2015
Revised: April 4, 2016
Accepted: April 7, 2016
Published: May 19, 2016

REFERENCES

Alder, N.N., Shen, Y., Brodsky, J.L., Hendershot, L.M., and Johnson, A.E. (2005). The molecular mechanisms underlying BiP-mediated gating of the Sec61 translocon of the endoplasmic reticulum. J. Cell Biol. *168*, 389–399.

Balch, W.E., Morimoto, R.I., Dillin, A., and Kelly, J.W. (2008). Adapting proteostasis for disease intervention. Science *319*, 916–919.

Chen, Q.-Y., Liu, Y., and Luesch, H. (2011). Systematic chemical mutagenesis identifies a potent novel apratoxin A/E hybrid with improved in vivo antitumor activity. ACS Med. Chem. Lett. *2*, 861–865.

Chen, Q.-Y., Liu, Y., Cai, W., and Luesch, H. (2014). Improved total synthesis and biological evaluation of potent apratoxin S4 based anticancer agents with differential stability and further enhanced activity. J. Med. Chem. *57*, 3011–3029.

Cross, B.C.S., McKibbin, C., Callan, A.C., Roboti, P., Piacenti, M., Rabu, C., Wilson, C.M., Whitehead, R., Flitsch, S.L., Pool, M.R., et al. (2009). Eeyarestatin I inhibits Sec61-mediated protein translocation at the endoplasmic reticulum. J. Cell. Sci. *122*, 4393–4400.

Gutiérrez, M., Suyama, T.L., Engene, N., Wingerd, J.S., Matainaho, T., and Gerwick, W.H. (2008). Apratoxin D, a potent cytotoxic cyclodepsipeptide from Papua New Guinea collections of the marine cyanobacteria *Lyngbya majuscula* and *Lyngbya sordida*. J. Nat. Prod. *71*, 1099–1103.

Hall, B.S., Hill, K., McKenna, M., Ogbechi, J., High, S., Willis, A.E., and Simmonds, R.E. (2014). The pathogenic mechanism of the *Mycobacterium ulcerans* virulence factor, mycolactone, depends on blockade of protein translocation into the ER. PLoS Pathog. *10*, e1004061.

Hamman, B.D., Hendershot, L.M., and Johnson, A.E. (1998). BiP maintains the permeability barrier of the ER membrane by sealing the lumenal end of the translocon pore before and early in translocation. Cell *92*, 747–758.

Junne, T., Wong, J., Studer, C., Aust, T., Bauer, B.W., Beibel, M., Bhullar, B., Bruccoleri, R., Eichenberger, J., Estoppey, D., et al. (2015). Decatransin, a new natural product inhibiting protein translocation at the Sec61/SecYEG translocon. J. Cell. Sci. *128*, 1217–1229.

Liu, Y., Law, B.K., and Luesch, H. (2009). Apratoxin A reversibly inhibits the secretory pathway by preventing cotranslational translocation. Mol. Pharmacol. *76*, 91–104.

MacKinnon, A.L., and Taunton, J. (2009). Target Identification by Diazirine Photo-Cross-Linking and Click Chemistry (Hoboken, NJ: John Wiley & Sons, Inc).

MacKinnon, A.L., Garrison, J.L., Hegde, R.S., and Taunton, J. (2007). Photoleucine incorporation reveals the target of a cyclodepsipeptide inhibitor of cotranslational translocation. J. Am. Chem. Soc. *129*, 14560–14561.

MacKinnon, A.L., Paavilainen, V.O., Sharma, A., Hegde, R.S., and Taunton, J. (2014). An allosteric Sec61 inhibitor traps nascent transmembrane helices at the lateral gate. Elife *3*, e01483.

Plath, K., Mothes, W., Wilkinson, B.M., Stirling, C.J., and Rapoport, T.A. (1998). Signal sequence recognition in posttranslational protein transport across the yeast ER membrane. Cell *94*, 795–807.

Schäuble, N., Lang, S., Jung, M., Cappel, S., Schorr, S., Ulucan, Ö., Linxweiler, J., Dudek, J., Blum, R., Helms, V., et al. (2012). BiP-mediated closing of the Sec61 channel limits Ca^{2+} leakage from the ER. EMBO J. *31*, 3282–3296.

Sharma, A., Mariappan, M., Appathurai, S., and Hegde, R.S. (2010). In vitro dissection of protein translocation into the mammalian endoplasmic reticulum. Methods Mol. Biol. *619*, 339–363.

Shen, S., Zhang, P., Lovchik, M.A., Li, Y., Tang, L., Chen, Z., Zeng, R., Ma, D., Yuan, J., and Yu, Q. (2009). Cyclodepsipeptide toxin promotes the degradation of Hsp90 client proteins through chaperone-mediated autophagy. J. Cell Biol. *185*, 629–639.

Tidgewell, K., Engene, N., Byrum, T., Media, J., Doi, T., Valeriote, F.A., and Gerwick, W.H. (2010). Evolved diversification of a modular natural product pathway: apratoxins F and G, two cytotoxic cyclic depsipeptides from a Palmyra collection of *Lyngbya bouillonii*. Chembiochem *11*, 1458–1466.

Tyedmers, J., Lerner, M., Bies, C., Dudek, J., Skowronek, M.H., Haas, I.G., Heim, N., Nastainczyk, W., Volkmer, J., and Zimmermann, R. (2000). Homologs of the yeast Sec complex subunits Sec62p and Sec63p are abundant proteins in dog pancreas microsomes. Proc. Natl. Acad. Sci. USA *97*, 7214–7219.

Vermeire, K., Bell, T.W., Van Puyenbroeck, V., Giraut, A., Noppen, S., Liekens, S., Schols, D., Hartmann, E., Kalies, K.-U., and Marsh, M. (2014). Signal peptide-binding drug as a selective inhibitor of co-translational protein translocation. PLoS Biol. *12*, e1002011.

Villar, E.A., Beglov, D., Chennamadhavuni, S., Porco, J.A., Jr., Kozakov, D., Vajda, S., and Whitty, A. (2014). How proteins bind macrocycles. Nat. Chem. Biol. *10*, 723–731.

Wacker, S.A., Houghtaling, B.R., Elemento, O., and Kapoor, T.M. (2012). Using transcriptome sequencing to identify mechanisms of drug action and resistance. Nat. Chem. Biol. *8*, 235–237.