# Chapter 4
# RNA Related Pathology in Huntington's Disease

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**Abstract** This chapter summarises research investigating the expression of huntingtin sense and anti-sense transcripts, the effect of the mutation on huntingtin processing as well as the more global effect of the mutation on the coding and non-coding transcriptomes. The huntingtin gene is ubiquitously expressed, although expression levels vary between tissues and cell types. A SNP that affects NF-κB binding in the huntingtin promoter modulates the expression level of huntingtin transcripts and is associated with the age of disease onset. Incomplete splicing between exon 1 and exon 2 has been shown to result in the expression of a small polyadenylated mRNA that encodes the highly pathogenic exon 1 huntingtin protein. This occurs in a CAG-repeat length dependent manner in all full-length mouse models of HD as well as HD patient post-mortem brains and fibroblasts. An anti-sense transcript to huntingtin is generated that contains a CUG repeat that is expanded in HD patients. In myotonic dystrophy, expanded CUG repeats form RNA foci in cell nuclei that bind specific proteins (e.g. MBL1). Short, pure CAG RNAs of approximately 21 nucleotides that have been processed by DICER can inhibit the translation of other CAG repeat containing mRNAs. The HD mutation affects the transcriptome at the level of mRNA expression, splicing and the expression of non-coding RNAs. Finally, expanded repetitive stretched of nucleotides can lead to RAN translation, in which the ribosome translates from the expanded repeat in all possible reading frames, producing proteins with various poly-amino acid tracts. The extent to which these events contribute to HD pathogenesis is largely unknown.

**Keywords** Huntingtin transcripts · Antisense RNA · Non-coding RNA
Huntingtin splicing · RAN translation

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Huntington's disease (HD) was first described in 1872 by George Huntington as a 'dancing' disorder due to involuntary movements, the chorea being the most obvious motoric symptom [1]. However, HD had been known since the Middle Ages under the name 'St. Vitas dance'. This name also included dancing and St. Vitas, the saint to whom people prayed for help. Their grimaces, the chorea and mental impairments were the reasons people believed HD patients were possessed by the devil. Some of the victims burned as witches in the Middle Ages were probably suffering from HD.

The discoveries of DNA and genes as units of hereditary information were milestones that ultimately led to the identification of a marker on chromosome 4 linked to HD in 1983 [2]. This discovery allowed the eventual identification of the disease causing gene in a large-scale collaborative effort a decade later in 1993 [3]. The publication describes an unstable CAG trinucleotide expansion in the huntingtin gene (*HTT*) and shows that this expansion is the sole genetic cause of HD. Genetic testing in the following years established that patients with a repeat length of 40 or more CAGs inevitably develop HD, with a mean age of onset in the 40 s. There is also a more severe, fast progressing form of HD with an age of onset in childhood or adolescence caused by longer CAG repeats in the juvenile onset range.

The CAG repeat encodes for a stretch of glutamines (polyQ) in the HTT protein. This polyQ tract in turn leads to the appearance of proteinaceous, very large aggregates, the so called inclusion bodies, a pathological hallmark in a variety of diseases. A wide variety of proteins co-localise with these aggregates and the proteins that are sequestered are most probably not able to fulfil their normal role in cell homeostasis. The observation of aggregates forming and the absorption of other proteins into those led to the hypothesis of a 'toxic gain of function' of mutant huntingtin. In contrast to this, the poly-glutamine stretch could disrupt the normal function of HTT leading to a 'loss of function' [4].

There were very early indications that N-terminal fragments of HTT, that include the polyQ tract, were an integral unit of the aggregates. In *post mortem* HD brains nuclear aggregates could only be detected with antibodies against the N-terminus of HTT [5, 6]. Furthermore, when aggregated proteins were released by formic acid treatment, N-terminal fragments could also be identified [7]. In cell models HTT can be cleaved to release the small N-terminal fragments cleavage products (cp)-A and (cp-B) [7] or cp-1 and cp-2 [8, 9]. A comprehensive study in a knock-in mouse model revealed 14 N-terminal fragments of HTT [10], the smallest of which was encoded by exon 1 of the *Htt* gene. Exon 1 HTT is extremely aggregation prone and is the most toxic species of naturally occurring HTT fragments [11].

## 4.1 Regulation of *HTT* Transcription

Although *HTT* is ubiquitously expressed [12], neuronal tissue is especially vulnerable in HD. A major determining factor of protein expression is the promoter driving expression of the respective gene. A difference in the promoter activity and

by this expression levels of *HTT*, which in turn would lead to higher concentration of the mutated protein and an increase in disease burden, could be one explanation for this selective vulnerability. We can deduce from the functional expression of the exon 1 *HTT* transgene in the R6/mouse model that an upstream sequence of approximately 1 kilobase of the *HTT* gene is sufficient to induce transcription of the transgene in an animal model [13]. The human *HTT* promoter has a CpG island [14], lacks the canonical TATA and CAAT boxes, but retains the SP1, AP-2 and AP-4 core transcription factor binding sites [15]. An in vitro analysis of different *HTT* promoter constructs driving a luciferase assay revealed a selectively higher reporter signal in a neuronal versus a non-neuronal cell line [16]. A recent study identified an additional NF-κB binding site in the *HTT* promoter and could show that a single nucleotide polymorphism (SNP) in this binding site correlated with the age of disease onset [17]. Reduced binding of NF-κB to the promoter due to the transition of a G to A led to reduced expression of HTT and a delayed onset of HD. Additional detailed studies of the interplay of transcription factors inducing expression of *HTT*, identification and analysis of possible enhancer regions of the *HTT* gene, as well as identification of other cis and trans acting factors influencing expression of *HTT* could lead to the development of new therapeutic avenues in the future. As proof-of-principle, decreasing the levels of NF-κB resulted in lower levels of *HTT* expression [17]. One could also imagine allele specific *HTT* lowering therapies by targeting SNPs [18, 17].

CAG trinucleotide expansions have the propensity to form highly stable self-complementary structures. Newly synthesised mRNA could interact with the DNA template and thus might require additional DNA-dependent RNA polymerase II (PolI) subunits for efficient transcription. These DNA/RNA hybrid structures are called R-loops, which play an important role e.g. in transcription regulation, DNA replication and genome stability including repeat instability. They have already been shown to exist in several repeat expansion diseases where it was proposed that they induce gene silencing [19, 20]. In HD, R-loops have not yet been identified. However, the yeast transcription factor Spt4 and its mammalian homologue Supt4 h were found to be required for efficient transcription through the CAG repeat [21]. Inhibition of this transcription factor led to reduced expression of *Htt*, while the transcription of other genes seemed to be largely unaffected.

## 4.2 Alternative Splice Forms of the *HTT* mRNA

Perhaps the most striking evidence for the extreme pathogenicity of the exon 1 HTT protein as compared to other HTT fragments, is the rapidly progressing phenotype displayed by the R6/mouse lines [13], in particular line R6/2. These were created by transgene integration into the mouse genome; the transgene consisting of about 1 kb of the human *HTT* promoter, human *HTT* exon 1 with an elongated CAG repeat and part of human *HTT* intron 1. R6/2 mice have been shown to develop very comparable molecular and phenotypic signatures at later stages of disease

progression when compared to full-length knock-in mouse models such as the *Hdh*Q150 mice [22], in which an expanded CAG repeat is integrated into the full-length endogenous mouse gene [23]. However, the length of time to reach the end stage of the disease is more than 20 months in homozygous *Hdh*Q150 mice as compared to approximately 14 weeks in the R6/2 model. The comparably higher levels of exon 1 HTT in the R6/2 mice, as compared to that of HTT fragments in the knock-in lines, was therefore sufficient to cause this vastly accelerated disease progression.

When exon 1 HTT was first detected in brain lysates from knock-in mice, it was assumed that it was generated through proteolytic cleavage of full-length HTT, similar to the generation of other HTT fragments [10]. However, more recent evidence showed that exon 1 HTT is generated through the incomplete splicing of the *HTT* mRNA [24]. In all full-length knock-in mouse models of HD with pathogenic repeat sizes, a transcript could be detected that consists of *Htt* exon 1 and a few hundred base pairs of intron 1 (HTT exon 1 mRNA). The production of the HTT exon 1 transcript was shown to be CAG repeat length dependent, with longer repeats producing higher levels of HTT exon 1 mRNA. The authors further showed that a general, serine, arginine rich splicing factor (SRSF6) bound tightly to the elongated CAG repeat. Serine, arginine rich splicing factors interact with U1 RNA containing small nuclear spliceosomal ribonucleoprotein complexes (U1 snRNP), also parts of the general splicing machinery [25]. U1 snRNP, in addition to initiating the formation of the spliceosome at the 5′ splice site, protects cryptic polyadenylation sites (polyA sites) from being recognised and thus inhibits the formation of aberrantly spliced, shorter transcripts [26]. A shortage in the levels of U1 snRNP therefore leads to the generation of shorter mRNAs, a phenomenon that can be observed during organismal development [27]. However, in a fully developed organism, generation of these aberrant transcripts is in the best case unproductive leading to mRNA decay, or in the worst case produces the message for toxic proteins, as is the case in HD. The increased binding of SRSF6 to the mutated CAG repeats very likely depletes the local pool of U1 snRNP by sequestering the spliceosomal component. This in turn could inhibit the formation of the spliceosome at the 5′ splice site and not mutually exclusive, also expose cryptic polyA sites in *HTT* intron 1. The reduced efficiency in splicing and the partly exposed cryptic polyA sites open a kinetic window in which cleavage and polyadenylation factors can recognise the normally protected sites in *HTT* intron 1, cleave and synthesise a polyA tail to generate a functional 3′ mRNA end. Moreover, the HTTexon 1 transcript was shown to be associated with poly-ribosomes indicating nuclear export and functional integrity. Finally, the authors showed that in all mouse models that produced the small transcript, the presence of an exon 1 HTT protein could be detected.

The incomplete splicing of the *Htt* message to create the HTT exon 1 mRNA is not limited to the mouse *Htt* gene. There are several mouse models of HD in which the mouse sequence of exon 1 and short sequences of intron 1 have been exchanged with their human counterparts. These chimeric models are based on two genetic constructs, which slightly differ in the amount of human *HTT* intron 1 insertion/

deletion of mouse *Htt* intron 1 [28–30]. Independently of these small differences, an incompletely spliced HTT exon 1 mRNA and exon 1 HTT protein was detected in all chimeric models with pathogenic repeat sizes [24]. Taking it one step further to the full length human gene, the authors also analysed BAC (bacterial artificial chromosome) and YAC (yeast artificial chromosome) mouse models of HD. Both models express a full length copy of the human *HTT* gene from artificial chromosomes that also includes large up- and downstream sequences. Strikingly, the same transcript of *HTT* exon 1 and partial intron 1 could also be found in these models. To exclude that the generation of the HTT exon 1 mRNA for some strange reason was restricted to mouse models of HD, Sathasivam et al. analysed samples from human HD patients. They identified usage of the cryptic polyA site in HTT intron 1 for patient derived fibroblasts and *post mortem* brain samples. Intronic sequences, consistent with the incomplete splicing of exon 1 to exon 2 have recently been detected in *post mortem* brain samples and fibroblast lines from juvenile HD patients (Neueder et al. unpublished).

In addition to the generation of HTT exon 1 mRNA through incomplete splicing, novel isoforms of mouse and human *HTT* have been identified [31–33]. These murine isoforms seem to be ubiquitously expressed and either lack exon 28 or 29, or retain part of intron 28. Interestingly, the *Htt* isoform lacking exon 29 was under-represented in the cerebellum of the *Hdh*Q150 HD mouse model [31]. Various isoforms have been identified in human brains [32] and cell lines [33]. The differences from the canonical full length HTT mRNA were: inclusion and exclusion of exons, retention of intronic sequences and inclusion of a novel hominid specific exon. Furthermore, the authors used protein homology modelling of the novel isoforms and suggested that the splice changes would lead to loss of protein-protein interactions and potential alterations in post-translational modifications of HTT. In any case, the impact of the respective isoforms on HD pathogenesis needs to be determined.

## 4.3 Implications of Aberrant Splicing of the *HTT* mRNA

These findings have major implications for our understanding of the molecular mechanisms that initiate the occurrence of HD symptoms. The HTT exon 1 mRNA that is present in all mouse models, as well as in human patients, is almost identical to the transgene expressed in the R6/mouse lines. Given the very rapid progression of the phenotype in these mouse models, the production of exon 1 HTT through the generation of an incompletely spliced *HTT* message might be expected to make a significant contribution to disease onset and progression in humans. One of the main questions which need to be answered is therefore: how much does generation of exon 1 HTT contribute to HD pathogenesis [34]? Novel mouse models in which the production of exon 1 HTT is inhibited or increased will certainly help to answer this question. It will also be very interesting to see if there is a correlation of HTT exon 1 mRNA levels in human patients with age of disease onset, rate of disease

progression, or severity of symptoms. Additional factors acting in trans, which could influence the rate of HTT exon 1 mRNA production, might be uncovered by genome wide association studies (GWAS). The first high powered GWAS analysis in HD found a locus on chromosome 15, which could either hasten or delay, and a locus on chromosome 8, which hastened the age of onset of HD (Genetic Modifiers of Huntington's Disease [35]. Additionally, in their analysis of over-represented pathways, the authors found that DNA maintenance and repair mechanisms were enriched. It has been shown that these pathways influence somatic instability of the CAG repeat, with striatum and cortex, the two most affected tissues in HD, showing the largest instability [36]. Since the production of HTT exon 1 mRNA is clearly CAG repeat length dependent [24], somatic instability would enhance the generation of exon 1 HTT.

One of the most promising strategies to counter HD is to lower HTT levels, either by targeting both the normal and mutated alleles, or preferably only the levels of the mutated allele [37, 38]. There are mounting data to suggest that by lowering HTT levels many phenotypical symptoms in HD model systems can be improved [39–42]. Lowering HTT levels in a non-allele specific way seemed to be well tolerated for at least 6 months in a non-human primate [43]. The first clinical trial to lower mutant huntingtin levels with an antisense oligonucleotide was initiated during 2015 (Ionis Pharmaceuticals in collaboration with Roche). However, these strategies do not target the production of the HTT exon 1 mRNA. While a reduction of mutated HTT in general has therapeutic potential, inhibiting the generation of exon 1 HTT and by this the probable source for nucleation of aggregation might have an even greater therapeutic value.

## 4.4 Antisense Transcription from the *HTT* Locus

Naturally occurring antisense transcription is a very common phenomenon acting as an important transcriptional regulator [44]. An antisense RNA (*HTTAS*), which is produced from the *HTT* locus and was identified in human brain tissue, influences sense *HTT* transcript levels [45]. Chung et al. showed that *HTTAS* is comprised of 3 exons, with the transcription start site at +300 base pairs relative to the transcription start site of the *HTT* gene. In addition *HTTAS* contains a 5′-cap and is polyadenylated, giving it all the essential features of a mature mRNA. The antisense RNA is differentially spliced into two isoforms: a longer one including the first exon, which is mostly complementary to *HTT* exon 1 including the CAG repeat, and a shorter one without this exon. Interestingly, only the longer isoform was able to repress *HTT* transcription in a reporter cell line in a CAG repeat length dependent manner. Its levels were also reduced in the frontal cortex of HD patients. The long isoform of HTTAS contains a CUG repeat, the same repeat as in patients of myotonic dystrophy type 1 and spinocerebellar ataxia type 8. These CUG repeats sequester proteins like CUG-BP (hNab50) [46] and prominently MBNL1 [47, 48] resulting in the inhibition of its function and induction of wide spread pathogenic

splicing changes [49]. RNA binding proteins like MBNL1 that bind to double stranded RNA hairpins recognize an RNA structure rather than a defined RNA sequence [50]. CAG repeats, as CUG repeats, seem to adopt a double stranded structure and were shown to also co-localize with MBNL1 [51, 52]. A comparison of splicing changes between *myotonic* dystrophy type 1 and induction of changes through expression of long CAG repeat containing reporter RNAs revealed a high similarity in the cellular response to both [53]. The existing evidence of RNA binding proteins being sequestered to the mutated CAG repeat in *HTT* suggests that there is a high probability of additional factors being involved. These experiments will certainly shed further light on the possibility of HD belonging to the class of 'RNAopathies' [54].

## 4.5 Small RNAs Are Produced from the *HTT* mRNA

Cellular compartmentalization and transport between these compartments constitutes an important regulatory layer in cellular homeostasis. Full length HTT mRNA, as well as HTT exon 1 mRNA are exported from the nucleus and are translated in the cytoplasm. However, elongated CAG repeat containing reporter RNAs [51, 53], *as well as full length HTT RNA* can form nuclear foci [51, 55]. These nuclear foci co-localize with MBNL1 and by increasing the levels of MBNL1, the amount of retention of HTT in the nucleus is increased as well. Higher levels of U2AF2, part of the U2AF splicing RNP, counteract this phenomenon and lead to more efficient export of *HTT* mRNA [55]. Whether these nuclear RNA foci are a quality control step in the maturation of the *HTT* mRNA, or whether they are a pathological feature induced by the elongated CAG repeat still needs to be clarified. Once the *HTT* mRNA is exported it can be engaged by Dicer, which normally processes double stranded (ds) pre-RNA into short (21-24 nucleotides) ds-RNAs that regulate gene expression [56]. Dicer recognizes the secondary structure of the elongated CAG repeat, which resembles the hairpin structure of its normal substrates [57]. It then processes the elongated CAG repeat into short, pure CAG RNAs of about 21 nucleotide length (sCAG RNAs) in a CAG repeat length dependent manner [58]. The authors could furthermore show that the levels of these sCAG RNAs were increased in post mortem brain tissue of HD patients. Since these sCAG RNAs potentially act as transcriptional repressors of other CAG containing mRNAs in the cell they can induce cytotoxicity. It is also conceivable that they are part of a feedback loop reducing the expression of *HTT* itself.

## 4.6 Mutant *HTT* Affects General Splicing

Transcriptome wide dysregulation is one of the hallmark features of HD and has been extensively studied in HD models and *post mortem* HD brains [59]. Historically, the striatum has been considered to be the most affected tissue.

However, transcriptional dysregulation is qualitatively very similar between different tissues (caudate nucleus, frontal cortex and cerebellum) in *post mortem* brains of HD patients, although its effect size is different [54]. An interesting possibility how mutant HTT could induce transcriptional dysregulation has been raised by the group of Jang-Ho Cha [60]. They found that HTT was bound to promoter DNA in a CAG repeat length dependent manner. Although wild type HTT also occupied some promoters, mutant HTT bound more strongly and to promoters of different genes. The elongated polyQ region is sufficient to confer DNA binding competence on exon 1 of HTT. The DNA binding competence seems to distort DNA structure, as well as interfere with the binding of other transcription factors.

As mentioned before, abnormal binding of the general splicing factor SRSF6 to the elongated CAG repeat is an important step in the induction of incomplete splicing of *HTT* mRNA and production of exon 1 HTT. Furthermore, MBNL1 and possibly U2AF2 bind to the elongated CAG repeat and regulate the localization of *HTT* mRNA. It is therefore easy to imagine that the mutant transcript sequesters additional RNA binding factors leading to attenuation of their function and eventually splicing changes. SRSF6 is, besides its role in *HTT* splicing, is implicated in the alternative splicing of tau where it regulates the inclusion of exon 10 coding for the fourth microtubule binding domain of the microtubule associated protein tau (MAPT, henceforth tau) [61]. Tau is a protein that is essential to stabilize microtubules, especially in the brain and its function is closely tied into its phosphorylation state [62]. There is a large, diverse group of diseases linked to the function and dysfunction of tau, the tauopathies, in which Alzheimer's disease is the most prominent one. In most tauopathies, toxicity is induced by tau forming self-propagating fibrils. However, dysregulation of its microtubule stabilizing function also leads to disease. The four repeat tau isoforms (tau 4R) exhibit a higher propensity to stabilize microtubules than the three repeat iso forms (tau 3R). A switch in the ratio of the 3R to 4R isoforms is sufficient to cause neurodegeneration [63]. This switch could contribute to disease pathogenesis in HD, as a greater amount of the four repeat isoforms and filamentous tau structures were observed in the striatum and cortex of HD patients [64]. Furthermore, it has recently been shown that an aberrant interaction of tau with mutated HTT in vitro and in HD mouse models led to hyperphosphorylation and mis-localization of tau [65]. Intriguingly, the same phenomenon has been observed in HD patients [66]. The authors also proposed that mutations in the *MAPT* gene are a clinical risk factor for increased disease progression as measured by cognitive decline; however, not for the age of onset of HD.

An indirect way of analysing the dysregulation of splicing changes and identifying the causative factors is motif analysis of transcriptome wide alternative splicing events [67]. Lin and colleagues used transcriptome wide sequencing data from the BA4 region of HD patients and controls to map alternative splicing events in HD. Bioinformatics analysis revealed a list of 15 potential regulatory factors,

including SRSF6, many of which are themselves dysregulated in HD. Another factor, polypyrimidine tract binding protein 1 (PTBP1), involved in repression of neuronal specific genes, was transcriptionally dysregulated in grade 2 [68] HD brains.

## 4.7 Mutant HTT Affects the Balance of Non-coding RNAs

In addition to the gain of novel interactions through the mutated polyQ domain, mutant huntingtin also loses the capability to bind to some of its normal interacting proteins. One example is the loss of interaction with REST (R element-1 silencing transcription factor) [69, 70]. REST is usually kept in an inactive state in the cytoplasm through its sequestration into a complex including wild type HTT [71, 72]. The loss of binding to the inhibitory complex leads to translocation of REST into the nucleus and repression of a variety of neuronal genes, amongst those e.g. neurotrophic factors like brain derived neurotrophic factor (BDNF) [73, 70]. Ever since the identification of BDNF dysregulation in HD [74], it has been a prominent target of investigations, partly because it could explain the higher vulnerability of neurons observed in HD. Increasing the levels of BDNF in the forebrain [75, 76] and inhibiting REST [77, 78] has conferred some therapeutic value in models of HD.

Abnormal regulation of REST is also linked to changes in microRNA (miRNA) levels in HD [79, 80]. MicroRNAs are non-coding RNAs that can bind to other transcripts and induce degradation of the miRNA/RNA hybrid, thus representing an important post-transcriptional regulatory mechanism [81]. They are important regulators of a variety of genes that are dysregulated in HD, e.g. genes encoding for synaptic proteins [82]. Dysregulation of various miRNA species has been observed in HD mouse models [83], monkeys [84] and more recently in human *post mortem* brains [85–87] and in peripheral tissue [88, 89]. However, maybe due to technical difficulties, or representing a real variance in the expression levels of the population, the overlap between the individual studies is only small and in need of further evaluation.

MicroRNAs and other small non-coding RNAs need to be in a protein complex with argonaute proteins to be catalytically active [90]. A pull-down experiment of wild type or mutated HTT expressed in a HELA cell line showed co-purification of two argonaute proteins (AGO1, AGO2) independent of the elongated CAG repeat [91]. Moreover, HTT co-localized with AGO2 in cytoplasmic foci, so called processing-bodies or p-bodies. P-bodies are a rendezvous point for ribonucleoprotein complexes and accessory factors playing a role in mRNA surveillance, degradation or silencing [92]. Despite its co-localization with p-bodies, the possible roles of HTT for p-body assembly, function or mRNA stability remain unknown.

## 4.8 Mutant *HTT* Affects Translation

Interestingly, the expanded CAG repeat in the *HTT* mRNA can act as a binding platform for a protein complex that enhances the translation of the mutant mRNA [93]. This regulatory complex consists of midline 1 (MID1), protein phosphatase 2A (PPP2A) and ribosomal protein S6 kinase (S6 K). Krauss and colleagues could show that binding of this complex was CAG repeat length dependent and its stimulatory effect on translation increased with CAG repeat length. Furthermore, a knockdown of MID1 resulted in decreased protein levels of mutant HTT, suggesting that targeting this mechanism could prove to be a valuable therapeutic approach. Expanded, repetitive stretches of nucleotides confuse the translation machinery in a fascinating way. They lead to so called repeat associated non-ATG (RAN) translation, where the ribosome starts translating from the expanded repeat in all possible reading frames creating proteins with different amino-acid expansions [94]. This phenomenon was discovered in spinocerebellar ataxia type 8 [95], but has now been described for a multitude of repeat expansion diseases [94], including for HD very recently [96]. The group could show that all four possible RAN proteins—polyAla (sense and antisense), polySer (sense), polyCys (antisense) and polyLeu (antisense)—were present in post mortem brain tissue of HD patients and a HD mouse model (N171 [97]; expresses the first 171 amino acids of human HTT with about 82 glutamines. The production was CAG repeat length dependent and the expression levels correlated with the severity of HD symptoms in the different tissues. Since several of these RAN proteins are implicated in disease [94] and some of them like polyAla show higher toxicity than polyGln in cell models [96], it is likely that they also contribute to HD pathogenesis. The extent of their contribution however, needs to be addressed.

## 4.9 Summary

Classically, polyglutamine expansion diseases like HD are categorized as ‘proteinopathies’, disorders in which abnormally folded proteins cause the disease by loss-of-function and/or gain-of-toxic-function mechanisms. It is without doubt that pathological symptoms of HD are caused by the mutant HTT protein, however, as described in this book chapter, HD shares some features with disorders in which the pathology is caused by mutations in non-coding regions, in particular through RNA gain-of-toxic-function mechanisms. Members of these ‘RNAopathies’ include myotonic dystrophy type 1 (DM1) and type 2 (DM2) [98] and the repeat expansion in *C9orf72*, which is the most common cause of familial and sporadic ALS and frontotemporal lobar degeneration [99, 100]. An unusual feature in HD is that *HTT* pre-mRNA splicing itself is altered in a way that multiple shorter versions of the HTT are produced; amongst these, exon 1 HTT is the most toxic N-terminal fragment. Understanding the underlying molecular mechanisms of RNA related

pathology in HD will certainly help to gain novel insights into the processes that cause and drive the disease. In the future these findings could then help to design new drugs and avenues of clinical intervention to treat Huntington's disease.

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