RNA Biology Provides New Therapeutic Targets for Human Disease

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RNA is the messenger molecule that conveys information from the genome and allows the production of biomolecules required for life in a responsive and regulated way. Most genes are able to produce multiple mRNA products in response to different internal or external environmental signals, in different tissues and organs, and at specific times in development or later life. This fine tuning of gene expression is dependent on the coordinated effects of a large and intricate set of regulatory machinery, which together orchestrate the genomic output at each locus and ensure that each gene is expressed at the right amount, at the right time and in the correct location. This complexity of control, and the requirement for both sequence elements and the entities that bind them, results in multiple points at which errors may occur. Errors of RNA biology are common and found in association with both rare, single gene disorders, but also more common, chronic diseases. Fortunately, complexity also brings opportunity. The existence of many regulatory steps also offers multiple levels of potential therapeutic intervention which can be exploited. In this review, I will outline the specific points at which coding RNAs may be regulated, indicate potential means of intervention at each stage, and outline with examples some of the progress that has been made in this area. Finally, I will outline some of the remaining challenges with the delivery of RNA-based therapeutics but indicate why there are reasons for optimism.

Keywords: mRNA processing, RNA editing, RNA export, RNA therapeutics, ncRNA, splicing, RNA epitranscriptomics, therapeutics

INTRODUCTION

The fundamental importance of RNA not only as a messenger molecule, but as a regulator of genes in its own right is increasingly being recognized. The production of mature messenger RNA (mRNA) is dependent on a plethora of processing and regulatory steps involving a complicated repertoire of sequence elements, RNA binding proteins and other regulatory RNA species. Given the complexity of the regulatory machinery, defects in non-coding regions of genes and regulatory genomic regions are common in genetic disease, being present in up to 50% of cases (Yang et al., 2013; Beaulieu et al., 2014) and are also the most common site of genetic variation conferring susceptibility to common, complex disease (Manolio et al., 2008). There is, however, a silver lining. The complexity that causes errors in gene expression or mRNA processing to be such a common occurrence, also provides multiple and differential points of potential therapeutic intervention. Over the past decade, there have been a number of examples, where the specifics of RNA regulatory machinery have been harnessed to produce novel therapeutics that are now in phase III clinical trials [e.g., Patisiran for Familial amyloid polyneuropathy (Rizk and Tuzmen, 2017), Custirsen for

prostate cancer (Edwards et al., 2017) and AGS-003 for renal cell carcinoma (Figlin, 2015)]. This review aims to explore the potential for intervention in mRNA processing or post-transcriptional regulation with selected examples for future therapeutic benefit.

THE LIFECYCLE OF A CODING RNA

The processes involved in the production of a mature mRNA, and its subsequent fate are multifaceted and complicated (**Figure 1**). The life of an RNA molecule starts upon transcription, which is controlled by tissue specific promoters and enhancers. The immature primary RNA transcript (heterogeneous nuclear RNA (hnRNA) or pre-mRNA) then undergoes a series of modifications that involve the addition of the 5′ cap structure, removal of the intronic sequences by constitutive or alternative splicing and 3′ end processing events that include the addition of the poly-A tail (Chen et al., 2017; Sperling, 2017; Zhang and Tjian, 2018). These processes are not a linear pipeline and occur co-transcriptionally (Beyer and Osheim, 1988; Bentley, 2002). Newly processed RNA may also undergo RNA editing, which is mostly A to G or A to I substitution in humans (Chen, 2013). RNAs may also undergo epitranscriptomic decoration, whereby different RNA modifications such as methylation of adenosine residues (m^6A) may be added. Such modifications are added by a series of RNA readers, writers and erasers (Helm and Motorin, 2017). Mature mRNAs are then exported from the nucleus to the cytoplasm. This is an active and regulated process, and one of the primary safeguards against the translation of aberrant mRNAs (Williams et al., 2018). The spatial and temporal expression of newly exported RNAs can also controlled at the level of specific localization within the cell. This can be passive, or an active process involving transport on cytoskeletal tracks (Suter, 2018). Gene expression can also be controlled at the level of translation. This can occur by virtue of selective degradation of specific RNAs by mRNA surveillance pathways such as nonsense-mediated decay, no-go decay and non-stop decay (Harigaya and Parker, 2010; Klauer and van Hoof, 2012; Lejeune, 2017), or it can be by regulation of the rate of translation itself (Gorgoni et al., 2014). The half-life of any given mRNA is then determined by a number of RNA decay pathways, most of which involve successive decapping and deadenylation of RNA molecules, which then renders them susceptible to exonucleases (Wahle and Winkler, 2013; Borbolis and Syntichaki, 2015). Finally, the fate of the RNA may also be influenced by the action of both short and long non-coding RNAs and RNA binding proteins which can result in degradation or translational blocking (Fukao et al., 2015; Iadevaia and Gerber, 2015; Fukao and Fujiwara, 2017).

POTENTIAL POINTS OF THERAPEUTIC INTERVENTION

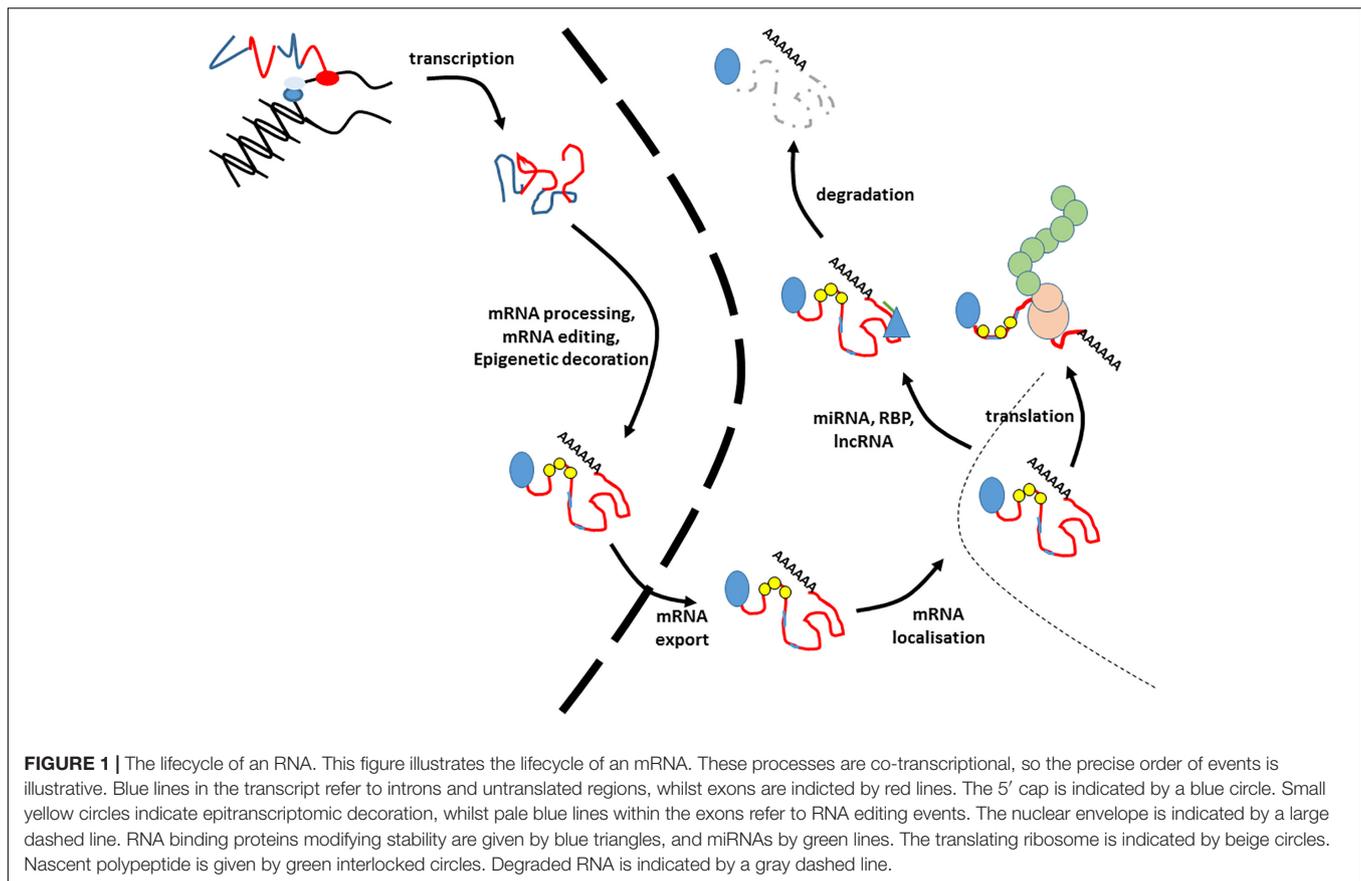Knowledge of the processes by which mature mRNAs are expressed, processed and regulated opens up the possibility of targeting the molecule with specific interventions for future therapeutic benefit.

Therapeutic Modulation of Transcription

Therapeutic modulation of gene activity can be achieved through several mechanisms which include triplex-forming oligonucleotides (TPOs) synthetic polyamides (SPs) and artificial transcription factors (ATFs) (Uil et al., 2003). These approaches work by altering the expression level of a gene, rather than restoring its sequence *per se*. TPOs and SPs work by binding the major and minor groove, respectively, of the genomic DNA in specific regions of the gene, with the consequence of modulating gene activity at the level of transcription. This can be achieved by using steric hindrance to block transcription elongation for down-regulation of gene activity or conversely, blocking access to naturally occurring repressor molecules to bring about gene activation. ATFs are custom molecules designed with DNA binding domains specific to the gene in question, coupled to a *trans*-regulatory domain to produce the desired activity. Although there have been some promising *in vitro* studies, such as reactivation of the *EPB41L3* gene, usually silenced by methylation, to promote tumor suppression in breast, ovarian, and cervical cell lines (Huisman et al., 2015), they have not yet reached prominence in the clinic.

Therapeutic Modification of Splicing

RNA splicing is controlled by a complex interplay between ribonucleoprotein complexes and sequence elements in the pre-mRNA. The splicing process consists of two phosphodiester transfer reactions; the first being an interaction between the 5′ splice site and the branch site, and the second comprising cleavage at the 3′ splice site, and joining of the released exons. This occurs due to the action of a family of small nuclear ribonucleoproteins (snRNPs) named U1, U2, U4, U5, and U6, which together with a battery of approximately 80 other ancillary proteins form the core spliceosome and orchestrate the splicing process (Will and Luhrmann, 2011). The spliceosome is a dynamic machine that undergoes structural remodeling and conformational change to bring about the excision of introns and the joining of introns (Makarov et al., 2002). This machinery is necessary but sometimes not sufficient for splice site usage to occur; 98% of the genome produces multiple RNA transcripts in a process termed alternative splicing (Pan et al., 2008). The precise nature of transcripts produced under different circumstances is under tight spatial and temporal regulation. This is facilitated by the combinatorial control of a series of splice site activators and inhibitor proteins that together determine whether or not a given splicing event occurs in a given circumstance. Serine Arginine rich proteins (SRSF) splicing factors usually (but not exclusively) promote splice site usage, whereas heterogeneous nuclear ribonucleoproteins (hnRNPs) usually (but not exclusively) promote splice site silencing, as well as having roles in nuclear export and other aspects of RNA metabolism (Smith and Valcarcel, 2000; Cartegni et al., 2002). Splicing defects can arise from single base pair changes to the core and regulatory sequence elements, but can also arise from insertion or deletion events and frameshifts, or from activation of

FIGURE 1 | The lifecycle of an RNA. This figure illustrates the lifecycle of an mRNA. These processes are co-transcriptional, so the precise order of events is illustrative. Blue lines in the transcript refer to introns and untranslated regions, whilst exons are indicted by red lines. The 5′ cap is indicated by a blue circle. Small yellow circles indicate epitranscriptomic decoration, whilst pale blue lines within the exons refer to RNA editing events. The nuclear envelope is indicated by a large dashed line. RNA binding proteins modifying stability are given by blue triangles, and miRNAs by green lines. The translating ribosome is indicated by beige circles. Nascent polypeptide is given by green interlocked circles. Degraded RNA is indicated by a gray dashed line.

cryptic splice sites by other sequence changes. Similarly, changes occurring in exon and intron splicing enhancer and silencer elements can elicit dysregulation of splicing patterns of specific genes (Blencowe, 2000). Dysregulation of the splicing regulatory machinery by cellular stress has been reported in more complex phenotypes such as cellular senescence (Holly et al., 2013; Latorre et al., 2017) and altered global alternative splicing profiles are a key characteristics of many complex diseases such as dementia, cancer and type 2 diabetes (Tollervey et al., 2011; Berson et al., 2012; Cnop et al., 2014; Love et al., 2015; Lu et al., 2015). The complexity of splicing regulation offers several points of potential intervention.

Moderation of the Core Spliceosome

The global dysregulation of splicing patterns that occur in complex disease may be addressed by targeting the core spliceosome. There are several compounds of bacterial origin that affect the function of the SF3B component of the U2 snRNP, which are showing promise as anti-cancer agents by causing stalling of the cell cycle at the G1/S or G2/M checkpoints (Nakajima et al., 1996). Although these approaches show promise, to date most remain some distance from the clinic.

Moderation of Splicing Regulation

It may be possible to globally restore splicing patterns by targeting the splicing regulatory proteins themselves. This could be done at the level of mRNA expression, or at the level of activation

or cellular localization. Splicing factor expression has recently been described to be negatively regulated at the mRNA level in senescent primary human dermal fibroblasts by the constitutive activation of the ERK and AKT pathways. Targeted inhibition of either ERK or AKT, as well as gene knock down of their effector genes *FOXO1* and *ETV6* was associated with restoration of splicing factor expression and rescue from cellular senescence (Latorre et al., 2018). Similarly, splicing factor activity and localization is controlled at the protein level by the action of a series of kinases and phosphatases including SRPK1, SRPK2, CLK1 - CLK4, DYRK1-2, PIM1-2, and PRP4. The action of these regulators ensures the correct localization of splicing factors for action at the correct time and in the correct place. Several small molecule inhibitors of SRPK1 or SRPK2 are in development currently and show promise as anti-cancer agents for prostate malignancy in humans (Mavrou et al., 2015; Bates et al., 2017). Similarly, CLK protein kinase inhibitors have been demonstrated to suppress cell growth in human mammary tumor cell lines (Araki et al., 2015).

Moderation of Splice Site Choice

If monogenic disease is due to dysregulated splicing, in some cases it may be possible to correct or reverse the defect by restoration of correct splicing patterns. There are several means of accomplishing this, including antisense oligonucleotides (AONs), or steric hindrance agents such as morpholino

oligonucleotides or similar to occlude specific splicing regulatory sequences. This potential of this approach is best exemplified by novel treatments for spinal muscular atrophy (SMA) and Duchenne Muscular dystrophy (DMD) for which therapies for manipulation of splicing have been developed and are now licensed for clinical use. SMA is characterized by progressive neuromuscular disorder caused by mutations in the Survival Motor Neuron (*SMN1*) gene (Lefebvre et al., 1995; Lorson et al., 1999). These are often deletion events. The human genome contains a second SMN gene, *SMN2*, which due to the presence of a single C-to-T transition at codon 280 which disrupts a splicing enhancer site produces an unstable *SMN* transcript lacking exon 7 (SMNΔ7). This transcript is present at only 10% of *SMN1* levels (Lorson et al., 1999) but has potential to compensate for mutation-related reduced activity of *SMN1*. This has formed the basis for a novel therapeutic strategy whereby an AON (Nusinersen) has been designed to influence splicing patterns of *SMN2*. Nusinersen targets the N1 (ISS-N1) motif in *SMN2*, and promotes the inclusion of exon 7 and increases levels of compensatory *SMN2*. Several clinical trials have now been undertaken (Parente and Corti, 2018) and Nusinersen, also known as Spinraza, has now been approved by both US and EU regulatory authorities for clinical use.

Similar strategies have also been employed for Duchenne Muscular dystrophy, an X-linked neuromuscular disorder that affects 1:5000 newborn boys (Mendell et al., 2012), and is primarily caused by deletions, frameshift or nonsense mutations in the dystrophin (*DMD*) gene (Monaco et al., 1988). The majority of these mutations yield mRNAs containing premature termination codons, which trigger nonsense-mediated decay and degradation of affected *DMD* transcripts. Several strategies involving AONs targeted to specific splice sites have now been employed to bring about exon skipping to remove the offending exon(s) and lead to the production of a truncated, but still partially functional DMD protein (Aartsma-Rus, 2010; Niks and Aartsma-Rus, 2017). Similar approaches have been employed to modify the effects of duplication mutations in cell lines (Wein et al., 2017). Most AONs under assessment as DMD therapeutics are chemically modified 2′-*O*-methyl-phosphorothioate oligonucleotides (2OMePS) or phosphorodiamidate morpholino oligomers (PMOs) which can be administered systemically (Goemans et al., 2011). One of these, eteplirsen, a PMO which brings about skipping of exon 51, a hotspot for DMD mutations, has demonstrated promising results in a number of clinical trials and been designated 'reasonably likely to predict a clinical benefit' by the FDA (Goemans et al., 2011). Other approaches have employed 'readthrough' agents such as ataluren that allow bypass of the premature termination codon and are now in Phase III clinical trials (Namgoong and Bertoni, 2016).

Therapeutic Moderation of Polyadenylation

Polyadenylation is an essential step in mRNA processing, with a pivotal role in maintenance of RNA stability and management of RNA turnover. Many genes contain more than one polyadenylation site and display alternative polyadenylation, producing mRNA transcripts with novel 3′ untranslated regions. These may be differentially targeted by non-coding RNAs such as miRNAs or RNA binding proteins, or have differential translation efficiency (Elkon et al., 2013). Control of polyadenylation is mediated by a number of sequence elements such as the polyadenylation site itself, but also a series of upstream (U and UGUA rich) and downstream (U and GU rich) elements (Tian and Graber, 2012) that bind the protein complexes that orchestrate the process. These sequence elements bind the polyadenylation machinery that include the cleavage and polyadenylation specificity factors, the cleavage stimulation factors and the polyadenylate polymerase itself (Shi et al., 2009). Differential choice of polyadenylation site is linked to the proliferation and differentiation capacity of the cells; transcripts in highly proliferative cells tend to have shorter 3′UTRs (Sandberg et al., 2008). Differential use of polyadenylation sites may also have impacts on mRNA stability, mRNA export and localization, translation rates and protein localization (Tian and Graber, 2012). Patterns of alternative polyadenylation are also regulated by differential binding of RNA binding proteins; CSTF2 and CFIm subunits of the main polyadenylation machinery have been shown to have effects on relative expression of alternatively polyadenylated isoforms (Zheng and Tian, 2014). Other RNA binding proteins such as HNRNPs H and I (Katz et al., 2010), as well as CPEB1 (Bava et al., 2013) have also been associated with alternative isoform choice. RBPs such as these may in the future form the basis of therapies to influence the 3′ end processing of alternatively polyadenylated transcripts as therapeutic agents.

Therapeutic Modification of RNA Editing

RNA editing is a mechanism of generating further transcriptomic diversity and can impact the final sequence or structure of both encoded proteins and non-coding RNAs (ncRNAs) (Ganem and Lamm, 2017; Yablonovitch et al., 2017). RNA editing is an extremely common event, occurring in the many dynamically regulated mRNA transcripts and can comprise a variety of modifications, the most common of which is adenosine to inosine (A to I), which is eventually read as guanosine (Peng et al., 2012). RNA editing is especially prevalent in Small Interspersed Repetitive Elements (SINE) elements such as Alu, and also in transcripts in the brain (Jepson and Reenan, 2008; Osenberg et al., 2010). RNA editing events have been implicated in control of mRNA splicing and miRNA regulation (Farajollahi and Maas, 2010; Nishikura, 2010). RNA editing events are primarily mediated by a family of adenosine deaminases acting on RNA (ADARs), of which there are three major members; ADAR1, ADAR2, and ADAR3. The three ADARs have common functional domains, but differential structural features and some degree of site specificity (Nishikura, 2016). ADAR expression is itself regulated by transcription factors such as CREB and activated by kinases such as JNK1 (Peng et al., 2006; Yang et al., 2012). Dysfunction of ADAR1 is associated with diseases such as Aicardi-Goutières syndrome (Rice et al., 2012), with psychiatric disorders due to attenuated 5-HT$_{2C}$R levels (Eran et al., 2013), and also with cancer (Ganem et al., 2017), whereas ADAR2 is linked to circadian rhythm and epilepsy (Gallo et al., 2017).

Although less advanced than therapies targeting splicing defects, strategies to target ADARs to influence RNA editing are beginning to be evaluated for future clinical benefit. ADAR1 has been demonstrated to target let7, a miRNA involved in many processes including control of cell cycle (Roush and Slack, 2008). Over-expression of ADAR1 and subsequent down-regulation of Let7 has been shown to drive the self-renewal of leukemic stem cells in human blood, an observation that can be reversed by inhibition of ADAR1-mediated RNA editing (Zipeto et al., 2016). Very recently, techniques for directing ADARs to specific points of intervention have been developed. This system, named RESTORE, uses a plasmid-borne guide RNA coupled to an ADAR recruiting domain to deliver ADAR2 directly to the region of interest. This approach has been used to successfully edit phosphotyrosine residues in STAT1 with resultant changes to the activity of this signaling protein (Merkle et al., 2019). There are also newly emerging techniques based on modified Cas technologies utilizing catalytically inactive Cas13-ADAR2 fusion proteins to bring about RNA editing (Cox et al., 2017). These early observations suggest that in the future, targeting ADARs or other regulators of RNA editing may prove promising points of traction for neurodevelopmental disorders and for cancer.

Modification of RNA Based Epitranscriptomics

Epitranscriptomic modification of DNA is well known, but it is now becoming increasingly evident that RNA is also epigenetically modified. RNA is subject to decoration with over 130 different modifications. Most of these map to very abundant RNAs such as rRNAs and tRNAs, but a subset are seen in mRNA, circRNA and lncRNA (Schaefer et al., 2017). The most common marks are N(6)-methyl-adenosine (m^{6}A), 5-methylcytosine (m^{5}C), 5-hydroxymethylcytosine (hm^{5}C) and N1-methyladenosine (m^{1}A), which have been shown to be widely present throughout the transcriptome by high throughput sequencing (Jung and Goldman, 2018). M^{6}A is enriched in the last exon of genes and also occurs preferentially at 5′ untranslated regions (UTRs) (Ke et al., 2015; Meyer et al., 2015), whereas m^{1}A is enriched in promoters and 5′ UTRs (Dominissini et al., 2016). m^{5}C marks are often located at both 5′ and 3′ UTRs (Squires et al., 2012). RNA modifications can influence gene expression by a number of mechanisms, including influencing RNA structure, recruiting other regulatory proteins (e.g., splicing factors, RNA binding proteins involved in control of stability) or moderation of translation (Nachtergaele, 2017). RNA epitranscriptomic marks are added and removed by a series of writers (METTL3, METTL14, WTAP, KIAA1429, RBM15/15B, and METTL16) and erasers (FTO and ALKBH5) (Tong et al., 2018). Disruption of m^{6}A disrupts RNA metabolism; m^{6}A depleted transcripts have been reported to be unstable (Tang et al., 2018). Accordingly, mutations in the writer or eraser machinery have been associated with cancers such as hepatocellular carcinoma and acute myeloid leukemia (AML) (Vu et al., 2017; Chen et al., 2018), and with memory, fertility and metabolic phenotypes (Fischer et al., 2009; Zheng et al., 2013; Nainar et al., 2016). The RNA epigenomic writers and erasers are therefore promising future therapeutic

targets. At present, the work in this area is mainly in cell and animal models. Silencing the METTL14 'writer' led to restoration of differentiation of myeloid cells in AML and inhibited AML cell survival and proliferation (Weng et al., 2018). Similar strategies targeting ALKBH5 have showed promise as anti-tumor agents in glioblastoma stem cells (Schonberg et al., 2015). Studies have suggested that small molecule inhibitors of FTO may have potential utility as anticonvulsants in mouse models of epilepsy *in vivo*, by suppression of 2-oxoglutarate (2OG) through altering m^{6}A levels (Zheng et al., 2014).

Modulation of RNA Export

The activity of genes is also dependent on the correct positioning of mRNAs within the cell. Once processed, RNAs are usually exported through the nuclear membrane into the cytoplasm ready to be translated. This is not a passive process; it is orchestrated by a portfolio of RNA export proteins which escort the RNA molecule through the nuclear pore. Messenger RNAs are primarily transported by Nxf1 and Xpo1, whereas miRNAs are exported by Xpot and Xpo5. The transcription Export complex 1 (Trex1) facilitates binding of Nxf1 to the processed mRNA, and together with a collection of other proteins such as karyopherins or importins causes the processed mRNA to associate with and transit through the nuclear pore (Viphakone et al., 2012). The nuclear pore itself is composed of a collection of nucleoporins, and comprises a multi-subunit structure consisting of a nuclear ring, a central transport channel and a basket-like structure (Kabachinski and Schwartz, 2015). Small molecules can diffuse across this barrier, but larger ones such an mRNA cannot. Some of the specificity of transport is achieved by the interaction of the nuclear transport machinery with specific signal sequences in the mRNA itself (Lee et al., 2006; Hutten and Kehlenbach, 2007), whereas other mRNAs rely upon adaptor proteins (Huang et al., 2017). The expression and localization of nuclear transporters is altered in certain cancers (Zhou et al., 2013; Talati and Sweet, 2018), and have been linked with some neurodegenerative disorders (Grima et al., 2017) and comprises important components of inflammatory and apoptotic response (Aggarwal and Agrawal, 2014; Kopeina et al., 2018). Individual components of the nuclear export machinery are currently under investigation as therapeutics. One of the most promising, Selinexor, targets exportin 1 (Xpo1) and is currently in pre-clinical trials and has shown efficacy against acute myeloid leukemia and multiple myeloma (Kashyap et al., 2016; Mahipal and Malafa, 2016).

Therapeutic Modulation of Non-coding RNA Regulators of Gene Expression

The repertoire of genes expressed by any given cell in any given circumstances is influenced by non-coding RNA (ncRNA) regulators of gene expression. These ncRNA genes do not encode proteins, but rather encode RNAs that contribute to the regulation of other RNAs. They are classified into 2 broad classes, short ncRNAs such as microRNAs (miRNAs) and longer ncRNAs such as long non-coding RNAS (lncRNAs) and circular RNAs (circRNAs).

Modulation of Small Non-coding RNAs

MicroRNAs (miRNAs) and siRNAs are short non-coding RNAs 20–25 bp in size. They interact with components of the RNA-induced silencing complex (RISC) to bring about translational blocking or RNA degradation. Each miRNA interacts with specific binding sites in the 3′ UTR of its target genes, which are 6–8 nt in length and are commonly found in the genome; each miRNA is thus capable of targeting hundreds of mRNA target genes simultaneously (Carthew and Sontheimer, 2009). Several classes of miRNAs have been associated with disease; these include the 17/92 cluster, the miR-24 cluster or miR-3676, all of which are associated with chronic lymphocytic leukemia (CLL) (Van Roosbroeck and Calin, 2016). Other examples include miR-21, miR-10b, miR-155, and Let-7a, which are associated with breast cancer (Khalighfard et al., 2018), and miR-192, miR200c and miR-17 which are associated with colon cancer (Ast et al., 2018). Similarly miR-33a and miR-33 have been associated with metabolic disease and atherosclerosis (Marquart et al., 2010; Rayner et al., 2011) and miR-155 has links with inflammatory diseases (Dorsett et al., 2008). The use of miRNAs as anti-tumor therapeutics is currently receiving much interest. Specific miRNAs can target the tumor suppressor machinery, and are commonly referred to as onco-miRs, or they may target the controls of cell cycle and act as tumor suppressors in their own right. The small size and relative stability of miRNAs and siRNAs, together with the observation that they are readily taken up in endosomes and microvesicles (Rani et al., 2017) renders them excellent candidates for therapeutic modulation or use as biomarkers of disease. This can take the form of antagomiRs that can target and silence endogenous miRNAs or chemically modified miRNA mimics that can increase regulation of their specific targets (Khvorova and Watts, 2017). To date, 20 clinical trials have been undertaken that exploit miRNA biology (Chakraborty et al., 2017), the first of which miravirsen, which is targeted to miR-122, is in phase II clinical trials for Hepatitis C (Lindow and Kauppinen, 2012). In August 2018, the first siRNA-related therapy, patisiran, was approved by the FDA for the treatment of peripheral nerve disease by targeting an abnormal form of the transthyretin (*TTR*) gene.

Modulation of LncRNAs

Long non-coding RNAs comprise a heterogeneous class of non-coding RNAs, which are longer than 200bp in length. They do not encode proteins, and originate from all most genomic regions. They can originate from the locus that they regulate, usually from the antisense strand, and regulate their target in *cis* (Natural antisense Transcripts (NATs), or they can map to entirely different genomic regions form their targets (introns, pseudogenes, and non-coding DNA) and cause regulation in *trans*. LncRNAs can also be associated with promoters, enhancers or other regulatory regions and do not have a homogeneous mode of action. They can activate or repress their targets and can work by a number of mechanisms. They are commonly involved in genomic imprinting; one of the first lncRNAs discovered, *XIST*, coordinates X chromosome inactivation (Brown et al., 1991). Other lncRNAs can act as guides. This class of lncRNA includes *ANRIL*, which directs the polycomb repressive complex to the site of action in the case of the *CDKN2A* and *CDKN2B* genes (Kotake et al., 2011) and the lncRNA *HOTAIR*, which has roles in colorectal cancer (Kogo et al., 2011). They can also act as scaffolds, directing the assembly of specific protein or RNA complexes to their sites of action. For example, one function of the lncRNA *NEAT1,* a multifunctional lncRNA with several roles in tumorigenesis (Ghaforui-Fard and Taheri, 2018) is to bring together the microRNA biogenesis machinery to enhance pri-miRNA processing (Jiang et al., 2017), and the lncRNA *LINP1*, which regulates the repair of DNA double strand breaks in breast cancer by acting as a scaffold for the ku80 and DNA-dependent protein kinase proteins (Zhang Y. et al., 2016). They can also repress expression by acting as decoys, co-regulators and inhibitors of RNA polymerase II. For example, the lncRNA *PANDA* acts by sequestering its transcription factor target NF-YA away from its site of action (Hung et al., 2011). They have roles in regulators of subcellular compartmentalization; the lncRNA *MALAT* is responsible for localizing splicing factors to the nuclear splicing speckles where they can be stored and regulated by phosphorylation (Bernard et al., 2010).

In accordance with their pivotal role in regulating gene expression, lncRNAs have been reported to be associated with several diseases such as cancer (Huarte, 2015; Parasramka et al., 2016; Peng et al., 2016), diabetes (Akerman et al., 2017; He et al., 2017; Leti and DiStefano, 2017), neurodegenerative disease (Riva et al., 2016) and cardiovascular disease (Hou et al., 2016; Haemmig et al., 2017; Gangwar et al., 2018). LncRNAs may represent promising therapeutic targets; they are responsive to small molecule therapeutics; a recent study documented 5916 lncRNAs that responded to 1262 small molecule drugs (Yang et al., 2017). Although progress toward the clinic has been slow, perhaps because of the diverse modes of actions of lncRNAs, there are some promising candidates. Several lncRNAs have been reported to be dysregulated in osteoarthritis (OA), including *HOTAIR, RP11-445H22.4, GAS5, PMS2L2, H19,* and *CTD-2574D22.4* (Xing et al., 2014). At the present time, the majority of studies have not progressed beyond cell or animal models, several potential future therapeutic candidates have emerged; the lncRNA *PCGEM1* was demonstrated to inhibit synoviocyte apoptosis on OA by moderation of its target miR-770 (Kang et al., 2016). Similarly, many lncRNAs have been identified as potential therapeutic targets in cardiovascular disease or cancer, including *GAS5, LIPCAR, SENCR, ANRIL, SMILR,* and *MALAT* (Gomes et al., 2017). ASP and siRNA approaches to therapeutically manipulate *MALAT* levels are in development in human cancer cells and in animal models (Arun et al., 2016). Targeting lncRNAs is subject to more difficulty than miRNAs, because of their larger size and the heterogeneity of their mode of action, which may explain why their evaluation is not as advanced as that of miRNAs. Nevertheless, they have significant potential as future therapeutic targets.

Modulation of CircRNAs

Circular RNAs (circRNAs) are a relatively newly discovered class of non-coding RNA regulators found in multiple species (Haque and Harries, 2017). They are formed from 'backsplicing' events of linear genes, and comprise circular molecules, which are therefore relatively immune to exonucleases (Cocquerelle et al., 1993; Schwanhausser et al., 2011; Jeck et al., 2013; Lan et al., 2016; Lasda and Parker, 2016). Like lncRNAs, circRNAs have been reported to influence gene expression by a variety of mechanisms including action as miRNA sponges or mRNA traps, as well as comprising modifiers of transcription. translation, or splicing (Haque and Harries, 2017). Circular RNAs have been suggested to have roles in many cellular processes, including embryonic development (Xia et al., 2016), metabolism (Xu et al., 2015), regulation of cell cycle (Zheng et al., 2016) and regulation of cellular stress (Burd et al., 2010). In accordance with this observation, dysregulated circRNA expression has been associated with multiple human diseases such as cancer (Yao et al., 2017), neurological disease (Khoutorsky et al., 2013), osteoarthritis (Liu et al., 2016), cardiovascular disease (Taibi et al., 2014; Wang et al., 2016), type 2 diabetes (Gu et al., 2017), pre-eclampsia (Zhang Y.G. et al., 2016) and impaired immune responses

(Ng et al., 2016). Although the study of circRNAs is in its infancy compared with other ncRNAs, they too have potential as future therapeutic targets.

REMAINING BARRIERS AND FUTURE PROSPECTS

This is an exciting time for RNA-based therapeutics, with several notable examples making it as far as license for clinical usage. Over the next decade, it is likely that there will be a large expansion in the breadth and scope of human disorders that can be treated using these, and similar approaches. Most developed at the present time, are interventions targeted at specific splice events and those involving small RNAs, but future work may harness the potential of targeting other parts of the RNA regulatory milieu (**Figure 2**).

Several barriers do, however, remain to the wide implementation of these opportunities which are focused mainly on delivery, specificity and duration of treatment. Firstly, delivery of specific molecules to their site of action may be challenging. For some applications, such as skin, which may be treated topically or lung, which may be treated via inhalation,
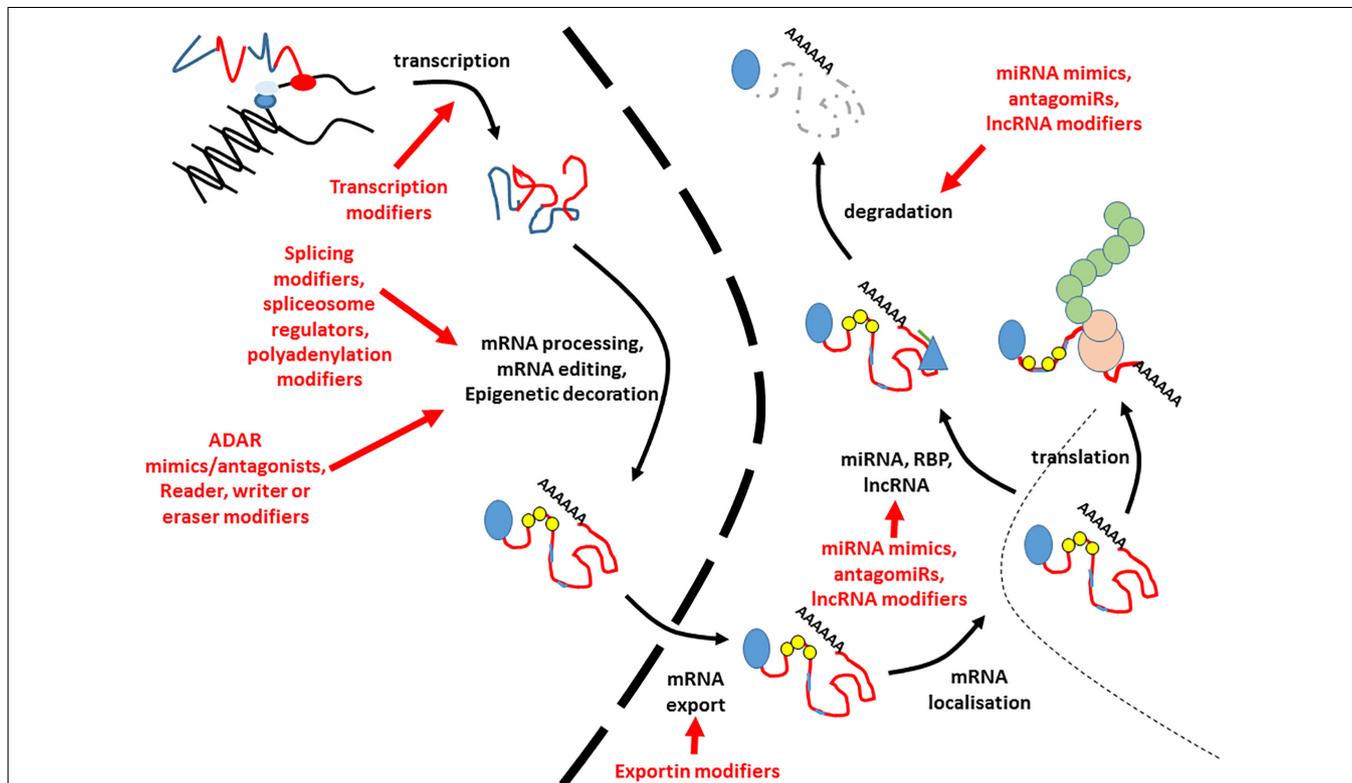
FIGURE 2 | Potential points of intervention for RNA based therapies. This figure indicates the potential points at which interventions could be made to alter the amount or nature of expressed RNA. Blue lines in the transcript refer to introns and untranslated regions, whilst exons are indicted by red lines. The 5′ cap is indicated by a blue circle. Small yellow circles indicate epitranscriptomic decoration, whilst pale blue lines within the exons refer to RNA editing events. The nuclear envelope is indicated by a large dashed line. RNA binding proteins modifying stability are given by blue triangles, and miRNAs by green lines. The translating ribosome is indicated by beige circles. Nascent polypeptide is given by green interlocked circles. Each potential point of intervention is given by a red arrow. Degraded RNA is indicated by a gray dashed line.

therapeutic delivery of interventions may be easier. Delivery to internal organs such as brain, liver or pancreas will require different and systemic approaches. One reason why AONs, readthrough agents and small RNAs have been at the forefront of this emerging field is that their small size and relative stability means that they can be more easily introduced into cells. This may not be true of entities such as lncRNAs or large circRNAs, which may be large molecules with potentially challenging secondary or tertiary structure. Small molecules can readily be introduced into cells using lipid-mediated transfer agents, or endogenous structures such as endosomes or microvesicles, which could be harnessed to deliver cargoes. Secondly, there are questions of specificity. One feature of the therapies that are in clinic currently is their specificity to their sites of action. Gene expression and the regulation thereof is highly tissue specific, and genes may often be required to be expressed only at a specified time, or in response to specific circumstances. It may not be advantageous to produce changes in all tissues or at all times, and effects must of course be limited to their intended targets. Specificity of effect can be achieved by choosing targets that are only present at their sites of action, or by modifying delivery so that cargoes are only delivered to their intended place of action. For example, strategies are emerging now which allow selective delivery of senolytic cargoes to senescent cells only using galactosaccharide nanoparticles, which harness the observation that senescent cells harbor large quantities of lysosomal β-galactosidase (Munoz-Espin et al., 2018). Similarly, strategies could be developed that introduce therapeutic oligonucleotides under the control of gene regulatory elements expressed only in the intended target tissues. Lastly, one needs to consider the potential need

for repeated treatments. The approaches discussed here differ from emerging "gene editing" technologies such as CRISPR, in that they are not transmitted to future generations, and may require repeated treatments. This can be considered both a caveat and an advantage. The need for repeated treatments may be burdensome for patients, but in reality, the vast majority of currently available treatments for human disorders fall into this category. Conversely, the need to deliver repeated doses introduces a degree of flexibility, and allows treatments to be quickly discontinued or changed if adverse effects occur. We are at a time of huge advances in our understanding of how our genome is curated and regulated and how our genes are expressed.

The multifactorial control of gene expression, and the complexity of this progress offers multiple points of potential intervention for therapeutic benefit. Over the coming decades, there is likely to be a huge increase in the number of therapies for human diseases that target not the genes themselves, but the expression and regulation of those genes. We are at the dawning of the era of genomic medicine, and the future looks bright.

AUTHOR CONTRIBUTIONS

LH planned and wrote the manuscript.

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