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Along the Central Dogma—Controlling Gene Expression with Small Molecules

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Keywords

chemical biology, gene expression, natural products, transcription, translation, RNA metabolism

Abstract

The central dogma of molecular biology, that DNA is transcribed into RNA and RNA translated into protein, was coined in the early days of modern biology. Back in the 1950s and 1960s, bacterial genetics first opened the way toward understanding life as the genetically encoded interaction of macromolecules. As molecular biology progressed and our knowledge of gene control deepened, it became increasingly clear that expression relied on many more levels of regulation. In the process of dissecting mechanisms of gene expression, specific small-molecule inhibitors played an important role and became valuable tools of investigation. Small molecules offer significant advantages over genetic tools, as they allow inhibiting a process at any desired time point, whereas mutating or altering the gene of an important regulator would likely result in a dead organism. With the advent of modern sequencing technology, it has become possible to monitor global cellular effects of small-molecule treatment and thereby overcome the limitations of classical biochemistry, which usually looks at a biological system in isolation. This review focuses on several molecules, especially natural products, that have played an important role in dissecting gene expression and have opened up new fields of investigation as well as clinical venues for disease treatment.

Contents

INTRODUCTION

To this day, plant and microbial metabolites constitute a major source of medicinal drugs as well as biological probes for research use (1, 2). The utilization of natural products for medical and recreational purposes dates back tens of thousands of years and, despite ever more sophisticated synthetic methodology, continues to this day. The likelihood of finding an active molecule in a library of natural products is usually much higher than the chance of finding an equally effective compound in a purely synthetic collection (3, 4). As the high hopes put in large combinatorial libraries of synthetically produced molecules remain unfulfilled, as they have yielded only a small number of active compounds, the use of naturally occurring molecular scaffolds as the basis for synthesis is increasingly garnering interest (5). We focus on natural products in our discussion of molecules that inhibit or modulate gene expression (**Table 1**), although we also mention synthetic compounds when appropriate. Hence, we also do not discuss molecules that affect transcription via signal transduction, such as inhibitors of JAK/Stat or mammalian target of rapamycin (mTOR) signaling pathways.

At the core of molecular biology lies DNA as information storage, which is then copied into RNA to produce a template for protein synthesis (**Figure 1**). Hence, it seemed that three macromolecules and the processes of transcription and translation could explain most of life itself and that there was not much left to discover (6). Fortunately, such pessimistic thought did not prevail and it became clear that life at a molecular level was not only more fascinating but also much more complicated than originally anticipated. While molecular biology originally concerned itself primarily with the manipulation of DNA, RNA started to take on an increasingly central role in the view of life. In some cases, the direction postulated by the central dogma may be reversed, as in several families of RNA viruses that force their host to produce DNA "hard copies" of their genomes, whereas others function just with RNA genomes and omit the DNA stage altogether (7). Furthermore, in eukaryotes especially, a series of many processes lies between transcription and protein production, which in many stages involves RNA regulators. To produce usable protein, the nascent messenger RNA (mRNA) receives a cap and a poly-A tail. Additionally, it is spliced, checked for premature stop codons, and finally exported. Even then, small silencing RNAs may command destruction of the final transcript or alter its expression level via microRNAs before the mRNA actually reaches the ribosome (8). Although one could still claim that these events are more or less details following the outline of the central dogma, the expression and organization of the genes themselves depend on epigenetic regulators, whose information content is passed on

Table 1 List of molecules targeting mechanisms of gene expression

Name	PubChem ID	Type	Origin	Target	Use(s)
Parthenolide	7251185	Plant natural product	*Tanacetum parthenium*	NF-κB pathway	Bioprobe
Actinomycin D	2019	Bacterial natural product	*Streptomyces parvulus*	RNA synthesis	Therapeutic and bioprobe
α-Amanitin	73755106	Fungal natural product	*Amanita phalloides*	RNA polymerase II	Bioprobe
Flavopiridol	5287969	Semisynthetic	Derivative of rohitukine from *Aphanamixis polystachya*	Cdk9	Experimental therapeutic
DRB	5894	Synthetic	NA	Cdk9	Bioprobe
Triptolide	107985	Plant natural product	*Tripterygium wilfordii*	XPB subunit of TFIIH	Therapeutic
5-Azacytidine	9444	Synthetic	NA	DNA methyltransferase	Therapeutic
Decitabene	451668	Synthetic	NA	DNA methyltransferase	Therapeutic
Zebularine	100016	Synthetic	NA	DNA methyltransferase	Therapeutic
SGI-1027	24858111	Synthetic	NA	DNA methyltransferase	Bioprobe
Trichostatin A	444732	Bacterial natural product	*Streptomyces* spp.	Histone deacetylase	Bioprobe
SAHA	5311	Synthetic	NA	Histone deacetylase	Therapeutic
Trapoxin B	395803	Fungal natural product	*Helicoma ambiens*	Histone deacetylase	Bioprobe
Apicidin	6918328	Fungal natural product	*Fusarium* spp.	Histone deacetylase	Bioprobe
Chlamydocin	124134	Fungal natural product	*Diheterospora chlamydosporia*	Histone deacetylase	Bioprobe
CHAP 31	56603758	Synthetic	NA	Histone deacetylase	Bioprobe
FK228	5352062	Bacterial natural product	*Chromobacterium violaceum*	Histone deacetylase	Therapeutic
HC-toxin	107864	Fungal natural product	*Cochliobolus carbonum*	Histone deacetylase	Bioprobe
Sirtinol	5717148	Synthetic	NA	Sirtuins	Bioprobe
Splitomicin	5269	Synthetic	NA	Sirtuins	Bioprobe
EX-527	5113032	Synthetic	NA	SIRT1	Bioprobe
SirReal2	1096292	Synthetic	NA	SIRT2	Bioprobe
Suramin	5361	Synthetic	NA	Sirtuins	Therapeutic (unrelated application)
Garcinol	174159	Plant natural product	*Garcinia indica*	Histone acetyltransferases	Bioprobe
Curcumin	969516	Plant natural product	*Curcuma longa*	Histone acetyltransferases	Bioprobe
Anacardic acid	167551	Plant natural product	*Ginkgo biloba*	Histone acetyltransferases	Bioprobe
C646	1285941	Synthetic	NA	CBP/p300	Bioprobe
Chaetocin	11657687	Fungal natural product	*Chaetomium minutum*	Histone methyltransferases	Bioprobe
BIX-01294	25150857	Synthetic	NA	G9a	Bioprobe
UNC0638	46224516	Synthetic	NA	G9a	Bioprobe
GSK126	68210102	Synthetic	NA	EZH	Experimental therapeutic
EPZ-6348	66558664	Synthetic	NA	EZH	Experimental therapeutic

(*Continued*)

Table 1 (*Continued*)

Name	PubChem ID	Type	Origin	Target	Use(s)
EPZ-5676	57345410	Synthetic	NA	DOT1L	Experimental therapeutic
JQ1	46907787	Synthetic	NA	Bromodomains	Bioprobe
I-BET	46943432	Synthetic	NA	Bromodomains	Bioprobe and experimental therapeutic
Spliceostatin A	10673568	Semisynthetic	Derivative of FR901464 from *Pseudomonas* spp.	Spliceosome	Bioprobe
Pladienolide	16202130	Bacterial natural product	*Streptomyces platensis*	Spliceosome	Bioprobe
Isoginkgetin	5318569	Plant natural product	*Ginkgo biloba*	Spliceosome	Bioprobe
E7107	16202132	Synthetic	NA	Spliceosome	Experimental therapeutic
TG003	1893668	Synthetic	NA	Clk	Bioprobe
NVS-SM1	86710591	Synthetic	NA	U1snRNP and SMN2 mRNA	Experimental therapeutic
Leptomycin B	57459335	Bacterial natural product	*Streptomyces* spp.	Exportin 1	Bioprobe
KPT-330	71481097	Synthetic	NA	Exportin 1	Experimental therapeutic
NMD I	12733992	Synthetic	NA	Nonsense-mediated RNA decay	Bioprobe
Pateamine A	10053416	Marine natural product	*Mycale hentscheli*	eIF4A	Bioprobe
Hippuristanol	9981822	Marine natural product	*Isis hippuris*	eIF4A	Bioprobe
Rocaglamide	331783	Plant natural product	*Aglaia elliptifolia*	eIF4A	Bioprobe
Allolaurinterol	470278	Marine natural product	*Laurencia filiformis*	eIF4A	Bioprobe
Elisabatin A	397069	Marine natural product	*Pseudopterogorgia elisabethae*	eIF4A	Bioprobe
Reveromycin A	9939559	Bacterial natural product	*Streptomyces* spp.	Ile tRNA synthetase	Bioprobe
Febrifugine	63224	Plant natural product	*Dichroa febrifuga*	Glu Pro tRNA synthetase	Bioprobe and experimental therapeutic
AN2690	11499245	Synthetic	NA	Leu tRNA synthetase	Therapeutic
Homoharringtonine	285033	Plant natural product	*Cephalotaxus harringtonia*	Ribosome	Therapeutic
Cytotrienin	11966097	Bacterial natural product	*Streptomyces* spp.	tRNA binding	Bioprobe
Agelastatin A	177936	Marine natural product	*Agelas dendromorpha*	Ribosome	Bioprobe
Lycorine	72378	Plant natural product	*Clivia miniata*	Ribosome	Bioprobe
Narciclasine	72376	Plant natural product	*Narcissus* spp.	Ribosome	Bioprobe
Cycloheximide	6197	Bacterial natural product	*Streptomyces griseus*	Ribosome	Bioprobe
Lactimidomycin	11669726	Bacterial natural product	*Streptomyces amphibiosporus*	Ribosome	Bioprobe
Mycalamide A	10345974	Marine natural product	*Mycale* spp.	Ribosome	Bioprobe

Abbreviations: Cdk9, cyclin-dependent kinase 9; NA, not applicable; NF-κB, nuclear factor κB; TFIIH, transcription factor IIH ; tRNA, transfer RNA; XPB, xeroderma pigmentosum type B.

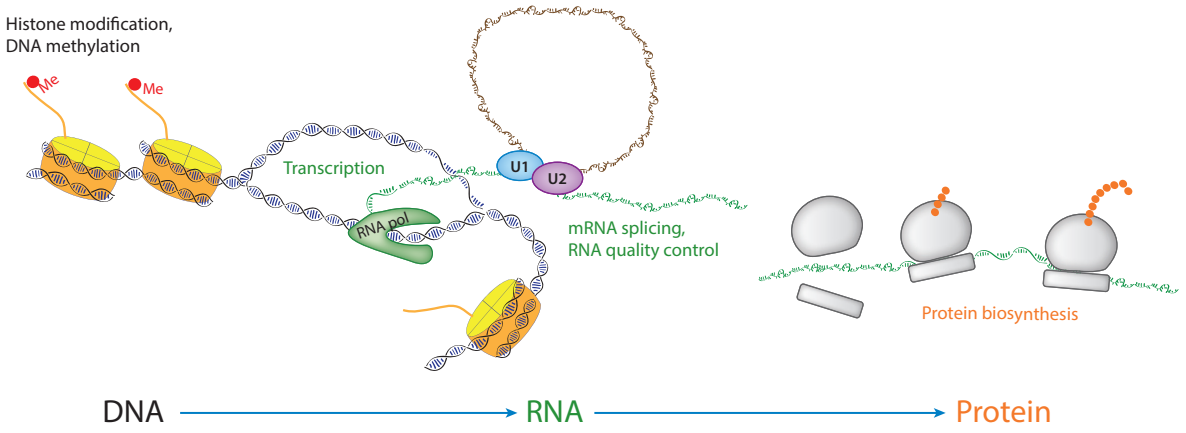


Figure 1

A simplified overview of the central dogma of molecular biology. Information contained in the DNA sequence is transcribed into RNA. The RNA message, after modification, including capping, splicing, and poly-A tailing, is exported into the cytoplasm, where the RNA sequence is translated into the sequence of a polypeptide by the ribosome. Abbreviations: mRNA, messenger RNA; RNA pol, RNA polymerase; U1, U2, small ribonuclear proteins of the spliceosome.

to daughter cells by nongenetic means (9). Small-molecule modulators have become important tools in research and clinical development, allowing the dissection and close scrutiny of individual steps in gene expression and regulation. In some cases, such as the histone deacetylase (HDAC) inhibitors, the small molecules even opened new fields of investigation (10). Many processes from DNA to protein can be manipulated by means of small molecules, as we discuss below.

TRANSCRIPTION INHIBITORS

Gene expression begins with transcription, the recruitment of RNA polymerase to the transcription start site (**Figure 2*b***). This process in itself depends on a myriad of transcriptional regulators binding to distal and proximal promoters as well as the work of mediator complexes (11). Transcriptional activation constitutes the last step in most signal transduction cascades, and naturally, inhibitors of signaling kinases greatly affect transcriptional output (12).

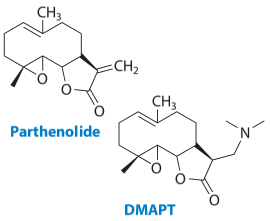
This process has been the subject of many reviews, and we shall limit our discussion to molecules more directly involved in RNA generation and metabolism. Hence, we only discuss parthenolide (PRT) as an example of a natural product interacting with a transcription factor itself. This sesquiterpene originates from the feverfew plant *Tanacetum parthenium* (13). As the plant's common name indicates, its medical use long predates studies on its mechanism. It was utilized in folk remedies to treat fever and pain and as a digestive aid, among other uses.

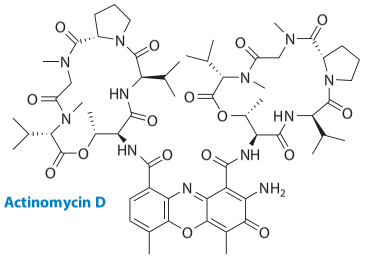
PRT inhibits the nuclear factor κB (NF-κB) signaling pathway, which is involved in cell proliferation and survival as well as in immune activation. Containing both an epoxide and an α-methylene group, PRT is a fairly reactive electrophile. This may explain why it appears to interfere with at least two steps in NF-κB signaling. In the canonical NF-κB pathway, the transcription factor itself, which consists of a dimer of two proteins, commonly p50 and p65 (RelA), is kept in the cytoplasm by its own inhibitor IκB. Upon pathway activation, IκB kinase (IKK) phosphorylates IκB, which marks the protein for degradation by the ubiquitin-dependent machinery. The actual transcription factor can now translocate to the nucleus and bind DNA (14). Gel shift assays and mutational studies indicated that PRT interacts with a cysteine residue on the

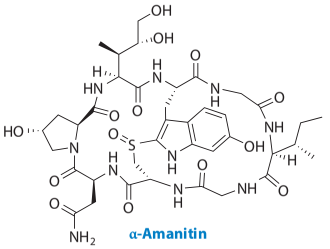
PRT: parthenolide

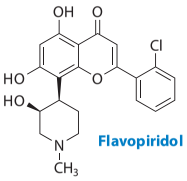
a

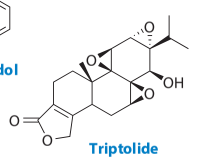
Transcription inhibitors

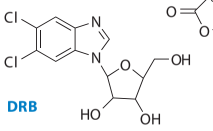




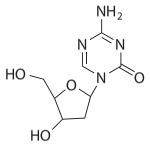


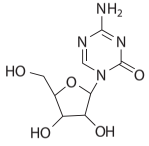


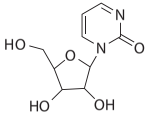


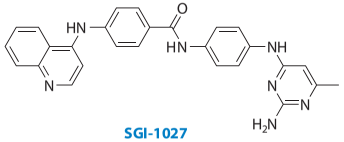


DNA methyltransferase inhibitors

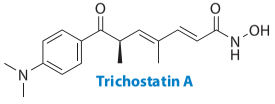


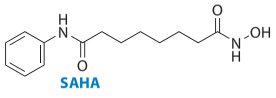




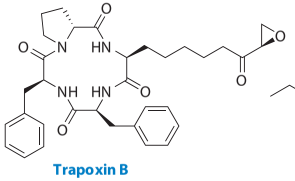


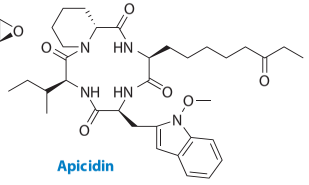
HDAC inhibitors

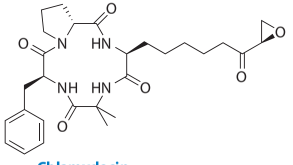


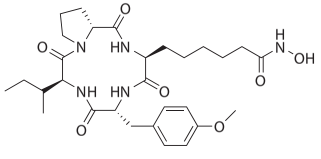


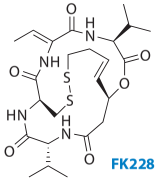
Peptide HDAC inhibitors

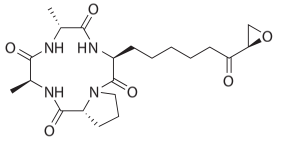












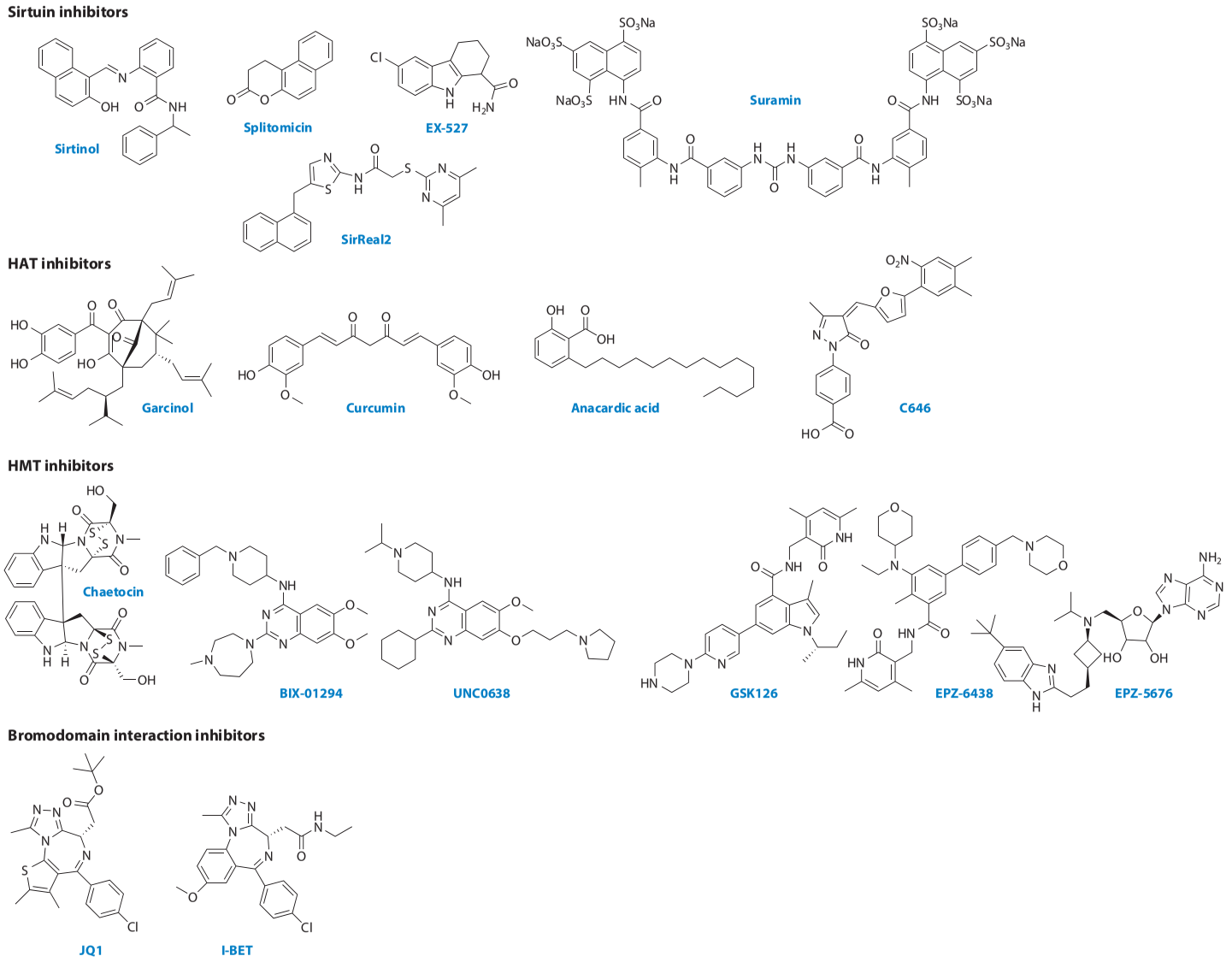


Figure 2

(*Continued*)

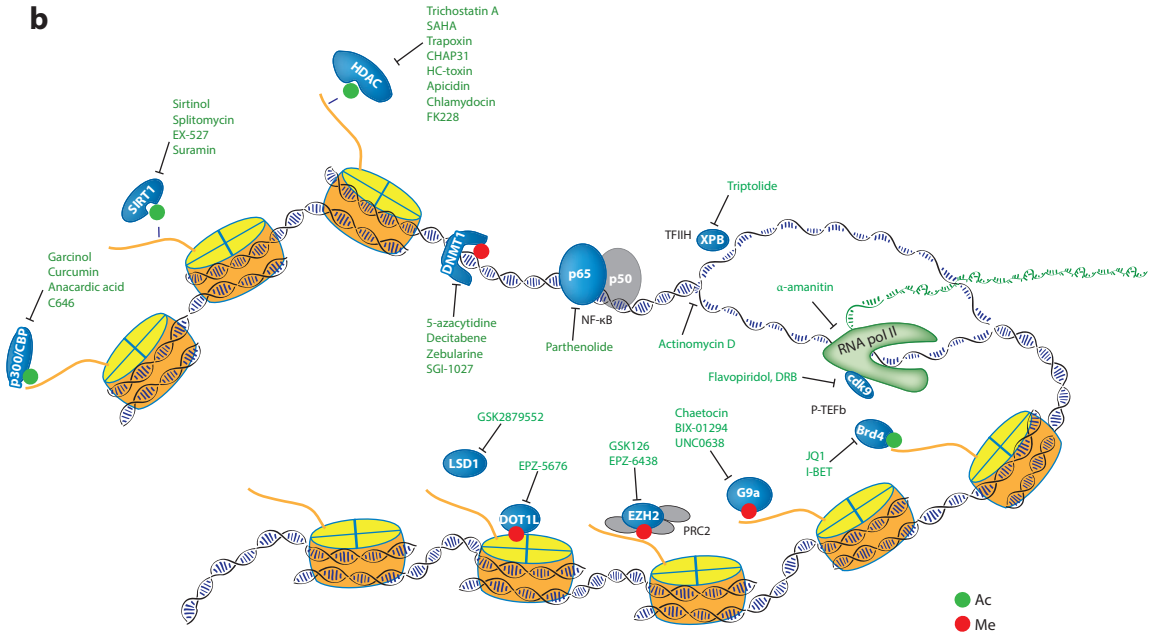


Figure 2

Transcription, DNA methylation, and histone modification. (*a*) Small-molecule inhibitors of the transcription process and its regulation. (*b*) Histone acetylation levels can be chemically modulated by inhibition of either the acetyltransferase, such as p300/CBP, or the deacylase enzymes of the sirtuin and the Zn^{2+}-dependent HDAC families. Increased acetylation levels generally result in increased transcription, whereas DNA methylation of CpG islands has the opposite effect. Aza-nucleotide analogs, including decitabine, reduce DNA methylation levels. The transcription machinery itself can be perturbed in many ways, including inhibition of transcription factor binding. Examples include parthenolide interfering with the p65 subunit of NF-κB's ability to bind DNA or triptolide blocking the XPB helicase of the TFIIH core transcription factor. Actinomycin D intercalates between DNA bases to block RNA polymerase progression, and α-amanitin targets the large subunit of RNA pol II itself. Flavopiridol and DRB block P-TEFb from functioning properly, leading to abortive transcription. Furthermore, P-TEFb recruitment can be disrupted by JQ1 or I-BET through prevention of bromodomain protein Brd4 (and also Brd2 and Brd3) from interacting with histone acetyl-lysine residues. Repressive histone methylation can be prevented by inhibiting HMTs G9a and EZH2. Similarly, an inhibitor for the transcription-activating HMT DOT1L has become available. Abbreviations: DMAPT, dimethylamino-parthenolide; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; NF-κB, nuclear factor κB; P-TEFb, positive transcription elongation factor b; RNA pol, RNA polymerase; SIRT, sirtuin.

p65 subunit of the transcription factor itself, thereby interfering with DNA binding (15). This aspect would make it fairly unique in interfering directly with transcription factor function, rather than with signaling or protein degradation. However, using a PRT–biotin conjugate, IKKβ of the IKK complex was identified as a binding partner, which of course suggests a function in signaling (16, 17). Again, a cysteine residue appeared crucial in allowing PRT binding. Furthermore, reduced PRT lacking the methylene group did not interact with the kinase. However, DMAPT (dimethylamino–parthenolide), a more hydrophilic derivative of PRT also lacking the extracyclic double bond, appears to retain its NF-κB inhibitory activity (18). In addition, the inhibition of IKK appeared to require higher concentrations than the inhibition of p65 binding to DNA. Therefore, abrogating DNA binding may constitute the primary mechanism of action. Owing to its reactive groups, PRT may interact with several partners more or less specifically, because incubation with excess cysteine seems to abolish its effect on NF-κB signaling altogether (13). Most of PRT's antitumor activity can be explained by inhibition of the NF-κB pathway; however, p65 may not be the

only relevant target. Part of its cytotoxic effect relates to the generation of reactive oxygen species (ROS) and PRT-mediated inhibition of thioredoxin reductase, and consequent increase in ROS has been reported (19). In this case, PRT appears to bind not to a cysteine but to a selenocysteine residue on the enzyme. Although PRT seems to have seen limited experimental use in humans, it appears that clinical trials for acute myeloid leukemia halted in phase I (18, 20).

ActD: actinomycin D

Whereas preventing transcription factor binding specifically interferes with expression of target genes, building a roadblock for RNA polymerase naturally has a more general effect on RNA polymerization. The cyclic peptide actinomycin D (ActD) intercalates between the two DNA strands (21, 22). It seems to prefer binding adjacent to G nucleotides and appears to insert itself into so-called β-DNA, which is duplex DNA at the transition between the duplex and single-stranded states, and even to form complexes with single-stranded DNA as one would encounter around active transcription sites (23–25). This intercalation prevents RNA polymerase progress and by virtue of its mechanism does not show any specificity for a particular isoform of RNA polymerase. Hence, ActD especially impacts transcription of ribosomal RNAs (rRNAs) as they represent the most heavily synthesized transcripts. The inhibitory effect on transcription was appreciated from fairly early on, whereas the identification of the mechanism followed in the mid-1990s, when the crystal structures were solved (26, 27). Long before we understood its mode of action, ActD already saw clinical use against a variety of neoplasms, especially Wilms tumors. It also remains a useful laboratory reagent to suppress de novo RNA synthesis (28).

While actinomycin has helped extend the life of cancer patients, α-amanitin, the poison of the toadstool *Amanita phalloides*, has been long known to significantly shorten the lifespan of unlucky mushroom pickers. In contrast to ActD, α-amanitin specifically inhibits RNA polymerase II and, to a much lesser extent, RNA polymerase III (29). It therefore halts canonical gene expression while leaving rRNA and transfer RNA (tRNA) transcription alone. The difference in α-amanitin sensitivity allowed distinguishing and characterizing the three RNA polymerases and their functions, making α-amanitin a useful tool compound. It was appreciated that α-amanitin binds to the largest subunit of RNA polymerase II and appears not only to inhibit its catalytic function but also to induce degradation of its target subunit while not affecting the other RNA polymerase II polypeptides (30). In contrast to actinomycin, the crystal structure of α-amanitin was relatively recently solved (31). The compound appears to lock the enzyme in a translocation intermediate, interfering with incorporation of both nucleotides and translocation. However, α-amanitin does not appear to completely block polymerase activity but rather to significantly slow the rate of nucleotide addition.

Beyond targeting the main enzyme, interfering with parts of the core machinery constitutes another means of preventing RNA polymerase II transcription. After leaving the transcription start site, the polymerase often becomes stuck on the template in the process of abortive transcription and requires accessory factors to continue RNA polymerization. The positive transcription elongation factor b (P-TEFb) is a protein complex containing cyclin-dependent kinase 9 (Cdk9), which catalyzes the phosphorylation of several regulatory proteins, including the negative elongation factor (NELF) and the 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF), as well as the C-terminal tail of RNA polymerase itself. The Cdk inhibitors flavopiridol and DRB block Cdk9 function with some specificity and stall RNA polymerase II. Their mechanism of action mirrors that of signaling inhibitors rather than that of molecules specifically interfering with RNA processing. Flavopiridol competitively inhibits several cyclin-dependent kinases but appears to bind Cdk9 so tightly that it behaves like a noncompetitive inhibitor in biological assays (32, 33).

In contrast, the plant natural product triptolide directly interferes with the function of the transcriptional core machinery. Triptolide has long found application in traditional Chinese medicine

and has been used in treatments for a variety of diseases including arthritis and inflammation. The diterpene triepoxide compound is isolated from *Tripterygium wilfordii*, a plant also known as the thunder god vine, which suggests that dose-limiting toxicity may present itself rather violently. The molecule also stands out for containing three adjacent epoxide groups and hence, not surprisingly, covalently binds its target protein (34). Triptolide specifically inhibits the XPB helicase, a subunit of the core transcription factor TFIIH, required both for basal transcription and nucleotide excision repair (35). Surprisingly, triptolide inhibits only XPB's ATPase activity and does not interfere with its function as a helicase.

SAM: *S*-adenosylmethionine

DNMT: DNA methyltransferase

TSA: trichostatin A

HDAC: histone deacetylase

EPIGENETIC INHIBITORS: DNA METHYLATION

In addition to interfering with the transcription machinery, altering the epigenetic marks that determine whether transcription can occur at a particular locus also proves a valuable target for bioprobe application and pharmacological intervention. After all, these processes are of the utmost importance for complex organisms. Each cell in an organism contains the same genome, yet during growth and development, distinct tissues and cell types differentiate, each utilizing only a subset of its available genes. A system of confounding complexity regulates gene accessibility of genomic loci by chemically modifying DNA itself and the histone proteins around which it is wrapped. A breakdown in this control system can promote pathologies either by shutting down required genes or by aberrantly expressing others. The most-studied mechanisms of epigenetic control are DNA methylation and posttranslational modification of histones, especially methylation and acetylation (36, 37). Utilizing *S*-adenosylmethionine (SAM) as a cofactor, DNA methyltransferases (DNMTs) methylate genomic DNA at the 5 position of cytidine in the context of CpG islands. Of genomic CpG sites, 70–80% are methylated through DNMTs, with DNMT3A and DNMT3B being responsible for de novo methylation and DNMT1 acting to maintain methyl marks. Methylation of promoter CpG islands has long been identified as a negative epigenetic marker to prevent gene expression and was, until the recent discovery of DNA demethylases, believed to be irreversible (38). 5-Azacytidine and 5-aza-2′-deoxycytidine (decitabene) nucleotide analogs carry a nitrogen instead of a carbon atom in their 5 position, which leads methyltransferases, especially DNMT1, to become covalently stuck. This inhibition triggers proteasome-mediated degradation of DNMT1, thereby decreasing the DNA methylation level over time (39–41). 5-Azacytidine and decitabene are approved clinical drugs that have shown efficacy against myelodysplastic syndromes and acute myeloid leukemia; however, they suffer from low chemical stability in the body and generation of toxic metabolites. Other nucleotide analog compounds, such as zebularine, lack specificity and potency (42, 43). Attempts to produce synthetic non-nucleotide analog inhibitors have made some progress with SGI-1027 and analogs inhibiting DNMT by competing with SAM (40, 44).

EPIGENETIC INHIBITORS: HISTONE MODIFIERS

Even though epigenetics has become a well-established field of biology, the role of histone proteins and their posttranslational modifications remained elusive for a long time. That histones experience acetylation and that increased acetylation levels generally correlated with increased transcription had been known for decades. In the late 1960s, it was also observed that treatment with *n*-butyrate increased the level of histone acetylation, but as butyrate was far from specific and affected many cellular systems, the observation did not lead to identification of the proteins responsible (45, 46). The antifungal antibiotic trichostatin A (TSA) was shown to specifically increase the level of histone acetylation. A resistance mutation was identified in a HDAC protein, demonstrating that histone proteins are dynamically acetylated and deacetylated (47). Containing

a hydroxamic acid moiety, TSA chelates a catalytic zinc ion from the enzyme's catalytic site, while the rest of the molecule binds to the HDAC protein. A number of additional hydroxamates have since been discovered and developed, including SAHA, which was first synthesized to develop agents that induce cell differentiation (48). Zinc chelation proved a common feature among diverse groups of HDAC inhibitors, which generally consist of three molecular substructures: a cap that binds around the substrate-binding pocket, a group interacting with the zinc ion, and a spacer between the cap and zinc ligand.

TPX: trapoxin

Although HDAC inhibition by TSA is easily reversible, the cyclic peptide trapoxin (TPX) binds its target so tightly that it allowed isolation of the first HDAC by affinity pulldown using a bead-conjugated derivative (49–51). The isolated protein named HDAC1 turned out to be a homolog of the yeast transcriptional regulator RPD3. Identification of the drug's target allowed searching for further enzyme homologs. Following TPX, a number of cyclic peptide HDAC inhibitors were identified, including apicidin, chlamydocin, HC-toxin, and FK228. These all represent nonribosomal peptide natural products, isolated from microbial cultures and often containing noncanonical amino acids (52–54). Even though one can see at least some structural similarities among the trapoxins, chlamydocin, and apicidin, FK228 and HC-toxin stand out. Although HC-toxin does contain the same epoxide as TPX and chlamydocin, its constituent amino acids and their order bear little resemblance with the rest. FK228, also known as romidepsin, contains an intramolecular disulfide bond, which relies on intracellular reduction by glutathione for activity, and hence constitutes a prodrug (55). The reduced thiol group is thought to interact with the catalytic zinc ion. The mechanisms of the hydroxamic acid and the cyclic peptides may be similar, as hybrid molecules such as CHAP31, synthesized to contain both TSA's hydroxamic acid and a cyclic peptide, proved to potently inhibit HDAC activity (56, 57).

To date, 11 human HDACs have been identified and fall into 3 classes: class I contains RPD3-like enzymes HDAC1, HDAC2, HDAC3, and HDAC8; class II is further subdivided into classes IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and IIb (HDAC6 and HDAC10); and class IV contains HDAC11, whose catalytic activity is poorly understood. Almost all HDAC proteins are sensitive to TSA, but owing to structural differences, class IIb enzymes are resistant to some HDAC inhibitors including cyclic peptides (58, 59). Although all family members are zinc-dependent deacetylating enzymes, despite the name HDAC, they do not exclusively deacetylate histone proteins. Even though HDAC1 and HDAC2 are part of large nuclear chromatin remodeling complexes involved in gene silencing, HDAC3 not only modifies chromatin in response to nuclear receptor signaling but also seems to deacetylate the RelA (p65) subunit of the NF-κB transcription factor, thereby acting as an intranuclear signaling switch, as acetylated RelA does not bind well to DNA (60, 61). HDAC6 is able to deacetylate tubulin and Hsp90. It stands out from the rest in having two catalytic domains, with one appearing to have specificity for substrates bearing C-terminal acetyl-lysine residues (58). Most HDAC proteins are essential for life, and knockout experiments in mice usually result in embryonic or perinatal lethality. Only knockout HDAC6, HDAC10, and HDAC11 animals are viable; HDAC6 deletions even survive with no obvious phenotype.

Class III HDACs constitute a completely different and mechanistically distinct class of deacetylases. Named sirtuins after the *Sir2* (silencing information regulator 2) gene in yeast, which had been identified in a screen for genes controlling silent mating loci, deacetylases in this class depend on NAD^+ to effect removal of their target's acetyl groups. Current mechanistic models involve attack of the acetamide carbonyl oxygen to cleave the NAD^+ glycosidic bond, thereby generating *O*-acetyl ADP-ribose and nicotinamide. Whether the cleavage of NAD^+ proceeds through a dissociative S_N1 reaction or a coordinated attack with S_N2 character remains a matter of debate. In mammals, seven sirtuins are known (SIRT1–SIRT7) and largely work on a variety of nonhistone

substrates, although SIRT7 specifically deacetylates histone H3K18Ac. SIRT1, like SIRT6 and SIRT7, is localized in the nucleus and acts not only on modifications of histones H1, H3, and H4 but also on a variety of epigenetic regulators and transcription factors (for a detailed review see 62). SIRT2 mainly resides in the cytoplasm but can translocate to the nucleus during mitosis (63). Meanwhile, SIRT3–SIRT5 are localized in mitochondria. Sirtuins are reportedly involved in a number of diseases, ranging from Parkinson's to diabetes, inflammation, and cancer, as well as in the control of life span. Unlike class I and II HDACs, knockout mice are, with the exception of SIRT1 and SIRT6, viable.

Owing to their different mechanisms, sirtuins are resistant to TSA or cyclic peptide inhibitors of zinc-dependent HDACs. In contrast to the other HDACs, fewer sirtuin inhibitors are known to date. The nicotinamide released in the reaction works as a sirtuin inhibitor and has been traditionally used at high concentration to block sirtuin activity. Alternatively, nonhydrolyzable analogs of NAD^+ act as sirtuin inhibitors, but given NAD^+'s wide range of cellular functions, their application yields complex results. Generally, sirtuin inhibitors act either on the NAD^+ binding site or on the interaction between the sirtuin protein and acetyl-lysine.

Phenotypic screens in yeast yielded the discovery of sirtinol and splitomicin, which compete with NAD^+ and acetyl-lysine, respectively, although neither is particularly potent with IC_{50} values in the mid-micromolar range. Owing to the connection between sirtuins and several disease states, significant screening and chemical optimization efforts were undertaken to identify more potent, and to some extent more selective, sirtuin inhibitors. EX-527 lowered the IC_{50} for sirtuin inhibition into the mid-nanomolar range with approximately 20-fold preference for SIRT1 (64). Perhaps most remarkably, SirReal (sirtuin-rearranging ligand) family members achieve more than 1,000-fold selectivity for SIRT2 over the other sirtuin family members. These proteins lock SIRT2 in an open, unproductive conformation and exploit small structural differences between otherwise closely conserved enzymes to specifically bind SIRT2 (65). Even suramin, an agent used since 1916 to treat sleeping sickness, was reported to inhibit sirtuins at IC_{50} values in the low micromolar range and has been crystallized with SIRT5 (66). Whether this binding plays a role in suramin's antiprotozoal activity is unclear, as the compound also seems to act on G-protein coupled receptors (67). In general, molecules blocking the function of class I and II HDACs have been identified, but far fewer potent and selective sirtuin inhibitors have been found to date. Perhaps there are more chemical routes to chelate a zinc ion than there are to interfere with the fairly unique mechanism of NAD^+-dependent deacetylation.

The discovery of HDAC inhibitors greatly improved our understanding of histone modification and its role in transcriptional control in health and disease. SAHA and FK228, the first two specific HDAC inhibitors, have won US Food and Drug Administration (FDA) approval and have found their way into the clinic as therapeutics for certain types of lymphoma. Several reasons may explain why increasing histone acetylation levels has an antitumorigenic effect. Most commonly, it is assumed that increased histone acetylation may reactivate epigenetically silenced tumor-suppressor genes, thereby increasing expression of proapoptotic factors and cell cycle regulators. In addition, hyperacetylation of nonhistone proteins may also have cytotoxic effects. HDAC inhibitor treatment can lead to the reexpression of p53, whereas acetylation of Hsp90 decreases the chaperone's activity, resulting in destabilization of a number of Hsp90 client proteins (68).

In recent years, an additional aspect of HDAC activity has emerged. Both zinc-dependent and sirtuin-type deacetylases appear to remove not only acetate groups from histones and nonhistone target proteins but also larger acyl groups. Beyond acetylation, other short fatty acid additions have been identified as specific and spatially regulated posttranslational modifications of histones and other proteins (69, 70). An in vitro study demonstrated that sirtuins could also

remove propionyl and butyryl groups from target protein peptides (71). Studies thereafter showed the ability of sirtuins to remove charged groups such as succinate, malonate, or glutarate (72, 73). The discovery that SIRT5 could efficiently perform desuccinylation, while only showing weak deacetylation activity, particularly suggested that the roles of sirtuins were more diverse than originally thought. Similarly, the role of class I and II HDACs appears to extend beyond acetylation. For instance, histone crotonylation is enriched in active promoter and enhancer regions of sex-chromosome associated genes (69). HDAC1–HDAC3 are considered likely responsible for the removal of crotonyl groups. These recent discoveries, of course, mean that the impact of HDAC and sirtuin inhibitors extends beyond protein acetylation, opening a whole new layer of complexity.

Although many known molecules inhibit histone deacetylation, inhibitors of the reverse process remain scarce, and clinically relevant molecules have thus far not emerged. Several food-derived inhibitors of the p300/CBP histone acetyltransferase enzyme have been reported, including curcumin from turmeric; garcinol from the *Garcina indica* fruit rind; and anacardic acid, found in cashew nuts and ginkgo (74–76). However, these compounds proved to be of only modest potency and limited specificity. For instance, anacardic acid seems to more potently inhibit the SUMO E1 enzyme than it does p300/CBP (77). So far C646, which emerged from a virtual screen against p300/CBP, presents a more usable and specific HAT (histone acetyltransferase) inhibitor (78). C646-mediated p300 inhibition reduces colony formation and leads to apoptosis in acute myeloid leukemia cells harboring the AML1-ETO fusion protein. In addition, C646 increases sensitivity to DNA-damaging agents in melanoma and nonsmall cell lung carcinoma cells (79, 80). These findings suggest that not only inhibition of acetyl removal but also blocking its addition can have clinical benefits.

READER INHIBITORS

Inhibiting global HDAC activity of course affects many cellular systems and constitutes a fairly large intervention. Interfering with proteins that interact with acetylated lysines presents a different venue of chemically altering transcriptional output. Bromodomains are protein folds that specifically bind acetylated lysines, many of which act as transcriptional regulators, especially members of the BET (bromodomain and extraterminal) domain family for proteins. For instance, BET family member Brd4 plays a key role in recruiting the aforementioned positive transcriptional regulator P-TEFb. Furthermore, Brd4 has been identified as a component of a chromosomal translocation in aggressive human squamous carcinoma. In these tumors, the N-terminal two bromodomains of Brd4 are fused with the NUT (nuclear protein in testis) protein. The Brd4-NUT oncoprotein drives cell proliferation in this fatal malignancy. Hence, preventing interaction between chromatin and Brd4 has proven a promising means of stalling tumor cell proliferation. Several synthetic compounds, most famously JQ1 and I-BET, were produced to fit into the acetyl-lysine binding pocket of bromodomain proteins with particular emphasis on Brd4, though they also bind Brd2 and Brd3 (81, 82). These molecules have shown to delay or prevent disease onset in mouse models but do not appear to be curative. Even though HDAC inhibitors increase transcriptional output by preventing promoter deacetylation, whereas BET inhibitors prevent transcriptional output by interfering with binding to acetylated lysine, the two classes of molecules appear somewhat synergistic in mouse models (83). Although effects of HDAC inhibitors in cells can be readily monitored by immunological methods using site-specific anti-acetyl-histone antibodies, it was hard to observe the interference with BET protein binding to acetylated histones in cells. Recent development of FRET (Förster resonance energy transfer) probes using BET bromodomains enabled live cell bioimaging of their interaction (84, 85).

HISTONE METHYLTRANSFERASE INHIBITORS

HMT: histone methyltransferase

DLBCL: diffuse large B-cell lymphoma

***MLL*:** mixed-linkage leukemia gene

Acetylation, of course, is not the only means by which gene expression can be regulated on the histone level, and histone methylation is at least as well studied as addition and removal of acetate groups. Methylation allows an even more differential marking of histone tails, as one lysine ε-amine can accommodate up to three methyl groups and a mono-, di-, or trimethylated lysine may recruit different sets of regulatory and effector proteins. Perhaps best studied are H3K4m3, a mark for active promoter regions, and the repressive marks H3K9m2, H3K9m3, and H3K27m3 (36). The H3K9 monomethyl- and dimethyl-specific lysine histone methyltransferase (HMT) G9a attracted attention as a target for therapy, as it is upregulated in various cancers and its expression correlates with poor prognosis (86, 87). Chaetocin, a metabolite of the fungus *Chaetomium minutum*, was first discovered as an inhibitor competitive for SAM of the H3K9m3-specific HMT Su(var)3–9 but proved to also inhibit G9a at comparable concentrations but not other HMTs (88, 89). Chaetocin's utility was however hampered by high cytotoxicity, which also raised questions of its specificity, as its internal disulfide bond renders the molecule fairly reactive (90). Total synthesis of chaetocin and analogs allowed reducing the molecule to an active core structure, which kept treated cells viable (91). A screen of synthetic molecules at high SAM concentration tried to overcome this limitation and led to the discovery of BIX-01294, which specifically inhibited G9a but not Su(var)3–9 (92). BIX-01294 in turn served as the starting point to optimize the compound, eventually yielding UNC0638, a G9-aspecific molecule with an inhibitory constant of only 15 nM (93).

The HMT EZH2 of the polycomb repressive complex 2 (PRC2) acts upon the H3K27m3 mark for inactive chromatin and is distinct from the methylase complexes working on H3K9. EZH2 is also commonly overexpressed in a variety of neoplasms including follicular lymphoma and diffuse large B-cell lymphoma (DLBCL) (94). A combination of screening and chemical optimization led to development of GSK126. Even though it competes with SAM, GSK126 is highly specific for EZH2 over all other HMTs (95). Having shown efficacy in mouse tumor, GSK126 failed phase I clinical trials for DLBCL. However, a structurally similar compound, EPZ-6438, proved more specific and efficacious. A few cases of responding patients have been reported in the current phase I/II trials (94, 96). Rearrangements of the mixed-linkage leukemia (*MLL*) gene present in a subset of acute leukemias correlate with aggressive disease progression and poor outcome. These neoplasms rely on methylation of H3K79, catalyzed by the HMT DOT1L, for disease maintenance. Similar to the molecules mentioned above, a SAM-competitive inhibitor specific to the corresponding HMT could be developed (97). EPZ-5676 showed great selectivity for DOT1L and appears to have favorable pharmacokinetic properties. In phase I clinical trials, the molecule displayed acceptable toxicity and seemed to inhibit its target in vivo (98).

In the field of histone modification, much remains to be discovered. Small-molecule inhibitors are of great help in dissecting the process, but many more compounds are necessary to understand epigenetic genome organization. Histone modifications extend beyond methylation and acetylation to serine and threonine phosphorylation, as well as addition of *N*-acetylglucosamine (GlcNAc) residues. To complicate matters, several histone acetylases and methylases, deacetylases, and demethylases obtain their specificity for only a particular amino acid in the context of large multiprotein complexes. Although chemical biology provides valuable tools for illuminating epigenetics and has already yielded clinically relevant compounds, we are still far from a fully comprehensive understanding of histone modification and function.

SPLICING INHIBITORS

The discovery of splicing, the process of removing intronic sequences from primary transcripts, came as a bit of a surprise. When the central dogma was formulated, nobody imagined that RNA

transcripts would undergo such dramatic editing. It soon became clear that splicing not only could result in removal of most of the primary transcript but also allowed the assembly of different mature mRNAs from the same message. These splice variants can yield dramatically different gene products, whose functions might include determining the sex of an entire organism (99). Furthermore, splice isoforms of the same gene may perform opposing biological roles such as promoting or inhibiting blood vessel formation (100). However, the possibility of deriving several mRNA messages from the same gene explains, at least in part, how relatively complex organisms, such as mammals, could function with a relatively small total number of genes. Although in vitro experimentation allowed a fairly detailed understanding of the spliceosome and the intricate action of ribonucleoproteins in coordinating the excision of intronic sequences, test tube experiments did not allow an investigation of splicing on a cellular scale (**Figure 3*b***).

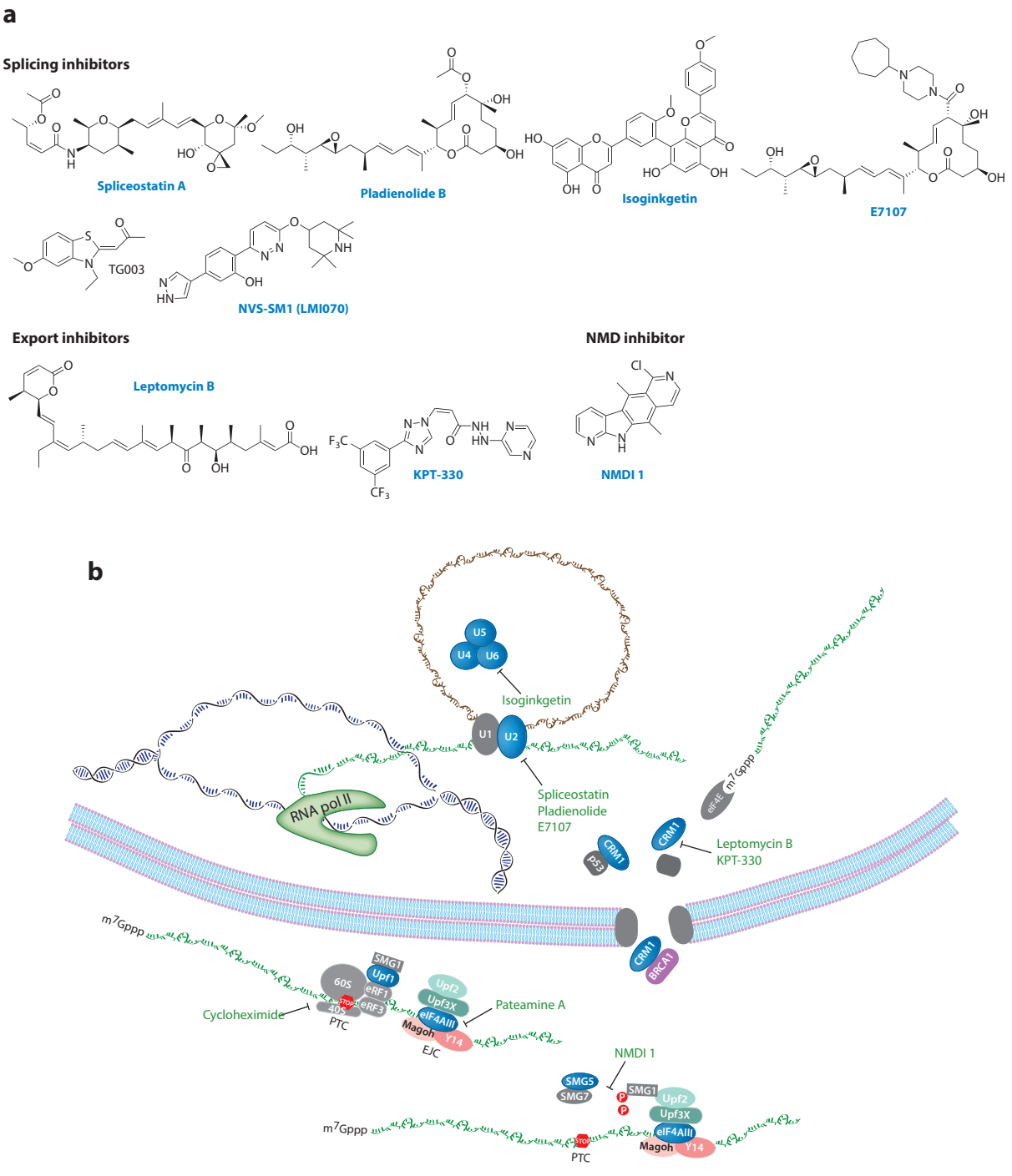
SSA: spliceostatin A

The splicing process itself, of course, could be recapitulated in in vitro experiments and revealed an intricate series of interactions between the pre-mRNA message and five small nuclear ribonucleoprotein (snRNP) complexes. In brief, an intron begins at the 5′ splice site (5′ss) and ends at the 3′ splice site (3′ss). Close to the 3′ss lies the branch point sequence (BPS), which contains the adenosine nucleotide whose 2′-hydroxyl group is required for attacking the 5′ss and thereby initiating the actual splicing process. Between the BPS and 3′ss lies the polypyrimidine tract (PPT), a stretch of pyrimidine residues required for the binding of accessory factors. The U1 snRNP binds at the 5′ss, while accessory factors bind BPS and PPT and recruit the U2 snRNP, which attaches at the BPS via base pairing. This assembly, termed the A complex, attracts the U4/U5/U6 tri-snRNP to form the B complex. After U1 and U4 leave, this assembly in turn becomes the C complex, the catalytically active spliceosome. Now the BPS adenosine 2′-OH can attack the 5′ss thereby freeing the 3′-OH at the end of the 5′ exon, which can then perform a nucleophilic attack on the 3′ss, cutting out the intron and linking the 5′ and 3′ exons with each other.

When screens first identified molecules activating viral promoters and inhibiting cell cycle progression, nobody imagined a relation to RNA splicing. In case of the natural product FR901464, isolated from a broth of *Pseudomonas* sp., a truncated and constitutively active form of the cell cycle regulator p27 was detected, which could account for the observed effect on the cell cycle. The transcript of the truncated protein, dubbed p27*, appeared to result from an unspliced transcript in which part of an intron became translated until a premature stop codon. A biotinylated form of the inhibitor allowed isolation of the SF3b subcomplex of the U2 snRNP subunit of the spliceosome, determining that FR901464 and its methyl-ketal derivative termed spliceostatin A (SSA) specifically targeted the spliceosome. FR901464 and SSA prevented the splicing process before any cleavage of phosphodiester bonds could occur (101). In the presence of SSA, the U2 snRNP only weakly interacts with the BPS and may bind in the wrong position, preventing constructive spliceosome formation (102). Around the same time, an independent group of researchers realized that the *Streptomyces* natural product pladienolide inhibited the same target and seemed to work by virtually the same mechanism as SSA (103).

The newly discovered inhibitors opened several novel venues of investigation. First of all, it became clear that splicing inhibition had a profound effect on several cellular systems. Upon inhibitor addition, the size and number of nuclear speckles seemed to increase, indicating an accumulation of unspliced RNA and splicing machinery inside the nucleus. Yet, some unspliced messages, such as p27*, evidently escaped from the nucleus and were translated, thereby circumventing RNA quality control mechanisms. Surprisingly, transcription as such seemed only somewhat affected with approximately 15–20% of genes experiencing downregulation, whereas a small percentage of genes were even increasingly transcribed (104, 105).

Splicing inhibition appeared to slow down the kinetics of RNA processing, leading to changed transcript fates including transcript shortening (106). Furthermore, splicing inhibition also



(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

Splicing, export, and nonsense-mediated mRNA decay (NMD). (*a*) Small-molecule inhibitors of RNA metabolism and quality control. (*b*) The excision of intronic sequences can be inhibited with spliceostatin A or pladienolide B and related molecules, which interfere with snRNP U2 and prevent formation of a functional splicing complex. However, isoginkgetin allows U1 and U2 to productively bind to the 5′ and 3′ splice sites, respectively, but blocks the tri-snRNP U4/U5/U6 from binding. Protein export from the nucleus and to some extent RNA export rely on the CRM1 export factor. Leptomycin B and KPT-330 prevent CRM1 from binding its cargo proteins, which include the breast cancer–associated BRCA1 protein and the tumor suppressor p53, as well as the cap-binding factor eIF4E. NMD depends on an initial round of translation. Therefore, general translation inhibitors, such as cycloheximide, also prevent destruction of mRNA containing a premature stop codon. Although also a translation inhibitor, pateamine A binds eIF4AIII, part of the EJC, and prevents destruction of the message. In contrast, NMDI 1 blocks NMD at a later stage and prevents phosphorylated Upf1 from interacting with SMG5, which in turn cannot recruit nucleases or decapping enzymes. Abbreviations: CRM1, chromosomal maintenance 1; EJC, exon junction complex; mRNA, messenger RNA; PTC, premature termination codons; RNA pol, RNA polymerase; snRNP, small nuclear ribonucleoprotein; U1, U2, small ribonuclear proteins of the spliceosome.

appeared to affect the chromatin state. SSA application was observed to induce a redistribution of the histone H3 lysine 36 trimethyl (H3K36m3) marker, often associated with alternative splicing (107). The specific effect on the cell cycle and selective toxicity against cancer cells also generated some interest in developing splicing inhibitors into clinical therapeutics. E7107, a urethane-derivative of pladienolide, has entered limited phase I clinical trials. Although it appeared efficacious in several patients, dose-limiting neurotoxicity has thus far prevented clinical application (108). With H3B-8800 the next generation of synthetic SF3b inhibitors has entered clinical testing.

Pladienolide and SSA are not the only known splicing inhibitors, though perhaps they are currently the most potent ones available. Isoginkgetin, a molecule found in *Ginkgo biloba*, among other plant species, also blocks mRNA splicing though at higher concentration. It prevents the recruitment of the U4/U5/U6 tri-snRNP complex, therefore preventing formation of a catalytically active spliceosome (109). Depending on a cell's stage in growth and development, different splice isoforms of the same gene may be required. The inclusion or skipping of certain exons can have drastic consequences for the function of the resultant protein and eventually for an entire organism. The process of alternative splicing is not well understood. It appears that SR proteins, a family of highly phosphorylated serine- and arginine-rich proteins, play an important role in spliceosome assembly and the regulation of alternative splicing. The benzothiazole compound TG003, which inhibits upstream kinases Clk1 and Clk4, alters the splicing pattern of several genes including tumor suppressor p53. In a case study, it was observed that TG003 led to exon skipping in cells with a nonsense mutation in the *dystrophin* gene (110). *Dystrophin* is commonly mutated in patients suffering from Duchenne muscular dystrophy, leading to premature termination codons (PTCs) and destruction of the transcript. In at least two observed cases, TG003 appeared to induce skipping of mutated exons. It was speculated that the mutations changed interaction sites for splicing factors, as TG003 had no effect on wild-type *dystrophin* splicing. However, the scenario in which TG003 leads to productive skipping of mutated exons may be too rare an occurrence for the development of TG003 as a clinical drug. A cross-species comparison of TG003-sensitive genes found that in both mice and humans short exons with relatively short PPTs were more likely to be skipped in the presence of compound (111).

Naturally, inhibiting a global process also results in global toxicity. Although certain types of tumors may be selectively susceptible to splicing inhibitors, a more specific form of therapy, which leaves the bulk of mRNA processing unchanged, would be preferable. In this context, the first molecules targeting specific RNA–RNA or RNA–protein interactions have been discovered. A splice modulator that was recently reported enhances the splicing of a small subset of genes with promise in the therapy of spinal muscular atrophy (SMA). SMA presents a relatively common lethal

SMA: spinal muscular atrophy

LMB: leptomycin B

genetic disease resulting from the death of α-motor neurons. In most cases, insufficient levels of the SMN (survival of motor neurons) protein are responsible for neuronal death. The *SMN* gene is present in two copies, *SMN1* and *SMN2*; however, a nucleotide transition in the *SMN2* gene results in inefficient splicing and frequent skipping of exon 7. Only 10–20% of SMN2 mRNA molecules become properly processed, whereas the remainder result in a truncated and unstable protein. In healthy individuals with an intact *SMN1* gene, low levels of *SMN2* expression have no effect on neuronal survival, but in patients with mutated *SMN1*, the other allele cannot compensate for the loss. Screening a large compound selection for molecules able to enhance *SMN2* splicing with subsequent lead optimization allowed development of NVS-SM1 (112). Although not a natural product, the mechanism of NVS-SM1 is quite remarkable as it enhances the interaction between the SMN2 pre-mRNA and the U1 snRNP around the exon 7 5′ss, resulting in more efficient exon inclusion and elevated levels of SMN protein. NVS-SM1 is currently undergoing phase II clinical trials. Recently, a first therapy for SMA received FDA approval, but this therapy is based not on a small-molecule modulator but on a stabilized antisense oligoribonucleotide to stabilize SMN2 exon 7 and requires intrathecal injection (113, 114).

NUCLEAR EXPORT INHIBITORS

After transcription and splicing, the mRNA still needs to reach the cytoplasm to be translated into protein. The nuclear export factor CRM1 (chromosomal maintenance 1), also known as exportin 1, is a Ran-GTP–dependent cargo adaptor involved in regulating the transport of a large variety of nuclear proteins to the cytoplasm. It was originally identified as a gene product required to maintain higher chromosomal structure in the fission yeast *Schizosaccharomyces pombe* (115). *CRM1* was then rediscovered as a gene that confers resistance to the *Streptomyces* secondary metabolite leptomycin B (LMB), which was later shown to inhibit nuclear export of NES (nuclear export signal)–bearing proteins (116–118). CRM1 cargoes include a number of cancer-associated proteins, such as BRCA1, p27, p53, and APC, as well as several mRNA carrier proteins (115, 119). Although the majority of capped RNA polymerase II transcripts leave the nucleus by the bulk export path, a subset of mRNAs rely on CRM1 for export to the cytosol (120). For instance, HuR, an RNA-interacting protein associated with AU-rich RNA elements, and the cap-binding protein eIF4E appear to be CRM1 cargoes. eIF4E association seems to fast-track certain transcripts for translation, whereas mRNAs exported via the bulk pathway are thought to bind eIF4E after arriving in the cytoplasm (121). LMB covalently modifies a cysteine residue inside CRM1's NES site in a Michael-type addition reaction, therefore obstructing the Ran-GTP–dependent binding between CRM1 and its cargo (122, 123). Later, the direct interaction between LMB and CRM1 could be demonstrated via affinity purification using an LMB-biotin conjugate (124). As many known oncogenes rely on CRM1-dependent export for their function, LMB showed promise in chemotherapy and selectively killed tumor cells. However, its mechanism of covalently modifying its target protein is not ideal for a therapeutic, and LMB has shown unacceptable toxicity in phase I clinical trials against a variety of tumors but without visible benefit to the patients (125). Nevertheless, CRM1 remains an attractive drug target, and the noncovalent inhibitor KPT-330 (selinexor) appears to have tolerable toxicity and has entered phase II clinical trials against myelodysplastic syndromes. Although LMB inspired the search for CRM1 inhibitors, KPT-330 is chemically unrelated to LMB and was developed from an in silico screen of virtual molecules binding to the CRM1 protein (126, 127). A recent study demonstrated a synthetic lethal relationship between *KRAS* mutants and nuclear export in lung cancer cells. KPT-330 and related KPT-185 could exploit this vulnerability and greatly relieve the tumor burden of model mice (128).

NONSENSE-MEDIATED mRNA DECAY INHIBITORS

EJC: exon junction complex

NMD: nonsense-mediated mRNA decay

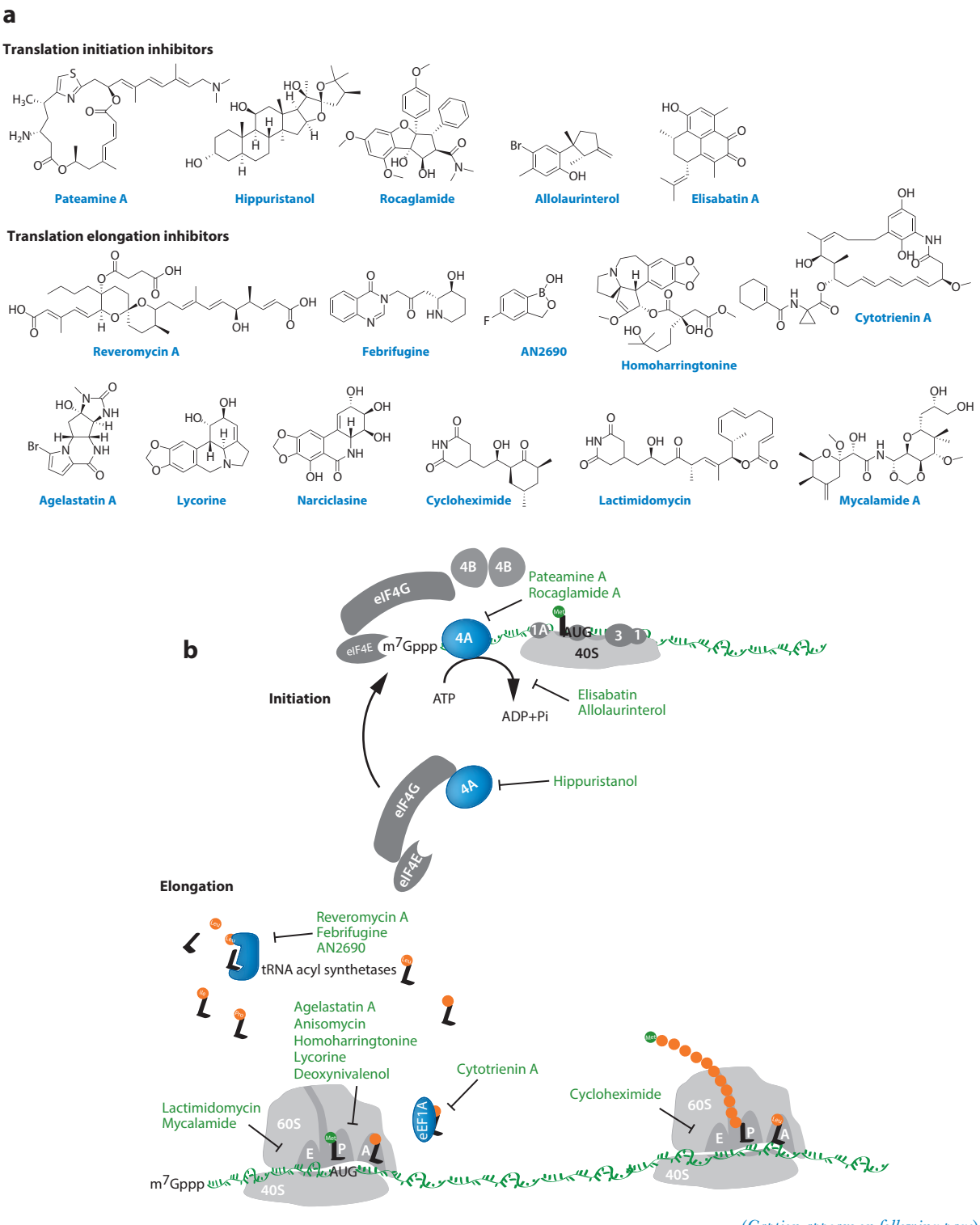
Before the spliced transcript reaches the translation machinery, one more round of quality control takes place in the form of nonsense-mediated decay. Transcripts containing PTC are degraded, which decreases the likelihood of producing aberrant or even toxic polypeptides. It seems that a pioneer round of translation takes place in which the ribosome displaces exon junction complexes (EJCs) deposited 24 nucleotides upstream of the splice site during the splicing process. The core of the EJC consists of Y14, Magoh, and eIF4AIII, an isoform of the translation factor eIF4A not involved in protein synthesis. This complex binds tightly to the mRNA and is associated with a multitude of other factors, which seem to include SR protein splicing factors (129). The terminating ribosome is thought to nucleate a surveillance complex dubbed SURF on the mRNA that comprises ribosome release factors eRF1 and eRF2, the protein kinase SMG1, and most importantly, the nonsense-mediated mRNA decay (NMD) factor Upf1 (130). Meanwhile, NMD factors Upf3X and Upf2 bind the EJC. Upon Upf1 binding, Upf3 and Upf2 stimulate Upf1's ATP-dependent helicase activity. Furthermore, SMG1 phosphorylates Upf1, which in turn recruits the SMG6 endonuclease and either SMG5–SMG7 or SMG5–PNRC2 complexes. SMG1 then brings in deadenylation and decapping enzymes, whose activity precedes the actual destruction of the transcript, in addition to phosphatase 2A, which acts on Upf1. This cycling of phosphorylation states appears to be important for proper Upf1 function and NMD activity.

The interaction between phosphorylated Upf1 and SMG5 plays an especially crucial role in efficient transcript destruction. Although neither binding partner nor precise mechanism have been identified so far, the NMD inhibitor, somewhat unsurprisingly named NMDI 1, seems to block Upf1 binding to SMG5, which greatly decreases nonsense-mediated RNA destruction (131). NMDI 1 was identified in a library of synthetically produced indoles and is hence not a natural product; however, it has the advantage of inhibiting NMD alone and not interfering with protein synthesis. General inhibitors of protein synthesis, such as cycloheximide (CHX), also block NMD, likely by preventing the initial round of translation (132).

TRANSLATION INHIBITORS

Once in the cytosol, the mRNA still remains subject to expression control mechanisms, including the process of translation initiation and translation itself. A large number of antimicrobial agents target protein synthesis, and many act on the ribosome. Although the prokaryotic and the eukaryotic ribosomes fulfill essentially the same function, it is remarkable that most of the compounds interfering with their function have very different mechanisms and are specific for either eukaryotes or prokaryotes (**Figure 4*b***).

One way of preventing protein synthesis lies in depleting available building blocks. The *Streptomyces* natural product reveromycin A (RevA) fits this description. It inhibits isoleucyl-tRNA synthetase, thereby starving protein synthesis of a common amino acid (133). Reveromycin preferentially kills osteoclasts. This preference likely relies on pH trapping. RevA itself is a fairly acidic molecule and would require a low-pH environment to neutralize its carboxylic acid groups to traverse the cell membrane. As osteoclasts create an acidic environment to dissolve bone matrix, this may allow RevA to enter the cell. Once inside, the higher internal pH should deprotonate RevA and prevent the molecule from leaving. It has shown promise in animal models against bone metastasis but appears not to have advanced into clinical trials so far (134). In recent years, more compounds that act on other tRNA synthetases have attracted attention. In the case of RevA, the target protein is known although the precise mechanism of action still awaits elucidation. For the plant natural product febrifugine and some of its derivatives, more detailed mechanistic data have become available (135). Febrifugine was identified as an active component in traditional



(Caption appears on following page)

Figure 4 (*Figure appears on preceding page*)

Translation. (*a*) Inhibitors of translation initiation and elongation. (*b*) The helicase and ATPase eIF4A component of the eIF4F complex present a key target in translation initiation and are inhibited in various ways. Hippuristanol interferes with eIF4A's ability to bind RNA. Pateamine A stimulates eIF4A's catalytic activities but appears to diminish its interaction with the other eIF4F subunits. Rocaglamide A displays sequence selectivity in binding eIF4A and arresting the enzyme on the RNA message, thereby turning an activating protein into a repressor. Recently characterized elisabatin A and allolaurinterol inhibit eIF4A's ATPase activity. One way of blocking protein synthesis lies in starving the translation machinery of amino acid–charged tRNA, which reveromycin A, febrifugine, and AN2690 accomplish with specificities for different aminoacyl-tRNA synthetases. Cytotrienin A blocks eEF1A-mediated delivery of acyl-tRNA to the ribosome. Working directly on the ribosome, lactimidomycin and mycalamide bind the large subunit's E site, preventing deacylated tRNA from entering and thereby arresting the ribosome on the start codon. While sharing the same binding site, cycloheximide binds together with the deacylated tRNA and can therefore inhibit translation of ribosomes on any position along the mRNA. Most known eukaryotic translation inhibitors, however, appear to prevent peptide bond formation by binding between the P site and the A site of the large ribosomal subunit, close to the position usually occupied by the tRNA's aminoacyl end. Abbreviations: A site, acceptor site; E site, exit site; mRNA, messenger RNA; P site, peptide site; tRNA, transfer RNA.

Chinese medicine and has been used to treat a variety of diseases from malaria to cancer. It appears to bind the catalytic pocket of glutamyl-prolyl tRNA-synthetase in an ATP-dependent manner, competing with the proline substrate. Febrifugine treatment seems to mimic reduced proline availability in cells, thereby triggering the amino acid response stress pathway. The febrifugine derivative halofuginone is currently undergoing phase I and II clinical trials against Duchenne muscular dystrophy and HIV-related Kaposi sarcoma. Although not a natural product, the boron-containing broad-spectrum antifungal AN2690 likely has the best-explained mechanism of action against leucyl-tRNA synthetase, because both biochemical data and its crystal structure are available (136). AN2690 binds into the enzyme's editing site in the position of the noncognate amino acid, where it forms an adduct with the 3′-end of the leucyl-tRNA, trapping it in the editing site and thereby preventing catalytic turnover.

Over the past years, many projects have investigated the connection between mTOR signaling and translation initiation, which have been extensively reviewed elsewhere (137). However, mTOR inhibition results in only a 15–20% reduction in protein synthesis, whereas many natural products targeting the translation initiation process have a more profound and direct effect.

Particularly, the initiation factor eIF4A has been identified as the target of several natural products, each acting by a distinct mechanism. The DEAD box helicase eIF4A forms part of the eIF4F complex, together with the scaffold protein eIF4G and the cap-binding protein eIF4E. A weak helicase by itself, eIF4A is thought to unwind secondary structures in the 5′ UTR with the help of eIF4B and aid proper delivery of the mRNA to the small ribosomal subunit, thereby forming the 48S complex. The marine natural product pateamine A (PatA) derails proper eIF4F function and stalls translation initiation. Surprisingly, PatA enhances rather than inhibits eIF4A's RNA binding, helicase, and ATPase activity but may decrease its association with the other eIF4F members, bringing translation to a halt before forming a working 48S complex (138). Furthermore, PatA binds all three eIF4A isoforms. Although eIF4AI and eIF4AII seem to have essentially the same function, eIF4AIII plays a role not in protein synthesis but in NMD, which is consequently also inhibited by PatA (139). Nevertheless, dissecting NMD via PatA promises to be a difficult endeavor, as the inhibition of translation initiation triggers a cellular stress response. This stress response includes the formation of stress granules, which in turn changes the distribution and fate of mRNAs within the cell (140).

In contrast to PatA, hippuristanol, another marine natural product, has a more straightforward mechanism of eIF4A inhibition. Hippuristanol binds to eIF4A's C-terminal domain and interferes with its catalytic function and decreases its association with the mRNA (141). eIF4A is another protein targeted by a large variety of different inhibitors with very distinct mechanisms. Two new

PatA: pateamine A

marine natural products, elisabatin and allolaurinterol, were recently reported to competitively inhibit eIF4A's ATPase activity (142).

RocA: rocaglamide A

HHT: homoharringtonine

Originally, eIF4A inhibitors were considered fairly general inhibitors of all protein synthesis. A recent study of rocaglamide A (RocA) has changed this view (143). RocA belongs to the flavagline family of natural products, which also includes silvestrol, a previously characterized translation inhibitor (144). RocA appeared to specifically block protein synthesis from some transcripts; however, this selectivity for certain mRNAs did not depend on UTR secondary structure. Instead, RocA forced eIF4A to strongly bind polypurine sequences in the 5′ UTR in an ATP-independent manner. In this process, RocA turns an initiation factor into a strong, sequence-selective translational repressor interfering with eIF4A's normal function of scanning for the proper AUG start codon. In terms of increasing catalytic activity and enhancing RNA binding, RocA behaves similarly to PatA, though whether the latter displays any sequence selectivity is currently unknown. RocA treatment also increased the translation of upstream open reading frames, likely owing to inhibited scanning.

Translation initiation represents a well-regulated step with many signaling pathways influencing the rate of protein synthesis and selectivity for certain transcripts. The last opportunity to prevent expression of a gene product lies in translation elongation. By its nature, inhibition of the elongation phase is not specific to certain transcripts but blocks general protein output. In the process of elongation, the factor eEF1A delivers an acyl-tRNA to the ribosomal acceptor site (A site) and leaves its cargo if the correct anticodon is recognized in a GTP-dependent manner. The ribosome itself catalyzes peptide bond formation by transferring the nascent peptide chain from the peptide site (P site) onto the incoming A-site tRNA. Elongation factor eEF2 then pushes the assembly over by one codon, thereby moving the peptide chain on its tRNA from the A site into the P site and the deacylated tRNA from the P site onto the exit site (E site). Aside from the tRNA analog puromycin, which inhibits protein synthesis equally in prokaryotes and eukaryotes, most eukaryotic translation inhibitors appear to prefer acting primarily by blocking peptide bond formation or by interfering with the passage of deacylated tRNA via the ribosomal E site. Cytotrienin A makes an exception to this rule as it prevents eEF1A-dependent delivery of acyl-tRNA but does not interfere with nonenzymatic tRNA binding to the ribosome (145).

Originally isolated from *Cephalotaxus harringtonia*, homoharringtonine (HHT) presents another plant natural product (146). The finding that HHT blocks translational elongation emerged in the 1970s and 1980s. A more recent crystal structure supports a mechanism of preventing peptide bond formation by binding into the cleft of the large subunit A site (147). Its mechanism of action is similar to that of anisomycin, and both molecules bind the ribosome close to the 3′-end of tRNA, likely competing for space with the amino acid carried on the tRNA. Agelastatin A, a brominated alkaloid isolated from the marine sponge *Agelas dendromorpha*, is another peptidyl transfer inhibitor recently described (148). In a sense, the A-site portion of the peptidyl transfer center seems to be a preferred site for inhibitors, as it has also been identified as the target of plant alkaloids lycorine and narciclasine and the tricothecene class of inhibitors such as verrucarin A, deoxynivalenol, and T-2 toxin (149). This binding of structurally unrelated compounds to the same target site presents another example of structurally unrelated molecules from distinct sources acting via nearly identical mechanisms. Yet, their binding is not identical. Structural analysis has shown that the inhibitors induce different conformations of the rRNA bases in their binding site. As growing tumor cells especially rely on protein synthesis to proliferate, translation inhibitors have attracted interest as therapeutics (150). Under the name omacetaxine mepesuccinate, HHT recently received FDA approval to treat tyrosine kinase inhibitor–resistant chronic myeloid leukemia.

The actions of anisomycin and HHT appear to be quite similar to those of some antibacterial macrolide antibiotics that bind close to the peptidyl transfer center. The mechanism of molecules

acting via the ribosomal E site, however, appears unique to eukaryotic translation inhibitors and has thus far not been identified in antibacterial compounds. The *Streptomyces* natural product and polyketide CHX is a popular translation inhibitor for laboratory use and has even been used as a pesticide in the past. Although it was known to block eEF2-dependent elongation, its binding site and precise mechanism remained unknown for decades. In a study investigating another *Streptomyces* polyketide, lactimidomycin (LTM), it became clear that both molecules bind the E site of the large ribosomal subunit, though each acts in a slightly different way. The structures of both molecules share a cyclic imide ring, yet the larger LTM prevents deacylated tRNA from entering the E site, thereby stopping translation after the first peptide bond formation. In contrast, the smaller CHX seems to act together with the deacylated tRNA in blocking further tRNA movement, thereby preventing translation after formation of two peptide bonds (151). CHX appears to bind the ribosome at any time during the elongation phase in vivo, thereby "freezing" the ribosome in place along the mRNA. In contrast, LTM acts mainly at the start site but does not seem to interfere with elongation once the ribosome has cleared the first codon. CHX and LTM have the same binding site, which was first identified by biochemical means before a crystal structure of the eukaryotic ribosome with bound inhibitors became available. CHX may act in a slightly different manner depending on the species, as its binding constant seems to vary between yeast and rabbit ribosomes (149). Yet, CHX and LTM are not the only molecules binding at that position: The marine natural product mycalamide, originating from the same genus of marine sponges as PatA, acts via a mechanism nearly indistinguishable from LTM (152). Beyond mechanistic insight, LTM has proved to be a highly valuable tool compound. Because it reliably halts translation before the first elongation step, treatment with LTM allowed comprehensive mapping of translation start sites, including the identification of noncanonical translation initiation sites or genes with more than one utilized start codon (153). It furthermore helped dissect pausing events during early elongation (154). Despite no structural relationship, LTM and mycalamide bind the same site of the ribosomal E site and leave identical footprints in chemical protection assays.

CHX: cycloheximide

LTM: lactimidomycin

The binding of structurally distinct molecules to the same binding site constitutes a very common theme throughout chemical biology. It shows that the binding sites for chemical manipulation are not evenly distributed. Certain macromolecules, such as the HDACs, eIF4A, or the ribosome, are highly amenable to drug binding, whereas other factors in the same biological process, even enzymes with substrate binding pockets, are far less accessible. Therefore, finding molecules that bind to these less accessible partners, which would allow more detailed analysis of a given biological process, is a challenge that underlines the need to keep searching for interesting molecules, especially from untapped natural sources. In summary, specific small molecules greatly aid the study of gene expression and hold promise in the development of specific therapeutics. With the development of molecular biology, the basic mechanisms of gene control came to light. Chemical biology plays a perhaps equally important role in understanding the intricacies of these mechanisms, providing the tools to study one process at a time without having to alter an organism's genetic makeup. In a sense, the tools of chemical biology have become so commonplace that many investigators do not even pay much thought to whom they owe their reagents when adding CHX or actinomycin to their experiments. In that sense, cell biologists have long practiced chemical biology without a license. We expect that the existing small-molecule inhibitors will enable further discoveries in the future, and the molecules that yet await discovery should make the process even more exciting.

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LITERATURE CITED

1. Theis N, Lerdau M. 2003. The evolution of function in plant secondary metabolites. *Int. J. Plant Sci.* 164:S93–102
2. Newman DJ, Cragg GM. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 75:311–35
3. Li JWH, Vederas JC. 2009. Drug discovery and natural products: end of an era or an endless frontier? *Science* 325:161–65
4. Koehn FE, Carter GT. 2005. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov.* 4:206–20
5. Harvey AL, Edrada-Ebel R, Quinn RJ. 2015. The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Discov.* 14:111–29
6. Stent GS. 1968. That was the molecular biology that was. *Science* 160:390–95
7. Ahlquist P. 2002. RNA-dependent RNA polymerases, viruses, and RNA silencing. *Science* 296:1270–73
8. Filipowicz W, Jaskiewicz L, Kolb FA, Pillai RS. 2005. Post-transcriptional gene silencing by siRNAs and miRNAs. *Curr. Opin. Struct. Biol.* 15:331–41
9. Chi P, Allis CD, Wang GG. 2010. Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. *Nat. Rev. Cancer* 10:457–69
10. Yoshida M, Kudo N, Kosono S, Ito A. 2017. Chemical and structural biology of protein lysine deacetylases. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 93:297–321
11. Lemon B, Tjian R. 2000. Orchestrated response: a symphony of transcription factors for gene control. *Genes Dev.* 14:2551–69
12. Darnell JE. 2002. Transcription factors as targets for cancer therapy. *Nat. Rev. Cancer* 2:740–49
13. Bork PM, Schmitz ML, Kuhnt M, Escher C, Heinrich M. 1997. Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-κB. *FEBS Lett.* 402:85–90
14. Gilmore TD. 2006. Introduction to NF-κB: players, pathways, perspectives. *Oncogene* 25:6680–84
15. Garcia-Pineres AJ, Castro V, Mora G, Schmidt TJ, Strunck E, et al. 2001. Cysteine 38 in p65/NF-κB plays a crucial role in DNA binding inhibition by sesquiterpene lactones. *J. Biol. Chem.* 276:39713–20
16. Kwok BH, Koh B, Ndubuisi MI, Elofsson M, Crews CM. 2001. The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IκB kinase. *Chem. Biol.* 8:759–66
17. Hehner SP, Hofmann TG, Droge W, Schmitz ML. 1999. The antiinflammatory sesquiterpene lactone parthenolide inhibits NF-κB by targeting the IκB kinase complex. *J. Immunol.* 163:5617–23
18. Guzman ML, Rossi RM, Neelakantan S, Li X, Corbett CA, et al. 2007. An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. *Blood* 110:4427–35
19. Duan D, Zhang J, Yao J, Liu Y, Fang J. 2016. Targeting thioredoxin reductase by parthenolide contributes to inducing apoptosis of HeLa cells. *J. Biol. Chem.* 291:10021–31
20. Ghantous A, Sinjab A, Herceg Z, Darwiche N. 2013. Parthenolide: from plant shoots to cancer roots. *Drug Discov. Today* 18:894–905
21. Reich E, Franklin RM, Shatkin AJ, Tatum EL. 1961. Effect of actinomycin D on cellular nucleic acid synthesis and virus production. *Science* 134:556–57
22. Goldberg IH, Rabinowitz M, Reich E. 1962. Basis of actinomycin action. I. DNA binding and inhibition of RNA-polymerase synthetic reactions by actinomycin. *PNAS* 48:2094–101
23. Perry RP, Kelley DE. 1970. Inhibition of RNA synthesis by actinomycin D: characteristic dose-response of different RNA species. *J. Cell Physiol.* 76:127–39

24. Wadkins RM, Vladu B, Tung CS. 1998. Actinomycin D binds to metastable hairpins in single-stranded DNA. *Biochemistry* 37:11915–23
25. Rill RL, Hecker KH. 1996. Sequence-specific actinomycin D binding to single-stranded DNA inhibits HIV reverse transcriptase and other polymerases. *Biochemistry* 35:3525–33
26. Kamitori S, Takusagawa F. 1994. Multiple binding modes of anticancer drug actinomycin D: X-ray, molecular modeling, and spectroscopic studies of d(GAAGCTTC)2-actinomycin D complexes and its host DNA. *J. Am. Chem. Soc.* 116:4154–65
27. Hou MH, Robinson H, Gao YG, Wang AH. 2002. Crystal structure of actinomycin D bound to the CTG triplet repeat sequences linked to neurological diseases. *Nucleic Acids Res.* 30:4910–17
28. Maier JG, Harshaw WG. 1967. Treatment and prognosis in Wilms' tumor. A study of 51 cases with special reference to role of actinomycin D. *Cancer* 20:96–102
29. Schultz LD, Hall BD. 1976. Transcription in yeast: α-amanitin sensitivity and other properties which distinguish between RNA polymerases I and III. *PNAS* 73:1029–33
30. Nguyen VT, Giannoni F, Dubois MF, Seo SJ, Vigneron M, et al. 1996. In vivo degradation of RNA polymerase II largest subunit triggered by α-amanitin. *Nucleic Acids Res.* 24:2924–29
31. Brueckner F, Cramer P. 2008. Structural basis of transcription inhibition by α-amanitin and implications for RNA polymerase II translocation. *Nat. Struct. Mol. Biol.* 15:811–18
32. Chao SH, Fujinaga K, Marion JE, Taube R, Sausville EA, et al. 2000. Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. *J. Biol. Chem.* 275:28345–48
33. Chao SH, Price DH. 2001. Flavopiridol inactivates P-TEFb and blocks most RNA polymerase II transcription in vivo. *J. Biol. Chem.* 276:31793–99
34. He QL, Titov DV, Li J, Tan M, Ye Z, et al. 2015. Covalent modification of a cysteine residue in the XPB subunit of the general transcription factor TFIIH through single epoxide cleavage of the transcription inhibitor triptolide. *Angew. Chem. Int. Ed. Engl.* 54:1859–63
35. Titov DV, Gilman B, He QL, Bhat S, Low WK, et al. 2011. XPB, a subunit of TFIIH, is a target of the natural product triptolide. *Nat. Chem. Biol.* 7:182–88
36. Jenuwein T, Allis CD. 2001. Translating the histone code. *Science* 293:1074–80
37. Yang X, Lay F, Han H, Jones PA. 2010. Targeting DNA methylation for epigenetic therapy. *Trends Pharmacol. Sci.* 31:536–46
38. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, et al. 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324:930–35
39. Ghoshal K, Datta J, Majumder S, Bai S, Kutay H, et al. 2005. 5-Aza-deoxycytidine induces selective degradation of DNA methyltransferase 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal. *Mol. Cell. Biol.* 25:4727–41
40. Datta J, Ghoshal K, Denny WA, Gamage SA, Brooke DG, et al. 2009. A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Res.* 69:4277–85
41. Patel K, Dickson J, Din S, Macleod K, Jodrell D, Ramsahoye B. 2010. Targeting of 5-aza-2′-deoxycytidine residues by chromatin-associated DNMT1 induces proteasomal degradation of the free enzyme. *Nucleic Acids Res.* 38:4313–24
42. Erdmann A, Halby L, Fahy J, Arimondo PB. 2015. Targeting DNA methylation with small molecules: What's next? *J. Med. Chem.* 58:2569–83
43. Flotho C, Claus R, Batz C, Schneider M, Sandrock I, et al. 2009. The DNA methyltransferase inhibitors azacitidine, decitabine and zebularine exert differential effects on cancer gene expression in acute myeloid leukemia cells. *Leukemia* 23:1019–28
44. Rilova E, Erdmann A, Gros C, Masson V, Aussagues Y, et al. 2014. Design, synthesis and biological evaluation of 4-amino-*N*-(4-aminophenyl)benzamide analogues of quinoline-based SGI-1027 as inhibitors of DNA methylation. *Chem. Med. Chem.* 9:590–601
45. Allfrey VG, Faulkner R, Mirsky AE. 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *PNAS* 51:786–94
46. Riggs MG, Whittaker RG, Neumann JR, Ingram VM. 1977. *n*-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature* 268:462–64

47. Yoshida M, Kijima M, Akita M, Beppu T. 1990. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* 265:17174–79
48. Richon VM, Webb Y, Merger R, Sheppard T, Jursic B, et al. 1996. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *PNAS* 93:5705–8
49. Taunton J, Hassig CA, Schreiber SL. 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 272:408–11
50. Kijima M, Yoshida M, Sugita K, Horinouchi S, Beppu T. 1993. Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J. Biol. Chem.* 268:22429–35
51. Yoshida M, Horinouchi S, Beppu T. 1995. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays* 17:423–30
52. Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley TM, et al. 1996. Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *PNAS* 93:13143–47
53. Liesch JM, Sweeley CC, Staffeld GD, Anderson MS, Weber DJ, Scheffer RP. 1982. Structure of HC-toxin, a cyclic tetrapeptide from *Helminthosporium carbonum*. *Tetrahedron* 38:45–48
54. Closse A, Huguenin R. 1974. Isolation and structural clarification of chlamydocin. *Helv. Chim. Acta* 57:533–45
55. Furumai R, Matsuyama A, Kobashi N, Lee KH, Nishiyama M, et al. 2002. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res.* 62:4916–21
56. Komatsu Y, Tomizaki KY, Tsukamoto M, Kato T, Nishino N, et al. 2001. Cyclic hydroxamic-acid-containing peptide 31, a potent synthetic histone deacetylase inhibitor with antitumor activity. *Cancer Res.* 61:4459–66
57. Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S. 2001. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *PNAS* 98:87–92
58. Hai Y, Christianson DW. 2016. Histone deacetylase 6 structure and molecular basis of catalysis and inhibition. *Nat. Chem. Biol.* 12:741–47
59. Miyake Y, Keusch JJ, Wang L, Saito M, Hess D, et al. 2016. Structural insights into HDAC6 tubulin deacetylation and its selective inhibition. *Nat. Chem. Biol.* 12:748–54
60. Kiernan R, Bres V, Ng RW, Coudart MP, El Messaoudi S, et al. 2003. Post-activation turn-off of NF-κB-dependent transcription is regulated by acetylation of p65. *J. Biol. Chem.* 278:2758–66
61. Chen L, Fischle W, Verdin E, Greene WC. 2001. Duration of nuclear NF-κB action regulated by reversible acetylation. *Science* 293:1653–57
62. Bheda P, Jing H, Wolberger C, Lin H. 2016. The substrate specificity of sirtuins. *Annu. Rev. Biochem.* 85:405–29
63. North BJ, Verdin E. 2007. Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. *PLOS ONE* 2:e784
64. Napper AD, Hixon J, McDonagh T, Keavey K, Pons JF, et al. 2005. Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1. *J. Med. Chem.* 48:8045–54
65. Rumpf T, Schiedel M, Karaman B, Roessler C, North BJ, et al. 2015. Selective Sirt2 inhibition by ligand-induced rearrangement of the active site. *Nat. Commun.* 6:6263
66. Schuetz A, Min J, Antoshenko T, Wang CL, Allali-Hassani A, et al. 2007. Structural basis of inhibition of the human NAD^+-dependent deacetylase SIRT5 by suramin. *Structure* 15:377–89
67. Communi D, Robaye B, Boeynaems JM. 1999. Pharmacological characterization of the human P2Y11 receptor. *Br. J. Pharmacol.* 128:1199–206
68. Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, et al. 2005. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol. Cell* 18:601–7
69. Tan M, Luo H, Lee S, Jin F, Yang JS, et al. 2011. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* 146:1016–28
70. Chen Y, Sprung R, Tang Y, Ball H, Sangras B, et al. 2007. Lysine propionylation and butyrylation are novel post-translational modifications in histones. *Mol. Cell Proteom.* 6:812–19
71. Smith BC, Denu JM. 2007. Acetyl-lysine analog peptides as mechanistic probes of protein deacetylases. *J. Biol. Chem.* 282:37256–65

72. Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, et al. 2011. The first identification of lysine malonylation substrates and its regulatory enzyme. *Mol. Cell Proteom.* 10:M111.012658
73. Du J, Zhou Y, Su X, Yu JJ, Khan S, et al. 2011. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science* 334:806–9
74. Balasubramanyam K, Altaf M, Varier RA, Swaminathan V, Ravindran A, et al. 2004. Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. *J. Biol. Chem.* 279:33716–26
75. Balasubramanyam K, Varier RA, Altaf M, Swaminathan V, Siddappa NB, et al. 2004. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J. Biol. Chem.* 279:51163–71
76. Egawa N, Kitaoka S, Tsukita K, Naitoh M, Takahashi K, et al. 2012. Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci. Transl. Med.* 4:145ra04
77. Fukuda I, Ito A, Hirai G, Nishimura S, Kawasaki H, et al. 2009. Ginkgolic acid inhibits protein SUMOylation by blocking formation of the E1-SUMO intermediate. *Chem. Biol.* 16:133–40
78. Bowers EM, Yan G, Mukherjee C, Orry A, Wang L, et al. 2010. Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. *Chem. Biol.* 17:471–82
79. Yan G, Eller MS, Elm C, Larocca CA, Ryu B, et al. 2013. Selective inhibition of p300 HAT blocks cell cycle progression, induces cellular senescence, and inhibits the DNA damage response in melanoma cells. *J. Invest. Dermatol.* 133:2444–52
80. Oike T, Komachi M, Ogiwara H, Amornwichet N, Saitoh Y, et al. 2014. C646, a selective small molecule inhibitor of histone acetyltransferase p300, radiosensitizes lung cancer cells by enhancing mitotic catastrophe. *Radiother. Oncol.* 111:222–27
81. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, et al. 2010. Suppression of inflammation by a synthetic histone mimic. *Nature* 468:1119–23
82. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, et al. 2010. Selective inhibition of BET bromodomains. *Nature* 468:1067–73
83. Bhadury J, Nilsson LM, Muralidharan SV, Green LC, Li Z, et al. 2014. BET and HDAC inhibitors induce similar genes and biological effects and synergize to kill in Myc-induced murine lymphoma. *PNAS* 111:E2721–30
84. Ito T, Umehara T, Sasaki K, Nakamura Y, Nishino N, et al. 2011. Real-time imaging of histone H4K12-specific acetylation determines the modes of action of histone deacetylase and bromodomain inhibitors. *Chem. Biol.* 18:495–507
85. Nakaoka S, Sasaki K, Ito A, Nakao Y, Yoshida M. 2016. A genetically encoded FRET probe to detect intranucleosomal histone H3K9 or H3K14 acetylation using BRD4, a BET family member. *ACS Chem. Biol.* 11:729–33
86. Tachibana M, Sugimoto K, Fukushima T, Shinkai Y. 2001. Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *J. Biol. Chem.* 276:25309–17
87. Casciello F, Windloch K, Gannon F, Lee JS. 2015. Functional role of G9a histone methyltransferase in cancer. *Front. Immunol.* 6:487
88. Greiner D, Bonaldi T, Eskeland R, Roemer E, Imhof A. 2005. Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. *Nat. Chem. Biol.* 1:143–45
89. Cherblanc FL, Chapman KL, Brown R, Fuchter MJ. 2013. Chaetocin is a nonspecific inhibitor of histone lysine methyltransferases. *Nat. Chem. Biol.* 9:136–37
90. Cherblanc FL, Chapman KL, Reid J, Borg AJ, Sundriyal S, et al. 2013. On the histone lysine methyltransferase activity of fungal metabolite chaetocin. *J. Med. Chem.* 56:8616–25
91. Fujishiro S, Dodo K, Iwasa E, Teng Y, Sohtome Y, et al. 2013. Epidithiodiketopiperazine as a pharmacophore for protein lysine methyltransferase G9a inhibitors: reducing cytotoxicity by structural simplification. *Bioorg. Med. Chem. Lett.* 23:733–36
92. Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, et al. 2007. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol. Cell* 25:473–81

93. Liu F, Chen X, Allali-Hassani A, Quinn AM, Wigle TJ, et al. 2010. Protein lysine methyltransferase G9a inhibitors: design, synthesis, and structure activity relationships of 2,4-diamino-7-aminoalkoxy-quinazolines. *J. Med. Chem.* 53:5844–57
94. Kim KH, Roberts CW. 2016. Targeting EZH2 in cancer. *Nat. Med.* 22:128–34
95. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, et al. 2012. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* 492:108–12
96. Knutson SK, Warholic NM, Wigle TJ, Klaus CR, Allain CJ, et al. 2013. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *PNAS* 110:7922–27
97. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, et al. 2013. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood* 122:1017–25
98. Stein EM, Garcia-Manero G, Rizzieri DA, Tibes R, Berdeja JG, et al. 2015. A phase 1 study of the DOT1L inhibitor, pinometostat (EPZ-5676), in adults with relapsed or refractory leukemia: safety, clinical activity, exposure and target inhibition. *Blood* 126:2547
99. Casper A, Van Doren M. 2006. The control of sexual identity in the *Drosophila* germline. *Development* 133:2783–91
100. Harper SJ, Bates DO. 2008. VEGF-A splicing: the key to anti-angiogenic therapeutics? *Nat. Rev. Cancer* 8:880–87
101. Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, et al. 2007. Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nat. Chem. Biol.* 3:576–83
102. Corrionero A, Minana B, Valcarcel J. 2011. Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A. *Genes Dev.* 25:445–59
103. Kotake Y, Sagane K, Owa T, Mimori-Kiyosue Y, Shimizu H, et al. 2007. Splicing factor SF3b as a target of the antitumor natural product pladienolide. *Nat. Chem. Biol.* 3:570–75
104. Furumai R, Uchida K, Komi Y, Yoneyama M, Ishigami K, et al. 2010. Spliceostatin A blocks angiogenesis by inhibiting global gene expression including VEGF. *Cancer Sci.* 101:2483–89
105. Khan K, Schneider-Poetsch T, Ishfaq M, Ito A, Yoshimoto R, et al. 2014. Splicing inhibition induces gene expression through canonical NF-κB pathway and extracellular signal-related kinase activation. *FEBS Lett.* 588:1053–57
106. Koga M, Satoh T, Takasaki I, Kawamura Y, Yoshida M, Kaida D. 2014. U2 snRNP is required for expression of the 3′ end of genes. *PLOS ONE* 9:e98015
107. Kim S, Kim H, Fong N, Erickson B, Bentley DL. 2011. Pre-mRNA splicing is a determinant of histone H3K36 methylation. *PNAS* 108:13564–69
108. Folco EG, Coil KE, Reed R. 2011. The anti-tumor drug E7107 reveals an essential role for SF3b in remodeling U2 snRNP to expose the branch point-binding region. *Genes Dev.* 25:440–44
109. O'Brien K, Matlin AJ, Lowell AM, Moore MJ. 2008. The biflavonoid isoginkgetin is a general inhibitor of pre-mRNA splicing. *J. Biol. Chem.* 283:33147–54
110. Nishida A, Kataoka N, Takeshima Y, Yagi M, Awano H, et al. 2011. Chemical treatment enhances skipping of a mutated exon in the *dystrophin* gene. *Nat. Commun.* 2:308
111. Sakuma M, Iida K, Hagiwara M. 2015. Deciphering targeting rules of splicing modulator compounds: case of TG003. *BMC Mol. Biol.* 16:16
112. Palacino J, Swalley SE, Song C, Cheung AK, Shu L, et al. 2015. SMN2 splice modulators enhance U1-pre-mRNA association and rescue SMA mice. *Nat. Chem. Biol.* 11:511–17
113. Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, et al. 2010. Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev.* 24:1634–44
114. Wan L, Dreyfuss G. 2017. Splicing-correcting therapy for SMA. *Cell* 170:5
115. Nguyen KT, Holloway MP, Altura RA. 2012. The CRM1 nuclear export protein in normal development and disease. *Int. J. Biochem. Mol. Biol.* 3:137–51
116. Kudo N, Khochbin S, Nishi K, Kitano K, Yanagida M, et al. 1997. Molecular cloning and cell cycle-dependent expression of mammalian CRM1, a protein involved in nuclear export of proteins. *J. Biol. Chem.* 272:29742–51
117. Fornerod M, Ohno M, Yoshida M, Mattaj IW. 1997. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* 90:1051–60

118. Fukuda M, Asano S, Nakamura T, Adachi M, Yoshida M, et al. 1997. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature* 390:308–11
119. Culjkovic-Kraljacic B, Borden KL. 2013. Aiding and abetting cancer: mRNA export and the nuclear pore. *Trends Cell Biol.* 23:328–35
120. Gallouzi IE, Steitz JA. 2001. Delineation of mRNA export pathways by the use of cell-permeable peptides. *Science* 294:1895–901
121. Culjkovic B, Borden KL. 2009. Understanding and targeting the eukaryotic translation initiation factor eIF4E in head and neck cancer. *J. Oncol.* 2009:981679
122. Dickmanns A, Monecke T, Ficner R. 2015. Structural basis of targeting the exportin CRM1 in cancer. *Cells* 4:538–68
123. Sun Q, Carrasco YP, Hu Y, Guo X, Mirzaei H, et al. 2013. Nuclear export inhibition through covalent conjugation and hydrolysis of Leptomycin B by CRM1. *PNAS* 110:1303–8
124. Kudo N, Wolff B, Sekimoto T, Schreiner EP, Yoneda Y, et al. 1998. Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1. *Exp. Cell Res.* 242:540–47
125. Newlands ES, Rustin GJ, Brampton MH. 1996. Phase I trial of elactocin. *Br. J. Cancer* 74:648–49
126. Etchin J, Montero J, Berezovskaya A, Le BT, Kentsis A, et al. 2016. Activity of a selective inhibitor of nuclear export, selinexor (KPT-330), against AML-initiating cells engrafted into immunosuppressed NSG mice. *Leukemia* 30:190–99
127. Kalid O, Toledo Warshaviak D, Shechter S, Sherman W, Shacham S. 2012. Consensus Induced Fit Docking (cIFD): methodology, validation, and application to the discovery of novel Crm1 inhibitors. *J. Comput. Aided Mol. Des.* 26:1217–28
128. Kim J, McMillan E, Kim HS, Venkateswaran N, Makkar G, et al. 2016. XPO1-dependent nuclear export is a druggable vulnerability in *KRAS*-mutant lung cancer. *Nature* 538:114–17
129. Singh G, Kucukural A, Cenik C, Leszyk JD, Shaffer SA, et al. 2012. The cellular EJC interactome reveals higher-order mRNP structure and an EJC-SR protein nexus. *Cell* 151:750–64
130. Kashima I, Yamashita A, Izumi N, Kataoka N, Morishita R, et al. 2006. Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay. *Genes Dev.* 20:355–67
131. Durand S, Cougot N, Mahuteau-Betzer F, Nguyen CH, Grierson DS, et al. 2007. Inhibition of nonsense-mediated mRNA decay (NMD) by a new chemical molecule reveals the dynamic of NMD factors in P-bodies. *J. Cell Biol.* 178:1145–60
132. Ishigaki Y, Li X, Serin G, Maquat LE. 2001. Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. *Cell* 106:607–17
133. Miyamoto Y, Machida K, Mizunuma M, Emoto Y, Sato N, et al. 2002. Identification of *Saccharomyces cerevisiae* isoleucyl-tRNA synthetase as a target of the G_1-specific inhibitor reveromycin A. *J. Biol. Chem.* 277:28810–14
134. Osada H. 2016. Chemical and biological studies of reveromycin A. *J. Antibiot.* 69:723–30
135. Keller TL, Zocco D, Sundrud MS, Hendrick M, Edenius M, et al. 2012. Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase. *Nat. Chem. Biol.* 8:311–17
136. Rock FL, Mao W, Yaremchuk A, Tukalo M, Crepin T, et al. 2007. An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science* 316:1759–61
137. Fonseca BD, Smith EM, Yelle N, Alain T, Bushell M, Pause A. 2014. The ever-evolving role of mTOR in translation. *Semin. Cell Dev. Biol.* 36:102–12
138. Low WK, Dang Y, Schneider-Poetsch T, Shi Z, Choi NS, et al. 2005. Inhibition of eukaryotic translation initiation by the marine natural product pateamine A. *Mol. Cell* 20:709–22
139. Dang Y, Low WK, Xu J, Gehring NH, Dietz HC, et al. 2009. Inhibition of nonsense-mediated mRNA decay by the natural product pateamine A through eukaryotic initiation factor 4AIII. *J. Biol. Chem.* 284:23613–21
140. Dang Y, Kedersha N, Low WK, Romo D, Gorospe M, et al. 2006. Eukaryotic initiation factor 2α-independent pathway of stress granule induction by the natural product pateamine A. *J. Biol. Chem.* 281:32870–78

141. Bordeleau ME, Mori A, Oberer M, Lindqvist L, Chard LS, et al. 2006. Functional characterization of IRESes by an inhibitor of the RNA helicase eIF4A. *Nat. Chem. Biol.* 2:213–20
142. Tillotson J, Kedzior M, Guimarães L, Ross AB, Peters TL, et al. 2017. ATP-competitive, marine derived natural products that target the DEAD box helicase, eIF4A. *Bioorg. Med. Chem. Lett.* 27:4082–85
143. Iwasaki S, Floor SN, Ingolia NT. 2016. Rocaglates convert DEAD-box protein eIF4A into a sequence-selective translational repressor. *Nature* 534:558–61
144. Cencic R, Carrier M, Galicia-Vazquez G, Bordeleau ME, Sukarieh R, et al. 2009. Antitumor activity and mechanism of action of the cyclopenta[b]benzofuran, silvestrol. *PLOS ONE* 4:e5223
145. Lindqvist L, Robert F, Merrick W, Kakeya H, Fraser C, et al. 2010. Inhibition of translation by cytotrienin A–a member of the ansamycin family. *RNA* 16:2404–13
146. Kantarjian HM, Talpaz M, Santini V, Murgo A, Cheson B, O'Brien SM. 2001. Homoharringtonine: history, current research, and future direction. *Cancer* 92:1591–605
147. Gurel G, Blaha G, Moore PB, Steitz TA. 2009. U2504 determines the species specificity of the A-site cleft antibiotics: the structures of tiamulin, homoharringtonine, and bruceantin bound to the ribosome. *J. Mol. Biol.* 389:146–56
148. McClary B, Zinshteyn B, Meyer M, Jouanneau M, Pellegrino S, et al. 2017. Inhibition of eukaryotic translation by the antitumor natural product agelastatin A. *Cell Chem. Biol.* 24:605–13.e5
149. de Loubresse NG, Prokhorova I, Holtkamp W, Rodnina MV, Yusupova G, Yusupov M. 2014. Structural basis for the inhibition of the eukaryotic ribosome. *Nature* 513:517–22
150. Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I. 2015. Targeting the translation machinery in cancer. *Nat. Rev. Drug Discov.* 14:261–78
151. Schneider-Poetsch T, Ju J, Eyler DE, Dang Y, Bhat S, et al. 2010. Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. *Nat. Chem. Biol.* 6:209–17
152. Dang Y, Schneider-Poetsch T, Eyler DE, Jewett JC, Bhat S, et al. 2011. Inhibition of eukaryotic translation elongation by the antitumor natural product Mycalamide B. *RNA* 17:1578–88
153. Lee S, Liu B, Huang SX, Shen B, Qian SB. 2012. Global mapping of translation initiation sites in mammalian cells at single-nucleotide resolution. *PNAS* 109:E2424–32
154. Han Y, Gao X, Liu B, Wan J, Zhang X, Qian SB. 2014. Ribosome profiling reveals sequence-independent post-initiation pausing as a signature of translation. *Cell Res.* 24:842–51

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Errata

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