RNA-Mediated Disease Mechanisms in Neurodegenerative Disorders

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Abstract

RNA is accurately entangled in virtually all pathways that maintain cellular homeostasis. To name but a few, RNA is the "messenger" between DNA encoded information and the resulting proteins. Furthermore, RNAs regulate diverse processes by forming DNA::RNA or RNA::RNA interactions. Finally, RNA itself can be the scaffold for ribonucleoprotein complexes, for example, ribosomes or cellular bodies. Consequently, disruption of any of these processes can lead to disease. This review describes known and emerging RNA-based disease mechanisms like interference with regular splicing, the anomalous appearance of RNA–protein complexes and uncommon RNA species, as well as non-canonical translation. Due to the complexity and entanglement of the above-mentioned pathways, only few drugs are available that target RNA-based disease mechanisms. However, advances in our understanding how RNA is involved in and modulates cellular homeostasis might pave the way to novel treatments.

Introduction

Neurodegenerative diseases become one of the main causes of death due to increased general health and hence an increased life-span. National science academies from the G7 countries identified the "challenges of neurodegenerative disease in an aging population" in 2017. From a purely economic point of view, the associated financial burden due to treatments, care, and others, is predicted to reach only in the United States 1 trillion US$ per year by 2050 [1]. Consequently, there is an immense interest of pharmaceutical companies in these diseases because of the high profit margin.

The heterologous group of neurodegenerative diseases includes the two most common ones, which are Alzheimer's and Parkinson's disease, but also rarer ones like amyotrophic lateral sclerosis (ALS)/frontal lobe dementia (FTD), Huntington's disease (HD) and other microsatellite expansion disorders and prion-based disease. A description of the cause(s) of each disease, symptoms and pathological features is beyond the scope of this review and has been extensively reviewed elsewhere [2]. This review will focus on the fascinating world of RNA and how disease-related alterations in RNA biology contribute to neurodegenerative diseases. At that, important lessons can be learnt from studying other disorders that are caused by RNA-mediated disease mechanisms. First, I highlight the roles of RNA in disease. For example, dysregulated RNA levels are a prominent feature observed in many diseases. However, these changes in RNA biology per se do not cause the disease. I discuss disease-causing mechanisms in the second part of this review. Finally, I briefly summarize possible treatment avenues to counter RNA-based disease mechanism.

RNA in Disease

Classically, neurodegeneration has been tightly linked to protein aggregation. In most neurodegenerative diseases, certain types of aggregates or inclusions are visible under the microscope [3]. Interestingly, mutations in proteins like tau [microtubule-associated protein tau (MAPT)] are found in several of these diseases and most probably change the spectrum of clinical representation [4].

Another hallmark feature is transcriptional dysregulation, which leads to changes in the expression levels of proteins by directly changing the levels of mRNA. Changes in protein levels can also be mediated through an indirect mechanism by changing the levels of regulatory non-coding RNA (ncRNA) species. These include a variety of promoter-associated ncRNAs, short ncRNAs (e.g., microRNAs, PIWI-interacting RNA) and long non-coding RNAs (e.g., long intergenic non-coding RNA) [5]. More recently, the class of circular RNAs has been described [6]. This class of RNA most probably functions as regulators during diverse cellular process by interacting with other RNA species. This Special Issue "Dementia, Brain Disorders and Molecular Mechanisms" includes reviews focusing on the role of microRNAs in spinocerebellar ataxia 3 (SCA3; Evert and Krauss [118]) and dementia [119] and the regulation of gene expression through the RNA interference (RNAi) machinery in Myotonic Dystrophy [120].

During gene expression, DNA in the nucleus gets transcribed into RNA, which is processed to remove intronic sequences and finally translated into protein in the cytoplasm. This process involves the action of numerous proteins and RNAs in multiple complexes. Among those are notably the DNA-dependent RNA polymerases, the spliceosome and the ribosome. Therefore, functional mutations in constituents of these will lead to changes in transcript levels. For example, mutations in PRPF6, PRPF8 [7] and SNRNP200, all components of the spliceosome, are associated with retinitis pigmentosa. The mutation in PRPF6 leads to its abnormal cellular distribution and as a consequence disturbed spliceosome assembly and recycling [8]. Mutations in SNRNP200 alter its helicase and proof-reading activity resulting in mis-spliced mRNAs [9].

Transcription elongation and splicing are closely intertwined and their respective functions precisely fine-tuned. Therefore, changes in the rate of transcription elongation lead to changes in splicing, mainly with faster elongation resulting in exon exclusion and vice versa [10–12]. Furthermore, RNA polymerases are much more error prone than DNA polymerases. Stochastic mutations introduced by erroneous RNA generation can potentially also affect splicing if these mutations are located in regions that are necessary for correct splicing like the 5′ and 3′ splice sites, the branch points, and others [13]. The RNA component of ribosomes is generated by RNA polymerase I in a sub-compartment of the nucleus, the nucleolus. A review article in this special issue will highlight the importance of the integrity of ribosome biogenesis and nucleolar function in neurodegenerative disorders [121].

Ribosomal RNA, but also mRNA is post-transcriptionally edited. For mRNA, the predominant editing mechanism is the deamination of adenosine to inosine [14], which has been shown to occur extensively in the normal human transcriptome [15] and is altered in various diseases [16]. Most of the editing occurs in the chromatin associated nuclear RNA before polyadenylation and nuclear export. As mentioned above, if these editing sites are positioned in a core splice site, alternative splicing might occur [17]. Interestingly, one of the deaminases ADAR2 (adenosine deaminases that act on RNA) edits its own mRNA [18]. This auto-regulation also involves RNA polymerase II, in particular the C-terminal domain [19]. Recently, an integrative approach of RNA expression, alternative splicing and editing showed that RNA editing and alternative splicing were tightly linked [20].

An important quality control step is the export of the RNA from the nucleus into the cytoplasm. Only fully matured RNA, defined by the bound proteins, which are remnants of the processing and modifications, is exported [21]. However, this is changed in disease. For example, mutations in the mRNA export adapter GLE1 (GLE1 RNA export mediator) have been causally linked to ALS [22] and lethal congenital contracture syndrome-1 (LCSS1) [23]. The loss of function mutation in GLE1 leads to nuclear accumulation of mRNAs resulting in neuronal death [24]. Microsatellite expansion diseases are caused by repeat expansions of regular nucleotide patterns, for example, CAG in HD, CGG in Fragile X-associated mental retardations, CUG in myotonic dystrophy and G_4C_2 in ALS. These repeat expansions can be located in coding, as well as in non-coding regions (Fig. 2). In most of these diseases, disruption of nucleo-cytoplasmic transport has been observed. Furthermore, specific export adapters seem to be required for export of microsatellite expansion RNAs. Consequently, changing the expression levels of nuclear export factors modulates disease phenotypes [25].

All of the above-mentioned observations of alterations in RNA homeostasis could be considered "passive." In other words, the underlying causes of the alterations are most likely not due to the RNA itself. Therefore, the pathogenic mechanisms that lead to disease are not based on RNA. In the following, I will highlight several mechanisms in which the RNA plays an active role, that is, phenomena in which RNA itself induces pathology (Fig. 1).

RNA-based Disease Mechanisms

Altered protein homeostasis

The most straightforward mechanism of RNA-based pathology is loss of function. Here, mutations in the DNA (point mutations or insertions/deletions)

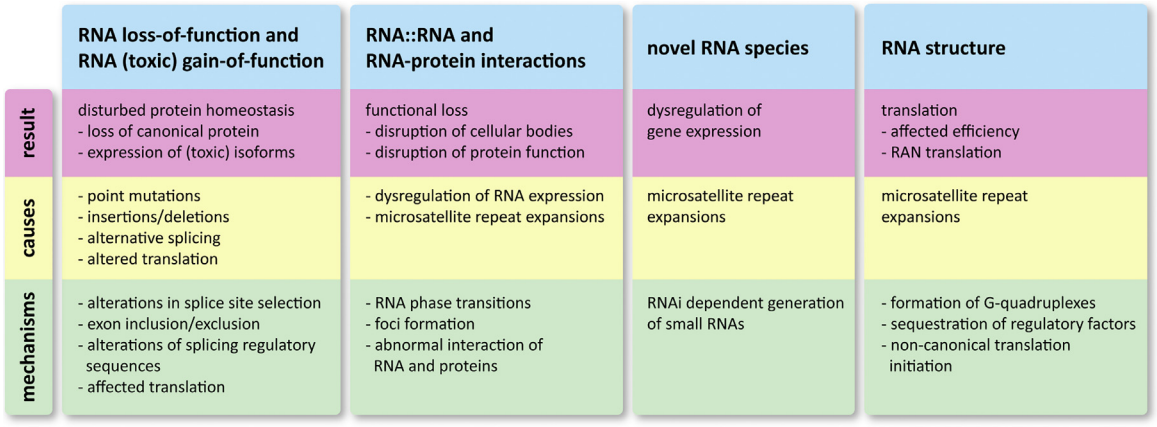

Fig. 1. RNA-mediated disease mechanisms. RNA-mediated disease mechanisms can be roughly grouped according to their final outcome. RNA loss- and toxic gain-of-function mechanisms mainly result in disturbed protein homeostasis. Abnormal interactions of RNA result in functional loss of the RNA itself or that of sequestered proteins. Disease-associated RNA species lead to dysregulation of gene expression. RNA structure alterations affect translation.

lead either to degradation of the RNA or expression of different protein isoforms. While this results in the same RNA loss-of-function phenotypes as caused by stochastic transcription errors introduced by RNA polymerases (see above), DNA mutations lead to 100% penetrance. This means that every single RNA molecule exhibits the mutation and thus the alteration in expression.

Degradation of the RNA is usually mediated by the nonsense-mediated RNA decay (NMD), a quality control pathway that recognizes premature termination codons (PTCs) [26]. These PTCs are generated through a frameshift introduced by the mutations. Some examples of mutations leading to NMD of the RNA transcripts are mutations in the dystrophin (*DMD*) and lamin A/C (*LMNA*) genes (Table 1). The

Table 1. Cis-acting mutations leading to disease-associated phenotypes through RNA mediated mechanisms

Gene	Mutation	Mechanism	Result	Disease	Reference
ATP6AP2	Exonic: c.345C > T	Abnormal isoform expression through altered splicing regulation	Altered protein homeostasis	X-linked parkinsonism with spasticity	[27]
DMD	Deletions	Exon skipping Exon deletion	RNA degradation by NMD	Duchenne muscular dystrophy	[28]
DMD	Exonic: c.4250 T > A	Abnormal isoform expression through altered splicing regulation	Altered protein homeostasis	Becker muscular dystrophy	[29]
HBB	Deletions, point mutations	See text	RNA and Protein dysregulation	β-Thalassaemia	[30]
IKBKAP	Intronic: c.2204 + 6 T > C	Changed U1 recruitment	Altered protein homeostasis	Familial dysautonomia	[31]
LMNA	Intronic: c.640-10A > G	Altered 3′ splice site selection	Altered protein homeostasis	Dilated cardiomyopathy	[32]
LMNA	Intronic: c.1488 + 5G > C	Novel 5′ splice site usage	RNA degradation by NMD	Familial partial lipodystrophy type 2	[33]
LMNA	Intronic: c.1608 + 5G > C	Novel 5′ splice site usage	RNA degradation by NMD	Limb girdle muscular dystrophy type 1B	[34]
LMNA	Exonic: c.1824C > T	Altered 5′ splice site selection	Altered protein homeostasis	Hutchinson–Gilford progeria syndrome	[35]
MAPT	Exonic: c.892A > G	Changes in splicing regulation	Altered protein homeostasis	Frontotemporal dementia with parkinsonism	[36]
PINK1	Exonic: c.1488 + 1G > A	Cryptic splice site selection	Altered protein homeostasis	Early-onset Parkinson's disease	[37]
mt tRNA$^{Leu(UUR)}$	Several	Changes in tRNA modification	Translational deficits	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes	[38]
mt tRNA^{Lys}	Several	Changes in tRNA modification	Translational deficits	Myoclonus epilepsy and ragged red fibres	[38]

Mutations can be exonic or intronic. NMD, nonsense-mediated RNA decay; mt, mitochondrial; tRNA, transfer RNA.

encoded proteins are not expressed and a loss-of-function phenotype is observed leading to Duchenne muscular dystrophy and familial partial lipodystrophy type 2 or limb girdle muscular dystrophy type 1B, respectively.

In cases in which mutations alter splicing regulation rather than leading to degradation of the transcript, non-canonical protein isoforms are expressed (Table 1). There have been several mechanisms described: (1) change of 5′ or 3′ splice site selection, (2) inclusion or exclusion of exons, and (3) alterations or *de novo* generation of splicing regulatory sequences (splicing enhancers or silencers). These mechanisms do not lead to a complete loss-of-function phenotype due to loss of protein expression. They generate, much like alternative splicing, protein isoforms with altered amino acid sequence. However, unlike isoforms generated through alternative splicing, the mutations are genomically encoded and thus lead to a complete isoforms change. A shift in isoform activity or toxic gain-of-function could lead to disease manifestation.

Two interesting cases of non-neurodegenerative diseases are the mutations in the haemoglobin subunit beta (*HBB*) and lamin A/C (*LMNA*) genes: There is a large number of mutations in the *HBB* gene described, all leading to the same disease—β-thalassaemia (Table 1). The severity of the disease correlates with the loss of HBB. Therefore, deletions or mutations that cause a (almost) complete loss of HBB lead to the most severe form β^0-thalassaemia. HBB expression can be shut down by introduction of PTCs and degradation of the RNA through NMD, or by inhibiting translation (nonsense codons or mutations in the start ATG codon). Less severe forms of β-thalassaemia (β^+ or β^{++}) are caused by mutations in the promoter region, the 5′ and 3′ untranslated regions or mutations affecting RNA processing. The latter can be affected by mutations in the consensus splice site sequences, activation of cryptic splice sites and mutations in the polyadenylation (polyA) signal [30].

LMNA-related diseases are part of the group of laminopathies. Lamins are constituents of the nuclear envelope, and mutations in lamins result in very heterogenic clinical representations [39]. Interestingly, in contrast to β-thalassaemia, quite similar mutations in the *LMNA* gene lead to different diseases (Table 1). Both the c.1488 + 5G > C and the c.1608 + 5G > C mutations result in changed 5′ splice site usage. However, the first mutation leads to familial partial lipodystrophy type 2 (FPLD2) [33] affecting body fat distribution, while the second leads to limb girdle muscular dystrophy type 1B (LGMD1B) [34] resulting in muscle weakness. Although neither β-thalassaemia nor laminopathies are classic neurodegenerative diseases, they illustrate the plethora of possible mechanisms leading to protein loss of function.

Other mutations rather affect translation. For example, mutations in the genes encoding for mitochondrial transfer RNAs (tRNAs) lead to several mitochondrial based diseases [40,41], some of which are highlighted in Table 1. The mitochondrial genome encodes for 22 tRNAs. The mutations affect the normal functions of the tRNAs to align the respective cognate codon with the correct amino acid. Intriguingly, the mechanism behind the deficit is very unique, as it seems to be related to RNA modification. Transfer RNAs usually are post-transcriptionally modified at the anticodon arm, and the correct recognition of the cognate codon depends on this modifications [42]. A special case seems to be taurine (2-aminoethanesulphonic acid) modification of the uridine at the wobble position in the two mt-tRNAs $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$ [43]. Mutations at this position (Table 1) lead to loss of taurine modification resulting in a deficit of mitochondrial translation and disease.

In summary, a variety of mechanism can lead to altered protein homeostasis through mutations in RNA. These can be caused by RNA loss of function: reduced protein expression through RNA degradation (frame shifts, nonsense codons, etc.) and translational deficits. Other mutations cause a shift in protein isoform ratio (alternative splice site selection, insertions/deletions, inclusion/exclusion of (cryptic) exons, etc.). These mutations could be considered as gain-of-function mutations, especially if leading to expression of toxic protein isoforms as described exemplary in the following.

Production of toxic protein isoforms by alternative splicing

Hutchinson–Gilford progeria syndrome (HGPS) is a rare dominant premature aging disease. The disease is caused by expression of progerin, a C-terminally truncated version of lamin A. An exonic mutation (exon 11) creates an alternative splice site resulting in the generation of a shortened *LMNA* mRNA (Table 1). This truncated mRNA encodes for progerin. In addition to loss of lamin A, an important constituent of the nuclear envelope, progerin expression exacerbates problems of HGPS cells to repair DNA double-strand breaks [44]. This acceleration of "molecular aging" could be the driver of the premature aging phenotype observed in patients with HGPS.

Polyglutamine diseases are caused by CAG repeat expansions in the coding regions of several genes. Expansions in the huntingtin (*HTT*) gene cause HD, expansions in the androgen receptor (*AR*) gene cause spinal and bulbar muscular atrophy (SBMA) and expansions in the atrophin 1 (*ATN1*) gene cause dentatorubropallidoluysian atrophy (DRPLA). There are also 6 types of spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, SCA17), which are caused by expansions in the genes *ATXN1*, *ATXN2*,

ATXN3, *CACNA1A*, *ATXN7* and *TBP*, respectively. The diseases are clinically very heterogeneous, while the underlying mutation is always a repeat expansion [45]. The CAG repeat expansion translates into a polyglutamine (polyQ) tract that causes the harboring proteins to aggregate by a toxic gain-of-function mechanism [46]. The most studied polyglutamine disease is HD. At least for huntingtin (HTT), but most probably also for the other polyQ proteins, smaller fragments containing the expanded polyQ repeat aggregate faster and are more toxic [47]. The most toxic fragment of HTT consists of only exon 1 HTT, which also contains the expanded polyQ tract. This protein is encoded by an mRNA that is generated through a block in splicing of *HTT* exon 1 to exon 2. This block occurs in all knock-in mouse models [48] and in human HD patient samples [49]. The genetic architecture of the *HTT* gene with a very long intron 1 [50,51], abnormal binding of a splicing factor to the expanded CAG repeats [48,52] and changes in transcription along the gene [52] all influence the amount of incomplete splicing of *HTT*. Because the disease-causing mutation is the same, one could image a similar mechanism of fragment generation for the other polyglutamine disorders. However, to date, only for SCA3, a truncated mRNA fragment of ATXN1 has been described [53]. Initial findings in a mouse model could not be replicated and most probably were due to genomic recombination events during the mouse model generation [54–56]. So the question remains whether splicing changes are a feature of all polyglutamine diseases. Furthermore, the contribution of the small fragments to disease pathogenesis needs to be determined.

RNA foci

There are several “assembly stations” during the generation, quality control and translation of RNAs [57]. These cellular bodies are defined compartments, usually by the localization of a marker protein, contain RNA and protein, but are not enclosed by membranes. Some examples are Cajal bodies, which are the site of small nuclear (sn) or small nucleolar (sno) ribonucleoprotein complex assembly [58]. In nuclear speckles, pre-mRNAs are modified/processed and stored [59]. The cytoplasmic P bodies (processing bodies) are sites of RNA degradation through exonucleases and repression of mRNAs [60]. In addition, recent data have shown that many of these structures have additional functions and are associated with disease.

Disturbances of nuclear bodies and consequently their function can be seen in several diseases. For example, various proteins or protein isoforms that are associated with disease co-localize with nuclear speckles: TARDP and FUS in ALS/FTD [61] and the Cγ fragment of amyloid precursor protein, as well as SRSF2 in Alzheimer's disease [62,63]. The survival of motor neuron protein (SMN), which is mutated in spinal muscular atrophy (SMA), even forms its own structures—nuclear gems or Gemini bodies [64,65]. The appearance of these structures also seems to correlate with disease severity [64].

Disruption of the RNA components of nuclear bodies is also apparent in disease. The long non-coding RNA *NEAT1* is an integral part of paraspeckles together with more than 40 proteins [66]. The function of paraspeckles seems to be gene regulation through dynamic exchange of RNA and proteins, and they might be involved in epigenetic regulation of some target genes [67,68]. *NEAT1* expression is dysregulated in several diseases [69–73]. Depending on the experimental settings, it is not clear if paraspeckles are protective or drive disease pathogenesis [66]. In the case of polyglutamine disorders, *NEAT1* (*NEAT1L*; long isoform) levels are upregulated in mouse models and patient samples [72]. This upregulation might pose a cellular defence mechanism against mutant HTT-induced pathogenic effects [72]. A similar upregulation of *NEAT1* has been observed in Parkinson's disease model mice; however, the higher expression levels seemed to correlate with increased apoptosis [73]. Clearly, more data are needed to unravel the (disease-specific) effects of nuclear bodies function.

RNA can establish extensive RNA::RNA intermolecule interactions. Especially in repeat expansions disorders (Fig. 2), this phenomenon leads to the formation of RNA foci. These foci consist of the expanded RNA and potentially proteins. The generation of RNA foci themselves seems to be well tolerated in a cell model, at least for the short time frame analyzed [74]. Therefore, the interaction and aggregation of RNA itself probably does not pose a toxic insult. An interesting phenomenon is the phase transition behavior of the foci. The extensive intermolecule interactions lead to a liquid–liquid and subsequent liquid–gel transition of the RNA molecules in the foci to form membrane-less compartments [74]. Hence, these phase transitions probably are the assembly platform for some of the cellular bodies mentioned above [75,76]. Alberti and colleagues propose that the high ratio of RNA/protein in the nucleus keeps RNA binding proteins soluble and prevents the pathogenic aggregation of, for example, FUS and TARBP [75].

While this might be a protective function of normal RNA, disease-associated RNA can form aberrant interactions with proteins. Here, a toxic gain of function mechanism is possible, for example, in the case of aberrant *HTT*-SRSF6 (serine arginine rich splicing factor 6) interactions. The increased binding of SRSF6 to the expanded CAG tract in the *HTT* pre-mRNA contributes to a block in cis-splicing and the production of a mRNA encoding a toxic fragment [48,52]. Sequestration of proteins into the RNA foci can also lead to a loss-of-function phenotype. The

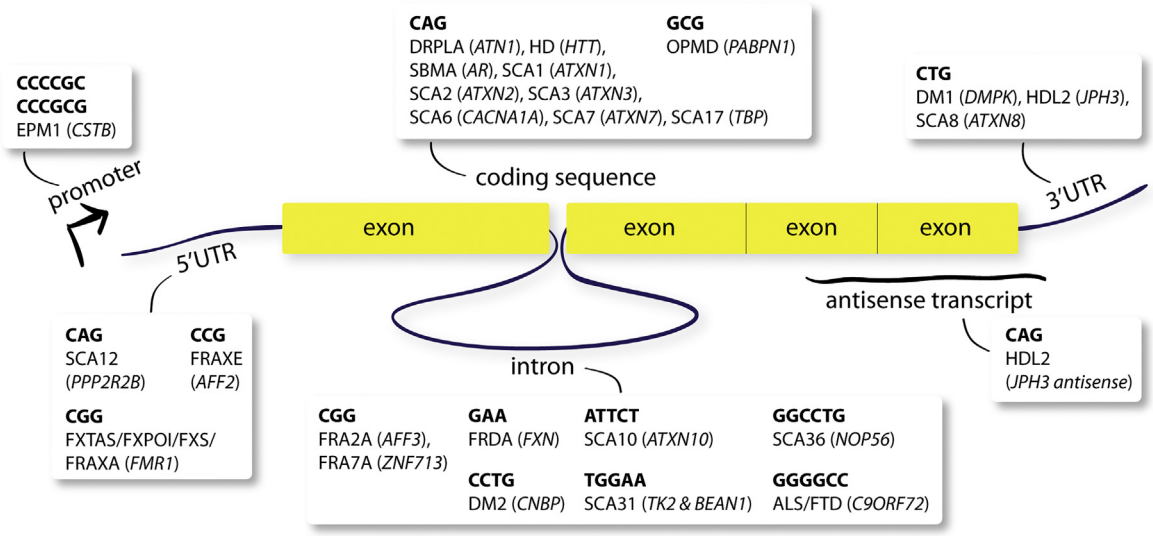

Fig. 2. Microsatellite repeat expansions. Microsatellite expansions are shown respective to their location. The repeat unit is highlighted in bold. The disease abbreviation is followed by the gene name that is affected by the expansion. UTR, untranslated region. Disease abbreviations: EPM1, progressive myoclonus epilepsy 1/Unverricht–Lundborg disease; SCAX, spinocerebellar ataxia X; FRAXE, fragile XE syndrome; FXTAS, fragile X-associated tremor/ataxia syndrome; FXPOI, fragile X-associated primary ovarian insufficiency; FXS/FRAXA, fragile X syndrome; DRPLA, Dentatorubral–Pallidoluysian atrophy; HD, Huntington's disease; SBMA, spinal–bulbar muscular atrophy; OPMD, oculopharyngeal muscular dystrophy; FRA2A, CGG expansion at fragile site 2A; FRA7A, CGG expansion at fragile site 7A; FRDA, Friedreich ataxia; DMX, myotonic dystrophy type X; ALS/FTD, C9ORF72-related ALS/FTD; HDL2, Huntington's disease-like 2.

classic examples for this is the sequestration of muscleblind like splicing regulator 1 (MBNL1) into CUG repeats containing foci of the *DMPK* gene (Fig. 2) [77,78]. This leads to a loss in the function of MBNL1 accompanied by severe splicing changes and causes myotonic dystrophy type 1 (DM1) [79–81]. The same mechanism contributes to fragile X-associated tremor/ataxia syndrome (FXTAS) in which a CGG repeat in the *FMR1* gene leads to SAM68 sequestration and splicing changes [82]. While there are probably many more diseases to be discovered in which abnormal RNA::protein interactions contribute to pathogenesis, in some cases, the appearance of RNA foci does not correlate with disease patterns [83].

Microsatellite-encoded ncRNAs

RNA can give rise to other, distinct RNA species. For example, intronically encoded microRNAs can be processed from introns derived from pre-mRNAs [84]. Furthermore, several snoRNAs are encoded in the introns of ribosomal genes [85]. Therefore, these non-coding RNAs share the same promoter and are tightly coupled to the expression of their host RNA. Micro RNAs act through the RNAi pathway to regulate their target genes [86]. One central player in this pathway is Dicer, an endonuclease that recognizes RNA hairpin structures and cleaves these precursor RNAs to form the mature RNA duplexes [86]. Several microsatellite expansions (Fig. 2) generate RNAs that can fold into hairpin structures [87–89]. These hairpins are subsequently recognized by Dicer, and small RNAs are generated in a repeat-length dependent manner [87–92]. The generation of these small RNAs potentially aggravates the repeat expansions induced phenotypes by (down-) regulating other genes. This has been shown for CAG repeat expansions, where CAG repeat-derived small RNAs interfered with the expression of CUG repeat containing genes in model systems [91,92]. However, similar experiments from another group led to no reproducible changes in endogenous CUG repeat containing genes [90].

Intriguingly, there is a potential inverse correlation of patients with certain microsatellite expansions and the occurrence of certain types of cancer in these patents. There are currently no studies directly focused on this correlation, but some larger existing clinical studies allow to draw some conclusions. While there are certainly conflicting data, the above inverse correlation holds true, for example, for SBMA and prostate cancer and HD and several types of cancer [93]. Moreover, screening dozens of trinucleotide expansion based siRNAs, Murmann colleagues [94] could show that CAG/CUG siRNAs were very effective in killing mouse and human cancer cells *in vitro* and *in vivo*. The detrimental effect to cancer cells was dependent on long complimentary sequences in target genes that were down-regulated. Furthermore, the down-regulation was mediated through the RNAi pathway [94]. An interesting thought

experiment is whether the small RNAs derived from microsatellite expansions might be beneficial in the cellular responses that have evolved to counter diseases like cancer [95].

Translation of expanded microsatellite RNA

RNA is highly structured and can form extensive secondary and tertiary domains. One of these structures are G-quadruplexes (G4s), which are stacks of G-nucleotides assembled into tetrads in a planar orientation [96]. G4s have shown to be involved in almost all RNA metabolic processes and are dysregulated in numerous diseases [96]. One of the disease-associated mechanism is an attenuation of translation efficiency if several G4s are clustered. One example is the downregulation of the *FMR1* gene-encoded FMRP protein in FXTAS. Splicing changes, as described above, and epigenetic dysregulation lead to a reduction in *FMR1* mRNA levels [97]. In addition, G4-induced translational attenuation could contribute to the loss of FMRP [98,99].

G4s have also been proposed as one of the factors modulating a non-canonical form of translation initiation at microsatellite repeats [100,101]. This repeat-associated non-ATG (RAN) translation has first been identified in 2011 [102]. Since then, it has been found in an ever-growing number of repeat expansion diseases [103]. A review article in this special issue describes RAN-translation products in the pathogenesis of ALS and HD [121]. RAN-translation, as the name suggests, initiates not at canonical ATG start-codons but at the structures formed by the repeat expansions. Mechanistically, RAN translation probably shares some IRES-like (internal ribosome entry site) features, but there is still need for further clarification [103]. Once RAN translation occurs, initiation is possible in all reading frames. This leads to the production of repeated amino acid tracts, other than the canonically encoded ones. For example, the CAG tract of *HTT* in HD canonically encodes for a stretch of glutamines. In the case of RAN translation, this tract leads to the production of polyalanine and polyserine in the sense direction [104]. Antisense transcription is very abundant [105] and also has been described for the *HTT* locus, where an antisense transcript including a CUG expansion (reverse complementary to the CAG tract) is generated [106]. RAN translation can also occur from this antisense transcript and creates polycysteine and polyleucine peptides [104]. As a side note, the ubiquitous nature of antisense transcription opens up the possibility that many, if not all of RNA-mediated (disease related) mechanisms occur from these transcripts as well. Furthermore, antisense transcripts themselves might have an impact on disease severity and/or progression. Some of peptides, which are produced through RAN translation, exhibit higher toxicity than the canonically expressed repeat containing proteins in model systems [107]. Spatial and temporal differences in expression of these RAN products therefore constitute an appealing hypothesis of selective vulnerability of different tissues and cell types that is often observed in neurodegenerative disorders.

The extent of translation of CAG repeat containing RNAs is altered in a very interesting way. The elongated CAG repeat leads to the binding of a protein complex that stimulates the translation of the respective RNA in a CAG repeat-length dependent way. This has first been described for *HTT* [108] and has now also been shown for three other CAG containing RNAs (*ATXN2*, *ATXN3*, *ATXN7*) [109]. The proteins binding to the repeat are 40S ribosomal S6 kinase (S6K), protein phosphatase 2A (PP2A) and midline 1 (MID1). MID1 has been implicated in neurodegenerative diseases [110]. It also negatively regulates PP2A, which in turn negatively regulates S6K. In conjunction with activation of mTOR (mechanistic target of rapamycin kinase) by MID1, S6K becomes over-activated leading to an increased translation of CAG repeat RNAs. Since higher levels of the disease-causing proteins lead to aggravated pathogenesis, this mechanism might mediate the manifestation of severity of clinical symptoms.

Therapies Targeting RNA-based Disease Mechanisms

Each newly identified mechanism of RNA-based toxicity potentially opens up a new therapeutic point of application. For example, if one could interfere with the just described mechanism of increased translation of CAG containing RNAs, one might alleviate the disease burden by reducing the disease-causing protein. So far, only *in silico* and first cell culture experiments have been published using an inhibitor of the MID1/CAG RNA complex [111]. Nonetheless, this shows the feasibility of such an approach.

Other approaches to reduce disease burden by lowering the expression of disease-causing proteins mainly target the mRNA through RNAi and antisense oligonucleotides (ASOs) [112]. These strategies hold great promise to improve or even modulate disease progression. In addition, ASOs, small molecules, snRNAs, and so on, can be used to alter splicing [113]. The use of ASOs to target splicing is particularly advanced in SMA. Patients suffering from this disease usually carry a mutated version of the *SMA1* gene resulting in a loss of the SMA protein and disease. Humans carry a second, often multiple times duplicated, almost identical gene—*SMA2*. However, in this gene, a single-nucleotide change leads to the exclusion of exon 7 from the mRNA rendering the resulting protein instable and less functional. With ASO therapeutics, the hope is to

increase levels of *SMA2* exon 7 inclusion resulting in higher levels of fully functional SMA protein [114]. Mechanistically, the ASOs can mask splicing silencer sequences, tether splicing proteins or target regulatory ncRNAs [114]. Using ASOs to mask splicing silencer sequences has been very effective and led to the development of a European Medicines Agency- and US Food and Drug Administration-approved ASO-based drug that significantly improved clinical measures in SMA patients [115]. However, the extreme cost associated with this ASO therapy and the lack of long-term data led the United Kingdom National Institute for Health and Care Excellence to not recommend the use of the drug by the National Institute for Health [116]. While the concerns about lack of long-term data are certainly true, the question remains how much money a disease-modifying treatment and thus a significant improvement of quality of life is worth. Further complicating facts of ASO-based therapy, as is true for any medication, are off target effects and the delivery problem into (deep) brain areas. The latter is currently partly circumvented by application of the drugs by intrathecal delivery. However, even with this route of application, the distribution of the drugs is challenging and might not reach deeper brain regions, which is often needed depending on the disease that is combated.

Conclusion

Since the discovery of differences between “animal” and “plant” nucleic acids and the coining of the terms DNA and RNA over 70 years ago [117], a wealth of new functions of RNA in cellular homeostasis has emerged. Despite decades of research, there are still novel roles of RNA to uncover. As an example, most recently, the analysis of the biophysical properties of RNA assemblies has shed new light onto possible ways in which RNA could lead to the formation of subcellular structures.

This review highlights some RNA-mediated pathological mechanisms. Extending our knowledge of these mechanisms will lead to a better understanding of the molecular causes of diseases and thus could lead to the development of drugs that target disease that are incurable so far. Moreover, the fundamental nature of RNA as one of the central macromolecules in a cell is in itself reason enough to study this fascinating entity.

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Abbreviations used:
ALS, amyotrophic lateral sclerosis; ncRNA, regulatory non-coding RNA; SCA3, spinocerebellar ataxia 3; NMD, nonsense-mediated RNA decay; PTC, premature termination codon; tRNA, transfer RNA; HGPS, Hutchinson–Gilford progeria syndrome; RNAi, RNA interference; G4s, G-quadruplexes; RAN, repeat-associated non-ATG; ASOs, antisense oligonucleotides; SMA, spinal muscular atrophy.

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