Human Molecular Genetics, 2011, Vol. 20, Review Issue 2 ***R116–R123***
doi:10.1093/hmg/ddr343
Advance Access published on August 5, 2011

Myotonic dystrophy, when simple repeats reveal complex pathogenic entities: new findings and future challenges

Géraldine Sicot, Geneviève Gourdon and Mário Gomes-Pereira*

INSERM U781, Université Paris Descartes, Hôpital Necker Enfants Malades, 156 rue de Vaugirard, 75730 Paris Cedex 15, France

Received June 30, 2011; Revised June 30, 2011; Accepted August 2, 2011

Expanded, non-coding RNAs can exhibit a deleterious gain-of-function causing human disease through abnormal interactions with RNA-binding proteins. Myotonic dystrophy (DM), the prototypical example of an RNA-dominant disorder, is mediated by trinucleotide repeat-containing transcripts that deregulate alternative splicing. Spliceopathy has therefore been a major focus of DM research. However, changes in gene expression, protein translation and micro-RNA metabolism may also contribute to disease pathology. The exciting finding of bidirectional transcription and non-conventional RNA translation of trinucleotide repeat sequences points to a new scenario, in which DM is not mediated by one single expanded RNA transcript, but involves multiple pathogenic elements and pathways. The study of the growing number of human diseases associated with toxic repeat-containing transcripts provides important insight into the understanding of the complex pathways of RNA toxicity. This review describes some of the recent advances in the understanding of the molecular mechanisms behind DM and other RNA-dominant disorders.

INTRODUCTION

Myotonic dystrophy (DM), a paradigm disease of RNA toxicity, is caused by a non-coding mutation. Toxic RNA transcripts accumulate in nuclear inclusions, interfering with the activity, localization and/or steady-state levels of RNA-interacting proteins. The most broadly accepted mechanism of disease predicts that toxic RNAs deregulate the splicing programme of a subset of developmentally regulated genes in multiple tissues, resulting in a truly multisystemic condition (1). However, recent findings suggest that DM molecular pathogenesis may be vastly more complex, going beyond spliceopathy, involving changes in gene expression and translation efficiency, antisense transcripts, non-conventional translation and micro-RNA (miRNA) deregulation. In this review, we will discuss these fascinating findings and their implications for the understanding of DM and other toxic RNA diseases.

DM: PARADIGM OF RNA TOXICITY

DM is a highly multisystemic neuromuscular disease characterized by a wide range of clinical manifestations, such as myotonia, muscle weakness and wasting, cardiac arrhythmias, cognitive and behavioural deficits, lens opacities, insulin resistance and other variable symptoms (2). Two DM loci are associated with two types of the disease. DM type 1 (DM1) is caused by the expansion of an unstable CTG trinucleotide repeat in the 3′ untranslated region of the DM protein kinase (*DMPK*) gene (3,4). The DM type 2 (DM2) mutation consists in the expansion of an unstable CCTG tetranucleotide within the first intron of the CCHC-type zinc finger, nucleic acid-binding protein (*CNBP*) gene (previously named myotonia) (5).

Longer CTG repeat expansions are associated with more severe symptoms and earlier disease onset (6). Nevertheless, the size of the repeat *per se* cannot explain disease variability, as illustrated by the clinical variability introduced by the presence of sequence interruptions within the DM1 CTG repeat tract (7,8).

Expanded CTG DNA repeats are highly dynamic, showing a marked tendency to further repeat gain in intergenerational transmissions and in somatic cells (reviewed in 9). The CCTG repeat is also unstable in the germline and somatic tissues (5).

Although genetically distinct, DM1 and DM2 share a common pathogenic mechanism.

*To whom correspondence should be addressed. Tel: +33 140615334; Fax: +33 140615445; Email: mario.pereira@inserm.fr

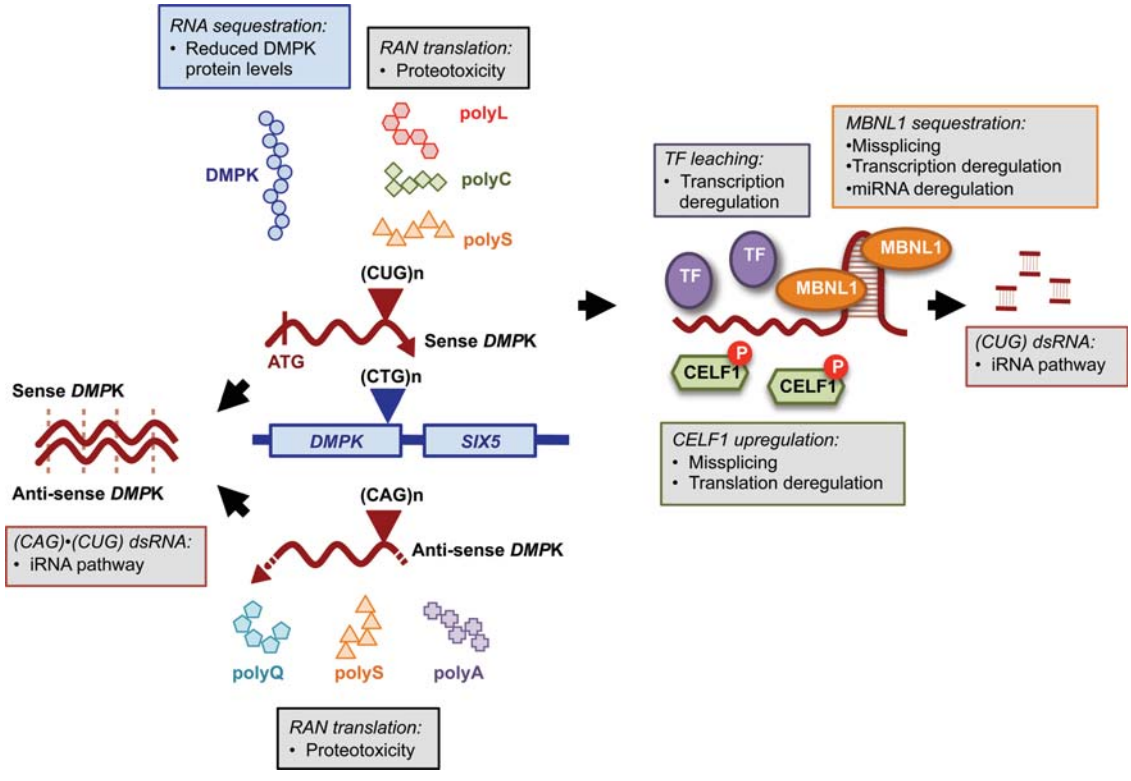

Figure 1. Molecular pathogenesis of DM1: mechanisms of RNA toxicity, spliceopathy, deregulation of gene expression and proteotoxicity. The expanded *DMPK* gene is transcribed into sense and anti-sense transcripts. CUG-containing *DMPK* transcripts form alternative RNA structures and accumulate in the nucleus of DM1 cells, resulting in reduced DMPK protein levels. Loss of function of MBNL1 (and other members of the MBNL protein family), through sequestration into RNA foci, deregulates not only alternative splicing, but also transcription and miRNA metabolism. CELF1 hyperphosphorylation and upregulation affect alternative splicing and translation efficiency. Leaching of transcription factors (TF) by expanded *DMPK* transcripts may mediate changes in gene expression. iRNA pathways might be activated by the processing of dsRNA structures, which can result from the folding of CUG-containing transcripts into hairpin structures, or from the hybridization of complementary sense and anti-sense *DMPK* transcripts. Both sense and anti-sense *DMPK* transcripts might be RAN-translated in all possible reading frames generating homopolymers, which might be deleterious to the cell.

Mechanisms of DM spliceopathy

Experimental evidence supports an RNA gain-of-function mechanism of expanded transcripts in DM1 and DM2 (1,10,11). Expanded CUG-containing transcripts interfere with RNA–protein interactions, resulting in decreased muscleblind (MBNL) and increased CUGBP/Elav-like family member 1 (CELF1) activities. MBNL proteins are depleted from the nucleoplasm through recruitment into ribonuclear foci (12–14). CELF1 stabilization by PKC phosphorylation results in increased steady-state levels and protein upregulation (15). A combined effect of decreased MBNL and increased CELF1 activity disrupts a tightly regulated developmental programme, leading to inappropriate expression of embryonic splicing isoforms in adult tissues (1,10) (Fig. 1). hnRNP H is also increased in DM1 cells, possibly contributing to the missplicing of target genes (16). In DM2, splicing abnormalities (1,17) are also associated with the sequestration of MBNL proteins by expanded transcripts (18). Although not detected in DM2 muscle biopsies (13), CELF1 is upregulated in the liver of DM2 transgenic mice (19).

RNA toxicity in other repeat expansion disorders

The molecular pathogenesis of spinocerebellar type 8 (SCA8), SCA10, SCA12, Huntington disease like 2 (HDL2) and fragile X tremor ataxia syndrome (FXTAS) likely involve toxic RNAs and abnormal RNA–protein interactions (Table 1).

The SCA8 CAG•CTG expansion is bi-directionally expressed. The CAG-containing *ATXN8* transcript produces polyglutamine tracts (20). As in DM1, the CUG-containing antisense *ATXN8OS* transcripts accumulate in nuclear foci, sequestering MBNL1 in selected neuronal populations and affecting alternative splicing and gene expression (21). The precise contribution of the polyglutamine peptide and the CUG toxicity to SCA8 pathology remains to be determined.

In FXTAS and HDL2, MBNL1 is also sequestered into intranuclear RNA inclusions, resulting in abnormal splicing (22–24). The recruitment of RNA-interacting proteins (Purα, hnRNP A2/B1 and Sam68) into FXTAS RNA inclusions might also contribute to disease (23,25,26). RNA toxicity in FXTAS is corroborated by the neuropathology of mice expressing CGG repeats within the murine *Fmr1* context (27,28) or in an unrelated gene (29).

SCA10 patients and transgenic mice expressing intronic ATTCT expansions in an unrelated gene accumulate nuclear and cytoplasmic foci. hnRNP K sequestration and loss of function disrupt RNA splicing (30), supporting a *trans*-dominant role of AUUCU RNA repeats. A toxic effect of non-coding CAG repeats has also been proposed in SCA12 (31,32).

Table 1. Toxic RNA diseases, protein recruitment to RNA foci and splicing abnormalities

Disease	RNA foci	Protein co-localization	CELF1	Missplicing	References
DM1	Nuclear	MBNL1 MBNL2 MBNL3 hnRNP H	Upregulated	Yes	(1,10,16,18,81,82)
DM2	Nuclear	MBNL1 MBNL2 MBNL3	Conflicting results	Yes	(1,5,13,18,19,79)
FXTAS	Nuclear	MBNL1 Sam68 hnRNP A2/B1 Purα	N/D	Yes	(23,26,83)
SCA8	Nuclear	MBNL1	N/D	Yes	(21)
SCA10	Nuclear Cytoplasmic	hnRNP K	N/D	Yes	(30)
SCA12	N/D	N/D	N/D	N/D	(31)

N/D, not determined.

In spite of shared key elements, the detailed mechanisms of RNA toxicity likely differ between individual repeat expansion disorders.

Toxic CAG repeats: it is not all about polyglutamines

Disease-causing CAG expansions are usually located within open-reading frames and result in a toxic gain-of-function of the expanded polyglutamine tract (33). The eye and neurological phenotypes of transgenic *Drosophila* expressing untranslated CAG-containing transcripts brought a new pathogenic element on stage: toxic CAG RNA repeats (34). CAG RNA can form small foci in a limited number of cells without affecting alternative splicing (34,35). MBNL1 interacts with CAG repeats and is sequestered into RNA foci in culture (35). In contrast to CUG toxicity, MBNL1 overexpression enhances CAG toxicity in CAG-expressing transgenic flies, whereas muscleblind deletion prolongs their lifespan (34). Given the protective effect of MBNL1 upregulation in DM1 transgenic mice expressing CUG repeats (36), these results suggest that different mechanisms operate in CUG and CAG RNA toxicity. It is conceivable that MBNL1 stabilizes CAG-containing pathogenic transcripts, increasing the steady-state levels of polyglutamine peptides.

The introduction of CAA interruptions within the expanded CAG sequence mitigates RNA toxicity in flies and abolishes phenotype enhancement by MBNL1 overexpression (34). These results are in line with a pathogenic role of the alternative structures adopted by pure CAG tracts, which may participate in deleterious protein–RNA interactions.

BREAKING THE RULES: ANTI-SENSE TRANSCRIPTION AND NON-CANONICAL TRANSLATION

The far-reaching consequences of the DM1 repeat expansion on cell biology have been illustrated by some exciting findings, which suggest that simple repeats are in fact increasingly complex.

Anti-sense *DMPK* transcription

Natural anti-sense transcripts are transcribed from the opposite DNA strand to a sense, usually protein-coding, strand and overlap in part with sense RNA. Both sense and antisense RNAs can encode proteins or be non-coding transcripts. In the mammalian genome, the most prominent form of anti-sense transcripts is a non-coding RNA partner of a protein-coding sense transcript (37). It is estimated that >70% of the mammalian genome is transcribed in both sense and anti-sense strands (38). Recently, anti-sense transcripts have been implicated in diverse transcriptional and post-transcriptional gene regulatory mechanisms operating in a wide variety of biological functions, such as the regulation of transcription, RNA–DNA interactions, RNA–RNA interactions in the nucleus, as well as RNA–RNA interactions in the cytoplasm.

In DM1, an anti-sense *DMPK* transcript emanating from the adjacent *SIX5* regulatory region has been described (39). This anti-sense transcript extends into the insulator element described in the 3′ region of *DMPK*, located between *DMPK* and *SIX5*. It has been proposed that the processing of anti-sense *DMPK* RNA into 21-nucleotide fragments might be involved in the regulation of the environmental chromatin structure. At the wild-type DM1 locus, CTCF-binding sites located on each side of the CTG repeat restrict the extent of the anti-sense RNA. In contrast, in congenital DM1 fibroblasts, the expanded allele is associated with loss of CTCF binding, spread of heterochromatin and regional CpG methylation. An attractive hypothesis resulting from this work is that insulator loss of function, due to impaired CTCF binding, results in higher *DMPK* expression in late embryogenesis, as a consequence of the high *SIX5* enhancer activity, thereby contributing to the earlier disease phenotype in congenital DM1.

Recently, bidirectional transcription of (CTG)•(CAG) repeat expansions was shown to stimulate repeat instability in human fibroblasts carrying 800 CTG (40), implying a contribution to somatic instability of highly expanded (CTG)•(CAG) repeats.

Identification of anti-sense transcripts and CTCF-binding sites flanking (CTG)•(CAG) repeats at other disease loci suggests that the features described at the DM1 locus might be present at other repeat-associated loci in the genome (41–43).

Table 2. miRNA and DM1 pathophysiology

mi-RNA	Physiological roles	Deregulation in DM1 tissues	References
miR-1 (miR-1a, miR-1b)	Myogenesis Cardiogenesis Control of cardiac conduction Inhibition of proliferation	Possible upregulation in DM1 skeletal muscle (conflicting results) Downregulation in DM1 heart Altered subcellular localization in DM1 myofibres	(56–58)
miR-29b, miR-29c	Cardiac adaption to stress	Downregulation in DM1 skeletal muscle	(56)
miR-33	Cholesterol metabolism Cell proliferation	Downregulation in DM1 skeletal muscle	(56,84)
miR-133 (miR-133a, miR-133b)	Myogenesis Control of cardiac conduction Muscle hypertrophy Apoptosis	Altered subcellular localization of miR-133b in skeletal muscle	(56)
miR-206	Myogenesis	Upregulation in DM1 skeletal muscle Altered subcellular localization in DM1 myofibres	(56,57)
miR-335	Muscle regeneration	Upregulation in DM1 skeletal muscle	(56)

Repeat-associated non-ATG translation

The non-canonical RNA translation of trinucleotide repeat-expanded transcripts unveiled other potential pathogenic entities. Repeat-associated non-ATG (RAN) translation occurs independently of an ATG initiation codon in all reading frames. RAN translation operates on CAG-containing transcripts to produce polyglutamine, polyserine and polyalanine homopolymeric peptides. RAN-translated peptides were detected in SCA8 mouse models and in the human cerebellum. In addition, antisense *DMPK* transcripts can also be RAN-translated to generate polyglutamine homopolymeric peptides in DM1 mouse models and in DM1 myoblasts, skeletal muscle and leucocytes (44). It is also possible that sense *DMPK* transcripts are RAN-translated to generate long polyleucine, polycysteine and polyalanine tracts (45) (Fig. 1). RAN translation can also operate on CAG repeats embedded in the HD, HDL2 and SCA3 flanking context (44).

The mechanism of RAN translation, as well as its contribution to disease, require future studies. The question is particularly challenging given the confounding and simultaneous effects of RNA and protein toxicity in the cell. In the case of SCA8, for instance, it will be important to distinguish between proteotoxicity resulting from orthodox ATG-dependent polyglutamine translation and proteotoxicity resulting from RAN-translated homopolymers. Preliminary evidence of a possible pathogenic role comes from the co-localization of RAN-translated peptides with indicators of apoptosis in transfected cells and transgenic DM1 mice (44). Cell culture and transgenic mouse model systems will help address these and other questions.

THE DM WORLD BEYOND SPLICEOPATHY

Misregulation of alternative splicing plays a central role in the development of important DM1 symptoms (1,10). However, DM1 mutation can affect gene expression in multiple ways. First, although primarily considered splicing regulators, MBNL and CELF proteins participate in mRNA transport, stability and translation (46,47). Their altered activity and/or localization in DM1 cells may alter transcription, translation and cell signalling. Second, the expanded CTG transcripts may affect the levels, availability and subcellular localization of transcription factors. Third, the disease may disturb highly regulated pathways of miRNA and double-stranded RNA processing (Fig. 1).

Deregulation of transcriptional factors

The effects of repeat expansion on gene expression were revealed by microarray analysis of DM1 and DM2 muscle biopsies (48), and confirmed by the mRNA profiling of transgenic mice expressing non-coding CUG repeats in skeletal muscle. Interestingly, most of the changes detected were also found in *Mbnl1* knock-out mice, indicating that transcriptional deregulation results, at least partially, from MBNL1 loss of function (49).

Altered gene expression may also result from a direct effect of the repeat expansion on transcription factors. Expanded *DMPK* transcripts bind SP1 and RARγ in DM1 myocytes, leaching them out of the chromatin, reducing their availability and the expression of target transcripts (50). NKX2-5 upregulation in DM1 skeletal muscle and heart affects the expression of downstream genes, possibly contributing to the muscle regenerative defects and cardiac conduction abnormalities (51). The mislocalization of SHARP (SMART/HDAC1-associated repressor protein) in the cytoplasm in DM1 myoblasts may alter the steady-state levels of a set of RNA transcripts implicated in muscle development and function (52).

The absence of co-localization of transcription factors with CUG-containing nuclear foci does not exclude a pathogenic role. It is conceivable that a fraction of expanded *DMPK* transcripts does not aggregate and remains soluble in DM1 cells, interacting with splicing regulators and transcription factors, thereby contributing to pathology (53). To this respect, it would be interesting to determine the extent of the soluble fraction of expanded *DMPK* transcripts and explore their pathogenic interactions.

miRNAs deregulation

MicroRNA deregulation can participate in muscular dystrophy and cardiomyopathies (54,55). In DM1, miRNAs appear to be affected in skeletal muscle and heart tissue (Table 2) (56–58).

Interestingly, mi-R1 downregulation in DM1 and DM2 hearts is mediated by the functional depletion of MBNL1, which affects the misprocessing of pre-miR-1 (58). Whether MBNL1 regulates the processing of other miRNAs remains to be determined.

DM-associated miRNA deregulation alters the expression of target transcripts, possibly contributing to disease pathology (56,58). The investigation of additional targets will help evaluate the pathophysiological impact of miRNA deregulation in DM1 skeletal muscle, heart and other tissues. It is noteworthy that some miRNAs deregulated in DM1 have also been identified in Duchenne muscular dystrophy and other muscular disorders (54), raising questions about the causative-consequential relationship between miRNA metabolism and muscular pathology.

miRNA changes have also been reported in other trinucleotide expansion disorders, such as Huntington disease (59) and SCA1 (60). The detailed understanding of the role of miRNAs in human disease will open new routes for their therapeutic manipulation.

RNA interference and double-stranded RNA pathways

In DM1 cells, CUG-containing RNAs form imperfect hairpin structures, which can be cleaved by the RNA interference (RNAi) machinery to generate CUG-containing small interference RNAs (siRNAs). CUG-derived siRNAs are capable of binding complementary sequences in target mRNAs, possibly interfering with their expression and contributing to disease pathogenesis (61). In contrast to the generation of siRNAs by hairpin-forming CUG RNA transcripts alone, the requirement of simultaneous expression of CUG and CAG has been suggested, following the development of transgenic DM1 *Drosophila*. The expression of non-coding CTG repeats in flies results in nuclear RNA foci accumulation, missplicing and eye phenotypes. The phenotype is enhanced by the co-expression of non-coding CUG and CAG expansions, supporting a pathogenic role for CUG and CAG interaction mediated by RNAi pathways. Co-expressed CUG and CAG-containing transcripts are processed into small triplet repeat-derived siRNAs capable of targeting downstream transcripts containing small CUG or CAG stretches (62). Blocking siRNA biogenesis alleviates repeat toxicity in flies co-expressing CUG and CAG RNA repeats.

In addition to the regulation of local chromatin reorganization and gene expression in the DM1 locus (39), anti-sense *DMPK* transcripts may act as *trans*-modifiers of the expression of other genes, through the activation of RNAi pathways, contributing to disease pathogenesis. RNAi targets relevant to DM1 pathogenesis remain to be identified.

Looking outside the nucleus: cell stress and translation inhibition

DM1 molecular pathogenesis may not be confined to the nucleus. CELF1-overexpressing mice recreate abnormal steady-state levels of MEF2A and p21 found in DM1 skeletal muscle (63), suggesting that CELF1 upregulation contributes to disease not only through splicing disruption in the nucleus, but also through stress-induced inhibition of translation in the cytoplasm (64). Cell stress and associated repression of protein translation may be relevant to the disease phenotype. In line with this view, polyubiquitin/proteasome stress-signalling pathways are activated in the skeletal muscle of a transgenic DM1 mouse model, showing progressive muscle atrophy (65).

Interestingly, MBNL1 may also participate in the cytoplasmic response to cell stress, given its accumulation in cytoplasmic stress granules (66). The cytoplasmic aspects of DM1 molecular pathogenesis have probably been overlooked and require additional studies.

REMAINING QUESTIONS AND FUTURE PERSPECTIVES

Our understanding of DM1 is improving fast, with the help of transgenic mouse models (11). Nevertheless, there are some key aspects of the disease that deserve further investigation.

Disease mechanisms in CNS

The neurological manifestations of DM1 have a dramatic impact on the quality of life, but this aspect of the disease was overlooked in the past. Our understanding of DM1 neuropathophysiology is falling behind, when compared with muscle and cardiac manifestations.

Expanded *DMPK* transcripts accumulate in the nucleus of cortical and subcortical neurons, sequestering MBNL1 and disrupting alternative splicing (12). MAPT/TAU missplicing results in the accumulation of pathological protein isoforms (67), which are usually hyperphosphorylated and accumulate in neurofibrillary tangles (68), supporting the classification of DM1 as a tauopathy. The pathophysiological consequences of MAPT/TAU hyperphosphorylation in DM1 require further studies. It is unclear whether brain spliceopathy is sufficient to fully explain DM neurological dysfunction; nor do we know whether the underlying mechanisms of missplicing are shared between the CNS, skeletal muscle and heart.

Other pathways might contribute to brain pathophysiology. Abnormal expression of microtubule-associated proteins (69) and altered functional expression of calcium channels (70) may account for disrupted neurite outgrowth in DM1 neuroblastoma cell models (71). Abnormal neuritogenesis has also been reported in pluripotent stem cell lines derived from DM1 embryos, and associated with downregulation of members of the *SLITRK* gene family (72). Transgenic mouse models will help elucidate the contribution of impaired neuronal differentiation to adult and congenital DM1.

In addition to the molecular signs of RNA toxicity, DM brains exhibit structural abnormalities in white and grey matter (73). In adult DM1 and DM2 patients, the parallel analysis of brain structure and cognitive dysfunction suggests that specific region-specific changes might explain neuropsychological deficits (74). In congenital and juvenile DM1, the overall extent of widespread white matter lesions correlates with cognitive dysfunction (75). In addition to structural changes, hypoperfusion and hypometabolism in frontal lobe may contribute to the frontal pattern of cognitive impairment in DM1 (76). Mapping the structural, cellular and metabolic

changes in DM brains, from an early age and throughout disease development, will help the challenging establishment of clinical and physiological correlations between brain abnormalities and neuropsychological manifestations.

Mechanisms behind the congenital DM1

DM1 patients carrying >500 CTG often develop the severe congenital form of the disease, mainly characterized by general hypotonia at birth and mental retardation. Splicing deregulation has been reported in congenital DM1 (77), but the mechanisms behind this form of the disease require further studies. Although *Mbnl1* knock-out mice did not re-create congenital phenotypes, CELF1 overexpression resulted in embryonic lethality and impaired mouse development (11). A role of CELF1 in congenital DM1 has therefore been suggested. In contrast, normal CELF1 levels in DM2 could explain, at least partially, the possible lack of congenital DM2 (13). Alternatively congenital DM1 might be mediated by increased expression of CUG-containing repeats in early stages of development as a result of the functional loss of the genetic insulator between *DMPK* and the downstream *SIX5* gene (39). Transgenic mice carrying large CTG repeat expansions within the genomic environment of the human DM1 locus (78) express anti-sense *DMPK* transcripts (44) and will help test this hypothesis.

Differences between DM1 and DM2

Clinical signs in DM1 and DM2 are similar, but there are some distinguishing features: DM2 is generally less severe and lacks a prevalent congenital form. Disease-specific manifestations may result from differences in spatial and temporal expression patterns of *DMPK* and *CNBP* genes. Similarly, changes in the expression of neighbouring genes may define disease-specific manifestations. RNA-interacting proteins may also act as modifiers of disease-specific phenotypes, through different affinities of MBNL1, CELF1 and other RNA-binding proteins for CUG versus CCUG RNA repeats. The role of CELF1 in DM2 is particularly intriguing with contradictory results being reported (13,19,79). It remains possible that CELF1 upregulation depends on the repeat flanking sequence, contributing to the singularity of the DM1 versus DM2.

In the wider context of toxic RNA diseases, the spatial (and temporal) expression profiles of expanded transcripts and their interacting proteins may delineate the susceptibility of specific cell populations to RNA toxicity and define disease-specific pathology.

Therapy development

Targeting toxic transcripts and their interactions provides a promising therapeutic approach in DM and other toxic RNA diseases (80). Nonetheless, additional strategies can be envisioned (11). As our understanding of the disease improves, it becomes clear that in addition to the reversal of missplicing, correction of gene expression will be required to fully rescue DM1 pathology. The implication of miRNAs, bidirectional transcription, RAN translation and cell stress-associated deregulation of translation in DM1 pathology does not invalidate neutralization of expanded transcripts. Instead, it imposes a requirement for therapeutic refinement to achieve maximum efficacy.

CONCLUSIONS

RNA toxicity mediates DM and other microsatellite expansion disorders. Key disease manifestations result from the missplicing of a subset of transcripts. However, spliceopathy may not fully explain the multisystemic disease spectrum. Disease mechanisms likely involve changes in gene transcription and translation.

Our understanding of RNA toxicity is expanding. Additional diseases that fit into the category of dominant toxic RNA disorders are likely to be identified. Recently, a toxic role of RNA transcripts was proposed in diseases previously regarded as entirely mediated by polyglutamine toxicity. Therefore, concomitant pathways of RNA and protein toxicity can operate simultaneously in the cell. Inversely, RAN translation of triplet repeat-containing transcripts implies proteotoxicity in diseases typically mediated by toxic RNA molecules.

In summary, the emerging pathways of molecular pathogenesis are far more complex than previously appreciated, but we believe to be in the right track towards the development of effective therapies.

Conflict of Interest statement. None declared.

FUNDING

Our work is supported by grants from AFM (Association Française contre les Myopathies, France), ANR (Agence Nationale de Recherche, France) and INSERM (Institut National de la Santé et Recherche Médicale, France). G.S. is funded by a PhD fellowship from the French Ministry for Higher Education and Research.

REFERENCES

1. Ranum, L.P. and Cooper, T.A. (2006) RNA-mediated neuromuscular disorders. *Annu. Rev. Neurosci.*, **29**, 259–277.
2. Harper, P.S. (2001) *Myotonic Dystrophy*. London: WB Saunders.
3. Brook, J.D., McCurrach, M.E., Harley, H.G., Buckler, A.J., Church, D., Aburatani, H., Hunter, K., Stanton, V.P., Thirion, J.P., Hudson, T. *et al.* (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell*, **69**, 385.
4. Mahadevan, M., Tsilfidis, C., Sabourin, L., Shutler, G., Amemiya, C., Jansen, G., Neville, C., Narang, M., Barcelo, J., O'Hoy, K. *et al.* (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science*, **255**, 1253–1255.
5. Liquori, C.L., Ricker, K., Moseley, M.L., Jacobsen, J.F., Kress, W., Naylor, S.L., Day, J.W. and Ranum, L.P. (2001) Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science*, **293**, 864–867.
6. Ashizawa, T. and Sarkar, P.S. (2011) Myotonic dystrophy types 1 and 2. *Handb. Clin. Neurol.*, **101**, 193–237.
7. Braida, C., Stefanatos, R.K., Adam, B., Mahajan, N., Smeets, H.J., Niel, F., Goizet, C., Arveiler, B., Koenig, M., Lagier-Tourenne, C. *et al.* (2010) Variant CCG and GGC repeats within the CTG expansion dramatically modify mutational dynamics and likely contribute toward unusual symptoms in some myotonic dystrophy type 1 patients. *Hum. Mol. Genet.*, **19**, 1399–1412.

8. Musova, Z., Mazanec, R., Krepelova, A., Ehler, E., Vales, J., Jaklova, R., Prochazka, T., Koukal, P., Marikova, T., Kraus, J. *et al.* (2009) Highly unstable sequence interruptions of the CTG repeat in the myotonic dystrophy gene. *Am. J. Med. Genet. A*, **149A**, 1365–1374.
9. Gomes-Pereira, M. and Monckton, D.G. (2006) Chemical modifiers of unstable expanded simple sequence repeats: what goes up, could come down. *Mutat. Res.*, **598**, 15–34.
10. Osborne, R.J. and Thornton, C.A. (2006) RNA-dominant diseases. *Hum. Mol. Genet.*, **15** (Spec no. 2), R162–R169.
11. Gomes-Pereira, M., Cooper, T.A. and Gourdon, G. (2011) Myotonic dystrophy mouse models: towards rational therapy development. *Trends Mol. Med.,* doi: 10.1016/j.molmed.2011.05.004. Epub ahead of print.
12. Jiang, H., Mankodi, A., Swanson, M.S., Moxley, R.T. and Thornton, C.A. (2004) Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons. *Hum. Mol. Genet.*, **13**, 3079–3088.
13. Lin, X., Miller, J.W., Mankodi, A., Kanadia, R.N., Yuan, Y., Moxley, R.T., Swanson, M.S. and Thornton, C.A. (2006) Failure of MBNL1-dependent post-natal splicing transitions in myotonic dystrophy. *Hum. Mol. Genet.*, **15**, 2087–2097.
14. Mankodi, A., Lin, X., Blaxall, B.C., Swanson, M.S. and Thornton, C.A. (2005) Nuclear RNA foci in the heart in myotonic dystrophy. *Circ. Res.*, **97**, 1152–1155.
15. Kuyumcu-Martinez, N.M., Wang, G.S. and Cooper, T.A. (2007) Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. *Mol. Cell*, **28**, 68–78.
16. Paul, S., Dansithong, W., Kim, D., Rossi, J., Webster, N.J., Comai, L. and Reddy, S. (2006) Interaction of muscleblind, CUG-BP1 and hnRNP H proteins in DM1-associated aberrant IR splicing. *EMBO J.*, **25**, 4271–4283.
17. Savkur, R.S., Philips, A.V., Cooper, T.A., Dalton, J.C., Moseley, M.L., Ranum, L.P. and Day, J.W. (2004) Insulin receptor splicing alteration in myotonic dystrophy type 2. *Am. J. Hum. Genet.*, **74**, 1309–1313.
18. Fardaei, M., Rogers, M.T., Thorpe, H.M., Larkin, K., Hamshere, M.G., Harper, P.S. and Brook, J.D. (2002) Three proteins, MBNL, MBLL and MBXL, co-localize *in vivo* with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells. *Hum. Mol. Genet.*, **11**, 805–814.
19. Salisbury, E., Schoser, B., Schneider-Gold, C., Wang, G.L., Huichalaf, C., Jin, B., Sirito, M., Sarkar, P., Krahe, R., Timchenko, N.A. *et al.* (2009) Expression of RNA CCUG repeats dysregulates translation and degradation of proteins in myotonic dystrophy 2 patients. *Am. J. Pathol.*, **175**, 748–762.
20. Moseley, M.L., Zu, T., Ikeda, Y., Gao, W., Mosemiller, A.K., Daughters, R.S., Chen, G., Weatherspoon, M.R., Clark, H.B., Ebner, T.J. *et al.* (2006) Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. *Nat. Genet.*, **38**, 758–769.
21. Daughters, R.S., Tuttle, D.L., Gao, W., Ikeda, Y., Moseley, M.L., Ebner, T.J., Swanson, M.S. and Ranum, L.P. (2009) RNA gain-of-function in spinocerebellar ataxia type 8. *PLoS Genet.*, **5**, e1000600.
22. Rudnicki, D.D., Holmes, S.E., Lin, M.W., Thornton, C.A., Ross, C.A. and Margolis, R.L. (2007) Huntington's disease-like 2 is associated with CUG repeat-containing RNA foci. *Ann. Neurol.*, **61**, 272–282.
23. Sellier, C., Rau, F., Liu, Y., Tassone, F., Hukema, R.K., Gattoni, R., Schneider, A., Richard, S., Willemsen, R., Elliott, D.J. *et al.* (2010) Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. *EMBO J.*, **29**, 1248–1261.
24. Tassone, F., Iwahashi, C. and Hagerman, P.J. (2004) FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). *RNA Biol.*, **1**, 103–105.
25. Khalili, K., Del Valle, L., Muralidharan, V., Gault, W.J., Darbinian, N., Otte, J., Meier, E., Johnson, E.M., Daniel, D.C., Kinoshita, Y. *et al.* (2003) Puralpha is essential for postnatal brain development and developmentally coupled cellular proliferation as revealed by genetic inactivation in the mouse. *Mol. Cell Biol.*, **23**, 6857–6875.
26. Iwahashi, C.K., Yasui, D.H., An, H.J., Greco, C.M., Tassone, F., Nannen, K., Babineau, B., Lebrilla, C.B., Hagerman, R.J. and Hagerman, P.J. (2006) Protein composition of the intranuclear inclusions of FXTAS. *Brain*, **129**, 256–271.
27. Brouwer, J.R., Huizer, K., Severijnen, L.A., Hukema, R.K., Berman, R.F., Oostra, B.A. and Willemsen, R. (2008) CGG-repeat length and neuropathological and molecular correlates in a mouse model for fragile X-associated tremor/ataxia syndrome. *J. Neurochem.*, **107**, 1671–1682.
28. Entezam, A., Biacsi, R., Orrison, B., Saha, T., Hoffman, G.E., Grabczyk, E., Nussbaum, R.L. and Usdin, K. (2007) Regional FMRP deficits and large repeat expansions into the full mutation range in a new Fragile X premutation mouse model. *Gene*, **395**, 125–134.
29. Hashem, V., Galloway, J.N., Mori, M., Willemsen, R., Oostra, B.A., Paylor, R. and Nelson, D.L. (2009) Ectopic expression of CGG containing mRNA is neurotoxic in mammals. *Hum. Mol. Genet.*, **18**, 2443–2451.
30. White, M.C., Gao, R., Xu, W., Mandal, S.M., Lim, J.G., Hazra, T.K., Wakamiya, M., Edwards, S.F., Raskin, S., Teive, H.A. *et al.* (2010) Inactivation of hnRNP K by expanded intronic AUUCU repeat induces apoptosis via translocation of PKCdelta to mitochondria in spinocerebellar ataxia 10. *PLoS Genet.*, **6**, e1000984.
31. Holmes, S.E., Hearn, E.O., Ross, C.A. and Margolis, R.L. (2001) SCA12: an unusual mutation leads to an unusual spinocerebellar ataxia. *Brain Res. Bull.*, **56**, 397–403.
32. Ranum, L.P. and Day, J.W. (2004) Pathogenic RNA repeats: an expanding role in genetic disease. *Trends Genet.*, **20**, 506–512.
33. Orr, H.T. and Zoghbi, H.Y. (2007) Trinucleotide repeat disorders. *Annu. Rev. Neurosci.*, **30**, 575–621.
34. Li, L.B., Yu, Z., Teng, X. and Bonini, N.M. (2008) RNA toxicity is a component of ataxin-3 degeneration in *Drosophila*. *Nature*, **453**, 1107–1111.
35. Ho, T.H., Savkur, R.S., Poulos, M.G., Mancini, M.A., Swanson, M.S. and Cooper, T.A. (2005) Colocalization of muscleblind with RNA foci is separable from mis-regulation of alternative splicing in myotonic dystrophy. *J. Cell Sci.*, **118**, 2923–2933.
36. Kanadia, R.N., Shin, J., Yuan, Y., Beattie, S.G., Wheeler, T.M., Thornton, C.A. and Swanson, M.S. (2006) Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy. *Proc. Natl Acad. Sci. USA*, **103**, 11748–11753.
37. Faghihi, M.A. and Wahlestedt, C. (2009) Regulatory roles of natural antisense transcripts. *Nat. Rev. Mol. Cell Biol.*, **10**, 637–643.
38. Katayama, S., Tomaru, Y., Kasukawa, T., Waki, K., Nakanishi, M., Nakamura, M., Nishida, H., Yap, C.C., Suzuki, M., Kawai, J. *et al.* (2005) Antisense transcription in the mammalian transcriptome. *Science*, **309**, 1564–1566.
39. Cho, D.H., Thienes, C.P., Mahoney, S.E., Analau, E., Filippova, G.N. and Tapscott, S.J. (2005) Antisense transcription and heterochromatin at the DM1 CTG repeats are constrained by CTCF. *Mol. Cell*, **20**, 483–489.
40. Nakamori, M., Pearson, C.E. and Thornton, C.A. (2011) Bidirectional transcription stimulates expansion and contraction of expanded (CTG)*(CAG) repeats. *Hum. Mol. Genet.*, **20**, 580–588.
41. Batra, R., Charizanis, K. and Swanson, M.S. (2010) Partners in crime: bidirectional transcription in unstable microsatellite disease. *Hum. Mol. Genet*., **19**, R77–R82.
42. Dion, V. and Wilson, J.H. (2009) Instability and chromatin structure of expanded trinucleotide repeats. *Trends Genet.*, **25**, 288–297.
43. La Spada, A.R. and Taylor, J.P. (2010) Repeat expansion disease: progress and puzzles in disease pathogenesis. *Nat. Rev. Genet.*, **11**, 247–258.
44. Zu, T., Gibbens, B., Doty, N.S., Gomes-Pereira, M., Huguet, A., Stone, M.D., Margolis, J., Peterson, M., Markowski, T.W., Ingram, M.A. *et al.* (2011) Non-ATG-initiated translation directed by microsatellite expansions. *Proc. Natl Acad. Sci. USA*, **108**, 260–265.
45. Pearson, C.E. (2011) Repeat associated non-ATG translation initiation: one DNA, two transcripts, seven reading frames, potentially nine toxic entities! *PLoS Genet.*, **7**, e1002018.
46. Pascual, M., Vicente, M., Monferrer, L. and Artero, R. (2006) The Muscleblind family of proteins: an emerging class of regulators of developmentally programmed alternative splicing. *Differentiation*, **74**, 65–80.
47. Barreau, C., Paillard, L., Mereau, A. and Osborne, H.B. (2006) Mammalian CELF/Bruno-like RNA-binding proteins: molecular characteristics and biological functions. *Biochimie*, **88**, 515–525.
48. Botta, A., Vallo, L., Rinaldi, F., Bonifazi, E., Amati, F., Biancolella, M., Gambardella, S., Mancinelli, E., Angelini, C., Meola, G. *et al.* (2007) Gene expression analysis in myotonic dystrophy: indications for a common molecular pathogenic pathway in DM1 and DM2. *Gene Expr.*, **13**, 339–351.
49. Osborne, R.J., Lin, X., Welle, S., Sobczak, K., O'Rourke, J.R., Swanson, M.S. and Thornton, C.A. (2009) Transcriptional and post-transcriptional impact of toxic RNA in myotonic dystrophy. *Hum. Mol. Genet.*, **18**, 1471–1481.

50. Ebralidze, A., Wang, Y., Petkova, V., Ebralidse, K. and Junghans, R.P. (2004) RNA leaching of transcription factors disrupts transcription in myotonic dystrophy. *Science*, **303**, 383–387.
51. Yadava, R.S., Frenzel-McCardell, C.D., Yu, Q., Srinivasan, V., Tucker, A.L., Puymirat, J., Thornton, C.A., Prall, O.W., Harvey, R.P. and Mahadevan, M.S. (2008) RNA toxicity in myotonic muscular dystrophy induces NKX2–5 expression. *Nat. Genet.*, **40**, 61–68.
52. Dansithong, W., Jog, S.P., Paul, S., Mohammadzadeh, R., Tring, S., Kwok, Y., Fry, R.C., Marjoram, P., Comai, L. and Reddy, S. (2011) RNA steady-state defects in myotonic dystrophy are linked to nuclear exclusion of SHARP. *EMBO Rep.*, **12**, 735–742.
53. Junghans, R.P. (2009) Dystrophia myotonia: why focus on foci? *Eur. J. Hum. Genet.*, **17**, 543–553.
54. van Rooij, E., Liu, N. and Olson, E.N. (2008) MicroRNAs flex their muscles. *Trends Genet.*, **24**, 159–166.
55. Latronico, M.V. and Condorelli, G. (2009) MicroRNAs and cardiac pathology. *Nat. Rev. Cardiol.*, **6**, 419–429.
56. Perbellini, R., Greco, S., Sarra-Ferraris, G., Cardani, R., Capogrossi, M.C., Meola, G. and Martelli, F. (2011) Dysregulation and cellular mislocalization of specific miRNAs in myotonic dystrophy type 1. *Neuromuscul. Disord.*, **21**, 81–88.
57. Gambardella, S., Rinaldi, F., Lepore, S.M., Viola, A., Loro, E., Angelini, C., Vergani, L., Novelli, G. and Botta, A. (2010) Overexpression of microRNA-206 in the skeletal muscle from myotonic dystrophy type 1 patients. *J. Transl. Med.*, **8**, 48.
58. Rau, F., Freyermuth, F., Fugier, C., Villemin, J.P., Fischer, M.C., Jost, B., Dembele, D., Gourdon, G., Nicole, A., Duboc, D. *et al.* (2011) Misregulation of miR-1 processing is associated with heart defects in myotonic dystrophy. *Nat. Struct. Mol. Biol.*, **18**, 840–845.
59. Johnson, R., Zuccato, C., Belyaev, N.D., Guest, D.J., Cattaneo, E. and Buckley, N.J. (2008) A microRNA-based gene dysregulation pathway in Huntington's disease. *Neurobiol. Dis.*, **29**, 438–445.
60. Lee, Y., Samaco, R.C., Gatchel, J.R., Thaller, C., Orr, H.T. and Zoghbi, H.Y. (2008) miR-19, miR-101 and miR-130 co-regulate ATXN1 levels to potentially modulate SCA1 pathogenesis. *Nat. Neurosci.*, **11**, 1137–1139.
61. Krol, J., Fiszer, A., Mykowska, A., Sobczak, K., de Mezer, M. and Krzyzosiak, W.J. (2007) Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. *Mol. Cell*, **25**, 575–586.
62. Yu, Z., Teng, X. and Bonini, N.M. (2011) Triplet repeat-derived siRNAs enhance RNA-mediated toxicity in a *Drosophila* model for myotonic dystrophy. *PLoS Genet.*, **7**, e1001340.
63. Timchenko, N.A., Patel, R., Iakova, P., Cai, Z.J., Quan, L. and Timchenko, L.T. (2004) Overexpression of CUG triplet repeat-binding protein, CUGBP1, in mice inhibits myogenesis. *J. Biol. Chem.*, **279**, 13129–13139.
64. Huichalaf, C., Sakai, K., Jin, B., Jones, K., Wang, G.L., Schoser, B., Schneider-Gold, C., Sarkar, P., Pereira-Smith, O.M., Timchenko, N. *et al.* (2010) Expansion of CUG RNA repeats causes stress and inhibition of translation in myotonic dystrophy 1 (DM1) cells. *FASEB J.*, **24**, 3706–3719.
65. Vignaud, A., Ferry, A., Huguet, A., Baraibar, M., Trollet, C., Hyzewicz, J., Butler-Browne, G., Puymirat, J., Gourdon, G. and Furling, D. (2010) Progressive skeletal muscle weakness in transgenic mice expressing CTG expansions is associated with the activation of the ubiquitin-proteasome pathway. *Neuromuscul. Disord.*, **20**, 319–325.
66. Onishi, H., Kino, Y., Morita, T., Futai, E., Sasagawa, N. and Ishiura, S. (2008) MBNL1 associates with YB-1 in cytoplasmic stress granules. *J. Neurosci. Res.*, **86**, 1994–2002.
67. Sergeant, N., Sablonniere, B., Schraen-Maschke, S., Ghestem, A., Maurage, C.A., Wattez, A., Vermersch, P. and Delacourte, A. (2001) Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. *Hum. Mol. Genet.*, **10**, 2143–2155.
68. Vermersch, P., Sergeant, N., Ruchoux, M.M., Hofmann-Radvanyi, H., Wattez, A., Petit, H., Dwailly, P. and Delacourte, A. (1996) Specific tau variants in the brains of patients with myotonic dystrophy. *Neurology*, **47**, 711–717.
69. Velazquez-Bernardino, P., Garcia-Sierra, F., Hernandez-Hernandez, O., Bermudez de Leon, M., Gourdon, G., Gomes-Pereira, M. and Cisneros, B. (2011) Myotonic dystrophy type 1-associated CTG repeats disturb the expression and subcellular distribution of microtubule-associated proteins MAP1A, MAP2, and MAP6/STOP in PC12 cells. *Mol. Biol. Rep.*, doi: 10.1007/s11033-011-0753-y. Epub ahead of print 13 May 2011.
70. Andrade, A., de Leon, M.B., Hernandez-Hernandez, O., Cisneros, B. and Felix, R. (2007) Myotonic dystrophy CTG repeat expansion alters Ca2+ channel functional expression in PC12 cells. *FEBS Lett.*, **581**, 4430–4438.
71. Quintero-Mora, M.L., Depardon, F., Waring, J., Robert, G.K. and Cisneros, B. (2002) Expanded CTG repeats inhibit neuronal differentiation of the PC12 cell line. *Biochem. Biophys. Res. Commun.*, **295**, 289–294.
72. Marteyn, A., Maury, Y., Gauthier, M.M., Lecuyer, C., Vernet, R., Denis, J.A., Pietu, G., Peschanski, M. and Martinat, C. (2011) Mutant human embryonic stem cells reveal neurite and synapse formation defects in type 1 myotonic dystrophy. *Cell Stem Cell*, **8**, 434–444.
73. Meola, G. and Sansone, V. (2007) Cerebral involvement in myotonic dystrophies. *Muscle Nerve*, **36**, 294–306.
74. Weber, Y.G., Roebling, R., Kassubek, J., Hoffmann, S., Rosenbohm, A., Wolf, M., Steinbach, P., Jurkat-Rott, K., Walter, H., Reske, S.N. *et al.* (2010) Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. *Neurology*, **74**, 1108–1117.
75. Wozniak, J.R., Mueller, B.A., Ward, E.E., Lim, K.O. and Day, J.W. (2011) White matter abnormalities and neurocognitive correlates in children and adolescents with myotonic dystrophy type 1: a diffusion tensor imaging study. *Neuromuscul. Disord.*, **21**, 89–96.
76. Romeo, V., Pegoraro, E., Squarzanti, F., Soraru, G., Ferrati, C., Ermani, M., Zucchetta, P., Chierichetti, F. and Angelini, C. (2010) Retrospective study on PET-SPECT imaging in a large cohort of myotonic dystrophy type 1 patients. *Neurol. Sci.*, **31**, 757–763.
77. Fugier, C., Klein, A.F., Hammer, C., Vassilopoulos, S., Ivarsson, Y., Toussaint, A., Tosch, V., Vignaud, A., Ferry, A., Messaddeq, N. *et al.* (2011) Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. *Nat. Med.*, **17**, 720–725.
78. Gomes-Pereira, M., Foiry, L., Nicole, A., Huguet, A., Junien, C., Munnich, A. and Gourdon, G. (2007) CTG trinucleotide repeat 'big jumps': large expansions, small mice. *PLoS Genet.*, **3**, e52.
79. Pelletier, R., Hamel, F., Beaulieu, D., Patry, L., Haineault, C., Tarnopolsky, M., Schoser, B. and Puymirat, J. (2009) Absence of a differentiation defect in muscle satellite cells from DM2 patients. *Neurobiol. Dis.*, **36**, 181–190.
80. Mulders, S.A., van Engelen, B.G., Wieringa, B. and Wansink, D.G. (2010) Molecular therapy in myotonic dystrophy: focus on RNA gain-of-function. *Hum. Mol. Genet.*, **19**, R90–R97.
81. Timchenko, N.A., Cai, Z.J., Welm, A.L., Reddy, S., Ashizawa, T. and Timchenko, L.T. (2001) RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. *J. Biol. Chem.*, **276**, 7820–7826.
82. Miller, J.W., Urbinati, C.R., Teng-Umnuay, P., Stenberg, M.G., Byrne, B.J., Thornton, C.A. and Swanson, M.S. (2000) Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. *EMBO J.*, **19**, 4439–4448.
83. Jin, P., Duan, R., Qurashi, A., Qin, Y., Tian, D., Rosser, T.C., Liu, H., Feng, Y. and Warren, S.T. (2007) Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a *Drosophila* model of fragile X tremor/ataxia syndrome. *Neuron*, **55**, 556–564.
84. Moore, K.J., Rayner, K.J., Suarez, Y. and Fernandez-Hernando, C. (2010) microRNAs and cholesterol metabolism. *Trends Endocrinol. Metab.*, **21**, 699–706.