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Mechanisms of Myotonic Dystrophies 1 and 2

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Myotonic Dystrophies type 1 and 2 (DM1/2) are neuromuscular disorders which belong to a group of genetic diseases caused by unstable CTG triplet repeat (DM1) and CCTG tetranucleotide repeat (DM2) expansions. In DM1, CTG repeats are located within the 3' untranslated region of myotonin protein kinase (DMPK) gene on chromosome 19q. DM2 is caused by expansion of CCTG repeats located in the first intron of a gene coding for zinc finger factor 9 on chromosome 3q. The CTG and CCTG expansions are located in untranslated regions and are expressed as pre-mRNAs in nuclei (DM1 and DM2) and as mRNA in cytoplasm (DM1). Investigations of molecular alterations in DM1 discovered a new molecular mechanism responsible for this disease. Expansion of un-translated CUG repeats in the mutant DMPK mRNA disrupts biological functions of two CUG-binding proteins, CUGBP and MNBL. These proteins regulate translation and splicing of mRNAs coding for proteins which play a key role in skeletal muscle function. Expansion of CUG repeats alters these two stages of RNA metabolism in DM1 by titrating CUGBP1 and MNBL into mutant DMPK mRNA-protein complexes. Mouse models, in which levels of CUGBP1 and MNBL were modulated to mimic DM1, showed several symptoms of DM1 disease including muscular dystrophy, cataracts and myotonia. Mis-regulated levels of CUGBP1 in newborn mice cause a delay of muscle development mimicking muscle symptoms of congenital form of DM1 disease. Since expansion of CCTG repeats in DM2 is also located in untranslated region, it is predicted that DM2 mechanisms might be similar to those observed in DM1. However, differences in clinical phenotypes of DM1 and DM2 suggest some specific features in molecular pathways in both diseases. Recent publications suggest that number of pathways affected by RNA CUG and CCUG repeats could be larger than initially thought. Detailed studies of these pathways will help in developing therapy for patients affected with DM1 and DM2.

Key Words: Myotonic dystrophy, CUG repeats, CCUG repeats, CUGBP1, MNBL

INTRODUCTION

DM1 and DM2 are primarily muscular diseases characterized by muscle weakness and myotonia (hyperexcitability of skeletal muscle) (Finsterer J, 2002; Harper PS, 2001; Ranum LP and Day JW, 2004). However, severity of muscle symptoms is different in two diseases. While patients with DM1 develop a severe muscle atrophy and progressive wasting, patients with DM2 show only mild proximal muscle weakness and slight wasting (Day JW et al, 2003; Finsterer J, 2002; Harper PS, 2001; Ranum LP and Day JW, 2004). Clinical myotonia is also milder in patients with DM2. In addition to skeletal muscle, both diseases affect other systems, including the eye, the heart and endocrine systems. An important distinction of DM2 is the lack of congenital form of disease (Finsterer J, 2002; Ranum LP and Day JW, 2004). In DM1, very long CTG repeats (more than 800) lead to the most severe and deadly form of disease, congenital DM1 (Harper PS, 2001). In newborn children long CTG expansions cause significant delay of muscle development, neonatal weakness and mental retardation (Harper PS, 2001). Despite of much larger expansions in DM2, there are no known cases of congenital disease. There is also lack of mental retardation in juvenile patients (Day JW et al, 2003). Both diseases are characterized by significant clinical heterogeneity with variable severity of symptoms.

DM1 is caused by untranslated polymorphic CTG triplet repeat in the 3' UTR of myotonin protein kinase gene on chromosome 19q (Aslanidis C et al, 1992; Brook JD et al, 1992; Fu YH et al, 1992; Mahadevan M et al, 1992). Because of high similarity between DM1 and DM2, DM2 was initially defined as DM disease, but without CTG expansion on chromosome 19q (Udd B et al, 1997). Recently, mutation for DM2 was discovered as untranslated tetranucleotide CCTG repeat in the first intron of ZNF9 gene on chromosome 3q (Liquori CL et al, 2001). While both expansions are polymorphic, CCTG expansion is proven to be extremely heterogeneous making it difficult to determine the length of CCTG repeats in DM2 patients by Southern blot hybridization (Day JW et al, 2003; Ranum LP and Day JW, 2004). DM1 is characterized by genetic anticipation when the number of CTG repeats is greater in offspring

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ABBREVIATIONS: cTnT, cardiac Troponin T; DM, Myotonic Dystrophy; DMPK, myotonin protein kinase; MNBL, RNA CUG, binding protein, named muscleblind; ZNF9, zinc finger nuclear factor 9.

(Harper PS, 2001). The expansions in children affected with DM2 are shorter than in their parents (Finsterer J, 2002). There is overall good correlation between size of repeats in DM1 patients, especially with adult form of disease, and age of onset (Harper PS, 2001). But in DM2, no significant correlation between age of onset and size of expansions has been found (Day JW et al, 2003; Finsterer J, 2002; Ranum LP and Day JW, 2004). Similarities and features of DM1 and DM2 suggest overlap of molecular mechanisms in both diseases as well as some differences in the mechanisms of expansion formation and inheritance and in the molecular expression of mutations.

RNA models for molecular mechanisms of DM1 and DM2

A number of hypotheses have been suggested for possible molecular mechanisms by which expansion of untranslated CTG repeats causes DM1 pathology. It was proposed that the CTG expansion located in DMPK gene affects DMPK levels disrupting DMPK signaling pathways (Fu YH et al, 1993). Another hypothesis suggested that excessive CTG repeats alter the adjacent chromatin structure (Otten AD and Tapscott SJ, 1995). We suggested that untranslated CTG repeats cause DM1 at the RNA level through binding to specific CUG-binding proteins functions of which, in turn, become misregulated in patient's tissues (Timchenko LT et al, 1996(a); Timchenko LT et al, 1996(b); Timchenko LT et al, 1999). Currently, this hypothesis has been proven in two mouse models in which expression of untranslated CTG repeats led to development of myotonia, muscular dystrophy and brain abnormalities (Mankodi M et al, 2000; Seznec H et al, 2001). Identification of DM2 specific mutation in intron of ZNF9 gene also argues in support of RNA mechanism for both DM1 and DM2. It was suggested that RNA CUG repeats are causing the disease through specific RNA-binding proteins interacting with CUG repeats. Search for CUG binding proteins resulted in the identification of two distinct families of RNA-binding proteins, CUGBP (Timchenko LT et al, 1996(a); Timchenko LT et al, 1996(b)) and MNBL (Miller JW et al, 2000).

The role of translational activity of CUGBP1 in DM1

In normal muscle cells a member of CUGBP family, CUGBP1, is located in cytoplasm and in nuclei. In cytoplasm, CUGBP1 regulates translation (Timchenko NA et al, 1999; Timchenko NA et al, 2001; Timchenko NA at al, 2004), and in nuclei it is involved in the regulation of splicing (Charlet-B N et al, 2002; Philips AV et al, 1998; Savcur RS et al, 2001). Several mRNAs have been identified as translational targets of CUGBP1. This group of mRNAs includes two transcription factors C/EBP β (Timchenko NA et al, 1999) and myocyte enhancer factor 2A (MEF2A) (Timchenko NA at al, 2004), and a cdk inhibitor, p21 (Timchenko NA et al, 2001).

Regulation of C/EBP β isoforms by CUGBP1: the role in cell proliferation

CCAAT/Enhancer Binding protein β, C/EBP β, is a transcription factor that plays a critical role in the regulation of cell proliferation and differentiation (Poli V, 1998). A single C/EBP β mRNA produces three proteins through the alternative translation from in frame AUG codons: the full-length protein and two truncated proteins, LAP and LIP (Calkhoven CF et al, 1994; Calkhoven CF et al, 2000; Descombes P and Schibler U, 1991). The translation of LIP is achieved by a "leaky scanning ribosome" mechanism which suggests that the 5'region of C/EBP β mRNA, designated as a short Open Reading Frame (sORF) (which is a binding site for CUGBP1) is a key sequence that directs ribosomes to the LIP AUG codon (Calkhoven CF et al, 1994; Calkhoven CF et al, 2000; Welm AL et al, 2000). Detailed studies of C/EBP β isoforms showed that the full-length and

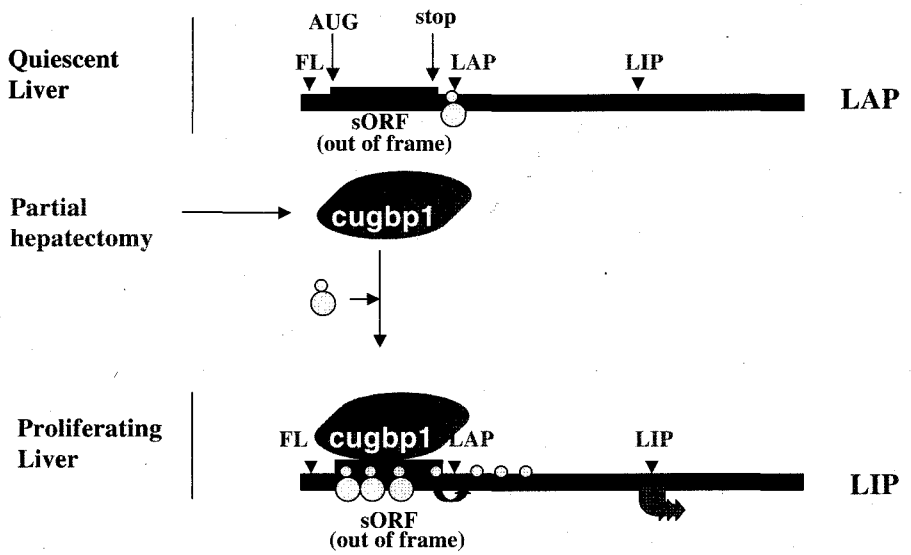

Fig. 1. Hypothetical model for the role of CUGBP1 in the induction of LIP in the liver proliferating after partial hepatectomy (see text).

truncated C/EBP β proteins have opposite functions. While the full-length C/EBP β inhibits cell proliferation, expression of LIP promotes cell growth (Baldwin BR et al, 2004; Luedde T et al, 2004). My laboratory has found that CUGBP1 binds to the 5' region of C/EBP β mRNA, sORF, which regulates translation of LIP (19, 29, see Fig. 1). It has been later shown that the binding of CUGBP1 to the 5' region of C/EBP β mRNA significantly increases synthesis of the truncated C/EBP β protein, LIP (Welm AL et al, 2000). Since C/EBP β isoforms regulate liver proliferation (Luedde T et al, 2004), we examined the role of CUGBP1 in the induction of LIP using the mouse model of liver proliferation after partial hepatectomy (PH). We have found that CUGBP1 binds to the 5' region of C/EBP β mRNA in the liver and that this binding correlates with induction of LIP translation after PH. CUGBP1-mediated induction of LIP expression correlated with the liver proliferation. Fig. 1 summarizes these studies. In quiescent livers, C/EBP β mRNA translates LAP, while LIP is not detectable. After partial hepatectomy, CUGBP1 binds to the 5'-region of C/EBP β mRNA and recruits more ribosomes to initiate translation of sORF sequence. These ribosomes translate 9 amino acids peptide, meet the stop codon of sORF and dissociate. Because of close proximity of the LAP AUG codon to the stop codon of the sORF, these ribosomes are unable to initiate translation of LAP and continue scanning until meet AUG codon of LIP and then initiate translation. Thus, the CUGBP1-mediated recruitment of ribosomes to sORF increases translation of LIP. Current investigations of the molecular mechanisms by which CUGBP1 activates LIP translation in proliferating hepatocytes suggest that this process requires a complex cascade of protein-protein and RNA protein interactions. Since LIP increases expression of PCNA, cyclins D1, A and E (Luedde T et al, 2004), this CUGBP1-mediated induction of LIP contributes to the liver proliferation. Thus, cells with elevated levels of CUGBP1 have increased amounts of LIP and, as the result, these cells have an accelerated growth.

In addition to liver proliferation, such biological situation was also observed in breast cancer where the increase of proliferation is associated with the activation of the CUGBP1-LIP pathway (Baldwin BR et al, 2004). It is expected that CUGBP1- C/EBP β pathway also increases proliferation in DM1 cells. Further studies are required to determine type of cells which are affected by this pathway in DM1 and symptoms associated with CUGBP1-LIP-dependent alteration of cell proliferation.

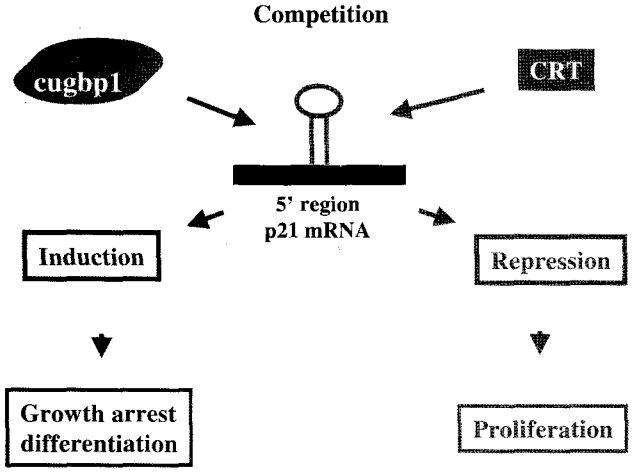

Fig. 2. The role of CUGBP1-mediated induction of p21 in the regulation of skeletal muscle differentiation and in senescence. The expression of p21 depends on the balanced binding of CUGBP1 and calreticulin (CRT) to the 5' region of p21 mRNA (see text).

Regulation of translation of p21 mRNA by CUGBP1: the role in skeletal muscle differentiation

In addition to C/EBP β, CUGBP1 regulates translation of myocyte enhancer factor 2A (MEF2A) and p21 mRNAs. Both MEF2A and p21 play a significant role in the control of myogenesis and muscle development (Molkentin JD et al, 1995; Zhang P et al, 1999). Expression of p21 has been shown to be regulated at different levels including rate of translation. We found that the rate of p21 translation is controlled by a competitive binding of two RNA-binding proteins, CUGBP1 and calreticulin (CRT) to the 5' region of p21 mRNA (Iakova P et al, 2004). Studies in senescent fibroblasts suggest that the competition of CRT and CUGBP1 for p21 regulation determines if cells proliferate (when CRT binds to p21 mRNA) or undergo growth arrest and differentiation (if CUGBP1 displaces CRT from p21 mRNA). Fig. 2 shows a hypothetical pathway by which CUGBP1 increases translation of p21. CRT is highly expressed in cells and it partially inhibits translation of p21 mRNA. Induction or activation of CUGBP1 during skeletal muscle differentiation and during senescence leads to the replacement of CRT and to a release of CRT-mediated inhibition of p21 translation. As the result, the increased levels of p21 inhibit cdk2 and cdk4 and arrest cell proliferation. This CUGBP1-p21-mediated growth arrest is required for the differentiation of myoblasts and for development of a senescent phenotype in human fibroblasts (Timchenko NA et al, 2001; Iakova P et al, 2004).

Examination of p21 in skeletal muscle of DM1 patients and in cultured cells derived from DM1 patients confirmed this hypothesis. Increase of p21 levels in proliferating myoblasts leads to irreversible arrest of proliferation and to initiation of differentiation (Zhang P et al, 1999). It was found that protein levels of p21 are significantly lower in DM1 differentiating myoblasts compared to normal cells (Timchenko NA et al, 2001). This reduction of p21 levels in differentiating cells is associated, at least in part, with reduction of CUGBP1 which participates in the regulation of p21 translation. As the result, DM1 proliferating myoblasts are unable to irreversibly withdraw from the cell cycle (Timchenko NA et al, 2001). Thus, alteration of CUGBP1-p21 pathway delays cell cycle withdrawal and differentiation in DM1 myoblasts. Misregulation of CUGBP1-p21 pathway might be responsible for delay of muscle development and differentiation in congenital DM1. This suggestion agrees with delay of muscle development in mice with elevated levels of CUGBP1. New born mutant mice with 8-fold elevation of CUGBP1 show significant under-development of myofibers (Timchenko NA at al, 2004). While hind-limb myofibers in wild type mice are very well developed, long and multinucleated, myofibers in CUGBP1 transgenic mice with 8-fold elevation of CUGBP1 are significantly shorter and contain enlarged, centered nuclei (Timchenko NA at al, 2004). This delay of muscle development suggests that myoblasts proliferation/ differentiation might be disrupted in CUGBP1 transgenic mice through

p21 and perhaps other regulatory factors.

The role of CUGBP1-MEF2A pathway in muscle differentiation

MEF2 family together with myogenic transcription factors (MyoD, MRF4, myogenin and Myf5) promotes myogenesis converting non-muscle cells (fibroblasts) into myoblasts (Molkentin JD et al, 1995). MEF2A protein expression is regulated in such way that during myoblast proliferation MEF2A protein levels are remaining low but during differentiation they are significantly increased. The elevation of MEF2A levels in differentiating myoblasts is required to activate transcription of muscle specific genes (Molkentin JD et al, 1995). This pattern of MEF2A expression allows regulation of specific muscle proteins during myogenesis when certain proteins have to be expressed at proper amounts and at proper stages of myogenesis. In DM1 muscle cells, MEF2A pattern of expression is significantly changed. MEF2A protein is highly expressed in DM1 proliferating myoblasts but it is reduced during differentiation (Timchenko NA at al, 2004). Such misregulation of MEF2A expression might result in misbalanced myogenesis leading to over-expression or under-expression of MEF2A-regulated proteins at wrong stages of muscle proliferation and differentiation. This suggestion agrees with the results of study of CUGBP1 transgenic mice, in which elevation of CUGBP1 perhaps disrupts a genetically programmed expression of MEF2A and delays myogenesis. A number of studies showed that, in muscle, MEF2 family of transcription factors is involved not only in muscle differentiation, but also in many other pathways. For instance, MEF2 activity is increased in slow myofibers in experimental mice with myotonia (Wu H and Olson EN, 2002). Increase of MEF2 levels in slow fibers in mice with myotonia suggests a possibility of molecular link between elevation of MEF2, increase of slow fibers and development of myotonia. Study of skeletal muscle from CUGBP1 transgenic mice indicated that elevation of CUGBP1 results in increased levels of MEF2 protein (Timchenko NA at al, 2004). Immunohistological analysis showed that the mice with elevated levels of CUGBP1 and MEF2 have increased number of slow myofibers. Physiological tests are required to study if increase of MEF2 expression in slow myofibers from CUGBP1 transgenic mice is associated with development of myotonia.

The role of translational activity of CUGBP1 in DM2 pathophysiology is unknown. CCTG expansion has been discovered recently; therefore, data on molecular pathways mediated by CCUG repeats are limited. If CUGBP1 is changed in DM2 muscle in the same fashion as in DM1, this protein might similarly affect translation of some mRNAs in cytoplasm of DM2 cells. However, the lack of congenital disease and mental retardation in DM2 suggest that if CUGBP1 is involved in DM2, its RNA-binding activity might be different or it might regulate different set of mRNAs leading to milder symptoms in DM2 muscle.

The role of CUGBP1 and MNBL in the regulation of splicing in DM1 and DM2

CUGBP1 is located in cytoplasm and in nuclei. A series of publications from Dr Cooper's laboratory presented strong evidence that CUGBP1 regulates splicing of several pre-mRNAs, including muscle specific chloride ion channel (Charlet-B N et al, 2002), cardiac troponin T (cTnT) (Philips AV et al, 1998) and insulin receptor (Savcur RS et al, 2001). It was shown that the patterns of cTnT splicing are different in embryonic and adult tissues (Cooper TA and Ordahl CP, 1984; Cooper TA and Ordahl CP, 1985). In embryonic cells, cTnT mRNA contains an alternative exon 5, which is excluded in adult cells. In DM1 adult muscle tissues, the levels of embryonic spliced isoform of cTnT are significantly increased while the amounts of adult form of cTnT are diminished (Philips AV et al, 1998). The increase of alternatively spliced cTnT mRNA in DM1 tissues is mediated by CUGBP1. This conclusion was made based on experiments in which overexpression of CUGBP1 in normal muscle cells led to the switch of cTnT splicing patterns from adult to embryonic. It has been suggested that alterations of splicing for cTnT are responsible for cardiac problems in DM1 (Philips AV et al, 1998). Analyses of cardiac function in CUGBP1 transgenic mice will help to study if increase of embryonic isoform of cTnT in vivo is sufficient to cause cardiac deficiency. CUGBP1 also regulates splicing of the chloride ion channel. It has been suggested that alterations of splicing of the chloride ion channel contributes to the development of myotonia (Savcur RS et al, 2001). Abnormal splicing of insulin receptor regulated by CUGBP1 might be involved in the unusual insulin resistance observed in DM1 patients (Charlet-B N et al, 2002). Recent results from Dr. Day's laboratory demonstrated that, similar to DM1, DM2 patients have abnormalities in the splicing of insulin receptor (Savcur RS et al, 2004). Thus, these studies show that misregulation of CUGBP1 splicing function contributes to DM2.

In addition to CUGBP1, a second family of nuclear CUG-binding proteins, MNBL, also possesses splicing activity (Kanadia RN et al, 2003). These proteins are bound to nuclear foci formed by mutant DMPK mRNA in DM1 patients (Mankodi A et al, 2001). Recent data suggest that, in DM2 patients, MNBL also binds to foci containing CCUG transcripts (Mankodi A et al, 2001). It was suggested that when MNBL proteins bind to RNA foci formed by CUG or CCUG repeats, their splicing function is changed. This suggestion has been made based on analysis of the splicing in mutant mice with deleted MNBL (Kanadia RN et al, 2003). The mutant mice with deleted MNBL show alterations of splicing for chloride ion channel and cTnT similar to those mediated by CUGBP1 (Kanadia RN et al, 2003). Abnormalities in splicing in chloride ion channel and in cTnT in mutant mice with deleted MNBL are accompanied by the development of myotonia and cataracts (Kanadia RN et al, 2003). Thus, it is expected that MNBL might regulate splicing of the same mRNAs as CUGBP1. Additional studies are needed to elucidate whether CUGBP1 and MNBL bind to the same sites on their RNA targets or interact with each other affecting splicing of chloride ion channel and cTnT.

The mechanisms by which untranslated CUG/CCUG repeats affect RNA-binding proteins, CUGBP1 and MNBL, in Myotonic dystrophy 1 and 2

Once RNA CUG-binding proteins have been identified, a number of studies have been focused on the molecular mechanisms by which CUG repeats expanded in DM1 cells

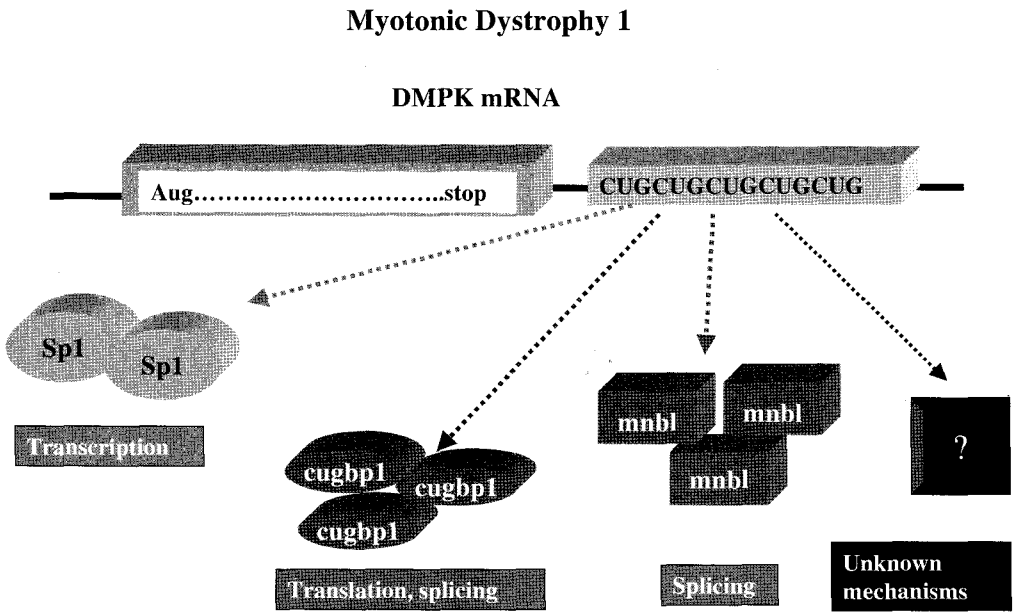

Fig. 3. An RNA model for DM1. Based on literature data, expansion of RNA CUG repeats in the 3' UTR of DMPK mRNA affects two RNA-binding proteins, CUGBP1 and MNBL, and DNA-binding protein, Sp1. Several mechanisms of the negative effect of CUG expansion are suggested. CUG repeats alter (a) translation of mRNAs through CUGBP1 protein; (b) splicing of pre-mRNAs through CUGBP1 and MNBL; and (3) transcription of genes through Sp1 transcription factor. Our model also suggests other pathways altered by CUG repeats which remain to be identified.

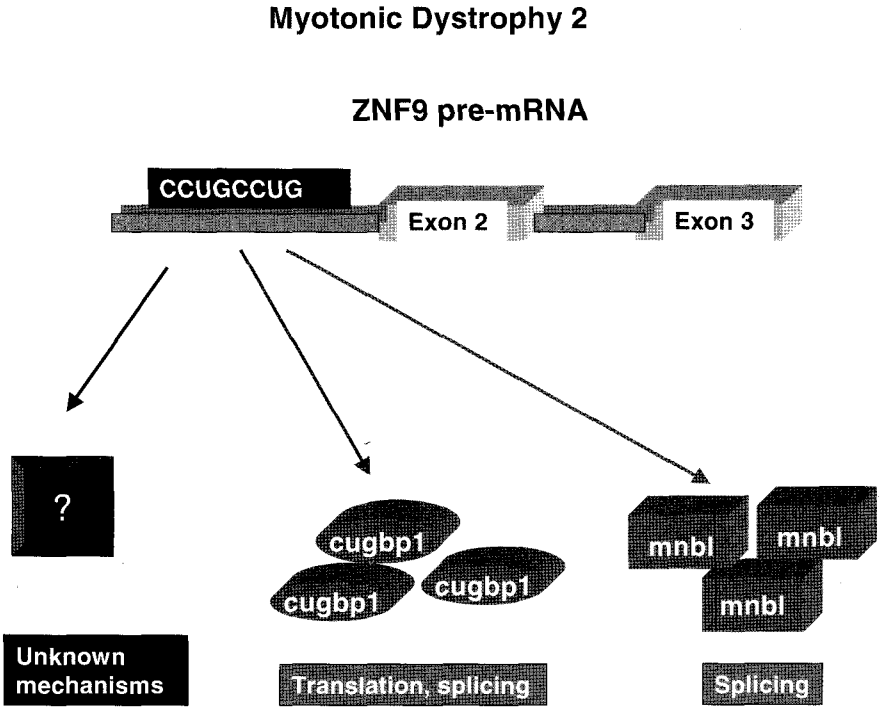

Fig. 4. A hypothetical RNA model for DM2. Based on similarity between CUG and CCUG repeats it is suggested that untranslated CCUG expansion affects RNA processing through CUGBP1 and MNBL proteins. As the result, translation and splicing of mRNAs regulated by these proteins will be misregulated. There are also other possible mechanisms of the negative effect of CCUG expansion.

affect CUGBP1 and MNBL. Using two different approaches, such as a combination of HPLC-based separation of RNA-protein complexes and free proteins (Timchenko NA et al, 2001) and chromatin immuno-precipitation (Ebralidze A et al, 2004), it has been demonstrated that in vivo CUGBP1 forms RNA-protein complexes with the mutant DMPK mRNA containing expanded CUG repeats. The initial hypothesis for the consequences of this interaction suggested that CUGBP1 should be titrated out of its biological functions. However, later investigations showed that this interaction leads to increased splicing and translational activities of CUGBP1. A detailed investigation of this finding using overexpression of untranslated RNA CUG repeats in cultured cells showed that the formation of CUGBP-CUGn complexes leads to elevation of CUGBP1 protein levels (Timchenko NA et al, 2001). Further studies suggested that CUGBP1 is stabilized within the complexes with RNA. When CUGBP1 is recruited into RNA-protein complexes, its half life is increased perhaps due to protection of CUGBP1 protein from degradation (Timchenko NA et al, 2001). This observation suggested a mechanism by which expansion of CUG repeats increases protein levels and binding activity of CUGBP1 in DM1.

Despite the significant progress, several important questions related to the interactions of CUGBP1 with mutant DMPK mRNA remain to be investigated. Mutant DMPK transcripts detected by FISH analysis are forming discrete foci in nuclei of DM1 cells (Taneja KL et al, 1995). These foci were mainly identified only in nuclei, while one report demonstrates the presence of mutant DMPK foci in both nuclei and in cytoplasm (Amack JD and Mahadevan MS, 2001). Immunofluorescent detection of CUGBP1 combined with identification of mutant DMPK foci by FISH showed multiple CUGBP1 complexes in nuclei and cytoplasm but they are not co-localized with DMPK foci (Fardaei M et al, 2001). The failure to co-localize DMPK foci and CUGBP1 by using FISH assay perhaps is associated with experimental problems such as disruption of the CUGBP1-RNA complexes during cell fixation. In addition, CUGBP1 is multifunctional protein which regulates several mRNAs and forms multiple complexes not only with DMPK mRNA, but also with other mRNAs including MEF2A, p21 mRNAs and yet to be determined CUGBP-dependent mRNAs (ref. 20 and Timchenko et al, unpublished data). Since molecular approaches preserving intact RNA-protein complexes (Chromatin-Co-IP and HPLC based chromatography) detected CUGBP1-CUGn complexes in vivo, the visualization of CUGBP1 RNA-protein complexes by IF with antibodies to CUGBP1 and FISH analysis with DMPK probes perhaps requires specific conditions to protect the disruption of the CUGBP1-DMPK complexes.

MNBL proteins are located in nuclei and in DM1/2 cells and they are co-localized with RNA foci formed by mutant DMPK mRNA (Mankodi A et al, 2001). Several important questions have to be answered to better understand the role of MNBL in DM1 and DM2 pathologies. It is unknown yet if this binding of MNBL results in a change of MNBL levels in DM1/2 cells, or it leads just in re-distribution of protein from free status to complexes with CUG/CCUG RNAs. Since deletion of MNBL in mice leads to development of myotonia and cataracts, it is expected that sequestration of MNBL by CUG repeats perhaps reduces free, unbound MNBL. Further experiments are required to elucidate how binding of MNBL to CUG/CCUG foci affects MNBL levels and its splicing activity. It is also important to better characterize biological activities of MNBL. Similar to CUGBP1, MNBL might be involved not only in the regulation of splicing, but also in the regulation of other biological functions.

CUG expansion in DM1 directly affects transcription factors

Although the role of sequestering RNA binding proteins by CUG repeats in DM1 is well established, recent data from Dr Junghans's laboratory showed that, in addition to CUGBP1, RNA CUG repeat expansion in DM1 patients sequesters several specific DNA-binding proteins such as a transcription factor Sp1 (Ebralidze A et al, 2004). This sequestration leads to a dramatic reduction of Sp1 factor in active chromatin. As a result, the transcription of Sp1 targets such as chloride ion channel is reduced (Ebralidze A et al, 2004). Since Sp1 regulates transcription of many genes, the sequestering Sp1 should have much bigger effect on the global expression of genes.

Conclusion and future directions

Myotonic dystrophy 1

Results of the current in vivo studies of molecular mechanisms of DM1 support the hypothesis that untranslated RNA CUG triplet repeats affect specific RNA-binding proteins and, perhaps, some transcription factors disrupting RNA processing and/or transcription of some mRNAs in patient's tissues. Fig. 3 summarizes these studies. Several mRNAs regulated by CUG-binding proteins in normal and in DM1 cells have been identified. Molecular pathways associated with CUG repeats, CUG-binding proteins and possible DM1 symptoms have been determined. It is expected that CUGBP1 and MNBL might regulate much greater number of mRNAs contributing to multisystem origin of DM1 phenotype.

Myotonic dystrophy 2

Data on the molecular mechanism of DM2 are limited, since the DM2 mutation was discovered only three years ago. Recent reports suggest the molecular mechanisms of DM1 and DM2 might be similar with CUGBP1 and MNBL as possible mediators of DM2 mutation. The hypothetical model for DM2 pathology is shown in Fig. 4. This model suggests a similarity in the DM1 and DM2 pathways and some differences in molecular pathologies of these two diseases. It is likely that CCUG repeats affect not only known CUG-binding proteins (CUGBP1, MNBL) but also novel CCUG-binding proteins. These novel RNA CCUG-binding proteins might be directly affected by CCUG repeats or might play a role of modifiers of CUGBP1 and/or MNBL activity. These novel proteins might be responsible for specific clinical features of DM2, including mild symptoms in skeletal muscle and the lack of congenital disease.

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