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Digest

The signal peptide as a new target for drug design

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ABSTRACT

Many current and potential drug targets are membrane-bound or secreted proteins that are expressed and transported via the Sec61 secretory pathway. They are targeted to translocon channels across the membrane of the endoplasmic reticulum (ER) by signal peptides (SPs), which are temporary structures on the *N*-termini of their nascent chains. During translation, such proteins enter the lumen and membrane of the ER by a process known as co-translational translocation. Small molecules have been found that interfere with this process, decreasing protein expression by recognizing the unique structures of the SPs of particular proteins. The SP may thus become a validated target for designing drugs for numerous disorders, including certain hereditary diseases.

Introduction

Most drugs used today target proteins, including receptors, enzymes, and transport proteins.¹ Traditional small-molecule drugs target a binding site and modify protein function, generally acting as agonists or antagonists.^{1–3} A modern alternative to this approach is to modify target protein concentration, for example by reducing translation with complementary RNA sequences,⁴ or by activating protein degradation pathways.⁵ We believe that a wide variety of current and future protein targets can be down-modulated by a mechanism that previously has not been recognized by most medicinal chemists: inhibition of co-translational translocation across the membrane of the endoplasmic reticulum (ER).

Most cell-surface and secreted human proteins, produced by more than 40% of the ~20,000 protein-encoding genes,⁶ are transported to the cell membrane by the Sec61 secretory pathway,^{7–15} which is highly conserved in eukaryotes, all the way back the evolutionary chain to bacteria.^{7–9} Such proteins in humans are translated by ribosomes on the surface of the ER, then transported to the cell membrane via the Golgi apparatus. In mammals, proteins containing more than 100 amino acids are generally targeted to the ER membrane during translation and enter the interior (lumen) of the ER by crossing its membrane during elongation, a process known as co-translational translocation.¹⁶ Successful translocation of these proteins is important for proper function of cells and defects in protein translocation have been linked to many diseases, including cancer and numerous hereditary diseases.^{14,17} Drugs

targeting the nascent protein chain during co-translational translocation can potentially reduce expression of many cell-surface and secretory proteins that are important therapeutic targets, such as type I and type II transmembrane receptors, G-protein coupled receptors (GPCRs), inflammatory cytokines, etc. In addition to drugs for treating human conditions arising from protein overexpression or malfunction, there are potential opportunities for new anti-infective drugs. Many viral proteins, such as the hepatitis C polyprotein¹⁸ and host proteins required for the life cycles of viruses, use the Sec61 pathway, and some bacterial toxins and pore-forming proteins are expressed by a similar process.⁹

Mechanism of co-translational translocation across the ER membrane

Except for smaller proteins that cross the ER membrane post-translationally, nascent membrane proteins are targeted co-translationally to the ER membrane channel (the translocon) by means of a signal sequence or signal peptide (SP) in mammalian cells. Smaller proteins bearing SPs and larger proteins with poorly functional SPs can be pulled through the channel by a chaperone in the ER lumen, such as BiP, a heat shock 70 (Hsp70) family protein.^{7,9} For most type I transmembrane proteins, such as the HIV entry receptor, cluster of differentiation 4 (CD4), the SP is the first 15–40 amino acids at the *N*-terminus of the nascent chain of the preprotein. About 5–10% of G-protein coupled receptors (GPCRs), which are the targets of about 30% of drugs on the market,¹ have *N*-terminal signal peptides.¹⁹ For the remainder,

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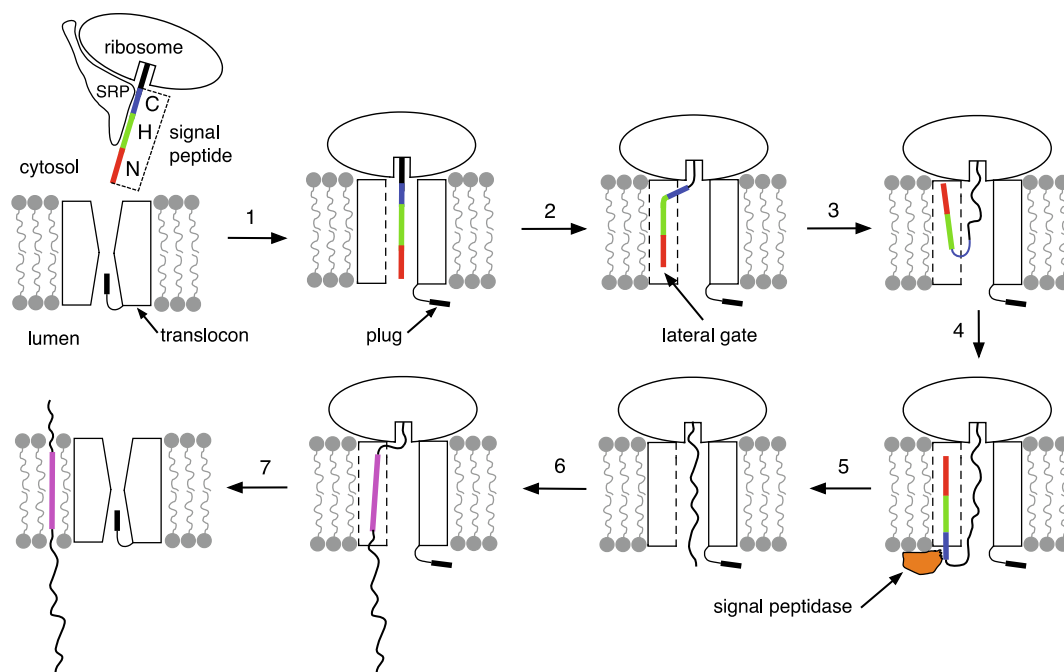


Fig. 1. Co-translational translocation of Type I transmembrane proteins across the ER membrane. 1: SRP transfers RNC to the translocon via the SRP receptor (not shown). 2: SP binds to hydrophobic pocket of the lateral gate. 3: translation continues, the SP inverts. 4: translation continues, the SP binds fully to the lateral gate. 5: SP is cleaved by SPase. 6: as a hydrophobic transmembrane domain emerges, it binds to the lateral gate. 7: translation ends, the ribosome departs, and the protein moves into the ER membrane.

the first transmembrane domain serves as the signal sequence for targeting to the translocon.

The dynamic mechanism for co-translational translocation of proteins with an *N*-terminal SP has not been completely elucidated at the molecular level. The model shown in Fig. 1 is based on previously reported models,^{7,8,20,21} on cryo-EM structures of ribosome-nascent chain complexes (RNCs),^{22–24} and on studies described below. The structure of the translocon is universally conserved,²³ but SPs have a wide variety of primary structures that are specific for every protein and are different for the same protein in different species. All SPs have a central, hydrophobic domain consisting of at least six non-hydrophilic amino acid residues forming about two α -helical turns (labeled H in Fig. 1), a cationic *N*-terminal domain (labeled N in Fig. 1), and a C-terminal segment between the H region and the cleavage site on the *N*-terminus of the mature protein (labeled C in Fig. 1). In eukaryotes, the main component of the heterotrimeric translocon termed Sec61 α consists of ten transmembrane α -helices and adopts the shape of an hourglass in its resting state.²³ Leakage of calcium and other constituents of the lumen are prevented by residues that form a central “pore ring” and by a short, terminal α -helix that serves as a plug. Protein translation begins in the cytosol. The emerging SP and ribosome are bound by the signal recognition particle (SRP), which temporarily arrests or slows translation.²⁴ The SRP then binds to the SRP receptor (SR) on the ER membrane, and the ribosome with the nascent protein chain (RNC) is transferred to the translocon, converting it to a “primed” state. A key feature of the translocon is its “lateral gate,” which is partially opened by RNC binding, exposing a hydrophobic pocket (Fig. 1, step 1).

To probe the orientation of the SP in the translocon, RNCs have been used in which translation is arrested and a glycosylation tag is added to the *N* terminus.^{20,25–27} Glycosylation shows that the *N* terminus initially faces the ER lumen. Studies suggest that the SP of CD4, and the transmembrane signal sequences of some Type II signal anchor proteins, initially bind to the translocon head-first, with their *N* termini facing the ER lumen. As translation pushes the nascent chain deeper into the translocon, the SP apparently does a flip-turn and reorients with its *N* terminus facing the cytosol (Fig. 1, step 3). The SP apparently

moves fully into the lateral gate, making room for the elongating chain to pass through the channel into the ER lumen. The SP is cleaved by a signal peptidase (SPase)²⁸ at the luminal surface of the ER membrane and chain elongation continues. For a Type I transmembrane protein such as CD4, when a hydrophobic transmembrane segment is produced, it binds to the lateral gate and passes into the ER membrane (Fig. 1, step 6). Finally, the protein is released when the mRNA stop codon is reached and the ribosome dissociates from the ER (Fig. 1, step 7).

Efficiency of protein co-translational translocation

The efficiency of protein translocation varies considerably from protein to protein.^{21,29–33} Most proteins apparently translocate without the help of chaperones, and the force of chain elongation during translation pushes the nascent protein through the translocon. Certain specific structural factors are known to facilitate translocation, including basic residues in the *N* region of the SP and hydrophobic residues, especially leucine, in the H domain. The positive charge of the *N* domain facilitates the cytosolic orientation shown for steps 3 and 4 in Fig. 1, and hydrophobicity of the H domain favors insertion of this α -helix into the lateral gate (gating), apparently making room for passage of the downstream residues through the channel. The human prion preprotein, which has a SP with minimal positive charge in the *N* region and with a weakly hydrophobic H region, undergoes BiP-assisted co-translational translocation.^{21,31,33,34} The *N*-to-cytosol orientation of the prion protein is apparently further destabilized by four positively charged residues at the *N* terminus of its mature domain.³³ Chaperone assistance is apparently required for translocation of proteins that cannot effectively be pushed through the translocon by the force of translational elongation, either because they enter the channel post-translationally or because they cannot easily achieve the required *N*-to-cytosol orientation. BiP is an ATPase, and its role facilitating translocation of a subset of proteins makes it a potential drug target.

In a number of hereditary diseases, suppressed protein expression is associated with single-site mutations that introduce a polar amino acid side chain into the SP H region of the preprotein.³⁵ Many of these

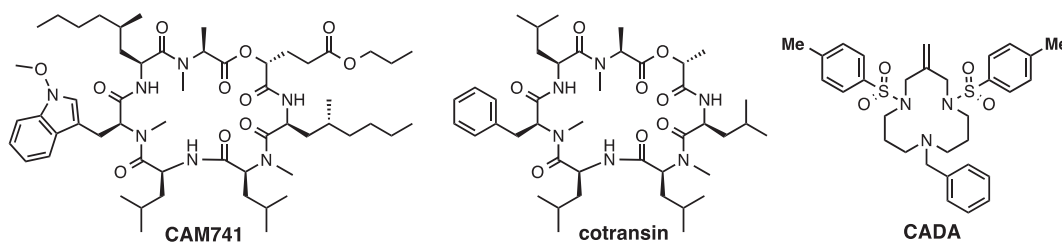


Fig. 2. Structures of co-translational translocation inhibitors CAM741, cotransin, and CADA.

mutations replace leucine with arginine, including the following seven disorders: Classic Ehlers-Danlos Syndrome,³⁶ Crigler-Najjar Disease,³⁷ Hereditary Angioedema,³⁸ Juvenile Hyperuricemic Neuropathy,³⁹ Hereditary Hypotrichosis Simplex,⁴⁰ Narcolepsy 1,⁴¹ and Permanent Neonatal Diabetes.⁴² We believe that these “signal peptidopathies” may introduce salt-bridge interactions with downstream residues in the nascent chain. For these disorders, it may be possible to develop small-molecule drugs that can inhibit these interactions and facilitate normal translocation and SP cleavage.

It is interesting that the SPs of many proteins have H regions containing some polar, uncharged amino acid residues, such as asparagine, glutamine, serine, threonine, tyrosine, and tryptophan. Some even contain a charged residue, such as aspartate, glutamate, lysine, or arginine, and many contain cysteine, which can potentially form covalent bonds. Are these the results of neutral mutations? The relatively slow rates of evolution of SPs and their variability in efficiency suggest not.²¹ Why aren't all SP H domains composed solely of hydrophobic residues, such as leucine, isoleucine, valine and phenylalanine? It was proposed that the structures of SPs are evolutionarily matched with those of the mature domains to facilitate efficient translocation,⁴³ but it has also been recognized that maximum translocation efficiency is not always optimal. SPs can be used by cells to control the efficiency of protein compartmentalization³⁰ and to attenuate protein translocation in response to ER stress,³⁴ so the translocation step can serve as a checkpoint for protein regulation.²¹ Nonoptimal H-residues may have been selected to reduce the efficiency of expression of some proteins. An interesting case is the lutenizing hormone receptor (LHR). A common human mutation (insLQ-LHR) introduces two additional amino acids into the H region of the LHR preprotein, which increases the efficiency of translocation and LHR expression. In women, this mutation is statistically correlated with shortening of disease-free breast cancer survival rates.⁴⁴ Polar residues in H regions may have a beneficial effect of regulating expression of many proteins and can potentially serve as handles for SP-targeting drugs.

Inhibitors of protein co-translational translocation

Substrate-nonspecific inhibitors of translocation

A number of small-molecule exotoxins, virulence factors, and plant secondary metabolites have been identified that inhibit protein co-translational translocation nonselectively by binding and blocking the translocon.^{45–47} Compounds including apratoxin A,⁴⁸ eeyerestatin I,⁴⁹ exotoxin A,⁵⁰ mycolactone,⁵¹ ipomoeassin F,^{52,53} and coibamide A⁵⁴ inhibit expression of many proteins, generally producing toxicity. A pharmacokinetic evaluation of mycolactone as a subcutaneous analgesic was recently reported.⁵⁵ The authors found that in mice the analgesic effects of mycolactone were dependent on type-2 angiotensin II receptors (AT₂R) and not related to its previously reported suppression of inflammation. This observation is consistent with the expectation that mycolactone inhibits translocation of the angiotensin precursor protein, angiotensinogen, which is expressed with a 33-residue SP on the N-terminus of its preprotein. Hence, the angiotensinogen SP could become a target for developing selective analgesic drugs, as well

as anti-hypertensive agents. The cytotoxic effects of substrate-nonspecific translocation inhibitors also suggest the translocon as a target for anticancer drugs. Interestingly, there are translocon mutations (channelopathies) that impair protein translocation and many are associated with certain diseases, such as diabetes.¹⁷ It is conceivable that small molecules acting as translocation facilitators could be used for treatment of such diseases.

Substrate-selective inhibitors of translocation

The first reported substrate-selective inhibitors of protein co-translational translocation are the cycloheptadepsipeptide fungal metabolite HUN-7293⁵⁶ and its analogs CAM741^{56–58} and cotransin⁵⁹ (Fig. 2). Photoaffinity labeling and resistance mutation studies have shown that these cycloheptadepsipeptides primarily bind to the Sec61 α subunit of the translocon,^{56,60,61} but they more potently inhibit translocation of certain preproteins in a SP-selective manner. Two proteins targeted by these cyclodepsipeptides are the vascular cell adhesion molecule 1 (VCAM1) and the vascular endothelial growth factor (VEGF). Mutagenesis studies of the SPs and N-terminal mature domains of these proteins have been performed, showing the importance of specific amino acids in the H and C domains.^{57,58} A cyclodecadsipeptide named decatransin has also been reported to inhibit protein co-translational translocation with a similar selectivity profile and resistance mutations occur in similar locations of Sec61 α .⁶² It is also of interest that the cyclodecadsipeptide ionophore antibiotic valinomycin has been reported to inhibit translocation of hamster but not human prion protein.³¹ Valinomycin is known to be a down-regulator of BiP, which assists translocation of the prion preprotein, so it has been suggested that the hamster prion nascent chain might require more assistance from BiP than the human variant.⁴⁶ On the other hand, valinomycin and decatransin are of similar size and hydrophobicity, suggesting that they may bind to the translocon in a similar manner, leaving the reason for substrate selectivity unclear.⁴⁷

CAM741 and cotransin are mechanistically classified as gating inhibitors because they bind the Sec61 α subunit of the translocon and apparently block access of nascent protein chains to the lateral gate. Their hydrophobic structures suggest that they may compete with the H domain of the nascent chain SP for binding to the lateral gate, and a rough correlation between sensitivity to their inhibitory effects and SP hydrophobicity has been observed.^{57,58} A mechanistic model has been reported in which cotransin binding allosterically prevents lateral gating of the SP or transmembrane domain, but “TMDs with increased hydrophobicity and helical propensity can override the cotransin-imposed block.”⁶¹ On the other hand, an H-region conformational consensus motif for sensitivity of signal sequences to cotransin has been identified.⁶³ It was reported that specific combinations of certain amino acids were important, rather than length or hydrophobicity of the signal sequence. The nonselective translocation inhibitor apratoxin A is a macrocyclic tetrapeptide that also acts as a gating inhibitor. Resistance mutations occur in a similar area of Sec61 α as for cotransin and decatransin, so it has been proposed that all of these compounds bind and act in a similar, but not identical, manner.⁴⁸

The macrocyclic triamine cyclotriazadisulfonamide (CADA) is a

small molecule that inhibits replication of various strains of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) by selectively down-modulating expression of cell-surface cluster of differentiation 4 (CD4).^{27,46,64–79} This type 1 integral transmembrane glycoprotein is expressed on immune cells and is the primary receptor required by HIV to enter host cells. CADA compounds are radically different from conventional anti-HIV agents that have viral targets and are highly susceptible to onset of resistance by mutation and selection mechanisms. CADA compounds target cellular machinery for expression of CD4, an essential receptor for HIV entry. Hence, it is quite difficult to generate a CADA-resistant virus *in vitro*; the resulting strain has relatively poor infectivity and is susceptible to neutralizing antibodies.⁶⁴ CADA has low cytotoxicity and has been shown to selectively down-modulate CD4, without affecting expression of 15 other surface proteins examined on T-cells.⁷² Available evidence indicates that CADA acts as a co-translational translocation inhibitor through direct interaction with the human CD4 SP (*vide infra*).

A proteomic survey using high throughput monoclonal antibody panel-based immunoblotting assay of cellular signaling proteins revealed sortilin as a second substrate of CADA.⁷⁰ The screen was based on a panel of 1000 monoclonal antibodies covering a wide array of intracellular pathways. Dose-response curves are shown in Fig. 3 for CADA-induced down-modulation of human CD4 and for a construct consisting of the sortilin SP with green fluorescent protein (GFP) fused to its C-terminus. The “absolute IC_{50} ” is the concentration at which protein is decreased by 50%. The values for sortilin and CD4 are $\sim 10 \mu\text{M}$ and $0.35 \mu\text{M}$, respectively. Absolute IC_{50} values are of clinical relevance, but the inflection points give “relative IC_{50} s,” which are of importance to the mechanism of action. When calculated as the concentration giving 50% of the maximum effect observed for each drug, the resulting relative IC_{50} values are 0.38 and $0.21 \mu\text{M}$ for sortilin and CD4, respectively. Under ideal conditions, the relative IC_{50} value is equal to the dissociation constant of a drug-target complex. Hence, CADA appears to bind almost as strongly to the sortilin SP as to the CD4 SP, yet the maximum down-modulation for sortilin is only $\sim 50\%$, while CD4 is decreased by $\sim 90\%$.

Sortilin has recently been identified as a potential drug target for many disorders,⁸⁰ including frontotemporal lobar degeneration,⁸¹ autism,⁸² Alzheimer’s disease,⁸³ atherosclerosis,^{84,85} and breast cancer. The last therapeutic application involves cancer stem cells (CSCs), which are subpopulations of cancer cells with similar properties to

normal stem cells in that they have the ability to self-renew and differentiate to form heterogeneous cancer cells. CSCs can perpetuate tumors, even after treatment, and lead to tumor aggression and heterogeneity, causing resistance to anti-cancer therapies.^{86,87} Progranulin has been identified as a secreted CSC modulator that contributes to breast cancer progression.⁸⁸ Progranulin’s role in tumor growth and therapy-induced resistance in various cancer types is associated with its binding to the neuronal receptor sortilin.^{89–91} Interest on the down-modulation of sortilin in breast cancer treatment is increasing as it is associated with breast cancer metastatic potential.⁹² Furthermore, sortilin is highly expressed in breast cancer cell lines compared to non-tumorigenic epithelial cells. Because sortilin binds to progranulin, its regulation could be beneficial to halting breast cancer progression.

CADA mechanism of action (MoA)

Proposed mechanism

The proposed MoA for down-modulation of cell-surface CD4 by CADA compounds is shown in Fig. 4. CADA has no effect on production of CD4 mRNA or on targeting of the RNC to the ER membrane.²⁷ In the absence of ER microsomes, CADA has no effect on CD4 synthesis *in vitro*. In the presence of ER microsomes, CADA prevents translocation of CD4 across the ER membrane. With RNCs bearing N-terminal glycosylation tags, CADA has been shown to inhibit inversion of the SP, blocking translocation of CD4, leading to its diversion to the cytosol (Fig. 4, step 4), where it is degraded by proteolysis.²⁷ It is not currently known whether CADA-displaced proteins are degraded by the ubiquitinating proteasome system. According to surface plasmon resonance (SPR) studies, CADA directly binds the 25-residue SP of human (and probably, more generally, primate) CD4, but not mouse CD4, which has less homology with the human CD4 SP.²⁷ We postulate that CADA binds the CD4 SP in a folded conformation, stabilizing an otherwise transitory state in the inversion process, as shown in Fig. 4. CADA is more specific than other selective co-translational translocation inhibitors and it has been proven to primarily target the SP.

Structure-activity relationships

To deduce physicochemical properties of the CADA binding site of the CD4 SP, we carried out a number of structure-activity relationship (SAR) studies. A quantitative SAR study showed the importance of a hydrophobic tail group for CD4 down-modulation and anti-HIV potency.⁷⁶ We also systematically varied substituents on one side arm to determine the influence of position and electron donating/withdrawing ability on potency.^{67,69} As seen in Fig. 5, replacing the *para*-methoxy group of VGD020 with dimethylamino in CK147 enhances potency.⁶⁹ In the benzyl tail series, moving *para*-dimethylamino to the meta position decreases potency. The *para*-nitro analog has very low potency, but moving nitro to the meta, then ortho position restores potency. These effects cannot be understood considering electron density alone (red, electron rich; blue, electron poor, Fig. 5). We calculated dipole moments of *N,N*-dimethylarenesulfonamides modeling each side arm and found a linear correlation between the pIC_{50} values for CD4 down-modulation and the component of the dipole moment in the plane of the benzene ring of the model compound (Fig. 5 inset).⁶⁹ The IC_{50} value is related to the dissociation constant of a drug-target complex, so this result indicates that interaction of the side arm dipole with the SP is a major determinant of binding energy.

The requirement for a hydrophobic tail group for potency of CADA compounds is of practical concern because the most potent down-modulators of CD4 and sortilin were poorly bioavailable because of low water solubility. Fortunately, the requirement for hydrophobicity does not extend to one or both arenesulfonamide side arms and potent CADA compounds have now been prepared with better solubility, due to greater polarity in the side arm regions. Synthetic studies, coupled with

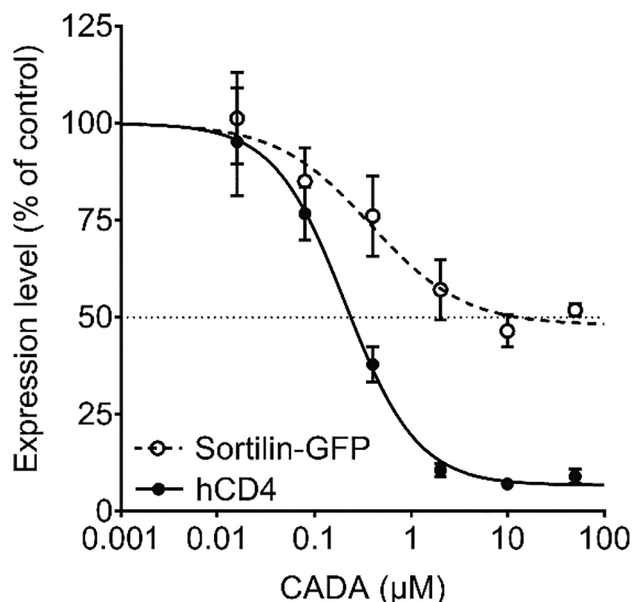


Fig. 3. Dose-response curves for down-modulation of sortilin vs. CD4.⁷⁰

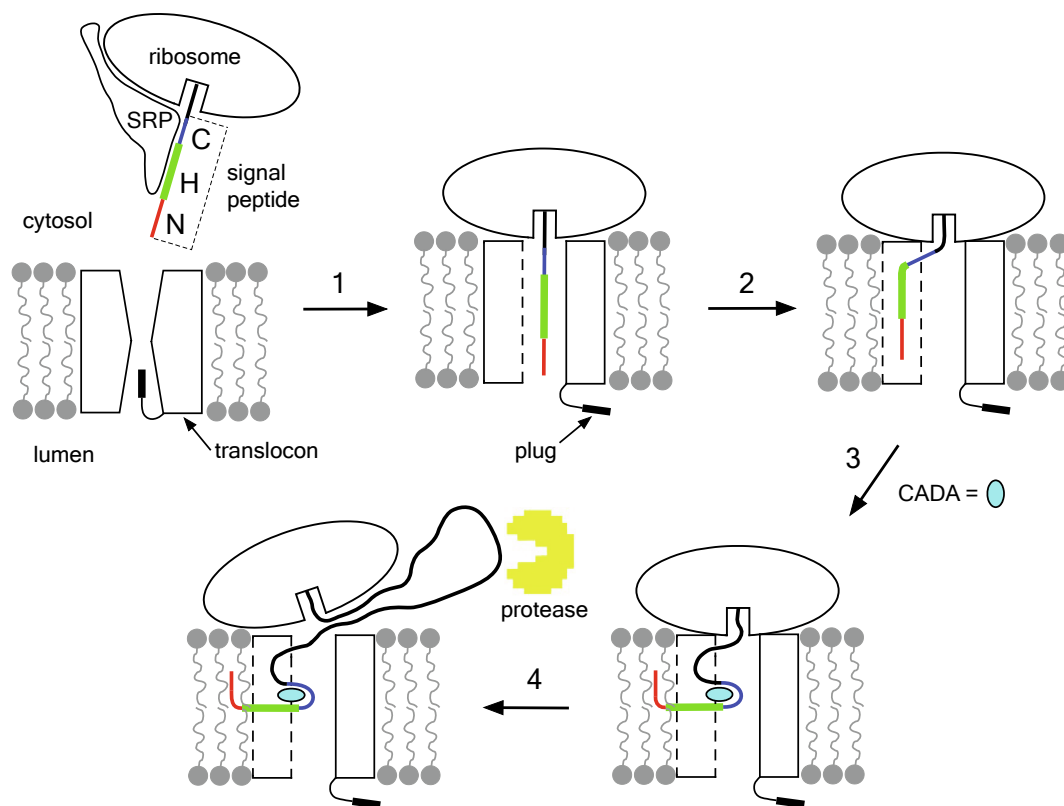


Fig. 4. Proposed mechanism for inhibition of CD4 co-translational translocation across the ER membrane. 1: SRP binds to signal receptor (not shown) and transfers RNC to the translocon. 2: SP binds to hydrophobic pocket of the lateral gate. 3: as translation continues, CADA binds to the SP and stabilizes a folded conformation, halting translocation. 4: as translation continues, the elongating protein chain loops into the cytosol and is degraded by proteolytic enzymes.

pharmacokinetic measurements, are underway to develop compounds with suitable drug-like properties for evaluation *in vivo* and for development of clinical candidates.

Alanine scan mutagenesis

To address the key amino acid residues in the human CD4 SP that interact with CADA, thorough alanine scan mutagenesis studies were conducted.⁷¹ The 32 amino acid N-terminal region of the CD4 preprotein, consisting of the 25 residues of the SP plus the first 7 residues of the mature protein, was previously determined to be essential for CADA sensitivity. The effect of each of these 32 residues on CADA sensitivity was investigated by analyzing two different protein structures: 1. full length (FL) huCD4 that is expressed on the cell surface; and 2. yellow fluorescent protein (YFP) carrying the 32 amino acid residues at the N-terminus.

Following are the first 40 N-terminal amino acids of human CD4 and sortilin, with the putative N, H, and C domains shown in red, green, and blue, respectively. Proposed critical glutamine residues in the H domains are shown in violet.

CD4: MNRGVPFRHL¹⁰ LVLQLALLP²⁰ AATQGGKVV³⁰ GKKGDTVELT⁴⁰...

Sortilin: MERPWGAADG¹⁰ LSRWPHGLGL²⁰ LLLLQLLPPS³⁰ TLSQDRDLAP⁴⁰...

Analysis of the hydrophobic region revealed four key amino acid residues of the CD4 preprotein that contribute to CADA sensitivity: Arg-8, Gln-15, Ala-17, and Pro-20. The Gln-15 mutation had the largest effect and made CADA potency immeasurable for both the FL and YFP constructs. Because of the correlation between side arm dipole moment with potency described above, we propose that CADA compounds recognize Gln-15 through a dipole-dipole interaction. Replacing Arg-8 with alanine had the second largest effect of reducing CADA potency, which can be attributed to either a secondary interaction with CADA or

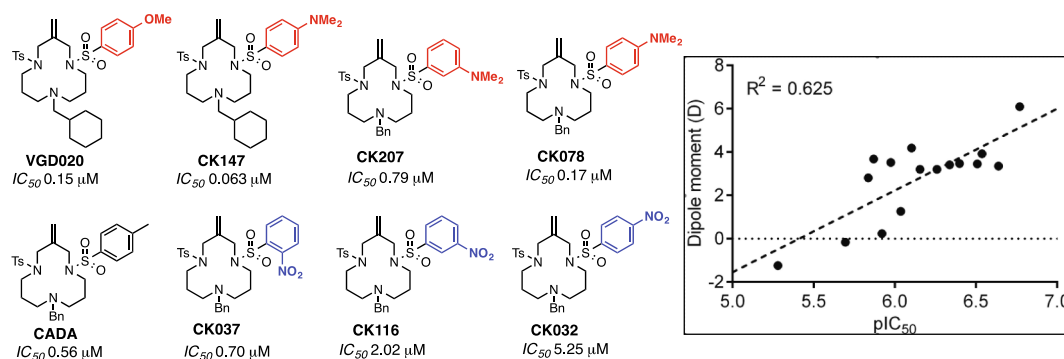


Fig. 5. Potencies of compounds bearing electron donating (red) or electron withdrawing groups (blue). IC₅₀ = concentration giving 50% CD4 downmodulation. *Inset:* correlation between potency and side arm dipole moment.⁶⁹

an effect of decreasing positive charge near the N region of the SP. Pro-20 was also replaced with alanine, resulting in greatly decreased sensitivity to CADA, especially in the YFP construct. Converting it to glycine fully restored CADA sensitivity, which is consistent with this residue forming a hairpin turn in our model and with both proline and glycine being known helix terminators. Exchanging Ala-17 with a valine residue resulted in decreased sensitivity to CADA, which can be attributed to increased hydrophobicity. A general trend observed is that lowering hydrophobicity in the H region increased sensitivity to CADA. Finally, replacing Lys-26 and Lys-27 with alanine also significantly decreased CADA potency, which can be attributed to decreased positive charge at the N terminus of the mature domain.

Comparing the 33-residue SP of sortilin with the 25-residue SP of CD4, we see some interesting similarities. First, both have a polar glutamine residue in the H region, which is consistent with our hypothesis that Q15 is important for binding by a dipole–dipole interaction between CADA and the CD4 SP. As noted above, the relative IC_{50} values suggest that CADA binds CD4 and sortilin with similar affinities. Also, the SPs of both CD4 and sortilin have H domains terminated by proline, actually two prolines in the case of sortilin. A turn at the end of the H-region α -helix may be important for stabilizing a folded structure of the drug–target complex that keeps the cleavage site away from the SPase active site on the luminal side of the ER membrane. A key difference between the SPs is that the N domain of sortilin is about twice as long as that of CD4. This may explain the lower efficacy for translocation inhibition by CADA for sortilin because its cleavage site may be statistically positioned much closer to the SPase active site. The emergent speculations so far are that for an SP to be druggable it must have: 1. One or more polar or otherwise recognizable amino acid in the H domain, which has the highest degree of secondary structure in the nascent chain; and 2. one or more proline or glycine residues terminating the H region. Polar groups in the H region may reside in the hydrophobic environment of the lateral gate, enhancing the binding energy of polar interactions, including dipole–dipole interactions, hydrogen bonds, and salt bridges.

Outlook

Toward the discovery of other druggable signal peptides

The discovery of CADA's ability to decrease expression of specific proteins in a SP-dependent manner has opened the door to the possibility that the signal peptide may become a validated target for drug design. It is certainly a challenge that the specific biomolecular structures targeted by CADA compounds have fleeting existences during dynamic processes, but there are a number of factors suggesting that many nascent proteins could be selectively targeted by the same mechanism. The SPs of proteins are unique and many contain residues in their H regions that might be targeted by ionic, hydrogen bonding, dipolar, or covalent interactions, which are already familiar to medicinal chemists. The co-translational translocation process is fallible, and handles for drug binding may have been introduced evolutionarily as adaptations for modulating protein expression.

How do we discover new drugs for inhibiting co-translational translocation of therapeutically interesting proteins? We speculate that there may already be drugs with poorly characterized mechanisms that bind SPs, and we believe that one example may be the “atypical” antidepressant tianeptine,⁹³ which is not a selective serotonin reuptake inhibitor, despite its tricyclic structure. Some studies found that tianeptine is a μ -opioid receptor agonist,^{94,95} but others showed recently that it inhibits neuronal inflammation by suppressing toll-like receptor TLR4-related pathways.^{96,97} It is a nonplanar sulfonamide with aromatic benzene rings that could bind an SP H-region α -helix with van der Waals interactions, as we propose for CADA compounds. Tianeptine might down-modulate receptors or cytokines on TLR4 inflammation pathways by inhibiting their co-translational translocation across the

ER membrane. In the search to develop new SP-binding drugs, we suggest focusing on proteins with recognized therapeutic potential that have SPs containing potentially recognizable residues in their H domains, including asparagine, aspartate, cysteine, glutamate, glutamine, histidine, lysine, methionine, serine, threonine, tryptophan, and tyrosine.

Following is a list of some proteins with potentially druggable SPs, including relevant therapeutic applications in parentheses: angiotensin-converting enzyme 2-ACE2 (SARS-CoV and SARS-CoV-2), hemagglutinin (H1N1 influenza), MARTX protein (cholera), plasmeprin X (malaria), GP160 (HIV Bal R5, NL4.3 X4), ANTR1 (anthrax), integrin α -IIb (anticoagulant), fibrinogen α chain (anticoagulant), sclerostin (osteoporosis), CX3CR1 (Alzheimers), amyloid beta (Alzheimers), complement 5 (rheumatoid arthritis), GM-CSF (rheumatoid arthritis), thyroglobulin (hyperthyroidism), myostatin/GDF-8 (muscle loss), growth arrest-specific protein-Gas1 (muscle loss), CAMPATH-1 antigen/CD52 (multiple sclerosis), glycoprotein B/gB (cytomegalovirus), kirrel 3 (autism/intellectual disability), candidalysin (*Candida albicans* toxicity), cadherin 10 (autism), thyroid stimulating hormone receptor-TSHR (Grave's disease), and toll-like receptor 4-TLR4 (anxiety, neurodegenerative diseases, stroke recovery). The structures of these proteins and references to related disorders may be found on the UniProt website at: <https://www.uniprot.org/uniprot/> The search is on!

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this publication.

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