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Review

Dysfunction of protein homeostasis in myotonic dystrophies

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Summary. Neuromuscular diseases Myotonic Dystrophies type 1 and type 2 (DM1 and DM2) are caused by unstable CTG and CCTG repeat expansions and have highly complex molecular mechanisms. DM1 is caused by the expansion of CTG repeats in the 3' UTR of the gene coding for Dystrophia Myotonica-Protein Kinase (*DMPK*). In DM2, intronic CCTG repeats are located in a gene encoding the Zinc Finger Protein 9 (*ZNF9*, also known as Cellular Nucleic Acid Binding Protein, CNBP). Both expansions cause pathologies through RNA CUG and CCUG repeats, which have toxic effects on the processing of many RNAs in the patients' tissues. The pathogenic role of CUG and CCUG repeats in the mis-regulation of alternative splicing, mediated by RNA-binding proteins CUGBP1 and MBNL1, has been discussed in a number of excellent reviews. Recent reports suggest that mutant RNA repeats affect several other RNA-binding proteins such as Staufen1 and the DEAD-box RNA helicase p68 (DDX5). Since CUGBP1, Staufen1 and p68 have many functions in cytoplasm, including regulation of protein translation, it is predicted that the alterations of these proteins in DM cells might have a toxic effect on global protein turnover. In this mini-review, we will summarize observations showing the role of RNA-binding proteins, CUGBP1 and ZNF9, in protein turnover in DM1 and in DM2. We will also discuss a possible role of misbalanced protein turnover in the age-dependent progression of DM1 and in a late onset of DM2.

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Introduction

Myotonic Dystrophy type 1 (DM1) and Myotonic Dystrophy type 2 (DM2) are complex genetic diseases, affecting many tissues with primary defects in skeletal muscle (Harper, 2001; Liquori et al., 2001). Both diseases are caused by expansions of non-coding CTG and CCTG repeats, respectively. There are four clinical forms of DM1: congenital, early childhood, adult-onset and oligosymptomatic late-onset. Adult-onset or classic DM1 is the most prevalent form among diagnosed patients. This form of DM1 is characterized by myotonia and skeletal muscle weakness and wasting. In addition to skeletal muscle, CTG repeat expansion affects heart, causing fibrosis in the conduction system and senoatrial node, resulting in cardiac conduction defects and tachyarrhythmia (Groh et al., 2008). DM1 patients also develop cataracts (Harper, 2001; Garrot et al., 2004). MRI analyses of DM1 patient brains show diffuse white matter with less evident atrophic changes (Minnerop et al., 2011). Endocrine functions are also impaired in patients with DM1, leading to insulin resistance, susceptibility to diabetes and hypothyroidism (Harper, 2001).

In contrast to DM1, there are no clinical subgroups in DM2. The clinical phenotype of DM2 is extremely variable ranging from disabilities in the 40s and early cardiac death to very mild proximal weakness or slightly elevated CK levels in elderly patients. Early muscle

symptoms in DM2 are either proximal lower limb weakness or myalgic pain. Myotonia, a cardinal feature of DM1, may be absent in DM2. In contrast to DM1, there is no prominent late weakness of the respiratory, facial or bulbar muscles. However, occasionally DM2 might be associated with severe muscular atrophy and disability (Udd et al., 2011). Despite the overall milder symptoms, DM2 patients may have severe myalgic pain as the major cause of dysfunction affecting professional performance (George et al., 2004).

It is well established that the main mechanism by which mutant CTG and CCTG repeats cause DM1 and DM2 pathologies is associated with the toxic effects of the CUG and CCUG RNA repeats on RNA-binding proteins (Schoser and Timchenko, 2010). The majority of the literature describes the effects of RNA repeats on splicing activities of two RNA-binding proteins, CUGBP1 (also known as CELF1, a member of the CUGBP and Embryonic Lethal abnormal vision-like Family of proteins) and MBNL1 (Ranum and Cooper, 2006). In addition to splicing, CUGBP1 has important functions in cytoplasm including regulation of translation, RNA deadenylation and RNA stability (Timchenko et al., 1996, 1999; Paillard et al., 1998; Philips et al., 1998; Savkur et al., 2001; Moraes et al., 2006; Vlasova et al., 2008; Huichalaf et al., 2010; Lee et al., 2010; Rattenbacher et al., 2010). Thus, it is predicted that the alteration of CUGBP1 activity and protein levels in DM1 and in DM2 could affect not only alternative splicing of CUGBP1 targets, but also translation and stability of mRNAs which are regulated by CUGBP1. It was initially suggested that the main function of MBNL1 is associated with the control of splicing. However, further studies revealed that this protein is also abundant in cytoplasm. In agreement with MBNL1's cytoplasmic location, a recent report described the identification of a large number of MBNL1 binding sites in the 3' UTRs of different mRNAs, suggesting that MBNL1 might regulate RNA stability and translation (Masuda et al., 2012). Other RNA-binding proteins affected by CUG repeats, are also multifunctional. Similar to MBNL1, an RNA helicase p68 is sequestered by the mutant CUG repeats (Laurent et al., 2012), suggesting that the activity of p68 is reduced in DM1. RNA helicase p68 regulates many stages of RNA metabolism, including mRNA export, RNA degradation, translation, microRNA processing, splicing and transcription (Janknecht, 2010). Protein levels of another CUG RNA-binding protein, Staufen1, are increased in DM1 cells (Ravel-Chapuis et al., 2012). Staufen1 regulates mRNA transport, translation and mRNA decay (Kim et al., 2005). Staufen1 also interacts with telomerase mRNA, suggesting that it may play a role in the processing of telomerase RNA (Bachand et al., 2001). Thus, these recent reports suggest that alterations of CUGBP1, MBNL1, Staufen1 and p68 in DM1 could change other biological processes which take place in cytoplasm, in addition to the alterations of splicing in the nucleus.

This review discusses the role of CUG and CCUG repeats in the misregulation of cytoplasmic functions of CUGBP1 and ZNF9 and their effects on global protein translation. We will also discuss the role of age-associated pathogenic alterations of protein homeostasis in DM1 and DM2.

CUGBP1 is a regulator of protein homeostasis

Translational function of CUGBP1 is required for normal development

CUGBP1 is a highly conserved protein that functions in many species. In *Drosophila*, the CUGBP1 homolog, named Bruno, is a critical translational regulator. Bruno plays a major role in the regulation of translation of *oskar* mRNA, encoding a protein which is required for proper embryonic development (reviewed in Kugler and Lasko, 2009). Bruno binds to the Bruno Response Element (BRE) within the 3' UTR of *oskar* mRNA. This binding leads to the recruitment of the protein *Cup* which inhibits *oskar* translation by interaction with eukaryotic initiation factor 4E, eIF4E. Interaction of *cup* with eIF4E prevents eIF4E from interacting with eukaryotic initiation factor 4G, eIF4G, and subsequent binding with the 43S initiation complex (Kugler and Lasko, 2009). It was shown that Bruno also inhibits translation of *oskar* independently of *Cup* through formation of *oskar* mRNA oligomers, producing large RNP "silencing" complexes that prevent access of *oskar* mRNA to the translational apparatus (Chekulaeva et al., 2006).

The CUGBP1 homolog in *Xenopus laevis*, EDEN-BP (deadenylation element-binding protein) binds to the U(A/G)-repeat site within maternal mRNAs and represses their translation during fertilization (Paillard et al., 1998). Studies of CUGBP1 homologs in other species demonstrated that CUGBP1 is required for normal development. Deletion of a CUGBP1 homolog in *C. elegans*, ETR1, disrupts muscle development (Milne and Hodgkin, 1999). Mice with fully deleted CUGBP1 die shortly after birth (Kress et al., 2007) showing that CUGBP1 is required for postnatal development. Such a crucial role of CUGBP1 in normal development is likely associated with the role of CUGBP1 in the regulation of processing of many mRNAs at different levels, including translation, RNA stability and alternative splicing.

Translational functions of CUGBP1 in human cells

Initial studies of CUGBP1 translational function in human cells showed that active CUGBP1 is a positive regulator of translation (further called $CUGBP1^{ACT}$). However, recent reports showed that inactive CUGBP1 might function as a negative regulator or repressor of translation (further called $CUGBP1^{REP}$). $CUGBP1^{ACT}$ binds to specific sites within the 5' UTRs of several mRNAs and increases their translation. These well studied mRNAs, translation of which is enhanced by $CUGBP1^{ACT}$, include mRNAs encoding a transcription

factor C/EBPß and a cdk inhibitor, p21 (Timchenko et al., 1999, 2001a,b). $CUGBP1^{ACT}$ also increases translation of myocyte enhancer 2A, MEF2A, cyclin D1 and HDAC1 (Timchenko et al., 2004; Salisbury et al., 2008; Wang et al., 2008). The increase of translation of these mRNAs by $CUGBP1^{ACT}$ plays a significant role in the pathogeneses of DM1 and DM2, normal skeletal muscle myogenesis, liver proliferation and differentiation, development of cancer and aging. The mechanism by which $CUGBP1^{ACT}$ stimulates protein translation includes physical associations with an eukaryotic initiation factor 2, eIF2, and subsequent delivery of mRNAs to polysomes (Timchenko et al., 2005, 2006). Recent studies revealed that CUGBP1 ($CUGBP1^{REP}$) can repress translation of mRNAs (Huichalaf et al., 2010). The ability of CUGBP1 to activate or repress translation depends on the phosphorylation of CUGBP1 at S302 by the cyclin D3-cdk4 complex and the subsequent capability of CUGBP1 to interact with the active form of eIF2α (un-phosphorylated at S51) or with the inactive form of eIF2α (ph-S51). The regulation of translation by $CUGBP1^{ACT}$ might be similar to that described for a member of the ELAV family of RNA-binding proteins, HuD. It was shown that the ability of HuD to regulate protein translation is controlled through RNA-binding domain 3 and the linker domain (Fukao et al., 2009). However, in contrast to ph-S302-CUGBP1, which interacts with eIF2, HuD interacts with eIF4A (Fukao et al., 2009).

It has been shown that CUGBP1 also regulates *cap*-independent translation, initiated from the internal ribosomal entry sites (IRES). Several mRNAs whose translation is regulated by CUGBP1 through IRES have been identified, including serine hydroxymethyl-transferase (SHMT) and a cdk inhibitor, p27 (Woeller et al., 2007; Fox and Stover, 2009; Zheng and Miskimins, 2011). CUGBP1 stimulates translation of SHMT through simultaneous binding to the 3' UTR of SHMT mRNA and to H ferritin, which binds to the 5' UTR of SHMT. It has been shown that CUGBP1 might also activate IRES-dependent translation of SHMT by interaction with hnRNP H2 (Fox and Stover, 2009). It has been proposed that the interactions between CUGBP1 and H ferritin or CUGBP1 and hnRNP H2 result in the circularization of the SHMT transcript and enhancement of the IRES activity due to recruitment of the translation initiation factors to the IRES (Woeller et al., 2007; Fox and Stover, 2009). These data show that CUGBP1 is an IRES-specific trans-acting factor (ITAF).

It has been shown that CUGBP1 reduces the IRES-dependent translation of p27 mRNA (Zheng and Miskimins, 2011). The impairment of the initiation of translation of p27 by CUGBP1 was implicated in X-linked dyskeratosis congenita, a disease with increased susceptibility to cancer (Kirwan and Dokal, 2008). It was found that only cytoplasmic CUGBP1 has the ability to regulate IRES-dependent initiation of translation of p27, suggesting that nuclear and cytoplasmic activities of CUGBP1 are controlled by different mechanisms. Although the mechanisms of positive and negative effects of CUGBP1 on IRES-dependent translation are not known, it is possible that these mechanisms are similar to those which CUGBP1 uses for regulation of *cap*-dependent translation. We suggest that ph-S302-CUGBP1 ($CUGBP1^{ACT}$) associates with active eIF2 and activates translation through binding to IRES, while un-ph-S302-CUGBP1 ($CUGBP1^{REP}$) associates with in-active eIF2 and represses translation through the subsequent binding to IRES.

Regulation of RNA stability by CUGBP1

A growing number of reports suggests that CBP1 is a main regulator of RNA stability (Vlasova et al., 2008; Zhang et al., 2008; Rattenbacher et al., 2010). It was shown that CUGBP1 binds to AU-rich elements (ARE) and GU-rich elements (GRE) in the 3' UTRs of mRNAs encoding short-lived proteins and causes their decay (Beisang et al., 2012). The predicted ARE CUGBP1 binding sites are located within the 3' UTRs of mRNAs, encoding proteins involved in cell growth, cell motility and apoptosis (Ratenbacher et al., 2010). It appears that CUGBP1 might reduce stability and cause decay of many short-lived mRNAs in mouse myoblasts (Lee et al., 2010).

Cellular stress and protein translation in DM1

An additional mechanism for the regulation of translation by CUGBP1 was suggested based on the studies of CUGBP1 as a repressor of "global" protein synthesis via recruitment of RNAs into Stress Granules (SGs) in DM1 myoblasts (Huichalaf et al., 2010). Mutant CUG repeats increase total levels of CUGBP1 in DM1 cells leading to the elevation of both $CUGBP1^{ACT}$ and $CUGBP1^{REP}$ (Fig. 1). The increase of $CUGBP1^{ACT}$ in DM1 occurs due to increased stability of CUGBP1 (Savkur et al., 2001; Timchenko et al., 2001a,b; Kuyumcu-Martinez et al., 2007). In agreement with elevation of $CUGBP1^{ACT}$, examination of CUGBP1 targets C/EBP, and MEF2A in DM1 muscle biopsies showed that levels of these proteins are increased in DM1. It has been shown that the total levels of CUGBP1, C/EBP, and MEF2A are also increased in muscle biopsies from patients with DM2 (Salisbury et al., 2009). Thus, it is expected that, in DM2 patients, CCUG repeats also increase the levels of $CUGBP1^{ACT}$ and the levels of downstream targets.

The elevation of $CUGBP1^{REP}$ in DM1 cells is associated with reduced levels of cyclin D3 (Salisbury et al., 2008). Since there is a reduction of cyclin D3 levels, $CUGBP1^{REP}$ does not bind to active eIF2, but instead interacts with in-active, ph-S51-eIF2 which is unable to stimulate translation (Huichalaf et al., 2010). The amounts of $CUGBP1^{REP}$ capable of interacting with inactive eIF2 are very low in normal cells and amounts

of CUGBP1^{REP}-eIF2 complexes are also low. It is likely that in normal cells the CUGBP1^{ACT} and CUGBP1^{REP} levels are balanced to maintain normal protein translation. In DM1 cells, both forms of CUGBP1 are increased and this might cause misbalance of protein synthesis in DM1 (Fig. 1).

Recent reports have shown that another component of CUGBP1-eIF2 complexes, eIF2α, is also affected in DM1 cells by the expansion of CUG repeats. This increase of in-active eIF2 usually occurs in healthy cells in response to the different cellular stresses and is aimed to preserve energy by reducing assembly of surplus ribosomes. One of the kinases that phosphorylates eIF2α at S51 is a double-stranded RNA activated protein kinase (PKR) (rev in Anderson and Kedersha, 2006). It was shown that long CUG repeats increase the levels and activity of PKR (Tian et al., 2000; Huichalaf et al, 2010). Elevation of PKR in DM1 might lead to the increase of in-active eIF2α. In agreement with these observations, the levels of in-active eIF2α are increased in atrophic fibers in patients with DM1 (Ikezoe et al., 2007). The increase of inactive eIF2α in DM1 suggests that the DM1 mutation might also cause cellular stress. In fact, studies of DM1 myoblasts show that these cells accumulate SGs containing CUGBP1 (Huichalaf et al., 2010).

It has been shown that CUGBP1 migrates to SGs in response to different stresses (Fujimura et al., 2008). One of the suggested functions for SGs is to re-program global translation in such a way that mRNAs encoding for housekeeping proteins would not have access to polysomes, whereas mRNAs encoding proteins required for cell survival and recovery after stress would be translated (Anderson and Kedersha, 2006; Fig. 2). It has been shown that CUGBP1 within SGs of DM1 myoblasts and DM1 cell culture models is un-phosphorylated at S302 and represents the CUGBP1^{REP} form (Huichalaf et al., 2010). The accumulation of CUGBP1^{REP} within SGs in response to other stresses, such as arsenite treatment, requires a linker domain of CUGBP1 which contains S302 (Fujimura et al., 2008). These observations suggest that a mechanism modifying the linker domain, such as phosphorylation at S302, is likely to be responsible for the location of CUGBP1 on polysomes or within SGs. It has been predicted that CUGBP1^{REP} within SGs is bound to its RNA targets, preventing their translation. In agreement with this suggestion, the CUGBP1 target mRNA, encoding for the DNA repair and remodeling factor MRG15 (MORF4L1), is located in SGs in cells expressing long CUG repeats (Huichalaf et al., 2010). Accordingly, protein levels of MRG15 are reduced in DM1 myoblasts. Thus, CUGBP1^{REP} within SGs of DM1 cells might re-program "global" translation by sorting mRNAs according to their roles in cell recovery after stress and their roles in protecting cells from apoptosis. Since DM1 cells constantly express CUG repeats, these cells remain under continuous stress. It is likely that stress-related re-programming of protein translation might affect global protein synthesis in DM1 cells. It remains to be

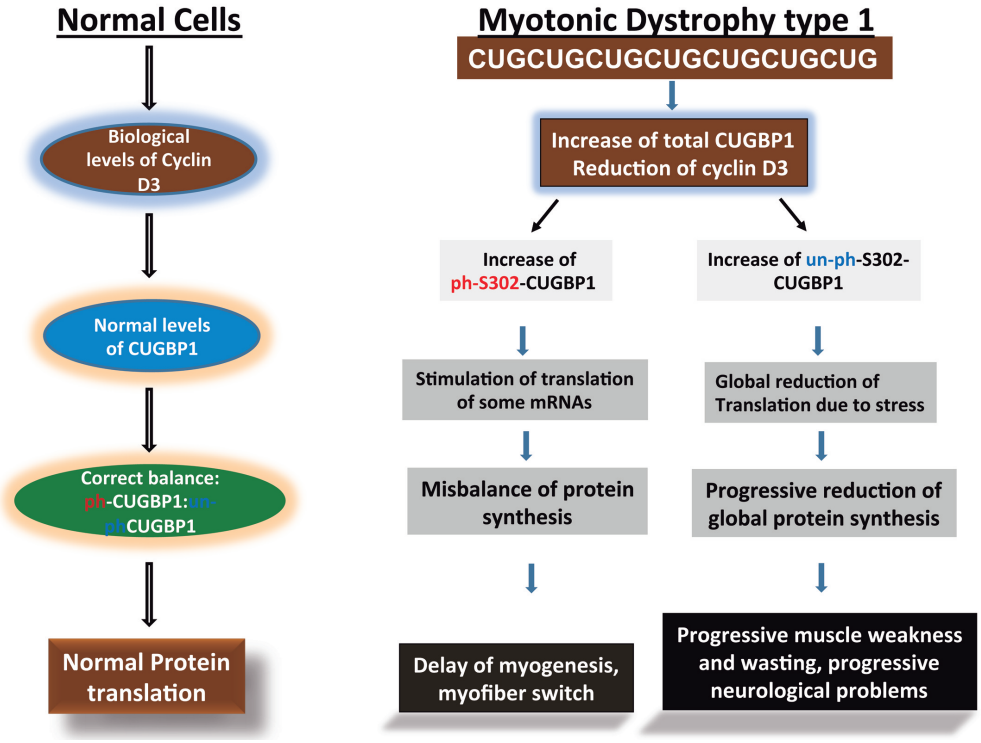

Fig. 1. CUGBP1 alters protein translation in DM1 (see text for details). The mutant CUG repeats increase total levels of CUGBP1 protein, including translation active ph-S302-CUGBP1 (CUGBP1^{ACT}) and translation repressor, un-ph-S302-CUGBP1 (CUGBP1^{REP}). While ph-S302-CUGBP1 increases translation of some mRNA targets, un-ph-S302-CUGBP1 causes a reduction of protein translation by trapping some mRNAs in SGs. We hypothesize that this reduction of global protein synthesis might contribute to the progressive muscle loss and age-associated neurological problems in DM1.

determined if the phosphorylation of CUGBP1 at S302 affects other key activities of CUGBP1 such as regulation of splicing and the regulation of RNA stability.

A recent report showed an additional and very interesting mechanism of the regulation of mRNA processing by CUGBP1 within SGs. It has been shown that CUGBP1, associated with SGs in stressed cells, might enhance translation of RNAs due to protection of RNAs from degradation by temporally trapping them within SGs. Such a mechanism was described for the regulation of p21 levels in cancer cells treated with an inhibitor of the 20S proteasome, bortezomib (Gareau et al., 2011). Since p21 protein plays a role as an anti-apoptotic factor, maintenance of p21 is beneficial for cancer treatment. However, p21 mRNA has a short half-life and quickly degrades. It was shown that treatment of myelomas and hematological tumors with bortezomib helps to maintain high levels of p21 because the ribo-protein complex containing CUGBP1 and p21 mRNA is trapped into SGs that are formed in response to inhibition of the proteasome by bortezomib. As a result, the accumulation of p21 mRNA within SGs in bortezomib treated cells preserves p21 mRNA from degradation. Subsequent dissociation of SGs releases p21 mRNA, promoting its translation and increasing p21 protein, which is required to protect cells from apoptosis (Gareau et al., 2011).

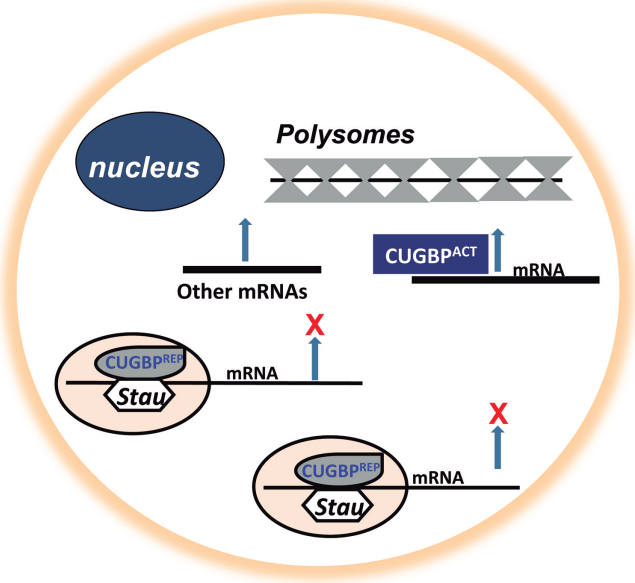

Fig. 2. A hypothetical model shows how un-ph-S302-CUGBP1 might block association of mRNAs with polysomes in DM1 cells. Un-ph-S302-CUGBP1 (CUGBP1^{REP}) binds to mRNAs and traps them in SGs. We hypothesize that other stress-responsive proteins such as Staufen1 might also participate in the repression of global translation in DM1 cells. In contrast to CUGBP1^{REP}, CUGBP1^{ACT} binds to some mRNA targets and delivers them to polysomes.

In addition to translation and stabilization of mRNAs, CUGBP1 might regulate translation of some mRNAs through microRNAs. For instance, CUGBP1 represses translation of CDK4 mRNA and this process is mediated by microRNA-222 (Xiao et al., 2011). Thus, microRNAs might be involved in the sorting of mRNAs regulated by CUGBP1 in SGs or Processing bodies (P-bodies). The ability of CUGBP1 to repress translation by trapping mRNAs in SGs is reminiscent of the role of the CUGBP1 homolog Bruno in repressing the translation of *oskar* in *Drosophila* (Chekulaeva et al., 2006). It was shown that Bruno forms heavy "silencing" complexes which repress translation of *oskar* mRNA (Chekulaeva et al., 2006). These heavy translation repressor complexes might be, to some extent, similar to SGs formed in human cells under stress.

Other CUG-binding proteins, such as MBNL1 and Staufen1, affected in DM1 are also predicted to be associated with SGs (Kiebler et al., 1999; Onishi et al., 2008). In *Drosophila*, Staufen cooperates with Bruno in the positioning and translation of *oskar* mRNA (Kugler and Lasko, 2009). We suggest that similar to *Drosophila,* Staufen1 and CUGBP1 might silence translation of some mRNAs within SGs in DM1 cells.

Although translational function of human p68 has not been investigated, p68 has been shown to shuttle between the nucleus and the cytoplasm (Wang et al., 2009). The yeast homolog of human p68, Dbp2p, is mainly a cytoplasmic protein associated with polyribosomes (Bond et al., 2001). In addition, Dbp2p plays a role in the regulation of nonsense-mediated RNA decay and rRNA processing. It was shown that the majority of DEAD-box proteins in *S. cerevisiae* control translation or ribosome biogenesis (de la Cruz et al., 1999). If p68 has the same functions in human cells, then

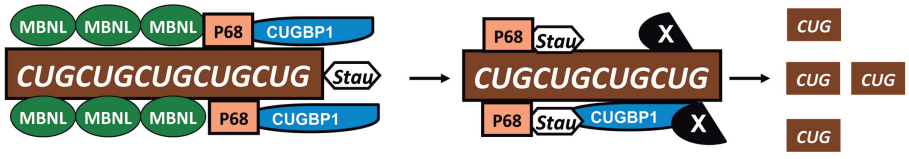

Fig. 3. A hypothetical model suggesting participation of different RNA-binding proteins in the molecular pathogenesis of DM1. The hypothesis suggests that different RNA-binding proteins might interact with mutant CUG repeats within DMPK mRNA during RNA processing. We suggest that RNA-binding proteins may enter and exit RNPs containing the mutant DMPK mRNA at different stages of processing. This model might explain the identification of a growing number of RNA-binding proteins (CUGBP1, MBNL1, RNP H1, p68, Staufen1 and, perhaps others) as altered in DM1 patients. X shows unknown RNA-binding proteins which might also be affected by mutant CUG repeats.

the binding of p68 to mutant CUG repeats in DM1 cells might affect global protein translation at the level of ribosome synthesis. P68 might also affect translation through the regulation of processing of rRNA and microRNAs because p68 is prevalent within the Drosha complex (Fukuda et al., 2007).

One important remaining question is related to the possible mechanisms by which several multifunctional RNA-binding proteins are affected by mutant RNA repeats. We suggest that mutant CUG RNA might form dynamic RNPs, as has been suggested for RNA-binding proteins, which control splicing and cytoplasmic transport (Farina and Singer, 2002). In this scenario, some RNA-binding proteins might be added or released from RNPs during processing of mutant CUG repeats. Such dynamic interactions of different RNA-binding proteins with mutant CUG RNA might change their normal functions, causing alteration of RNA processing in nucleus and in cytoplasm of DM1 cells (Fig. 3). This model could explain identification of the growing number of RNA-binding proteins affected by the mutant CUG repeats in DM1 cells.

ZNF9 and protein turnover in DM2

In patients with DM2, the expanded CCTG repeats are located within intron 1 of a gene encoding ZNF9 (Liquori et al., 2001). ZNF9 is a small, highly conserved protein, containing seven zinc-finger domains (Warden et al., 1994; Yasuda et al., 1995; Flink et al., 1998, Calcaterra et al., 2010). Biological functions of ZNF9 are not well understood but its crucial role in normal development has been demonstrated in many species (Chen et al., 2007; Calcaterra et al., 2010). It is known that ZNF9 binds to both DNA and RNA (Rajavashisth et al., 1989; Warden et al., 1994; Yasuda et al., 1995). Its ability to bind to DNA predicts the role of ZNF9 in the regulation of transcription (Rajavashisth et al., 1989; Edwards et al., 1992; Flink and Morkin, 1995; Michelotti et al., 1995). Since ZNF9 also binds to RNA, it is possible that it might be involved in the misregulation of RNA metabolism in DM2 cells. Cytoplasmic function of ZNF9 is associated with the regulation of translation at several levels. It has been shown that ZNF9 binds to the 5' UTRs of mRNAs containing highly conserved terminal oligopyrimidine (TOP) tracts (Pellizzoni et al., 1997; Huichalaf et al., 2009). TOP-containing mRNAs encode proteins of the translational apparatus, including ribosomal proteins and elongation translation factors (Meyuhas, 2000). ZNF9 binds to the 5' UTRs of TOP-containing mRNAs in different species (X. laevis and human cells) suggesting that this activity of ZNF9 is conserved (Pellizzoni et al., 1997; Huichalaf et al., 2009).

The translational function of ZNF9 has been demonstrated by its physical association with translating polysomes, synthesizing human ornithin decarboxylase (ODC) (Sammons et al., 2010). It was shown that ZNF9 binds to polysomes with the same affinity as other translation factors (Sammons et al., 2010). ZNF9 regulates translation of human ODC through binding to the IRES element in the 5' UTR of mRNA (Gerbasi and Link, 2007; Sammons et al., 2010). The ZNF9 homolog in *S. cerevisiae*, Gis2, co-purifies with translation factors and ribosomal subunits, suggesting that Gis2 is also a translation regulator (Sammons et al., 2011). The conservation of RNA-binding activity of ZNF9 in yeast and in human cells was shown by the binding of Gis2 and ZNF9 to the same RNA-binding site containing GWW repeats, where W is A/U (Scherrer et al., 2011). The analysis of mRNA targets containing this site demonstrated that ZNF9 might bind to hundreds of

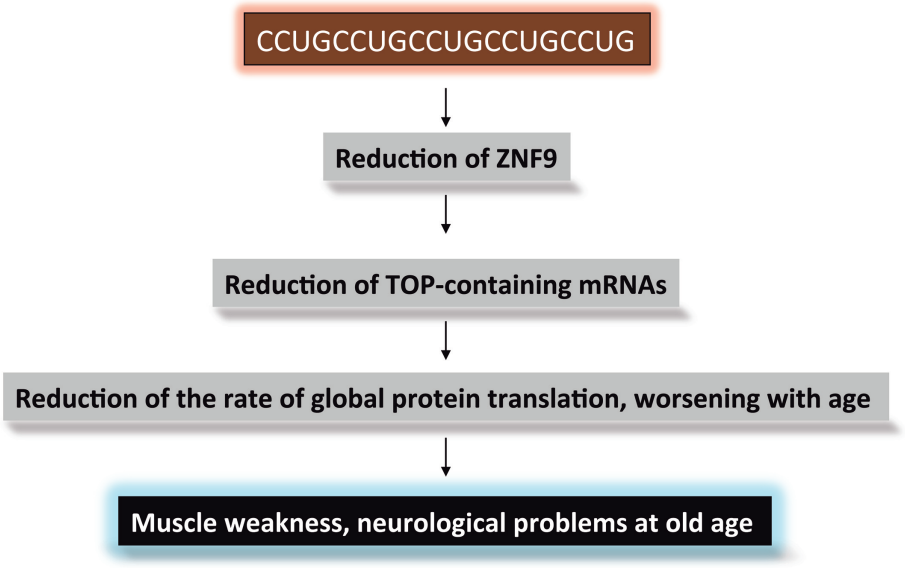

Fig. 4. The role of ZNF9 in the control of global protein translation in DM2. ZNF9 is normally bound to the 5' UTRs of the TOP-containing mRNAs and regulates global protein translation through synthesis of TOP proteins (see text). ZNF9 activity is reduced in patients with DM2. This leads to the reduction of the rate of global protein synthesis. It is possible that the reduction of the rate of global protein synthesis during aging might lead to the skeletal muscle weakness and neurological abnormalities in patients with DM2.

mRNAs, including mRNAs encoding proteins involved in the processing of ribosomal RNAs, components of the Ras pathway and proteins participating in muscle contraction (myosin heavy chains, components of the troponin complex and ion channels) (Scherrer et al., 2011). Gis2 interacts with many RNA-binding proteins, which regulate translation (including eIF2α), rRNA processing and RNA decay (Scherrer et al., 2011).

Several reports showed that ZNF9 levels and its activity are reduced in DM2 muscle biopsies and DM2 myoblast cell lines (Huichalaf et al., 2009; Raheem et al., 2010; Sammons et al., 2010). However, some patients with DM2 show unaltered levels of ZNF9 (Botta et al., 2006; Margolis et al., 2006). It is possible that the reduction of ZNF9 levels or reduction of its activity in DM2 patients is dependent on the length of CCUG expansions. Reduction of translational activity of ZNF9 in DM2 might have an effect on global protein translation due to reduced translation of TOP-containing mRNAs (Fig. 4) as well as reduced translation of other mRNAs involved in ribosome biogenesis. Such a reduction of the proteins of the translational apparatus might reduce the rate of global protein synthesis. This was in fact demonstrated in myoblast cell lines generated from muscle biopsies of patients with DM2 (Huichalaf et al., 2009). The reduction of ZNF9 translational activity in DM2 might have an additional toxic effect through reduction of synthesis of the proteins of the contractile apparatus since ZNF9 or its homologues bind to these mRNAs.

It has been shown that reduced synthesis of TOP proteins occurs during stress caused by amino acid starvation (Damgaard and Lykke-Anderson, 2011; Ivanov et al., 2011). Moreover, stress-responsive RNA-binding protein TIA-1 is involved in the repression of translation of TOP mRNAs (Damgaard and Lykke-Andersen, 2011; Ivanov et al., 2011). The reduction of TOP proteins in cells deficient in amino acids is an adaptive response preventing the energy-demanding assembly of surplus ribosomes. We suggest that the reduction of ZNF9 translational activity in DM2 cells and subsequent reduction of TOP proteins reducing rate of global protein synthesis might also be an adaptive response to stress caused by the accumulation of RNA CCUG repeats (Fig. 4).

The role of alterations of protein homeostasis in progressive disease in patients affected with DM1 and in the late onset of DM2

Both DM1 and DM2 are aging diseases. Symptoms in patients with the adult form of DM1 gradually progress with age, leading to disability. In early adulthood, muscle weakness in DM1 may be totally absent. With age, however, muscle weakness develops in facial, neck and distal limb muscles in parallel with muscle wasting. Skeletal muscle weakness, leading to immobility, respiratory insufficiency, dysarthria and dysphagia, is ultimately the major cause of severe disability and death at late stages of the disease (Mathieu et al., 1999). Myotonia is not apparent early in life in DM1. However, in late stages of the disease myotonia with muscle weakness cause difficulties with speech, swallowing, respiration and smooth muscle dysfunction (including abnormal intestinal motility and uterine dysfunction). It is important to note that minor brain abnormalities in patients with adult DM1 progress with age to mild cognitive impairment and later apathy (Meola et al., 2003). Daytime sleepiness is invariably present at later stages of disability. Dysphagia, associated with aspiration and pneumonia, may become a major concern at advanced stages of disease progression (Ashizawa and Sarkar, 2011). In contrast to DM1, a disease that is characterised by the progression of disease severity with age, DM2 mainly affects patients of advanced age (Udd et al., 2011).

The molecular mechanisms responsible for the progressive phenotype of adult DM1 and late-onset DM2 are not well understood. One of the reasons for the increase of disease severity with age is a well-documented increase of somatic instability of CTG repeats during life span (Harper, 2001). However, the increase of repeat instability might be only in part responsible for the age-dependent severity of DM1. We suggest that the stress-related re-programming of global protein translation in DM1 cells mediated by $CUGBP1^{REP}$ might be responsible for the progression of muscle weakness and wasting and age-dependent increase of severity of neurological symptoms in DM1 (Fig. 1). It is possible that at younger ages, DM1 cells better adapt to the toxicity of mutant CUG repeats. However, with age, stress-responsive system might be exhausted and, as a result, DM1 cells will not be able to cope with the increasing stress. This would lead to the progression of disease.

In patients with DM2, the reduction of the rate of protein synthesis at early stages of disease might have only minor effects on skeletal muscle and brain functions because the amounts of the proteins might be maintained at normal levels despite the reduced rate of protein synthesis. However, at advanced ages, a reduction in the rate of protein synthesis in combination with other factors (such as stress associated with the inhibition of the proteasome) might lead to global reduction of protein synthesis, causing muscle and brain atrophy. In agreement with this suggestion, it has been shown that mutant CCUG repeats bind to the 20S proteasome (Salisbury et al., 2009). As a result, the activity of the proteasome is reduced in DM2 patients leading to the increased stability of many short-lived proteins which are targets of the Ubiquitin-Proteasome System (UPS) (Salisbury et al., 2009). Such inhibition of the proteasome during aging might result in stress in DM2 cells. We predict that cellular stresses associated with the reduction of TOP proteins and with an inhibition of the proteasome might significantly misbalance protein homeostasis in DM2 cells, leading to muscle atrophy.

In summary, the detailed analysis of cellular functions of RNA-binding proteins affected in DM1 and in DM2 is necessary to identify all major biological processes disrupted by toxic CUG and CCUG RNAs. This knowledge can be used to develop effective treatments for these diseases.

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