Targeting eukaryotic translation elongation with natural products

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Abstract

The translation of mRNA into proteins is a precisely regulated, complex process that can be divided into three main stages, i.e. initiation, elongation and termination. This contribution is intended to highlight how natural products interfere with the elongation phase of eukaryotic protein biosynthesis. Cycloheximide, isolated from *Streptomyces griseus*, has been the prototype inhibitor of eukaryotic translation elongation for long. In the last three decades, a variety of natural products from different origin were discovered to also address the elongation step in different manners, including interference with the elongation factors eEF1 and eEF2 as well as binding to A-, P- or E-sites of the ribosome itself. Recent advances in the crystallization of the ribosomal machinery together with natural product inhibitors allowed characterizing similarities as well as differences of their mode of action. Since aberrations in protein synthesis are commonly observed in tumors, and malfunction or overexpression of translation factors can cause cellular transformation, the protein synthesis machinery has been realized as an attractive target for anticancer drugs. The therapeutic use of the first natural products that reached market approval, plitidepsin (Aplidin®) and homoharringtonine (Synribo®), will be introduced. In addition, we will highlight two other potential indications for translation elongation inhibitors, i.e. viral infections and genetic disorders caused by premature termination of translation.

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This highlight is intended to showcase how natural products interfere with the elongation phase of eukaryotic protein biosynthesis. After an introduction to eukaryotic protein biosynthesis in general and to the individual steps in the elongation phase, we will present a selection of important translation inhibitors from natural origin, with a focus on their binding modes and their mechanisms of action. Finally, existing and potential future uses of elongation inhibitors will be highlighted.

1 Introduction to eukaryotic protein biosynthesis

The translation of information stored by DNA into proteins is a vital process in biological systems. In brief, the base sequence of the double-stranded DNA is transcribed and further processed into a single-stranded RNA molecule, the mRNA. The base sequence of the mRNA is translated into an amino acid sequence of a protein on ribosomes in the cytoplasm of the cell.[1-4] In the mRNA, three consecutive bases, a base triplet, form a codon for a specific amino acid. On the ribosome, the codons are translated according to their sequence into amino acids, and these are sequentially linked to form a polypeptide. The structural relationship between a codon and its corresponding amino acid is mediated by tRNA molecules, that have two distinct binding sites for the specific attachment of the amino acid (catalyzed by aminoacyl-tRNA synthetases), and the specific binding to a codon (by a complementary, so-called anticodon sequence). In order to form a peptide bond between two amino acids, they must be brought into proximity to each other. This task is fulfilled by the ribosomes, composed of a small (40S) and a large subunit (60S) in *Homo sapiens* (Fig. 1). The 60S RNA unit has three joining binding sites, called acceptor site (A-site), peptidyl site (P-site), and exit site (E-site).

The translation itself is a highly regulated and complex process involving many factors. It can be divided into the three main stages initiation, elongation and termination (Fig. 1). For its initiation, the cell needs a special initiator tRNA, the two ribosomal subunits and the mRNA. The initiator tRNA binds to the start codon AUG, which transmits methionine in eukaryotes. In addition, a wide range of eukaryotic initiation factors (eIF's) are involved. After formation of a preinitiation complex of the small ribosomal subunit and the initiator tRNA with eIF-2 and other factors, the mRNA is screened in the 3' direction for an AUG sequence. Once an AUG base triplet of the mRNA is found, the initiator Met-loaded tRNA will bind to it. After the release of eIF2, the 60S subunit is bound, creating the elongation-competent 80S initiation complex (for detailed information about the initiation process see the contribution by Pelletier and collegues to this NPR special issue). The initiation phase is followed by the elongation of the amino acid chain as described in the next chapter, that continues

until a stop codon is reached, leading to the termination of the peptide formation. The ribosomal subunits are dissociated and recycled to re-enter the initiation phase.

2 The elongation phase of the translation system

The elongation phase begins when the initiator Met-tRNA is bound to the start AUG codon and positioned on the P-site of the ribosome, leaving the A-site free for the entry of the next aminoacyl tRNA. A single elongation cycle involves three steps: Amino acid incorporation (i.e. binding of the amino acid-loaded tRNA at the A-site), formation of the peptide bond, and ribosome translocation. The first step is mediated by the eukaryotic elongation factor eEF1A (Fig. 2). Mammalian eEF1A is a 50 kDa protein that possesses two paralogs, eEF1A1 and eEF1A2, with high amino acid sequence homology (approximately 90% identity). eEF1A, which is equivalent to bacterial EF-Tu, binds amino acyl-tRNA in a GTP-dependent manner and directs the tRNA to the A-site of the ribosome. A codon recognition between mRNA and tRNA causes GTP hydrolysis by eEF1A. eEF1A is released and the aminoacyl-tRNA can be accommodated into the A-site. The eEF1A-GDP is recycled to eEF1A-GTP with the help of the guanine exchange factor (GEF) function of the nucleotide exchange factor eEF1B complex, which is composed of the subunits α, δ, and γ.

The peptide bond formation between the amino acids located at the P-site and the A-site is catalyzed by ribosomal peptidyl transferases. The deacylated tRNA remains in a P/E hybrid position. In this state the acceptor end is bound to the E-site and the anticodon stem to the P-site, whereas the peptidyl tRNA (the tRNA on which the peptide chain has been loaded) lies in an A/P hybrid position. To continue with the next cycle of elongation, a translocation that places the deacylated tRNA completely in the E-site and the peptidyl tRNA in the P-site is required. This translocation is an energy dependent process that employs eEF2, which stabilises the hybrid state. eEF2-GTP hydrolysis induces a conformational change in the complex and promotes translocation, thereby creating an empty A-site, where a new tRNA can bind and initiate the next cycle of peptide chain elongation. Among all three binding sites, the E-site is the most diverse across species, showing different nucleotides and proteins in bacteria, archaea and eukaryotes.[5, 6]

3 Natural products as inhibitors of the elongation phase

Natural products have played an instrumental role to study the translation process, because they demonstrate how to interfere with elongation at very diverse stages and positions. This includes interactions with proteins involved in the elongation process or with the rRNA of the ribosome. The understanding of the underlying molecular details has been greatly enhanced in the past decade by structural biology studies, that provided high-resolution structures of ribosomes from yeasts, archaea and humans in complexes with translation inhibitors.[6, 7-10] The preferential binding sites of eukaryote-specific inhibitors at the ribosome are the tRNA E-site and the peptidyl transferase center (PTC) on the 60S subunit. Remarkably, the far majority of co-crystallized compounds is of natural origin. A selection of the most prominent natural products interfering with the elongation phase, that are highly diverse from a structural point of view, is given in Fig. 3 and Fig. 4.

Cycloheximide (CHX) represents the prototype inhibitor of eukaryotic translation elongation, as it was the only known inhibitor of this process for a long time. Its isolation from *Streptomyces griseus* was reported already in 1946. CHX inhibits eEF2-mediated translocation. Surprisingly, bound CHX still

allows one translocation cycle to proceed before the further elongation is stopped.[11] It binds to the 25S rRNA and a eukaryote-specific protein eL42 at the E-site on the large subunit of the ribosome, and interaction data at atomic resolution are available for *Saccharomyces cerevisiae* (Fig. 5).[6] Recent cryo-EM experiments on human ribosomes with an average resolution of 3.6Å indicated a similar binding site, but additional molecular interactions compared to the yeast ribosome.[8] Thus, it is possible that the overall mechanism of action of CHX is not identical across species.

CHX contains a glutarimide moiety, a motif that is also present in the side chain of the macrolactone lactimidomycin (LTM) isolated from *Streptomyces amphibiosporus.*[12] LTM inhibits the elongation step of eukaryotic translation through binding to the E-site of the 60S ribosome in a similar, but not identical fashion to CHX.[13] The binding of CHX and LTM to the E-site blocks eEF2-mediated tRNA translocation, and the glutatimide groups of CHX and LTM closely overlap in the binding site. CHX and LTM differ in the ability to bind the E-site together with the E-site tRNA: According to a model of Schneider-Poetsch *et al.*,[13] the presence of CHX at the E-site does not stall translocation, but occupation of the E-site by both CHX and deacylated tRNA does. Consequently, two translocation events can occur before the process stops. While LTM binds to the same site, the larger LTM blocks the access of deacylated tRNA to the E-site and prevents the ribosome from leaving the start site. When LTM is bound to an empty E-site immediately after initiation, it allows peptide bond formation, but blocks the translocation of the newly formed deacylated initiating tRNA from the P- to E-site, thereby arresting the ribosome at the AUG start codon. After elongation has been started and deacylated tRNA is bound to the E-site, it is more difficult for LTM to get access to the E-site. Unlike LTM, CHX can interrupt the elongation process at any time, as its binding to the E-site is regardless of whether the E-site is occupied by deacylated tRNA or not. The eukaryote-selective activity of E-site inhibitors is rationalized by the presence of two bacteria-specific rRNA residues (U2431 and A2432 in *E. coli*) that prevent drug binding to the bacterial ribosome.

The lissoclimides, which were isolated from shell-less mollusks, carry a succinimide rather than a glutarimide moiety. They were also shown to interfere with the elongation step of protein biosynthesis. Detailed investigations found that chlorolissoclimide did not affect the loading of aminoacyl tRNA onto ribosomes or the peptidyl transferase reaction, but interfered with ribosomal E-site function.[14] Recent X-ray co-crystal structure studies showed a similar binding of lissoclimides to the eukaryotic ribosome as found for CHX and LTM.[15]

Phyllanthoside is a translation elongation inhibitor first isolated from extracts of the Central American tree *Phyllanthus acuminatus.*[16] Although chemically unrelated, phyllanthoside was also found to bind to the same rRNA nucleotides and the eL42 protein as the glutarimide inhibitors (Fig. 5); the electron density in the crystal structure suggests a covalent bond between C2764 and the opened epoxide group of phyllanthoside.

Mycalamides A and B were originally isolated as cytotoxic compounds from the marine sponge *Mycale* spec. from the coast of New Zealand. They were shown to inhibit protein synthesis,[17] but their molecular mechanism of action remained unknown for long. In 2011 it was reported that by mycalamide B (MycB) inhibits translation elongation through blockade of eEF2-mediated translocation similar to CHX and LTM, in spite of no structural resemblance to the glutarimide-containing inhibitors. MycB did not affect the eEF1A-mediated loading of tRNA onto the ribosome, the AUG start codon recognition, or peptide bond formation, but competed with deacylated tRNA for E-site binding in a dose-dependent manner. As seen with LTM, MycB blocks tRNA binding to the E-site and arrests the ribosome on the mRNA one codon ahead of CHX.[18]

Cryptopleurine is a phenanthroquinolizidine alkaloid which was isolated from the bark of *Cryptocarya pleurosperma* trees. It was also shown to be an E-site inhibitor and described to be eukaryote specific.[19] X-ray crystallographic data showed that it binds to the mRNA channel at the 40S E-site of the small subunit. It shares the same binding site as pactamycine, an antibiotic which also inhibits prokaryotic translation. The structure of cryptopleurine bound to yeast ribosome did not provide hints for its specificity to eukaryotes.[6]

Sordarins are fungal terpenoid natural products with a tetracyclic core embedding a norbornene. They inhibit protein synthesis, but exhibit a notable, selective antifungal activity. Sordarin binds to eEF2 of *Saccharomyces cerevisiae*, but not to eEF2 from plants or mammals, though there is a high level of amino acid sequence conservation among these proteins. The efficacy of sordarin differs greatly in different species of fungi. It could be demonstrated that a "sordarin specificity region" between amino acid residues 517 and 525 within eEF2 of *S. cerevisiae* accounts for the special bioactivity profile of this class of compounds. A single substitution of serine at position 523 by glutamic acid, the corresponding residue in human eEF2 at this position, was sufficient to render *S. cerevisiae* insensitive to sordarin.[20]

Most elongation inhibitors target the peptidyl transferase center (PTC) of the ribosome located on the large subunit, which is composed of highly conserved rRNA nucleotides. For peptide bond formation, the amino acyl-tRNA and the peptidyl-tRNA must be properly aligned in the A-site and P-site of the PTC. The trichothecenes span a group of important mycotoxins that comprises more than 150 members, including T-2 toxin, deoxynivalenol, and verrucarin. They share a sesquiterpene core that mediates the major interactions with 25S rRNA residues in the binding pocket (Fig. 6a).[6] However, trichothecenes have also been shown to inhibit translation initiation and termination, and to have other effects beyond protein biosynthesis as well.[21] A second, structurally unrelated group of natural products that occupy the same binding site is formed by plant alkaloids like lycorine, narciclasine, and homoharringtonine, which share a dioxol-pyrroline group (Fig. 6b). These alkaloids are reported to be specific elongation inhibitors. Lycorine and narciclasine were isolated from Amaryllidaceae, and narciclasine showed potential as an anticancer or anti-inflammatory drug. However, clinical trials have not been conducted so far.[22, 23] Homoharringtonine is an alkaloid with a cephalotaxine scaffold that was first isolated from *Cephalotaxus harringtonii* and *C. fortunei* trees, whose bark extracts were used in Chinese traditional medicine to treat cancer. Because cephalotaxine itself is abundant in Cephalotaxus leaves, homoharringtonine can be conveniently obtained by a simple esterification from isolated cephalotaxine.

All A-site inhibitors block the access of the charged tRNA and consequently peptide bond formation. In contrast to the E-site inhibitors mentioned above, they do not interact with protein residues, but only bind to the RNA nucleotides of the ribosome. A-site inhibitors have been obtained from very diverse sources, as exemplified by anisomycin, an antibiotic produced by *Streptomyces griseolus,* nagilactones, diterpenoid lactones isolated from *Podocarpus nagi* trees, or agelastatins, brominated alkaloids isolated from the marine sponge *Agelas dendromorpha*. The potent antitumor activity of agelastatins[24] was only recently traced back to the inhibition of protein synthesis. Using a high-throughput chemical footprinting method, the AglA-binding site was mapped to the A-site.[9, 25]

Aminoglycosides exert a unique mechanism of action by perturbing nucleotides in the decoding centre that discriminates cognate tRNA from non-cognate tRNA. They bind within a loop of helix 44 of 18S rRNA, and they stabilize the flip-out conformation of the two essential and universally conserved nucleotides A1755 (A1492) and A1756 (A1493) in yeast (human numbering in brackets).

Because this conformational change is part of the proofreading process, its induction by aminoglycoside binding lowers the high fidelity of decoding, enables the binding of near-cognate tRNAs and thereby promotes the incorporation of incorrect amino acids into peptide chains. While most aminoglycosides show selectivity for bacterial ribosomes, congeners like geneticin (G418) bind with high affinity for the eukaryotic ribosome (Fig. 7a). The structural basis for this selectivity has been deciphered recently by X-ray crystallography[6] and ascribed to the residues G1645 and A1754, that are specific for eukaryotic ribosomes (Fig. 7b) and act as a barrier that prevents the binding of most aminoglycosides.

The polyketide myriaporone 3/4 was first isolated in 1995 from the false coral *Myriapora truncata*. The high cytotoxicity in the nanomolar range was attributed to its activity as a translation inhibitor. Myriaporone 3/4 also stalls protein synthesis at the elongation phase by interference with eEF-2 activity, but this is achieved in an indirect manner. eEF2 activity is inhibited by phosphorylation of Thr56 by eEF2 kinase (eEF2K). eEF2K is a Ca^{2+}-dependent kinase which is activated/deactivated by various stimuli. Phosphorylation of eEF2K at Ser 359 leads to its inactivation. The subsequent dephosphorylation of eEF2 then induces the continuation of translation. eEF2K activity was shown to be increased in several cancer cell lines and reported as a potential target in cancer treatment.[26-28] Myriaporone 3/4 was shown to bind to eEF2K, which in turn led to a phosphorylation of eEF2 and an inhibition of translation elongation.[29] Mycalamide (mentioned above) and myriaporone are members of a structurally related polyketide family, which also includes compounds like pederin, psymberin, tedanolid, and gephyronic acid. All these compounds are translation inhibitors, but each inhibitor acts in a different way.[30-33] This polyketide family is therefore an interesting example for the diversification of bioactivity associated with subtle structural changes.

Didemnins and the related tamandarins constitute another class of deeply studied translation elongation inhibitors. The cyclic depsipeptides were first isolated from tunicates and already reported in 1981;[34, 35] recent studies identified the marine α-proteobacteria *Tistrella mobilis* and *T. bauzanensis* as the producer of didemnins.[36] The compounds showed remarkable antitumor, antiviral and immunosuppressive properties. The analog didemnin B was shown to inhibit protein synthesis also by preventing the translocation step, but in contrast to CHX and LTM, didemnin B binds to a pocket of eEF1A that is normally used to bind either aa-tRNA or eEF1Bα, a factor that is needed for guanine nucleotide exchange upon GTP hydrolysis. Since eEF1Bα and didemnin B are mutually exclusive, the protein synthesis is inhibited by didemnin B because of its competition with this nucleotide exchange factor. The following mode of action was proposed: didemnin B binding to eEF1A takes place following delivery of aa-tRNA to the ribosome. The resulting didemnin B-eEF1A complex rests at the A-site of the ribosome, because binding of eEF1Bα is precluded by didemnin B. Stabilization of this complex at the A-site prevents displacement of eEF1A by eEF2 causing translational arrest.[37-40]

Nannocystins are macrocycles isolated from myxobacterial *Nannocystis sp.*[41] Mode of action studies using haploinsufficiency profiling (HIP) in yeast, mutational studies in human HCT116 cells, and target fishing with chemoproteomic probes established eEF1A as the primary target for this compound class.[42] Nannocystin A showed varying activities in different cancer cell lines, and the main differentiating factor was found to be the eEF1A expression level. Biochemical and genetic evidence support an overlapping binding site of nannocystin with didemnin B on eEF1A. Although a crystal structure is missing so far, structural determinants for the activity of nannocystins could be proposed based on rationally designed analogs from total synthesis.[43-45]

Other natural compounds like the polyketides cytotrienin and ansatrienin and the cyclic peptide ternatin have been shown to compete with didemnin binding to the ternary complex of eEF1A, GTP and aminoacyl tRNA.[46]

4 Inhibitors of elongation phase as therapeutics

Almost all inhibitors of eukaryotic translation isolated from nature were found to be cytotoxic in assays with mammalian cell cultures and were proposed to have potential antitumor activity. Aberrations in protein synthesis are often observed in established cancers, and perturbation by mutation or overexpression of translation factors can cause cellular transformation. Therefore, the protein synthesis machinery is a potential target for anticancer drugs. However, as the activity of protein elongation factors in healthy cells is ubiquitous and essential, the inhibition of these targets leads to a delicate balance between antitumor effects and toxic side effects.

Several inhibitors of eukaryotic protein synthesis have started to be developed as anticancer drugs, and a few advanced to phase I and II clinical trials. However, most of the translation inhibitors failed in clinical trials, mostly due to dose-limiting toxicity.[47] However, the two elongation inhibitors plitidepsin and homoharringtonine have obtained market authorization and will be introduced in more detail.

Didemnin B, the most active among nine natural didemnins, was the first marine natural product that entered clinical trials as an anticancer drug. It has completed phase II clinical trials against non-Hodgkin's lymphoma, kidney adenocarcinoma, advanced epithelial ovarian cancer, and metastatic breast cancer, but finally failed to demonstrate effective antitumor activity, while showing cardiac and neuromuscular toxicities.[36,48] Though the primary target of didemnins is eEF1A, they showed pleiotropic effects in cancer cells. Using gene expression signatures, didemnin B was identified as a persistent mTORC1 pathway agonist, and palmitoyl-protein thioesterase 1 (PPT1) could be identified as a second target. Didemnin induces cell-cycle arrest and a caspase dependent apoptotic process through dual inhibition of eEF1A and PPT1.[49]

The closely related compound plitidepsin (dehydrodidemnin B, aplidine, PLD) has an N-terminal pyruvate instead of a lactate residue (Fig. 4). Despite the minor structural difference, PLD was shown to be more potent and less toxic, and has therefore outpaced didemnin B in clinical development.[50-53]. PLD binds to eEF1A2 and induces oxidative stress, Rac1 activation and JNK1 phosphorylation, which leads to a rapid apoptotic process in tumor cells. While several clinical phase II studies showed only limited activity in solid tumors, PLD had significant beneficial effects in hematological cancers, particularly in multiple myeloma, where eEF1A2 is overexpressed.[54] The phase III trial ADMYRE, which ended in November 2017, showed that a combinatorial therapy led to a longer progression-free and overall survival of patients with multiple myeloma. Adverse effects like fatigue, myalgia and nausea were usually transient and manageable. Despite the negative opinion adopted by European Medicines Agency's (EMA) committee, PLD received orphan drug status for the treatment of multiple myeloma in Switzerland in 2017 and has also been recently approved by Australian regulatory authorities. PLD is marketed by PharmaMar S.A. under the trade name Aplidin.[36, 52, 53, 55-61]

Though it is proven that PLD inhibits protein biosynthesis, a secondary, important function of eEF1A could contribute to its beneficial effect in myeloma patients: eEF1A also interacts with polypeptides after their release from the ribosome. Specifically, it is involved in the elimination of misfolded proteins through the proteasome and aggresome process.[62] Inhibition of eEF1A by PLD led to an

increase in the levels of misfolded proteins, while concomitantly reducing the autophagic flux. These effects prevent PLD-treated cancer cells from reducing proteotoxic stress and lead to apoptosis.[63] We note in passing that most multiple myeloma therapies also target the proteasome.

The interest in homoharringtonine started following the disclosure of its potent antiproliferative activity against murine P-388 leukemia cells with IC_{50} values of 17 nM. Homoharringtonine or a mixture of cephalotaxine esters have been used to treat hematological malignancies in China since the 1970s.[64] After the development of the above-mentioned semisynthetic production, homoharringtonine attracted attention of Western medicine as well. The efficacy of the drug is traced back to the depletion of proteins with rapid turnover that are essential for cancer, such as the short-lived oncoproteins BCR-ABL1 and anti-apoptotic proteins (Mcl-1, Myc), which are upregulated in leukemic cells.[65] It was recently shown that homoharringtonine also affects signalling pathways like Jak-Stat5 by regulating protein tyrosine kinase phosphorylation[66] and by activating the TGF-β pathway through phosphorylation of smad3.[67] Homoharringtonine was approved by the U.S. Food and Drug Administration in 2012 for the treatment of chronic myeloid leukemia in patients with resistance and/or intolerance to two or more tyrosine kinase inhibitors. Homoharringtonine is the only natural product approved as a drug to treat chronic myeloid leukemia. It is marketed by Teva Pharmaceutical Industries under the trade name Synribo.[68]

The discovery of specific inhibitors of eukaryotic translation has improved our knowledge of the similarities and differences between the translational machinery of eukaryotes and bacteria. The characterization of new translation inhibitors has also improved our knowledge of the changed protein biosynthesis in malignant cells, and further studies will open new paths – although they might be narrow - to develop therapeutic agents against cancer.

In addition to cancer, two potential medical indications for translation elongation inhibitors are viral infections and genetic disorders. The antiviral spectrum of CHX included several DNA and RNA viruses,[69] and LTM was identified as be a potent inhibitor of dengue virus 2 infection in cell culture with an EC_{90} value of 0.4 μM. Antiviral activity was observed at concentrations that do not affect cell viability. Other fast replicating RNA viruses were also found to be sensitive to LTM.[70] Homoharringtonine showed a good (EC_{50} = 3.0 and 3.5 μM) inhibitory effect against two different virus strains of foot-and-mouth disease (FMDVs) in swine kidney cells. The compound did not affect virus attachment or entry, but the early phases of FMDV replication - consistent with its mechanism of action. Homoharringtonine has the potential to be an effective anti-FMDV drug but further studies to explore the antiviral activity *in vivo* are required.[71] Also mycalamides A and B have shown antiviral activities against coronaviruses, HSV and Polio virus, which have been ascribed to their ribosomal inhibition.[72] However, analogues of mycalamides inhibited influenza virus *in vitro* by binding to the viral nucleoprotein (NP), impeding its association with viral RNA.[73] Thus, whether mycalamides exert their antiviral action by targeting protein translation, viral components or both is not clear. To the best of our knowledge, no translation elongation inhibitor is in advanced studies as an antiviral drug.

As mentioned above, aminoglycoside binding to ribosomes lowers fidelity in cognate tRNA recognition and thereby promotes the incorporation of incorrect amino acids into peptide chains. This unique mode of action has a potential therapeutic use for the treatment of inherited disorders caused by nonsense mutations.[74, 75] In fact, studies with tissue culture as well as *in vivo* models have provided a proof of concept that aminoglycoside treatment can indeed induce reading through premature termination codon mutations and thereby restore the production of full length proteins in several genetic disorders. First clinical studies have been undertaken in patients with Duchenne

muscle dystrophy and cystic fibrosis.[76] However, the long-term use of aminoglycosides in this indication is limited by the inherent nephrotoxic and ototoxic side effects of this class of natural products that is (at least partly) ascribed to the inhibition of mitochondrial ribosomes. Therefore, the generation of novel analogs that have an increased premature termination codon (PTC) suppression at eukaryotic ribosome, but a reduced efficacy at bacterial and/or mitochondrial ribosomes, is a subject of current research.[77]

5 Conclusion

Small molecule inhibitors of bacterial protein synthesis have been shown to be powerful tools in the elucidation of prokaryotic protein biosynthesis. Compared to prokaryotes, fewer compounds that inhibit eukaryotic translation have been identified. The structural basis for their efficacy has been elucidated only recently, enabled by advances in the crystallization of the ribosomal machinery. Natural products have informed cell biology that interference with protein translation is possible by a variety of mechanisms, involving the two elongation factors eEF1 and eEF2 as well as the A, P or E-sites of the ribosome itself. Aplidin and homoharringtonine are the first compounds inhibiting translation elongation that are approved as anticancer drugs, albeit in very narrow indications or markets. Since translation plays an essential role in the proliferation and survival of fast-growing tumor cells, in particular for proteins with a rapid turnover that are essential for cancer, it is well-possible that further niches for translation inhibitors as new cancer therapeutics are carved. This is facilitated by a more detailed characterization mode of action of translation inhibitors, including secondary effects of the participating elongation factors and/or off-target effects of the natural products (e.g. protein degradation or PPT1 inhibition, as mentioned above). The same holds true for other indications like viral infections or the amelioration of genetic disorders caused by premature termination of translation. Thus, the study of natural product interfering with eukaryotic translation offers ample opportunities for future discoveries.

Conflicts of interest

There are no conflicts of interest to declare.

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Figure captions

Fig. 1 Schematic representation of the discrete steps of protein synthesis in eukaryotes. The translation from mRNA into polypeptides can be divided into the main phases initiation, elongation and termination. The latter is followed by a recycling step (modified from De Loubresse et al.[6]).

Fig. 2 The elongation phase consists of steps for the incorporation of amino acids loaded on tRNA to the ribosome, the peptide bond formation between amino acids, and the ribosome translocation by one mRNA triplet towards the 3' end. For all steps, natural products inhibitors have been identified (modified from Fritz & Boris-Lawrie[3]).

Fig. 3 Chemical structures of elongation inhibitors binding to the E- and A-site of the ribosome.

Fig. 4 Chemical structures of elongation inhibitors that interfere with elongation factors and amino acid selection.

Fig. 5 Structural details for binding of E-site inhibitors to yeast ribosomes. **a**, Cycloheximide and lactimidomycin share the same binding site, but the additional lactone ring in lactimidomycin obstructs the binding of the inhibitor to the E-site in the presence of bound tRNA. **b**, Binding site of phyllanthoside. The asterix indicates a covalent bond (De Loubresse et al.[6]).

Fig. 6 Structural details for binding of A-site inhibitors to yeast ribosomes. **a**, The structurally related trichothecenes T-2 toxin, verrucarin A and deoxynivalenol show an overlapping binding sites. **b**, The plant alkaloids lycorine, narciclasine and homoharringtonine occupy the same binding pocket. The arrows point to the location of the dioxol-pyrroline moieties (modified from De Loubresse et al.[6]).

Fig. 7 Aminoglycoside binding in the decoding center to yeast ribosomes. **a**, Geneticin binds to helix 44 of 18S rRNA and induces a flipping out of A1755 and A1756. **b**, Secondary structure diagrams of helix 44 from bacteria (16S rRNA, left) and from yeast and human (18S rRNA, right), demonstrating sequence differences in prokaryotes and eukaryotes at the aminoglycoside binding site (modified from De Loubresse et al.[6]).