Tapping the RNA world for therapeutics

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A recent revolution in RNA biology has led to the identification of new RNA classes with unanticipated functions, new types of RNA modifications, an unexpected multiplicity of alternative transcripts and widespread transcription of extragenic regions. This development in basic RNA biology has spawned a corresponding revolution in RNA-based strategies to generate new types of therapeutics. Here, I review RNA-based drug design and discuss barriers to broader applications and possible ways to overcome them. Because they target nucleic acids rather than proteins, RNA-based drugs promise to greatly extend the domain of 'druggable' targets beyond what can be achieved with small molecules and biologics.

Twenty-five years ago, a relatively simple model of gene expression prevailed: DNA is transcribed to mRNA and then translated to protein. Gene transcription was thought to be largely regulated by protein transcription factors. This model was supposed to explain how cells with the same genetic code could carry out diverse functional programs. However, this simple model of RNA as a relatively passive carrier of genetic information from DNA to protein has been overturned[1]. In particular, we now know that much of extragenic DNA is transcribed into noncoding RNAs (ncRNAs) and that multiple transcripts, both coding and noncoding, are produced, the latter in both directions, from the same genes[2,3]. Long and short ncRNAs regulate gene expression at almost every step. This can occur transcriptionally, for example, by regulating chromatin modification, and post-transcriptionally, for example, by affecting mRNA stability and translation.

This revolution in RNA biology began around the time that *Nature Structural & Molecular Biology* was born. *Xist*, the first well-described long noncoding RNA (lncRNA), responsible for X-chromosome inactivation in females, was identified a few years earlier[4,5]. In the same year as *NSB*'s first issue, Ambros, Ruvkun and colleagues identified *lin-4*, a microRNA (miRNA) gene encoding a precursor RNA processed into a short, 22-nucleotide double-stranded RNA, as an important regulator of *Caenorhabditis elegans* development[6,7]. Lin-4 suppresses the expression of a developmental gene by recognizing a partially complementary sequence in its 3′ UTR[6,7]. Since then, thousands of miRNAs have been shown to inhibit protein translation and enhance mRNA decay[8,9]. Just as protein transcription factors regulate the transcription of gene networks that function synchronously to change cellular states, miRNAs each potentially regulate hundreds of transcripts to control cellular responses to changes in the environment or to developmental cues[10]. Small interfering RNAs (siRNAs) in lower eukaryotes and the closely related Piwi-interacting RNAs (piRNAs), present in both invertebrates and vertebrates, also regulate transcription, mostly by enhancing formation of heterochromatin[11–15]. Small ncRNAs can also activate gene expression[16,17]. Interestingly, miRNA profiling more accurately predicts a cell's differentiation state than mRNA profiling, a clear sign of the importance of ncRNAs in regulating cellular function[18].
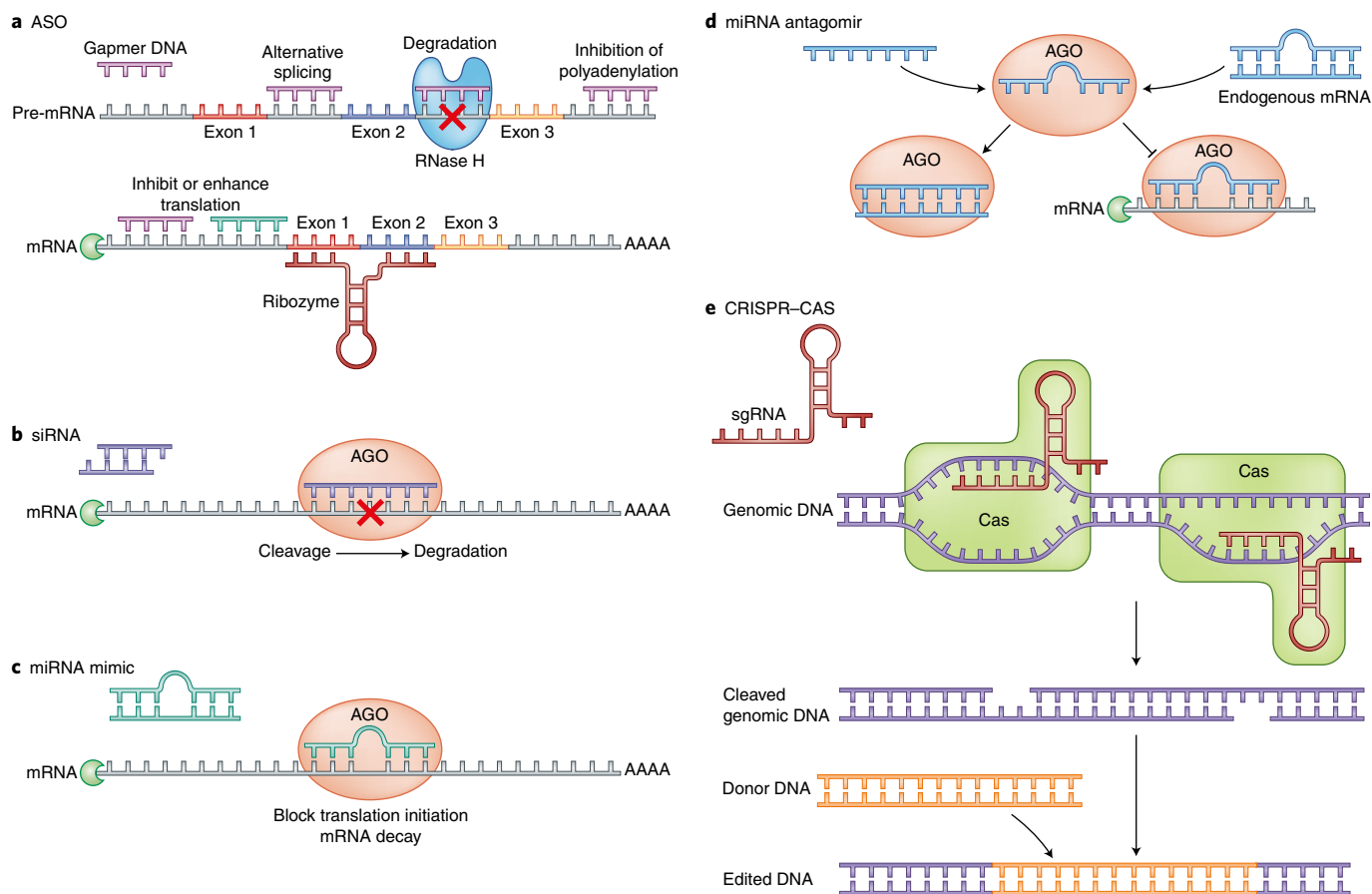
Even before the discovery of new classes of ncRNAs, critical roles in protein synthesis and mRNA splicing were well established for small ncRNAs, such as tRNAs, rRNAs and small nuclear RNAs (snRNAs), as well as for longer heterogeneous nuclear RNAs (hnRNA)[1]. RNAs can assume many functions, including acting as switches or as RNA enzymes, termed ribozymes, that can catalyze peptide bond formation or cleave and ligate DNA and RNA[19,20].

Although ribozymes are generally enzymatically inferior to protein catalysts, theorists of the origin of life have postulated an early self-replicating and self-sustaining 'RNA world' in which many of the functions now performed by DNA and proteins were entirely executed by RNA[21,22].

RNAs can fold into complex 3D structures, partly mediated by Watson–Crick pairing of short stretches of complementary bases within their sequences, but also by noncanonical hydrogen bonds formed by Hoogsteen base-pairing and by hydrogen bonds between ribose-phosphate backbone moieties[23]. Tertiary RNA structure can generate so-called aptamers, which recognize small-molecule ligands, other nucleic acids or proteins with high specificity, often with binding constants in the nanomolar range[24,25]. In naturally occurring bacterial riboswitches, the tertiary conformation of the aptamer is radically altered by ligand binding to generate RNAs that regulate protein expression in a ligand-gated manner[19,26]. They act via various mechanisms, such as catalyzing RNA cleavage or regulating translation, splicing or transcription. These tertiary structural interactions combined with the specificity conferred by base-pairing of linear sequences bestow on RNA the potential for incredibly precise interactions with its targets.

The revolution in RNA biology has been fueled in large part by the development of more sensitive and inexpensive methods to sequence RNAs expressed in cells and to isolate and characterize RNAs bound to DNA, protein and other RNAs[3]. These unbiased methods have revealed a myriad of ncRNAs, both small and long, transcribed in both sense and antisense directions from coding gene bodies and their regulatory regions, as well as thousands of transcripts from what was formerly considered 'junk DNA'[27]. The numbers of ncRNAs (and possibly their functions) has increased with evolution, perhaps explaining how organisms developed complexity without a corresponding increase in protein-coding genes. It is still uncertain, however, how many of these transcripts, especially those that are of very low abundance, are functional or just transcriptional noise. Nonetheless, the numbers of annotated ncRNAs continues to expand, and they will probably eventually surpass the numbers of protein-coding genes.

For coding RNAs, alternative splicing and the choice of multiple transcription start sites or polyadenylation sites generate multiple transcripts from the same gene, spawning even more biological complexity. In addition, nucleotides can be modified with over one hundred chemical modifications. We now know that modification of mRNA bases, such as by N^6-adenine methylation (6mA), play an important role in regulating mRNA stability and translation[28]. Some lncRNAs resemble mRNAs in that they are capped, polyadenylated and sometimes (although not often) evolutionarily conserved, even

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Fig. 1 | Antisense mechanisms of RNA-based drugs. Most RNA-based drugs take advantage of complementary base-pairing of RNA analogs. They are generally extensively modified to improve their resistance to RNases and innate immune sensors and to potentially increase binding affinity to their nucleic acid or protein targets, which mediate their intracellular activity. These drugs bind to complementary sequences in endogenous genomic DNA or its RNA transcripts. The major classes are ASOs (usually single-stranded with a central DNA gapmer region) (**a**), double-stranded siRNAs (**b**), miRNA mimics (**c**) or antagomirs (**d**) and, most recently, single guide RNAs (sgRNAs) for gene editing using CRISPR–Cas (**e**). Although the targets are depicted in the diagram as pre-mRNAs, mRNAs or genomic DNA, in principle, these strategies could also be used to target ncRNAs. Credit: Debbie Maizels/Springer Nature

though they do not contain open reading frames predicted to be translated into proteins longer than short peptides. The function and mechanism of action are understood for only a small number of lncRNAs[29]. Most of the mechanistic studies of lncRNAs involve lncRNAs that bind in cis, sometimes in trans, to regions of DNA to modulate the epigenetic landscape, often by recruiting or evicting transcriptional activators or repressors. However, lncRNAs can also act as extrachromosomal scaffolds that assemble proteins into functional complexes in the cytosol or nucleus. Some of these examples involve binding to proteins by short linear sequences, much as transcription factors bind to specific DNA sequence motifs. Understanding of the functions of lncRNAs is still at an early stage. It is, however, likely that they are just as diverse as those of proteins and will include functions that rely not only on binding to linear sequences but also on the ability of RNAs to assemble into tertiary structures capable of high-affinity interactions with other molecules. Undoubtedly, some lncRNAs will also utilize the ability of RNAs to function as ribozymes.

RNA-based therapeutic strategies

The explosion of new RNA classes has raised the possibility of new therapeutic strategies that mimic or antagonize the function of these novel RNAs. Attempts to therapeutically harness RNA pathways began as soon as they were discovered, even before their mechanism

of action was well understood. In the past year, several RNA-based drugs have shown clinical benefits for treating previously intractable diseases. We are on the verge of an era of new drugs that tap into RNA biology. Most of these drugs use nucleic acid analogs and take advantage of complementary base-pairing to mimic or antagonize endogenous RNA processes (Fig. 1). Turning RNAs into drugs has required overcoming two major hurdles: the poor pharmacological properties of RNA, which is rapidly degraded by RNases that are active in all body fluids, and the need to devise methods to deliver charged nucleic acid analogs across hydrophobic cell membranes into the cytosol or nucleus where they need to act (Box 1). It has taken almost 40 years of painstaking work to overcome these obstacles since the initial observation in 1978 that a 13-mer DNA oligonucleotide could inhibit Rous sarcoma virus translation and proliferation in a sequence-specific manner[30,31].

When *NSMB* was launched, virtually all drugs were small molecules that bound to druggable targets—the active-site pockets of protein receptors or enzymes—to inhibit or potentiate their function. However, only approximately one third of the roughly 20,000 proteins in the human genome are potentially druggable[32,33]. Biologicals (monoclonal antibodies (mAbs), immune modulators, replacement enzymes), which have become an increasing proportion of the pharmacopoeia, target these same druggable proteins. It has been only 30 years since the first mAb drug, anti-CD3 to treat

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Box 1 | Challenges for developing RNA-based drugs

- Intracellular delivery across cell and endosomal membranes
- Poor pharmacokinetic properties, partly due to urinary excretion and ubiquitous RNases
- Activation of innate immune nucleic acid sensors
- Off-target effects:
  Suppression of unintended homologous targets
  Activation of DNA repair pathways
  Translocations or imprecise gene editing

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transplant rejection, was approved. Now, new mAb drugs make up a large and growing proportion of new drugs. In 2016, the US Food and Drug Administration approved 22 new drugs (rather than variations of previously approved products). Of these 22 drugs, seven were mAbs and two (nusinersen (Spinraza) and eteplirsen (Exondys 51)) were antisense oligonucleotide (ASO) drugs that alter mRNA splicing of genes implicated in previously untreatable genetic neurodegenerative diseases (spinal muscular atrophy and Duchenne's muscular dystrophy, respectively)[34,35]. It is likely that these are just the first examples of RNA-based medicines. Conceptually, nucleic acid–based drugs can be divided into two groups: those whose specific activity is mediated by an antisense mechanism[36], namely by recognizing a complementary sequence on DNA or RNA and modulating its processing or function (Fig. 1), and all 'others' (Fig. 2).

ASO drugs

ASOs are usually single-stranded, highly modified and stabilized nucleic acid analogs. They can act on precursor mRNAs (pre-mRNAs) in the nucleus to modulate splicing (for instance, by binding to and blocking a splice junction, like the recently approved drugs mentioned above). They can cause sequence-specific degradation (by binding to an intronic sequence and recruiting nuclear RNase H) and potentially bind to polyadenylation recognition sites to block polyadenylation and accelerate RNA decay (Fig. 1a). ASOs could also be designed to bind to translation initiation sites on mRNAs in the cytosol to block translation.

Alterations in the chemistry of the basic nucleotide building blocks of ASOs were essential to turning the ASO concept into drugs, leading to more stable nucleic acid analogs that bind to their target with higher specificity and with improved cell penetration (reviewed in refs [37–41]). Advances in ASO chemistry culminated in the first approved ASO drug to treat cytomegalovirus retinitis, fomivirsen, in 1998. This drug was only briefly marketed because the disease declined precipitously when effective anti-HIV drugs became available. More recently, mipomersen, targeting *APOB*, a gene encoding the apolipoprotein B-100 in LDL cholesterol particles, was approved for treating familial hypercholesterolemia[42]. Although ASOs have mostly been designed to inhibit gene expression, a recent study suggests that ASOs that bind to and inhibit inefficient upstream open reading frames could be used to increase translation[43], potentially providing a way to address diseases caused by inadequate gene expression. It is also possible to design ASOs that cleave mRNAs at specific sites by inserting an RNA-cleaving ribozyme sequence between sequences complementary to the targeted cleavage site (Fig. 1a). However, this strategy is not actively being developed because of poor in vivo activity. ASOs complementary to mature miRNAs ('antagomirs') are also being developed to counteract miRNAs implicated in disease pathogenesis[44]. An example is miravirsen, an antagomir to miR-122, the most abundant liver miRNA, which binds to hepatitis C virus genomic RNA and protects it from degradation. Although a phase 2 study published in 2013 showed impressive viral suppression by miravirsen[45], the

clinical need for the drug was largely eliminated when potent curative hepatitis C small-molecule antivirals became available.

siRNA-based drugs

The discovery of miRNAs[6,7] and RNA interference (RNAi)[46] opened up a new mechanism for RNA therapeutics:gene silencing (reviewed in ref. [47]). In 2001, Tuschl and colleagues showed that RNAi operates in mammalian cells[48]. Previously, RNAi was thought to be limited to plants and invertebrates. Transfection of siRNAs, 21–23 nucleotides long containing an mRNA sequence (sense strand) and its complement (antisense active strand)[48], could harness this ubiquitous pathway to degrade target-gene mRNAs and suppress their expression with high specificity.

Soon after its discovery, RNAi became an important and widely used research tool by providing a relatively simple and fairly specific method to probe the importance of individual genes by knocking down gene expression. RNAi was also rapidly harnessed for unbiased genome-wide screening to identify genes important in biological processes, first in invertebrate cells[49–52] and then in mammalian cells[53–57], using transfection of siRNAs or vectored expression of short hairpin RNAs (shRNAs) that mimic endogenous miRNAs. These knockdown techniques provide a valuable way to identify novel drug targets.

The potential for siRNA therapeutics was demonstrated almost immediately after its discovery, first in vitro, in inhibiting HIV replication by depleting viral genes or host receptors[58–62], and soon thereafter in vivo, when injection of *Fas* siRNAs protected mice from autoimmune hepatitis[63]. Drug development since then has been rapid. The obstacles of turning siRNAs into drugs are similar to those faced with ASO drugs[41]. Some of the ASO chemical modification strategies and experience could be adapted to siRNA therapeutics, accelerating siRNA preclinical drug development and clinical evaluation. Simple chemical modifications of the 2′ position of the ribose and substitution of phosphorothioate linkages or DNA bases at the ends protected siRNAs from nuclease digestion and prolonged half-life in serum[64] and other bodily fluids. 2′ modifications also prevent recognition by innate immune receptors[64,65] and limit off-target effects, owing to suppression of partially complementary sequences[66].

Intracellular delivery of double-stranded siRNAs is more challenging than delivery of single-stranded ASOs. This difficulty is partly mitigated by the fact that RNAi is a catalytic mechanism in which the same siRNA is used over and over again to degrade many mRNA molecules. By contrast, most ASO drugs act on a one-to-one basis. As a consequence of this catalytic mechanism of action, the best estimates suggest that delivery of only a few hundred siRNAs into the cytosol of a target cell causes complete gene knockdown[67,68]. Moreover, once the active antisense strand is taken up by the RNA-induced silencing complex (RISC), it is remarkably stable under most circumstances, unless it is diluted by cell division. In recent human clinical studies, gene knockdown by highly chemically modified siRNAs persisted for many months, suggesting that siRNA therapeutics might only need to be given every 3–6 months[69]. The reasons for such clinical durability are not completely understood.

Delivery of siRNA-based drugs

Most of the initial clinical development of siRNA therapeutics was focused on developing lipid nanoparticles (LNP) to introduce siRNAs into hepatocytes. The liver is a major filtering organ that traps nanoparticles. It is also the primary site of synthesis of many circulating proteins. Therefore, the liver has been the target organ in most early clinical attempts at translating RNAi. Results of a recently completed phase 3 study that used LNP-encapsulated siRNAs to knock down transthyretin (*TTR*), which, when mutated, causes a fatal familial amyloidotic neurodegenerative syndrome, showed impressive improvement of neurological symptoms and safety
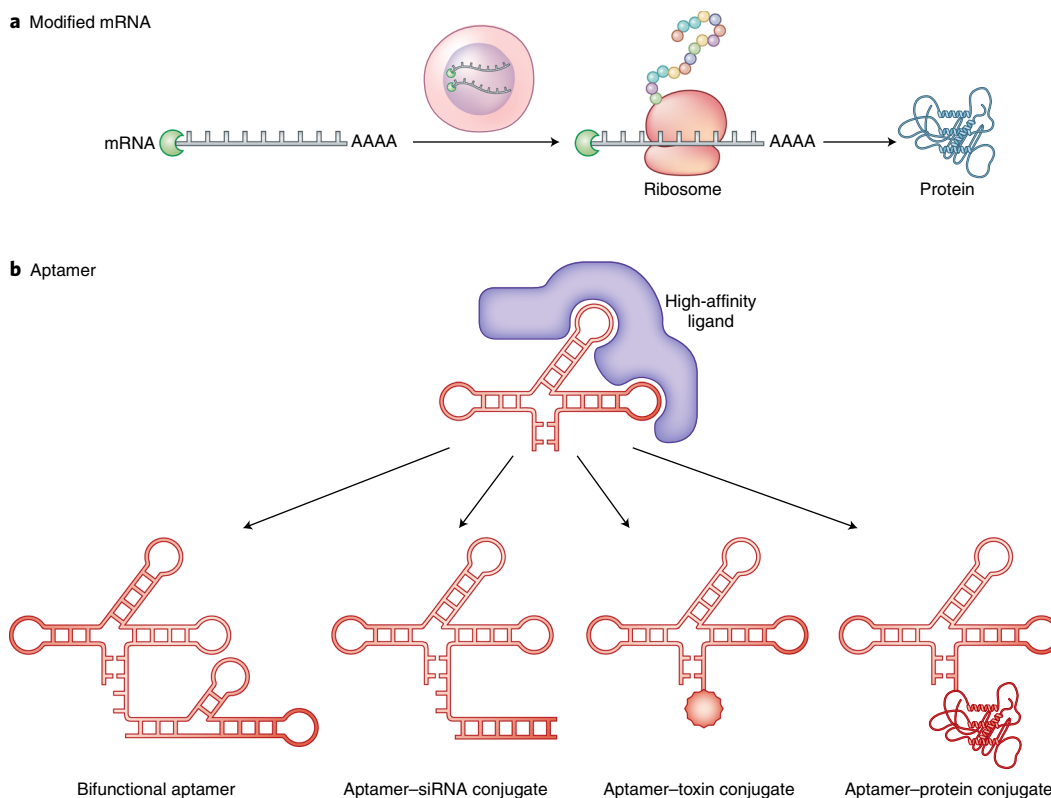
Fig. 2 | Additional RNA-based drug mechanisms. a, Sense-RNA drugs can be used for transient in vivo transcription of mRNAs to replace mutated proteins or for vaccination without the risk of genomic alteration. **b**, Aptamers take advantage of selection for high-affinity binding to molecular ligands, often in the nanomolar or subnanomolar range. They can be thought of as nucleic acid antibodies and have many of the advantages of conventional protein antibodies. They can be agonists or antagonists, linked for bifunctional targeting and conjugated to other RNAs, small-molecule drugs, toxins or peptides. However, unless modified, they are rapidly excreted and do not activate immune functions via binding to Fc receptors. Credit: Debbie Maizels/Springer Nature

(J. Gollob (Alnylam), personal communication). It is likely that this formulation (patisiran) will be approved as the first siRNA drug.

Nevertheless, siRNA therapeutics is rapidly moving away from LNPs, which are complicated to produce and have some immune-stimulatory side effects. Instead, next-generation drugs are targeted to the liver by chemical conjugation to trivalent *N*-acetylgalactosamine (GalNAc)[70–73], which is recognized by the hepatocyte-restricted asialoglycoprotein receptor (ASGPR) (Fig. 3). The GalNAc–ASGPR ligand-receptor pair works much better than other siRNA conjugates tested thus far. Although other conjugates were generally taken up into cells, they did not result in efficient knockdown, possibly because of inefficient endocytosis or endocytic release. The success of the GalNAc conjugate may be due to its hepatocyte specificity, the high cell surface expression of the receptor and rapid and continuous receptor recycling. Although a phase 3 study using GalNAc-conjugated siRNAs targeting *TTR* for cardiac amyloidosis[74] had to be terminated because of increased deaths of treated patients[75], multiple other GalNAc conjugates to siRNAs, further chemically modified for enhanced stability and activity and directed against other gene targets, have shown impressive results (~80–95% knockdown lasting for as long as 3–6 months after a single injection without significant safety concerns in thousands of patients[69]). This suggests that the platform as a whole will be adaptable for developing drugs against multiple liver targets.

So far, human clinical studies have shown that GalNAc-conjugated siRNAs have been able to strongly knock down genes in the liver, sometimes with impressive clinical benefit in early phase trials. Mainly, these studies evaluated treatment of genetic orphan diseases for which effective therapy is lacking or inadequate.

Examples include knockdown of *PCSK9* to treat hypercholesterolemia[69]; anti-thrombin III to improve clotting in patients with hemophilia[76]; *ALAS1*, the gene encoding the first enzyme in heme biosynthesis, to treat porphyria[77]; the complement component C5 to treat hemolytic-uremic syndrome and paroxysmal hemoglobinuria, and glycolate oxidase to treat primary hyperoxaluria[78,79]. It is likely that GalNAc–siRNA conjugates will eventually replace most therapeutic LNP efforts for liver targets. GalNAc conjugates have also been adapted for liver delivery of ASOs and antagomirs, which are currently in early phase clinical trials[80–82].

Therapeutic gene knockdown outside the liver is still an area of active development. Some of the most attractive approaches in mouse models include siRNA conjugation to RNA or DNA aptamers that recognize cell surface receptors[83–85], siRNA conjugation to CpG nucleotides that bind to an innate immune receptor on dendritic cells or macrophages[86], antibody fusion protein complexed siRNAs[87,88], antibody-coated LNPs[89–91] and siRNA conjugation to a fatty acid constituent of neuronal membranes for nerve cell delivery[92] (Fig. 3). Some of these strategies, which have not yet been tested in the clinic, show exquisite targeting specificity and provide the possibility of knocking down genes in narrowly defined subsets of immune cells (i.e., only activated lymphocytes or only regulatory T cells)[88,93] or only in cancer cells[94]. Targeted gene knockdown is also likely to reduce bystander cell toxicity and require lower doses. The same strategies that deliver siRNAs to the liver or elsewhere in the body can also be employed to deliver miRNA mimics, either as stem-loops or as double-stranded ~22-nucleotide modified RNAs that resemble the endogenous Dicer-cleaved miRNA[80,95]. The lessons learned about delivering siRNAs will probably facilitate the development of ways to deliver the larger cargo being contemplated
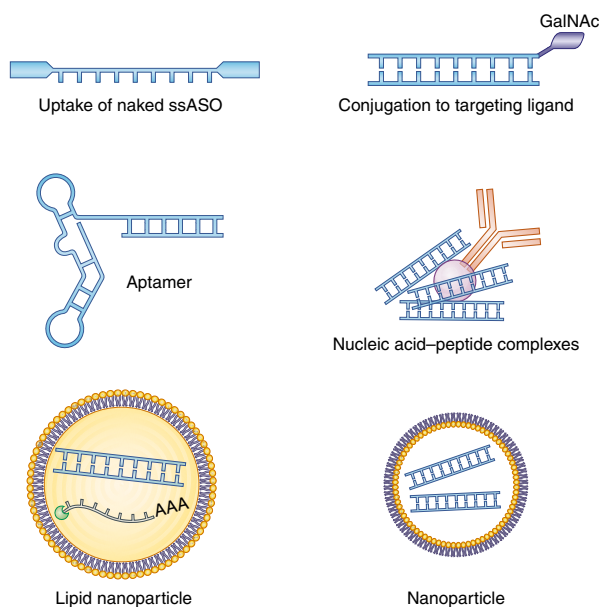
Fig. 3 | RNA-based drug delivery strategies. Intracellular delivery is the most challenging obstacle to RNA-based drug development. Most siRNA drugs need to act in the cytoplasm, where their target RNAs are located. ASOs, acting on pre-mRNA, and sgRNAs, acting on genomic DNA, have the additional challenge of getting into the nucleus. Drug development is moving away from using particles or complexes, which have a tendency to get trapped in the liver, are complicated to manufacture and have side effects. Instead, conjugation of nucleic acid analogs to sugars, lipids, peptide or nucleic acid ligands that bind to the cell membrane or surface receptors is used. Binding activates cellular uptake and provides the opportunity for cell-specific delivery. Credit: Debbie Maizels/Springer Nature

Box 2 | Advantages of RNA-based drugs

- Active on 'undruggable' targets
- Easy and rapid design
- Chemical synthesis without the variability of biologics
- Cost effective
- Stable without refrigeration
- Stable, unfluctuating suppression of target protein for up to 6 months
- Easy to combine into drug cocktails, providing flexibility for personalized drugs
- Low immunogenicity

for therapeutics using modified mRNAs[96,97] or CRISPR–Cas-mediated gene editing[98–104]. However, the delivery obstacles for these approaches are greater than those for siRNAs and should not be underestimated.

Advantages of RNA-based drugs

Some of the siRNA drug targets are also targeted by ASO or mAb drugs that are approved or under development. As they compete to treat the same diseases, it will become clearer which of these strategies offers better therapeutics. Examples are the approved ASO mipomersen and PCSK9 mAbs versus the siRNA-based drug inclisiran, now in phase 3 trials, all three designed to lower cholesterol by targeting the same enzyme[69,105,106], and the Ionis ASO inotersen versus Alnylam patisiran, both targeting *TTR* to treat amyloidosis (J. Gollob (Alnylam), personal communication and ref. [107]). For gene knockdown strategies, siRNAs will probably be more effective drugs than ASOs, provided that they can be effectively delivered to the tissue or cell of interest. siRNAs and ASOs that use RNase H to degrade target RNA are catalytic (i.e., one molecule can destroy multiple target RNAs), unlike ASOs that modulate splicing or translation, which only inactivate a single target RNA. This catalytic mechanism, particularly for siRNAs that harness the efficient RISC machinery, leads to high potency and unusually sustained activity. However, ASOs have more potential mechanisms of action and are easier to deliver to cells and the nucleus. The best class of nucleic acid therapeutic for addressing a particular disease and gene target will vary with the target. RNA-based drugs have several advantages compared to therapeutic antibodies directed against the protein product of the target gene (Box 2). For now, antibodies can only recognize targets that are both druggable and secreted or extracellular, because there is no good strategy to deliver them into cells.

By contrast, siRNAs and ASOs can in principle suppress any gene, even if it is highly expressed, including noncoding genes. Unlike antibodies, RNAs can be chemically synthesized, thus leading to cheaper and more easily manufactured drugs than biologics, which can have batch-to-batch variability. Antibodies need to be administered every few weeks, and patients often develop immunological responses, which can limit the effectiveness of antibody therapy with continued use. Thus far, there is no evidence of adaptive immune responses to RNA therapeutics. Another potential advantage of drugs that operate by an antisense mechanism, compared to antibodies, is that they can be rapidly identified, and antidotes are straightforward to design using the complementary sequence to specifically bind and inactivate the drug.

CRISPR–Cas gene editing

Like siRNA and shRNA research tools and drug candidates that rapidly evolved from the discovery of RNAi, the discovery of the CRISPR–Cas immune defense mechanism in bacteria and archaea[108–111] was swiftly harnessed to develop relatively facile and specific methods for genomic DNA editing. This includes activation and suppression of gene expression and genome-wide screening[98–104]. Like RNAi, the specificity of CRISPR–Cas relies on the antisense pairing of sgRNAs to specific genes, but on chromosomal DNA rather than RNA (Fig. 1e). Genomic targeting creates indels that provide the exciting possibility of stable genomic editing. This method could be used to correct mutations in coding or regulatory regions of genes in somatic and possibly eventually in germline cells in order to treat and cure disease. However, because CRISPR–Cas modifies the genome, ethical and safety concerns are amplified enormously. Like all antisense technologies, there is potential for both on- and off-target toxicity. Moreover, the Cas endonuclease creates double-stranded DNA breaks, which can possibly lead to oncogenic gene translocations and trigger a DNA damage response, causing cell-cycle arrest or cell death. Depending on which DNA repair pathway is activated, gene editing can be imprecise. Moreover, transfection of both sgRNAs and Cas mRNA or protein is currently not very efficient. However, in the 5 years since methods to harness this bacterial mechanism for mammalian gene manipulation were first reported, improvements that reduce off-target effects, offer better control and enhance the efficiency of gene editing have been rapidly developed[112].

Effective sgRNAs are easier to design than shRNAs. Furthermore, unlike siRNAs, sgRNAs induce stable changes in gene expression, which are invaluable for in vivo screening. Therefore, CRISPR–Cas-mediated gene knockout has rapidly replaced other techniques for many research uses, including screens to identify dependency genes, synthetic lethal interactions and novel gene targets. In addition, CRISPR–Cas gene knockout in zygotes provides a greatly accelerated method for making gene knockout or knock-in mice compared to homologous recombination. The method can also be

applied to develop knockout strains of almost any species, including non-human primates. The resulting animal models will be invaluable for preclinical drug development. However, clinical CRISPR–Cas studies, which will undoubtedly be initiated using ex vivo editing of differentiated cells that will then be infused into patients, will need to be both more efficient and better controlled for specificity and genetic modification. Yet the first gene editing drug strategies were approved this year for producing CAR-T cells[113,114] and for treating a congenital cause of blindness (Luxturna)[115] using an engineered adeno-associated virus. Experiences with non-RNA-based gene editing tools, such as zinc-finger nucleases, and with siRNA drug delivery will facilitate development of CRISPR–Cas-based drugs[112,116].

mRNA-based drugs

In addition to RNA drugs based on antisense mechanisms, several other mechanisms of action are also potential strategies for new classes of drugs. One approach is to introduce chemically modified, stabilized mRNAs into cells to be translated to protein[96,117–119] (Fig. 2a). mRNA therapies have the safety advantage of not modifying the genome, but also the disadvantages of transient expression and difficult in vivo intracellular delivery. If transfection of small RNAs in vivo is challenging, the problem is magnified for large mRNAs, which will probably require encapsulation into nanoparticles to avoid RNases present in bodily fluids and to aid cellular uptake. So far, despite intense efforts by the biotech and pharmaceutical industries to develop this approach, there are no truly convincing examples of high and sustained gene expression in vivo. The application that looks most promising is using stabilized mRNAs for vaccination, a setting for which high or sustained gene expression is not required because of the exquisite sensitivity of immune cells to low levels of foreign antigens[120–123].

Aptamer-based therapeutics

Aptamers are another potential class of RNA therapeutics. These folded RNAs behave like nucleic acid antibodies in that they bind with high affinity and specificity to target molecules. They can be selected from large libraries of short random nucleotide sequences for binding to all types of molecules by repeated rounds of enrichment by a process called SELEX[24,25,85,124,125]. Recent advances in SELEX technology, in particular the introduction of chemically modified bases and use of deep sequencing to analyze enriched RNAs in early rounds of selection, have greatly reduced the time needed and the likelihood of identifying high-affinity aptamers[85,124,125]. SomaLogic, which develops aptamer-based diagnostics to measure levels of many serum proteins simultaneously, has identified modified aptamers specific for thousands of human proteins[126,127]. Although drug development of aptamers is currently not very active, these RNAs or DNAs could substitute for some applications of therapeutic antibodies with reduced risk of developing immunological responses. They could also be used for targeted intracellular delivery of other molecules, including RNA-based drugs[85,124,128]. Because aptamers can be chemically synthesized, their manufacture is much cheaper and more reproducible than that of antibodies. Moreover, the flexibility of chemically linking RNAs should easily enable the design of combinations of aptamers, functionally resembling bifunctional antibodies, or their reversible linkage to more than one therapeutic small RNA, toxin, peptide or conventional small-molecule drug (Fig. 2b).

Concluding remarks

Our increased understanding of the versatile roles of RNAs has sparked the development of new classes of RNA-based drugs. Drugs in development are based on antisense mechanisms that can be used to inhibit gene expression (ASOs, siRNAs, miRNA mimics and antagomirs), alter splicing to produce functional proteins (ASOs) or edit mutated DNA (CRISPR–Cas). Novel approaches also utilize sense mechanisms (modified mRNA replacement therapy or vaccines) or tertiary structures of RNA that can be selected for specific binding (aptamers, riboswitches). The first RNA-based drugs to modify splicing were just approved, and the first siRNA-based drug is likely to be approved within the year. Although harnessing these RNA mechanisms and turning RNAs into drugs is challenging, it is likely that we are on the brink of a revolution in drug development. Expanding the range of targeted cells and tissues will require developing robust strategies for cytosolic delivery to tackle the twin hurdles of getting across the plasma membrane and out of the endosome. There is a lot of room for optimizing these steps and for improving the drug-like properties of therapeutic nucleic acids. As the first generation of nucleic acid therapeutics become drugs, the barrier for investing in nucleic acid therapeutics will be lowered, and resources will become available for exploring the option of harnessing other mechanisms of action aside from interfering with splicing and knocking down a single gene. The flexibility of RNA design should allow for the facile construction of potent multifunctional drugs that have more than one mode of action and disrupt multiple targets that could substitute for drug cocktails in the future. There is also the largely unexplored potential of targeting other RNA species and disrupting their functions. In the near future, RNA-based drugs may become an increasing component of the pharmacopoeia, greatly expanding the universe of druggable targets to provide treatment for previously untreatable diseases and potentially curing genetic diseases.

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Competing interests

J.L. is on the Scientific Advisory Board of Alnylam Pharmaceuticals.

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