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Post-transcriptional gene regulation: From mechanisms to RNA chemistry and therapeutics



Summary

A better understanding of the RNA biology and chemistry is necessary to then develop new RNA therapeutic strategies. This review is the synthesis of a series of conferences that took place during the 6th international course on post-transcriptional gene regulation at Institut Curie. This year, the course made a special focus on RNA chemistry.

Keywords RNA; Post-transcriptional regulation; RNA therapeutics

Abbreviations ASOantisense Oligonucleotide; dsRNAdouble-stranded RNA; EJCexon-junction complex; EMTepithelial-to-mesenchymal transition; FDAFood and Drug Administration; G4G4-quadruplex; m⁶AN6-methylAdenosine; mRNAmessenger RNA; miRNAmicroRNA; NMDnonsense-mediated decay; PTBpolypyrimidine tract-binding protein; PTCpremature termination codon; RNAPIIRNA polymerase II; RBPRNA-Binding protein; RNAiRNA interference; RNPRiboNucleoProtein; SELEXsystematic evolution of ligands by exponential enrichment; siRNAsmall interfering RNA; tRNAtransfer RNA; UTRuntranslated region; vRNAviral RNA; 5BrdU5-Bromo deoxyUridine

Introduction

Among the different layers regulating gene expression, the post-transcriptional gene regulation is an essential biological process that has gained interest in recent years because of its implication in many diseases. The aim of this review is to summarize the scientific presentations that took place during the 6th course on post-transcriptional gene regulation at Institut Curie. This course was focused on various aspects of RNA biology (chemistry and therapeutics). Here, we discuss some of the highlights of this five-day course, sponsored by the Institut Curie, the Graduate School Life Science and Health of the Université Paris-Saclay, the École normale supérieure, and that took place at the Institut Curie in Orsay and Paris, in April 24-28, 2023.

RNA splicing

One of the major gene regulatory processes used by the cell to acquire new phenotypic traits and gain protein diversity is pre-messenger RNA (pre-mRNA) alternative splicing, which generates different mature mRNAs from the same transcribed gene. It is performed by the splicing machinery, called the spliceosome,

and targets the genomic regions that must be excised or kept in the final mRNA via two alternative mechanisms, so-called exon or intron definition [1]. However, the choice between one and the other mechanism is not fully understood yet. The team of Gil Ast (Department of Human Molecular Genetics Medical School, Tel Aviv, IL) has been studying the impact of gene architecture and its 3D genomic organization in alternative splicing regulation and the choice of exon or intron definition. Compilations of genomic and transcriptomic datasets showed the appearance of two distinct exon-intron architectures during evolution of warm-blooded organisms from a common ancestor with a differential GC content between the exons and short introns [2]. One architecture, termed leveled architecture, is defined by short introns with overall higher GC content in the exons and flanking introns. Whereas the second one, named differential architecture, is preserving the differential exon-intron GC content, but with longer introns. His team has now identified a 3D spatial organization of the genome based on this GC content gradient that would impact the choice of exon or intron definition and thus the final splicing outcome [3]. They found that genes with high GC content and leveled architecture are located in the inner part of the nucleus where they are usually defined at the intron level, leading to more frequent intron retention, while low GC content genes with differential architecture are more in the nuclear periphery and associated with exon definition and thus exon skipping. Moreover, this spatial organization creates two distinct environments with specific sub-networks of splicing factors favoring one splicing definition or the other, depending on the given architecture of the gene and thus determining the final splicing outcome.

Delving deeper into the role of chromatin organization in splicing, the team of Reini Luco (Institut Curie, Orsay, FR) found that chromatin modifications, such as H3K36me3 and H3K27me3, can also deeply impact cell-specific alternative splicing by favoring the recruitment of the splicing factors, such as PTB (polypyrimidine tract-binding protein), to the pre-mRNA via direct interaction with specific chromatin-adaptor complexes [4,5]. To explore further the biological relevance of these splicing-associated histone marks, the team moved into a dynamic cell reprogramming system dependent on well-known changes in splicing essential for early development and tumor metastasis, called the epithelial-to-mesenchymal transition (EMT). They found that at several genes essential for EMT, such as *FGFR2* and *CTNND1*, well-defined changes in specific histone marks right at the regulated exon are essential for the dynamic splicing changes responsible for EMT's phenotype [6]. Looking at how these exon-specific histone marks are regulated, the Luco team

has uncovered a role for 3D chromatin organization in which alternatively spliced exons physically interact with long distant regulatory regions important for EMT's splicing regulation. Finally, upon splicing, a group of proteins are recruited at the exon-exon-junctions for stabilization of the mRNA and its transport to the cytoplasm for translation, called the Exon-Junction Complex (EJC) [7]. This protein complex is first assembled at the exon-junction by the spliceosome during splicing, and then is transported into the cytoplasm. Hervé Le Hir's team (Institut de biologie de l'école normale supérieure, Paris, FR) has identified the 4 proteins composing its core structure, and their 3D organization [8,9]. One of these core proteins is eIF4AIII, a helicase that binds to the RNA, more specifically to its sugar backbone, independently of its sequence. This complex can only be assembled on RNA. First, the EJC can regulate pre-mRNA splicing by changing RNA polymerase II elongation rate [10]. It can also modulate mRNA nonsense-mediated decay (NMD) that degrades transcripts containing a premature termination codon (PTC) or regulates gene expression levels. If a PTC is a few base pairs upstream of a splice site, the EJC can help recruit the proteins needed to trigger the NMD [11]. On the other hand, the EJC can mask mRNA post-transcriptional modifications that destabilize the mRNA [12]. Finally, they recently showed that EJC is present around centrosomes which allow an active transport of the messenger ribonucleoprotein (mRNP) to accumulate transcripts at a specific location where they are translated [13]. Interestingly, most functions of the EJC appear to be indirectly or directly linked to translational control. Future studies will undoubtedly reveal new mechanisms by which the EJC, in association with other proteins, activates or represses translation.

RNA translation

Throughout the course of evolution, species undergo adaptive transformations in response to their environment yielding, among various outcomes, the de novo emergence of translated open reading frames (ORFs) [14]. This discovery was facilitated by the use of ribosome profiling (Ribo-seq), enabling the monitoring of actively translated regions of mRNAs in vivo. This technique has revealed thousands of translated short ORFs (sORFs) that result in the production of microproteins, found in both coding and non-coding regions of the genome [15]. The research group led by Norbert Hübner (Max Delbrück Center for Molecular Medicine, Berlin, DE) has significantly advanced our understanding of micropeptides in humans. They identified many microproteins (< 100 amino acids) including 221 novel translated sORFs that are shorter than 16 amino acids in five human tissues (brain, heart, liver, kidney and testis) [16]. Most of these human microproteins recently emerged during evolution and show only limited sequence conservation, restricted to primates. The team investigated these microproteins in terms of their sequence (e.g. presence of a transmembrane domain),

cellular localization (e.g. nucleus, mitochondria) and interactions with other partners. They conducted a high-throughput protein interaction screen on peptide matrix (PRISMA) and discovered that small peptides interact with proteins involved in several cellular mechanisms such as mRNA splicing, translational regulation and endocytosis. For instance, they characterized a 5 amino acid-long peptide translated from an upstream ORF within the myotubularin related protein 3 (*MTMR3*) gene, which is associated with all four clathrins, the phosphatidylinositol binding clathrin assembly protein (PICALM) and the clathrin interactor 1 (CLINT1). Hence, their research not only offers insights for exploring evolutionary novel human microproteins but also provides tools to understand their cellular functions [16].

Furthermore, over the past few years, the translation of these non-canonical ORFs has been associated with tumorigenic mechanisms [17-19]. More broadly, perturbations in global translation can represent a major event that contributes to the development of cancer. Indeed, cancer cells often remodel post-transcriptional regulatory networks as they progress toward metastasis [20,21]. However, the identification of key post-transcriptional regulators that drive pathologic gene expression remains a challenge. Aiming to identify these regulators, Albertas Navickas (Institut Curie, Orsay, FR) and collaborators have identified oncogenic RNA-binding proteins (RBPs) through the detection of coordinated changes in their target regulons [22]. For example, they revealed that the RNA-binding motif single-strand interacting protein 1 (RBMS1) acts as a suppressor of colon cancer metastasis and as a post-transcriptional regulator of RNA stability by directly binding to its target mRNAs [22]. Moreover, in cell lines and patient-derived xenograft models of breast cancer metastasis, the Small Nuclear Ribonucleoprotein Polypeptide A' (SNRPA1) was revealed to mediate aberrant alternative splicing in highly metastatic breast cancer through direct interactions with the SNRPA1-associated structural splicing enhancers (S3Es) [21]. In the scope of translational regulation, ribosome profiling and alternative polyadenylation data of poorly and highly metastatic breast cancer cells and patient-derived xenografts revealed the Heterogeneous Nuclear RibonucleoProtein C (HNRNPC) as a translational controller [20]. In highly metastatic cells, HNRNPC is downregulated which causes its target mRNAs to undergo 3'UTR lengthening via alternative polyadenylation site selection and, subsequently, translation repression. Altogether, these results uncover previously unknown gene regulatory programs mediated by key RBPs involved in cancer metastasis. Thus, these RBPs, together with their target mRNAs, provide more knowledge to identify points of vulnerability to design new targets and to better determine the prognosis.

In line with the objective of enhancing therapeutic approaches, the laboratory of Reuven Agami (The Netherlands Cancer Institute NKI, Amsterdam, NL) has used the Ribo-seq technology to

study events regulated at the mRNA translation level, and consequently discovered a novel point of vulnerability in cancers. The mRNA translation is disrupted by oncogenes to facilitate cancer development, and his team found that this disruption affects the quality of proteins, and that cancer cells exhibit deficiencies in amino acids [23]. Indeed, in order to survive, tumors adapt to their abnormal growth by hijacking the pathways of amino acid production. An example of such metabolic alteration is the tryptophan shortage. Reuven Agami and his collaborators demonstrated that in melanoma, interferon- γ (INF- γ) released from T cells triggers the upregulation of the indoleamine 2,3-dioxygenase 1 (IDO1) enzyme, that catabolizes the tryptophan into kynurenine [24]. They found that the deprivation of this amino acid results in the synthesis of aberrant proteins by ribosomal frameshifting and substituents (tryptophan-to-phenylalanine), an event they referred to as "sloppiness in mRNA translation" [25,26]. Additionally, the tryptophan deficiency in melanoma cells is associated with the hyperactivation of the MAPK pathway. The sloppiness can therefore be eliminated through the pharmacological inhibition of the oncogenic MAPK signaling [26]. However, in cells resistant to these drugs, the sloppiness is restored, which can activate the response of T lymphocytes. Indeed, the sloppiness results in the presentation of aberrant peptides on the cell surface, targeted by T cells, and thereby expanding the antigen landscape. These abnormal neoepitopes thus represent a vulnerability that can be explored to improve anti-cancer strategies [26].

RNA degradation

Targeting RNA processing mechanisms and associated RBPs thus opens new therapeutic pathways for better treating human diseases. In addition to cancer, pathogenic viruses represent a threat to public health and therefore pose significant socio-economic challenges. In RNA viruses, the RNA has a central role serving both as the viral genome and mRNA for protein synthesis [27,28]. However, viral genome-encoded proteins are not sufficient to enable viral infection. To address this limitation, it is therefore unsurprising that these obligatory intracellular parasites heavily rely on host cell proteins for their life cycle [27,28]. Using novel multi-omics strategies, Alfredo Castello's team (MRC University of Glasgow Centre for Virus Research, Glasgow, UK) showed that RNA viruses hijack cellular RBPs to regulate their life cycle [27,28]. Through the development and use of the comparative RNA Interactome Capture (cRIC) approach, they identified RBPs that displayed differential interaction with RNA and altered localization upon sindbis virus (SINV) infection [29,30]. Those RBPs with enhanced activity were found to accumulate in the viral factories together with the viral RNA (vRNA), thereby regulating the viral fitness and infection capacity. For example, the 5'-3' exoribonuclease 1 (XRN1) was detected near viral replication factories and its knockdown induced cell resistance to SINV infection [29]. Accordingly,

infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human lung cancer epithelial cells led to remodeling of the cellular RNA-bound proteome [31]. Interestingly, the development and employment of a new technology, named vRNA Interactome Capture (vRIC), revealed shared host-virus interactions between SARS-CoV-2 and SINV, despite the absence of sequence homology between the two vRNAs [30,31]. Collectively, these findings uncover the existence of key RBPs involved in virus infection regulation, and therefore a new universe of host-virus interactions emerges, harboring the potential for the development of antiviral treatments. Indeed, experiments with available inhibitors revealed that cellular RBPs are promising targets for broad-spectrum antiviral therapies [30].

In mammalian cells, early resistance against viral replication relies on the rapid activation of innate immune responses that trigger interferon (IFN) production. However, the inducible protection conferred by these cytokines is much more pronounced in differentiated cells than in embryonic or adult stem cells [32]. In organisms in which the IFN system is absent (e.g. invertebrates and plants), the protection against virus infection is conferred by means of RNA interference (RNAi). This process is initiated by the protein Dicer, which recognizes and cleaves viral double-strand RNA (dsRNA) [33]. Using *in vitro* models and brain organoids, the laboratory of Enzo Poirier (Institut Curie, Paris, FR) demonstrated in an innovative way a new pathway in which mammalian stem cells protect themselves from RNA viruses, such as Zika virus (ZIKV) and SARS-CoV-2 [34]. These cells were shown to preferentially express an alternatively spliced isoform of Dicer, termed antiviral Dicer (aviD), which enhances antiviral RNAi by processing long double-strand viral RNAs into small interfering RNAs (siRNAs). These siRNAs guide a sequence-specific degradation of corresponding vRNAs through the RNA-induced silencing (RISC) complex. Notably, in cases where a viral suppressor of RNAi (VSR) is used in the context of aviD, the viral dsRNA is shielded from Dicer cleavage resulting in increased viral production [34]. Thus, this indicates that the degradation of viral dsRNA in mammalian stem cells relies on the RISC complex, shedding light on the molecular regulation of antiviral RNAi in mammalian innate immunity.

Beyond its importance in combating vRNA, the RISC complex is crucial in gene regulation by controlling the fate of the RNA at the post-transcriptional level. However, the timing and molecular mechanism involved in this control are not yet fully understood. These are the questions that Stefan Ameres' group (Max Perutz Labs, Vienna, AT) is trying to answer, and their research has contributed to a deeper understanding of the RNA silencing biology. By combining the technique of thiol (SH)-linked alkylation for the metabolic sequencing of RNA (SLAMseq) with bioinformatics approaches [35], they found that the identity of the Argonaute (Ago) protein, which is a core protein of the RISC complex in double-stranded microRNA (miRNA)

maturation, determines the stability and function of small RNAs [36]. For instance, in *Drosophila*, the Ago2-bound miRNA duplexes are approximately two times more stable than those bound to Ago1. Additionally, the Ago1-dependent pathway generates single-stranded miRNAs, whereas the Ago2-mediated pathway produces siRNAs. The balance between these two pathways thereby reflects specific developmental requirements. Indeed, Ago1 facilitates the selective and rapid renewal of miRNAs in response to environmental changes and cellular differentiation, while Ago2 plays a pivotal role in antiviral defense. Furthermore, their investigations have revealed that the Ago-mediated processing is a fast process, occurring in less than 5 minutes, which can be disrupted by the uridylation of pre-miRNAs. Indeed, Stefan Ameres and his collaborators have shown that 3' uridylation of RNA is a modification that helps to control the RNA quality and thus contributes to the regulation of the fate of RNA [37,38]. This RNA modification therefore represents a novel layer of gene regulation.

RNA modifications

RNA can be post-transcriptionally modified by more than a hundred distinct chemical modifications [39]. These post-transcriptional modifications play important roles in various biological processes [40]. Pierre Close's laboratory (GIGA Institute, Liège, BE) found that the RNA modification landscape has important functional consequences in cell physiology through its work on transfer RNA (tRNA) modification. Indeed, they have shown the importance of tRNA modifications and their control of protein synthesis in the growth, invasion, and therapeutic resistance of cancers. During tumorigenesis, oncogenic signalling can change the expression of tRNAs, and this is correlated with changes in mRNA translation of genes with specific codon usage. Specifically, enzymes regulating wobble uridine tRNA (U34-tRNA) modification are upregulated in melanoma. U34-tRNA modifications are required for specific codon decoding during translation to promote the expression of selected genes like HIF1A, which drives glycolysis and therapeutic resistance in melanoma [41,42]. Curiously, it has been discovered that gene codon content is necessary but not sufficient to predict protein fate. Indeed, most genes can escape regulation by U34-tRNA modifications whereas for several genes, these modifications are essential to maintain translation speed and protein output. It appears that the presence of specific hydrophilic motifs causes protein aggregation and degradation upon codon-dependent translation elongation defects [43]. Therefore, it is the combination of codon content and the presence of hydrophilic motifs that defines the proteome. Together, these results provide a better understanding of the impact of translation regulation by tRNA modification on proteome homeostasis. This highlights the importance of codon-specific mRNA translation and tRNA modifications as possible therapeutic targets in cancer.

Because of their cellular abundance, RNA post-transcriptional modifications were first identified in rRNA and tRNA. However, it is now clear that all species of RNA undergo dynamic modification, including mRNAs [44]. Considering all cellular RNA, pseudouridylation (the conversion of uridine into pseudouridine) is one of the most abundant post-transcriptional modifications, but its biological role remains poorly understood [45]. Marianne Farnebo's Group (Karolinska Institute, Stockholm, SE) has highlighted that pseudouridine is deposited onto mRNAs during their transcription through the action of the pseudouridine synthase dyskerin. Indeed, in cells depleted in dyskerin, the amount of pseudouridine in mRNAs is significantly reduced, suggesting that dyskerin is responsible for the majority of mRNA pseudouridylation. Moreover, by performing CHIP-seq and co-immunoprecipitation experiments, they have shown that dyskerin is associated with RNAPII and enriched in genes transcribed by RNAPII throughout the genome [46]. The consequences of this co-transcriptional mRNA pseudouridylation by dyskerin are a repression of translation allowing controlled protein synthesis. However, when the function of dyskerin is impaired, it has different outcomes on translation depending on the duration of impairment; short-term depletion enhances translation due to reduced mRNA pseudouridylation whereas long-term depletion attenuates translation due to defective rRNA pseudouridylation and processing. In fact, pseudouridine can also affect the structure and function of various non-coding RNAs and not only mRNAs. Thus, pseudouridylation of mRNAs by dyskerin appears to be part of a general mechanism enabling communication between transcription and translation, with important implications for both normal development and disease. Going beyond RNA modification, Farnebo's group has evidence that RNA itself could be a "modification" for the chromatin regulating its compactions. This effect on chromatin occurs only with single-stranded RNA and in a sequence-independent manner. Mechanistically, RNA binds histones and neutralizes their positively charged tails, which restricts histone self-association and interaction with DNA, thereby promoting an open chromatin state. Conversely, removal of the negatively charged RNA exposes the positive charges on histone tails, which stimulates electrostatic interactions with DNA, resulting in tighter packing of the chromatin [43]. This discovery provides a novel and conceptionally simple mechanism by which RNA is involved in the opening and closure of chromatin and establishes RNA as a key functional component of eukaryotic chromatin.

Another widespread RNA modification is *N*⁶-methyladenosine (m⁶A), a methyl modification of adenosine. Novel high-throughput m⁶A mapping methods (e.g. MeRIP-seq, miCLIP) developed in Samie Jaffrey's team (Cornell University, New York, USA) have contributed to a significant progress in uncovering the landscape of m⁶A modifications in mammalian mRNAs [44,45]. It is now known that m⁶A modifications in mRNAs influence different

gene expression processes in both physiological and pathological conditions, such as RNA splicing, nuclear transport, mRNA stability and translation [46,47]. m⁶A is installed co-transcriptionally in the nucleus by the m⁶A writer complex and is removed by erasers. Importantly, m⁶A functions in mRNAs are mediated by m⁶A binding proteins, also called as "readers". The most well-established readers are the nuclear YTHDC1, responsible for nuclear RNA processing, and the cytosolic YTHDF1, 2, and 3. How distinct cytosolic readers, sharing high amino acid similarity, may mediate different fates of m⁶A-modified mRNAs, such as decay or translational control, is a subject of debate. The aim of Jaffrey's lab is to elucidate this aspect. Starting from the observation that the amino acid sequence of the three YTHDF proteins contains low complexity domains, they found that the three readers, by preferentially interacting with mRNAs containing multiple m⁶A sites, may associate and undergo phase separation into P-bodies and stress granules [48], where they predominantly function, in a redundant manner, in mediating degradation of m⁶A-modified mRNA [49]. However, the mechanism by which m⁶A modifications dictate the fate of distinct m⁶A-modified mRNAs may be context-specific and influenced by the localization of m⁶A within mRNA body. Further analysis to clarify this aspect is needed. In addition to chemical modifications installed on transcript body, the 5' end of mRNA is modified by a so-called m⁷G cap structure that participates in different biological processes, including pre-mRNA processing and translation [50]. The mRNA cap structure can be additionally methylated. Depending on the presence of 2'-O-methylation on the first, or both the first and second transcribed nucleotide, mRNA caps exist in either the Cap1 or Cap2 form, respectively [51]. Although Cap1 methylation is present in all mRNAs, Cap2 methylation seems to be restricted to a subset of mRNAs [52]. Jaffrey's team latest work explored the molecular basis of Cap2 selectivity and functions. By developing novel methods (CLAM-Cap-Seq) to profile Cap2 transcriptome-wide as well as on specific mRNAs of interest, they found that Cap2 modification can be present in all mRNAs, but it increases throughout mRNAs lifetime, being thus more enriched as mRNAs age [53]. In addition, by performing experiments in cells with reduced level of Cap2, they found that this epitranscriptomic modification mainly functions in suppressing the activation of the innate immune response by inhibiting RIG-I binding to Cap1, thus providing a mechanism to distinguish self RNA from viral RNA.

Additionally, metabolite-derived cap structures have been discovered in both prokaryotes and eukaryotes. These caps originate from nucleotide-derived molecules, including nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) and dinucleoside polyphosphates [54]. The latter are small signalling molecules, also called as "alarmones", whose cellular concentrations increase during stress, hence the name. The molecular target of such "alarmones" was not

known. By developing a liquid chromatography-mass spectrometry technique for the detection of dinucleoside polyphosphates, the group of Hana Cahová (Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, CZ) has discovered that alarmones are a non-canonical form of 5' RNA caps in bacteria [55]. They found that dinucleoside polyphosphate capped RNA can be cleaved by two types of enzymes, RppH and ApaH, and thus degraded. However, in stationary phase of growth, and thus under starvation, the dinucleoside polyphosphate RNA caps increase and undergo methylation, protecting RNA from cleavage and further degradation during stress. Thus, the presence of dinucleoside polyphosphate caps, which is strictly dependent on the environmental conditions, may represent a way to preserve specific species of mRNAs during stress.

RNA therapeutics

More than 150 different RNA chemical modifications have been identified so far, characterized by a wide variety of chemical diversities. However, the function of most of these modifications is still unknown. Therefore, the development of reliable chemoenzymatic methods allowing to produce chemically modified RNA may help to elucidate the function of these native modified RNAs and their putative use as RNA tools (such as siRNA, miRNA, antisense nucleotides (ASOs) or aptamers). Introduction of modified nucleotides is a common approach used to increase RNA stability. For example, RNA molecules used to silence genes are quickly degraded by nucleases after cellular uptake and thus modifications make them more resistant to the attack of these enzymes. Aptamers, which are single-stranded oligonucleotides, can act as antagonists or agonists for specific proteins by binding their targets with potentially high affinity. These structured nucleic acid ligands require modifications not only to increase their resistance to nucleases, but also to increase the number of putative targets. Thus, to obtain more stable oligonucleotides, non-native modifications are introduced either at the level of the base, the ribose or the sugar/phosphate backbone. Two major methods already exist to modify nucleic acid: (i) the automated solid-phase synthesis and (ii) the enzymatic synthesis [56]. Due to inherent limitations of both approaches, chemoenzymatic methods are under development in Marcel Hollenstein's group (Institut Pasteur, Paris, FR). Currently, his group is working on the polymerization of modified dNTPs and ligation of oligonucleotides with modified fragments. They equipped the nucleobase of a nucleoside triphosphate with a vancomycin moiety and showed the ability of DNA polymerase to incorporate the modified analogue during DNA synthesis [57]. This modified nucleotide was compatible with SELEX methodology (systematic evolution of ligands by exponential enrichment), and therefore, it was employed to select new aptamers to tackle antimicrobial resistance and restore drug effectiveness. Our global recent experience has shown that nucleic acid

therapeutics are an invaluable alternative to classical vaccine strategies, as it has been highlighted by the development of the mRNA vaccines against SARS-CoV-2.

Indeed, oligonucleotide-based therapies have the potential to treat a wide range of diseases by preventing, replacing, adding, or editing RNA and DNA. Besides aptamers, these novel intervention strategies also comprise the use of antisense oligonucleotides, RNAi and the CRISPR/Cas9 technology. In this context, Jørgen Kjems' group (Interdisciplinary nanoscience center, Aarhus, DK) works on RNA chemical modifications and the design of efficient delivery systems for RNA therapeutics. For example, his group reported a 2'-fluoro protected RNA aptamer which has the ability to bind with high affinity to the receptor-binding domain of SARS-CoV-2 spike protein and inhibits its interaction with the host receptor ACE2 [58]. Impressively, a trimer of this RNA aptamer was able to enhance the binding affinity in the low picomolar range. In addition, Kjems' team recent work focused on the use of aptamers as diagnostic and predictive biomarkers for bladder cancer patients. They developed an RNA aptamer-

based strategy called APTASHAPE, which is based on serum-stable RNA oligonucleotides interacting with the proteome of plasma from bladder cancer or healthy control patients [59]. Thus, they were able to identify 33 aptamers that differentiate in abundance between plasma samples from patients with various stages of bladder cancer and controls. Differences in aptamer abundance reflected differences in global proteome of the plasma samples, highlighting how this aptamer-based strategy can be employed for the discovery of new disease biomarkers. Moreover, therapeutic targeting of highly expressed non-coding RNAs represents a novel approach for the treatment of diverse diseases such as cancer. The group of Maria Duca (Institute of Chemistry of Nice, FR) works on development of new RNA ligands able to target molecules such as miRNA. Ribosomal RNAs are well-established antibiotic targets; for example, aminoglycosides and tetracyclines have been already used for several decades. The importance and feasibility of RNA targeting by small molecules has been confirmed in 2021, when Risdiplam was approved by the FDA (Food and Drug Administration)

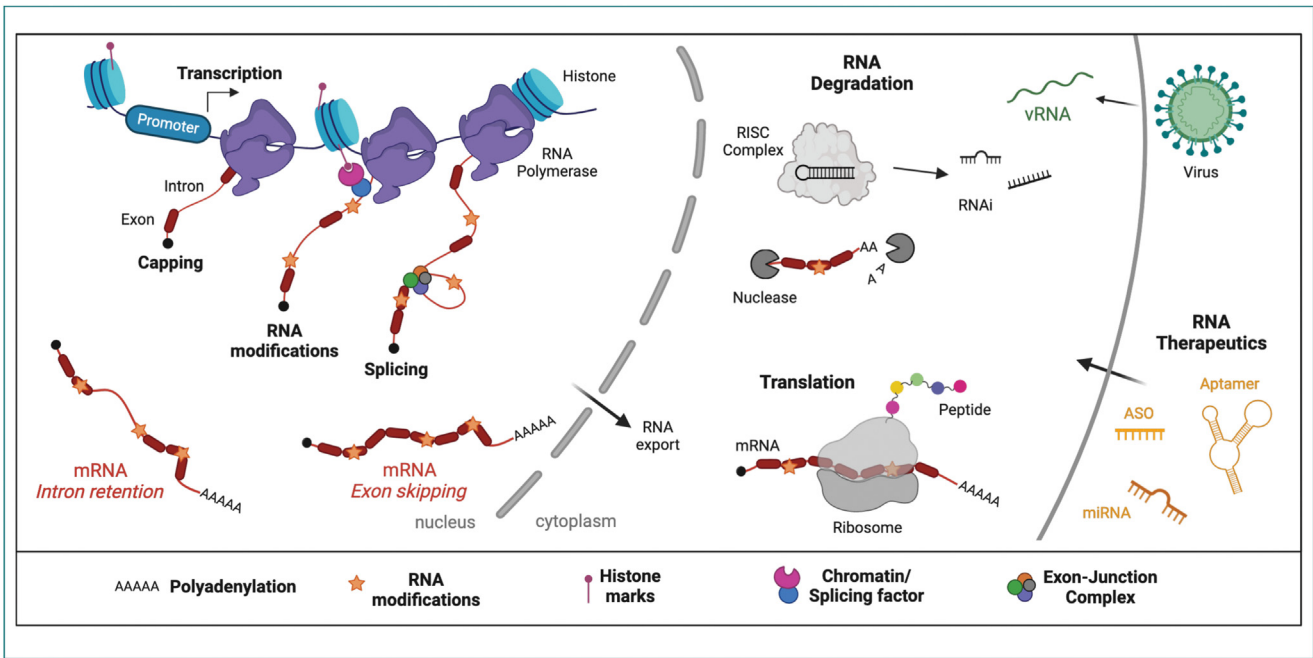


FIGURE 1

Copy of Curie Course. Illustration of the various aspects of the RNA biology and therapeutic aspects discussed in the course. During and after transcription, the molecule of RNA can undergo several regulatory processes important for gene expression that can be targeted with therapeutical purposes. There is (pre-)mRNA alternative splicing, which is the excision of intronic and/or exonic sequences and which is driven by the Exon-Junction Complex; RNA polyadenylation to mark the end of the transcript; RNA modification with the addition of post-transcriptional modifications along the RNA (marked by orange stars on the figure); mRNA export through nuclear pore complex to reach the cytoplasm to perform RNA translation in which the messenger RNA (mRNA) is translated into a peptide thanks to the ribosome complex; and RNA degradation either by nuclease or RISC complex (a ribonucleoprotein complex which forms RNA interference molecules (RNAi) to then target complementary mRNA). The study of these regulatory layers, combined with the chemistry of RNA, allow the design of new RNA therapeutic strategies, such as the use of sequence-specific antisense oligonucleotides (ASO), microRNAs (miRNA) or aptamers. RISC: RNA-induced silencing complex; vRNA: viral RNA. (created by BioRender.com)

for the treatment of the spinal muscular atrophy [60]. The drug modulates splicing and enables the SMN2 gene to produce a full-length SMN protein, which prevents the truncated protein-induced motor neuron deterioration and death. The Duca's lab developed a different approach for the discovery of new RNA ligands, in which they designed and synthesized multimodal RNA binders to increase affinity and selectivity toward the target. For example, her lab designed and synthesized new powerful RNA binders by conjugating the C-terminal lateral chain of bleomycin A5 to different chemical scaffolds known to be able to interact with RNA residues and inhibit the biogenesis of oncogenic pre-miRNAs [61]. In addition, her lab established several chemical library screenings to identify new potential bioactive compounds: they use approaches such as phenotypic screening, target-based screenings, Inforna strategy and RIBOTACS (Ribonuclease-targeted chimeras).

Likewise, emerging evidence has highlighted the involvement of non-canonical nucleic acid secondary structures called G-quadruplexes (G4s) in key cellular processes including translation regulation, 3'-end processing, alternative splicing, and mRNA localization. Importantly, specific G4 ligands not only stabilize G4s and may promote their formation but can modify RNA functions by increasing the lifetime of these structures and modulating their recognition by protein partners. This is why G4 ligands represent highly promising candidates for drug discovery able to act selectively at specific G-rich RNA loci. Daniela Verga (Institut Curie, Orsay, FR) works on the design, synthesis, biophysical and biochemical evaluation of G4 ligands. Her group exploited new methods to follow G4 ligand distribution in both metaphase chromosome spreads and in cells to associate ligand localization to specific cellular responses. For example, they functionalized a highly selective G4 ligand (PhenDC3) with a bromine and followed its distribution on metaphase chromosome spreads by using nanoscale secondary ion mass spectrometry (nanoSIMS) [62]. A more recent research has used other G4 ligands, belonging to the same family of PhenDC3, to develop a new immunofluorescence method allowing the detection of the compounds and, therefore, G4 structures in cells with increased resolution. The more promising compounds were functionalized in situ with 5BrDU (5-bromo deoxyuridine), a hapten which can be recognized by a commercially available antibody, by click reaction (CuAAC, copper catalyzed alkyne-azide cycloaddition reaction) [63].

Conclusion

In conclusion, this course has underscored the importance of post-transcriptional regulation of gene expression in both physiology and disease, such as cancer and viral infection. In particular, the course provided mechanistic insights into the many steps leading to the production of a mature RNA and the control of its translation, and highlighted how RNA chemistry plays a fundamental role in such processes and can be exploited for

therapeutics (figure 1). Indeed, all the steps involved in RNA metabolism, including pre-mRNA splicing, mRNA editing, translation, and degradation, together with RNA intrinsic features and trans-acting factors participating in such processes, are key contributors of cancer progression, viral infections, as well as immunological responses. This indicates how the post-transcriptional regulation of gene expression, as well as the RNA molecule itself, represents promising target in many pathologies. Moreover, the RNA molecule and chemistry may be useful tools for therapeutic applications, highlighting how advances in RNA biology and chemistry can be fully integrated to pave the way to new therapeutic strategies.

Disclosure of interest: the authors declare that they have no competing interest.

References

- [1] Ule J, Blencowe BJ. Alternative splicing regulatory networks: functions, mechanisms and evolution. *Mol Cell* 2019;76:329–45. <http://dx.doi.org/10.1016/j.molcel.2019.09.017>.
- [2] Amit M, Donyo M, Hollander D, Goren A, Kim E, Gelfman S, et al. Content between exons and introns establishes distinct strategies of splice-site recognition. *Cell Rep* 2012;1:543–56. <http://dx.doi.org/10.1016/j.celrep.2012.03.013>.
- [3] Tammer L, Hameiri O, Keydar I, Roy VR, Ashkenazy-Titelman A, Custódio N, et al. Gene architecture directs splicing outcome in separate nuclear spatial regions. *Mol Cell* 2022;82:1021–1034.e8. <http://dx.doi.org/10.1016/j.molcel.2022.02.001>.
- [4] Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM, Misteli T. Regulation of alternative splicing by histone modifications. *Science* 2010;327:996–1000. <http://dx.doi.org/10.1126/science.1184208>.
- [5] Gonzalez I, Munita R, Agirre E, Dittmer TA, Gysling K, Misteli T, et al. A lncRNA regulates alternative splicing via establishment of a splicing-specific chromatin signature. *Nat Struct Mol Biol* 2015;22:370–6. <http://dx.doi.org/10.1038/nsmb.3005>.
- [6] Segelle A, Núñez-Álvarez Y, Oldfield AJ, Webb KM, Voigt P, Luco RF. Histone marks regulate the epithelial-to-mesenchymal transition via alternative splicing. *Cell Rep* 2022;38:110357. <http://dx.doi.org/10.1016/j.celrep.2022.110357>.
- [7] Hir H, Le Saulière J, Wang Z. The exon junction complex as a node of post-transcriptional networks. *Nat Rev Mol Cell Biol* 2016;17:41–54. <http://dx.doi.org/10.1038/nrm.2015.7>.
- [8] Le Hir H, Izaurralde E, Maquat LE, Moore MJ. The spliceosome deposits multiple proteins 20–24 nucleotides upstream of mRNA exon–exon junctions. *EMBO J* 2000;19:6860–9. <http://dx.doi.org/10.1093/emboj/19.24.6860>.
- [9] Buchwald G, Schüssler S, Basquin C, Le Hir H, Conti E. Crystal structure of the human eIF4AIII-CWC22 complex shows how a DEAD-box protein is inhibited by a MIF4G domain. *Proc Natl Acad Sci* 2013;110:E4611–18. <http://dx.doi.org/10.1073/pnas.1314684110>.
- [10] Wang Z, Murigneux V, Le Hir H. Transcriptome-wide modulation of splicing by the exon junction complex. *Genome Biol* 2014;15:551. <http://dx.doi.org/10.1186/s13059-014-0551-7>.
- [11] Le Hir H, Gattfield D, Izaurralde E, Moore MJ. The exon–exon junction complex provides a binding platform for factors involved in mRNA export and nonsense-mediated mRNA decay. *EMBO J* 2001;20:4987–97. <http://dx.doi.org/10.1093/emboj/20.17.4987>.
- [12] Uzonyi A, Dierks D, Nir R, Kwon OS, Toth U, Barbosa I, et al. Exclusion of m6A from splice-site proximal regions by the exon junction complex

- dictates m6A topologies and mRNA stability. *Mol Cell* 2023;83:237-251.e7. <http://dx.doi.org/10.1016/j.molcel.2022.12.026>.
- [13] Kwon OS, Mishra R, Safieddine A, Coleno E, Alasseur Q, Faucourt M, et al. Exon junction complex dependent mRNA localization is linked to centrosome organization during ciliogenesis. *Nat Commun* 2021;12:1351. <http://dx.doi.org/10.1038/s41467-021-21590-w>.
- [14] Broeils LA, Ruiz-Orera J, Snel B, Hubner N, van Heesch S. Evolution and implications of de novo genes in humans. *Nat Ecol Evol* 2023;7:804-15. <http://dx.doi.org/10.1038/s41559-023-02014-y>.
- [15] Mudge JM, Ruiz-Orera J, Prensner JR, Brunet MA, Calvet F, Jungreis I, et al. Standardized annotation of translated open reading frames. *Nat Biotechnol* 2022;40:994-9. <http://dx.doi.org/10.1038/s41587-022-01369-0>.
- [16] Sandmann C-L, Schulz JF, Ruiz-Orera J, Kirchner M, Ziehm M, Adami E, et al. Evolutionary origins and interactomes of human, young micro-proteins and small peptides translated from short open reading frames. *Mol Cell* 2023;83:994-1011.e18. <http://dx.doi.org/10.1016/j.molcel.2023.01.023>.
- [17] Prensner JR, Enache OM, Luria V, Krug K, Clauser KR, Dempster JM, et al. Noncanonical open reading frames encode functional proteins essential for cancer cell survival. *Nat Biotechnol* 2021;39:697-704. <http://dx.doi.org/10.1038/s41587-020-00806-2>.
- [18] Suenaga Y, Islam SMR, Alagu J, Kaneko Y, Kato M, Tanaka Y, et al. NCYM, a Cis-antisense gene of MYCN, encodes a de novo evolved protein that inhibits GSK3 β resulting in the stabilization of MYCN in human neuroblastomas. *PLoS Genet* 2014;10:e1003996. <http://dx.doi.org/10.1371/journal.pgen.1003996>.
- [19] Buhl AM, Jurlander J, Jørgensen FS, Ottesen AM, Cowland JB, Gjerdrum LM, et al. Identification of a gene on chromosome 12q22 uniquely overexpressed in chronic lymphocytic leukemia. *Blood* 2006;107:2904-11. <http://dx.doi.org/10.1182/blood-2005-07-2615>.
- [20] Navickas A, Asgharian H, Winkler J, Fish L, Garcia K, Markert D, et al. An mRNA processing pathway suppresses metastasis by governing translational control from the nucleus. *Nat Cell Biol* 2023;25:892-903. <http://dx.doi.org/10.1038/s41556-023-01141-9>.
- [21] Fish L, Khoroshkin M, Navickas A, Garcia K, Culbertson B, Hänisch B, et al. A prometastatic splicing program regulated by SNRPA1 interactions with structured RNA elements. *Science* 2021;372. <http://dx.doi.org/10.1126/science.abc7531>.
- [22] Yu J, Navickas A, Asgharian H, Culbertson B, Fish L, Garcia K, et al. RBMS1 suppresses colon cancer metastasis through targeted stabilization of its mRNA regulon. *Cancer Discov* 2020;10:1410-23. <http://dx.doi.org/10.1158/2159-8290.CD-19-1375>.
- [23] Loayza-Puch F, Rooijers K, Buil LCM, Zijlstra J, Oude Vrielink JF, Lopes R, et al. Tumour-specific proline vulnerability uncovered by differential ribosome codon reading. *Nature* 2016;530:490-4. <http://dx.doi.org/10.1038/nature16982>.
- [24] Bartok O, Pataskar A, Nagel R, Laos M, Goldfarb E, Hayoun D, et al. Anti-tumour immunity induces aberrant peptide presentation in melanoma. *Nature* 2021;590:332-7. <http://dx.doi.org/10.1038/s41586-020-03054-1>.
- [25] Pataskar A, Champagne J, Nagel R, Kenski J, Laos M, Michaux J, et al. Tryptophan depletion results in tryptophan-to-phenylalanine substituents. *Nature* 2022;603:721-7. <http://dx.doi.org/10.1038/s41586-022-04499-2>.
- [26] Champagne J, Pataskar A, Blommaert N, Nagel R, Wernaart D, Ramalho S, et al. Oncogene-dependent sloppiness in mRNA translation. *Mol Cell* 2021;81:4709-4721.e9. <http://dx.doi.org/10.1016/j.molcel.2021.09.002>.
- [27] Dicker K, Järvelin AI, Garcia-Moreno M, Castello A. The importance of virion-incorporated cellular RNA-Binding Proteins in viral particle assembly and infectivity. *Semin Cell Dev Biol* 2021;111:108-18. <http://dx.doi.org/10.1016/j.semcdb.2020.08.002>.
- [28] Iselin L, Palmalux N, Kamel W, Simmonds P, Mohammed S, Castello A. Uncovering viral RNA-host cell interactions on a proteome-wide scale. *Trends Biochem Sci* 2022;47:23-38. <http://dx.doi.org/10.1016/j.tibs.2021.08.002>.
- [29] Garcia-Moreno M, Noerenberg M, Ni S, Järvelin AI, González-Almela E, Lenz CE, et al. System-wide Profiling of RNA-binding proteins uncovers key regulators of virus infection. *Mol Cell* 2019;74:196-211.e11. <http://dx.doi.org/10.1016/j.molcel.2019.01.017>.
- [30] Kamel W, Ruscica V, Garcia-Moreno M, Palmalux N, Iselin L, Hannan M, et al. Compositional analysis of Sindbis virus ribonucleoproteins reveals an extensive co-opting of key nuclear RNA-binding proteins. *BioRxiv* 2021;2021. <http://dx.doi.org/10.1101/2021.10.06.463336>.
- [31] Kamel W, Noerenberg M, Cerikan B, Chen H, Järvelin AI, Kammoun M, et al. Global analysis of protein-RNA interactions in SARS-CoV-2-infected cells reveals key regulators of infection. *Mol Cell* 2021;81:2851-2867.e7. <http://dx.doi.org/10.1016/j.molcel.2021.05.023>.
- [32] D'Angelo W, Gurung C, Acharya D, Chen B, Ortolano N, Gama V, et al. The molecular basis for the lack of inflammatory responses in mouse embryonic stem cells and their differentiated cells. *J Immunol* 2017;198:2147-55. <http://dx.doi.org/10.4049/jimmunol.1601068>.
- [33] Maillard PV, van der Veen AG, Poirier EZ, Reis e Sousa C. Slicing and dicing viruses: antiviral RNA interference in mammals. *EMBO J* 2019;38:e100941. <http://dx.doi.org/10.15252/emboj.2018100941>.
- [34] Poirier EZ, Buck MD, Chakravarty P, Carvalho J, Frederico B, Cardoso A, et al. An isoform of Dicer protects mammalian stem cells against multiple RNA viruses. *Science* 2021;373:231-6. <http://dx.doi.org/10.1126/science.abq2264>.
- [35] Herzog VA, Reichholf B, Neumann T, Rescheneder P, Bhat P, Burkard TR, et al. Thiol-linked alkylation of RNA to assess expression dynamics. *Nat Methods* 2017;14:1198-204. <http://dx.doi.org/10.1038/nmeth.4435>.
- [36] Reichholf B, Herzog VA, Fasching N, Manzenreither RA, Sowemimo I, Ameres SL. Time-resolved small RNA sequencing unravels the molecular principles of MicroRNA homeostasis. *Mol Cell* 2019;75:756-768.e7. <http://dx.doi.org/10.1016/j.molcel.2019.06.018>.
- [37] Reimão-Pinto MM, Ignatova V, Burkard TR, Hung J-H, Manzenreither RA, Sowemimo I, et al. Uridylation of RNA hairpins by tailor confines the emergence of MicroRNAs in drosophila. *Mol Cell* 2015;59:203-16. <http://dx.doi.org/10.1016/j.molcel.2015.05.033>.
- [38] Reimão-Pinto MM, Manzenreither RA, Burkard TR, Sledz P, Jinek M, Mechtler K, et al. Molecular basis for cytoplasmic RNA surveillance by uridylation-triggered decay in Drosophila. *EMBO J* 2016;35:2417-34. <http://dx.doi.org/10.15252/emboj.201695164>.
- [39] Rapino F, Delaunay S, Rambow F, Zhou Z, Tharun L, De Tullio P, et al. Codon-specific translation reprogramming promotes resistance to targeted therapy. *Nature* 2018;558:605-9. <http://dx.doi.org/10.1038/s41586-018-0243-7>.
- [40] McMahon M, Ruggero D. A wobbly road to drug resistance in melanoma: tRNA-modifying enzymes in translation reprogramming. *EMBO J* 2018;37:e99978. <http://dx.doi.org/10.15252/emboj.201899978>.
- [41] Rapino F, Zhou Z, Roncero Sanchez AM, Joiret M, Seca C, El Hachem N, et al. Wobble tRNA modification and hydrophilic amino acid patterns dictate protein fate. *Nat Commun* 2021;12:2170. <http://dx.doi.org/10.1038/s41467-021-22254-5>.
- [42] Pederiva C, Trevisan DM, Peirasmasmi D, Chen S, Savage SA, Larsson O, et al. Control of protein synthesis through mRNA pseudouridylation by dyskerin. *Sci Adv* 2023;9:eadg1805. <http://dx.doi.org/10.1126/sciadv.adq1805>.
- [43] Dueva R, Akopyan K, Pederiva C, Trevisan D, Dhanjal S, Lindqvist A, et al. Neutralization of the positive charges on histone tails by RNA promotes an open chromatin structure. *Cell Chem Biol* 2019;26:1436-1449.e5. <http://dx.doi.org/10.1016/j.chembiol.2019.08.002>.
- [44] Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 2012;149:1635-46. <http://dx.doi.org/10.1016/j.cell.2012.05.003>.
- [45] Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR. Single-nucleotide-resolution mapping of m6A and m6Am throughout

- the transcriptome. *Nat Methods* 2015;12:767-72. <http://dx.doi.org/10.1038/nmeth.3453>.
- [46] Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol* 2019;20:608-24. <http://dx.doi.org/10.1038/s41580-019-0168-5>.
- [47] He PC, He C. m6A RNA methylation: from mechanisms to therapeutic potential. *EMBO J* 2021;40:e105977. <http://dx.doi.org/10.15252/embj.2020105977>.
- [48] Ries RJ, Zaccara S, Klein P, Orlarier-George A, Namkoong S, Pickering BF, et al. m6A enhances the phase separation potential of Mrna. *Nature* 2019;571:424-8. <http://dx.doi.org/10.1038/s41586-019-1374-1>.
- [49] Zaccara S, Jaffrey SR. A unified model for the function of YTHDF proteins in regulating m6A-modified mRNA. *Cell* 2020;181:1582-1595.e18. <http://dx.doi.org/10.1016/j.cell.2020.05.012>.
- [50] Ramanathan A, Robb GB, Chan S-H. mRNA capping: biological functions and applications. *Nucleic Acids Res* 2016;44:7511-26. <http://dx.doi.org/10.1093/nar/gkw551>.
- [51] Furuichi Y, Shatkin AJBT-A in VR. Viral and cellular mRNA capping: past and prospects. Academic Press 2000;55:135-84. [http://dx.doi.org/10.1016/S0065-3527\(00\)55003-9](http://dx.doi.org/10.1016/S0065-3527(00)55003-9).
- [52] Furuichi Y, Morgan M, Shatkin AJ, Jelinek W, Salditt-Georgieff M, Darnell JE. Methylated, blocked 5 termini in HeLa cell Mrna. *Proc Natl Acad Sci* 1975;72:1904-8. <http://dx.doi.org/10.1073/pnas.72.5.1904>.
- [53] Despic V, Jaffrey SR. mRNA ageing shapes the Cap2 methylome in mammalian mRNA. *Nature* 2023;614:358-66. <http://dx.doi.org/10.1038/s41586-022-05668-z>.
- [54] Doamekpor SK, Sharma S, Kiledjian M, Tong L. Recent insights into noncanonical 5' capping and decapping of RNA. *J Biol Chem* 2022;298:102171. <http://dx.doi.org/10.1016/j.jbc.2022.102171>.
- [55] Hudeček O, Benoni R, Reyes-Gutierrez PE, Culka M, Čánderová H, Hubálek M, et al. Dinucleoside polyphosphates act as 5-RNA caps in bacteria. *Nat Commun* 2020;11:1052. <http://dx.doi.org/10.1038/s41467-020-14896-8>.
- [56] Flamme M, McKenzie LK, Sarac I, Hollenstein M. Chemical methods for the modification of RNA. *Methods* 2019;161:64-82. <http://dx.doi.org/10.1016/j.ymeth.2019.03.018>.
- [57] Figazzolo C, Bonhomme F, Saidjalolov S, Ethève-Quellejeu M, Hollenstein M. Enzymatic synthesis of vancomycin-modified DNA. *Molecules* 2022;27:8927. <http://dx.doi.org/10.3390/molecules27248927>.
- [58] Valero J, Civit L, Dupont DM, Selnhin D, Reinert LS, Idorn M, et al. A serum-stable RNA aptamer specific for SARS-CoV-2 neutralizes viral entry. *Proc Natl Acad Sci USA* 2021;118. <http://dx.doi.org/10.1073/pnas.2112942118> [e2112942118].
- [59] Fjelstrup S, Dupont DM, Bus C, Enghild JJ, Jensen JB, Birkenkamp-Demtröder K, et al. Differential RNA aptamer affinity profiling on plasma as a potential diagnostic tool for bladder cancer. *NAR Cancer* 2022;4:zcac025. <http://dx.doi.org/10.1093/narcan/zcac025>.
- [60] Ratni H, Scalco RS, Stephan AH. Risdipam, the first approved small molecule splicing modifier drug as a blueprint for future transformative medicines. *ACS Med Chem Lett* 2021;12:874-7. <http://dx.doi.org/10.1021/acsmchemlett.0c00659>.
- [61] Maucourt C, Bonnet M, Ortuno J-C, Tucker G, Quissac E, Verreault M, et al. Synthesis of bleomycin-inspired RNA ligands targeting the biogenesis of oncogenic miRNAs. *J Med Chem* 2023;66:10639-57. <http://dx.doi.org/10.1021/acs.jmedchem.3c00797>.
- [62] Verga D, Hamon F, Nicoleau C, Guetta C, Wu T, Guerquin-Kern J, et al. Chemical imaging by NanoSIMS provides high-resolution localization of the G-Quadruplex interactive drug (Br)-PhenDC3 on human chromosomes. *J Mol Biol Mol Imaging* 2017;4:1-6.
- [63] Masson T, Landras Guetta C, Laigre E, Cucchiarini A, Duchambon P, Teulade-Fichou M-P, et al. BrdU immuno-tagged G-quadruplex ligands: a new ligand-guided immunofluorescence approach for tracking G-quadruplexes in cells. *Nucleic Acids Res* 2021;49:12644-60. <http://dx.doi.org/10.1093/nar/qkab1166>.

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