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筋強直性ジストロフィーの病態解明と RNA を介した治療

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（総合）研究報告書

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主任研究者　石　浦　章　一
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研究要旨

筋強直性ジストロフィーは、CTGまたはCCTGリピートの伸長で発病するが、そこにはMBNLまたはCELFというRNA結合タンパク質ファミリーが関係している。平成17－19年度の研究により、これらの因子のRNA結合特異性、DM患者での発現、スプライシングへの関与、並びに薬剤を用いたスプライシング調節、特にトレハロースの効果について新しい知見を得た。また、線虫でDMモデル系を立ち上げることができた。

A．研究目的

筋強直性ジストロフィー（*Dystrophia Myotonica*, DM）は、我が国で一番多い筋ジストロフィーである。症状は致死性ではないものの、ミオトニア、禿頭、精神遅滞、糖耐能の異常、性腺萎縮など種々の全身症状が特徴で、生活の質を改善する薬剤もない。難治性疾患である本症に対する効果的な薬剤の開発は、厚生労働行政上、重要なものと考えられる。

本症には2つの型があり、1型はDMキナーゼ（DMPK）遺伝子の3'非翻訳領域中にあるCTGリピートの伸長、2型はジンクフィンガータンパク質ZNF9をコードする遺伝子のイントロン1にあるCCTGリピートの伸長である。平成17－19年の研究により、これらが転写されたCUGまたはCCUGのRNAリピートに結合するタンパク質MBNL1-3とCELF1-6が発症に関わることがわかり、その機能を明らかにするのが第一の目的である。

特に石浦と西野は、国立精神･神経センターに保管してある筋肉バンクの筋強直性ジストロフィー筋21例からRNAを抽出し、PCRによってスプライシングパターンを調べたところ、塩素チャネルやインスリン受容体が幼若型優位なスプライシングを行っており、これがDM特有のミオトニア、耐糖能異常の原因であることが示唆された。

また本研究の第二の目的はモデル動物を作ることであったが、線虫を使ってDMのモデルを作り出すことに成功した。CTGリピートが伸びた線虫、CCTGリピートが伸びた線虫、MBNLが欠損している線虫などを作出し、MBNLが欠損すると寿命が短縮することを発見した。これはヒトDMにおける早老症のモデルになると考えられた。

最終年度になって、塩素チャネル遺伝子に対するMBNL1依存性のスプライシングに焦点を絞り、スプライシングパターンを変える薬剤がないかどうかについてスクリーニングを行った。これは、機能を保持した塩素チャネルの発現を導く薬剤をスクリーニングするもので、MBNL1の発現を間接的に上昇させる化合物のスクリーニングでもある。この系により、トレハロースが症状の改善に効果があるかもしれないという結果を得た

B．研究方法

1）材料の調製

ヒトcDNAライブラリーより、MBNLとCELFあわせて9種のリピートRNA結合タンパク質（MBNL1、MBNL2、MBNL3、CUG-BP、CUG-BP2、CELF3、CELF4、CELF5、CELF6）をクローニングした。

スプライシングを調べるアッセイ系には、塩素チャネル、インスリン受容体、αアクチニン、c-srcなどのmini-geneを用い、HEK細胞にトランスフェクションした後、発現を確認した。

2）相互作用タンパク質の同定

酵母two-hybrid法と免疫沈降は、定法に従って行った。

3）リアルタイムPCRを用いたDM生検筋におけるMBNL1、CELF1の発現量の定量

まず、DMに特徴的なスプライシングが見られるかどうか、RNA結合タンパク質の量が変化しているかどうかの2点について、筋肉バンク中のDM筋からRNAを抽出し、発現量を、DM患者21例と疾患対象者12例で調べた。

4）線虫のモデル作製

線虫にGFPと融合させたCTG5、CTG130、CCTG100をインジェクションし、筋肉細胞に発現させた(myo3 プロモーター)。また、ヒトMBNLのホモログであるK02H8.1遺伝子をクローニングするとともに、K02H8.1が欠損した線虫を作出した。

5）スプライシングアッセイ

筋強直に一番関係が深いと考えられている塩素チャネルのミニジーンを用いて、試験管内スプライシングアッセイを行った。マウス塩素チャネルのエキソン6, 7A, 7を使ったこのアッセイは、エキソン7Aを含む胎児型(6-7A-7)と7Aを含まない成熟型(6-7)の比を検出するものである。胎児型では停止コドンが入るため、機能のない遺伝子が作られる。このミニジーンをトランスフェクトしたCOS細胞に各種因子を添加し、時間を追ってmRNAを抽出して、PCR法によってスプライシング活性を検討した。

6）筋分化促進物質の探索

もう1つのスクリーニングとして、筋芽細胞C2C12を用いて分化させ、そこで見られるマイオチューブラリン関連タンパク質1（MTMR1）のアイソフォームをPCR法で検討した。筋管細胞特異的なアイソフォームCの出現を分化の指標として、分化を促進させる因子の検討も行った。

（倫理面への配慮）

本実験では市販のヒトcDNAライブラリーを用いたため、倫理規定に抵触することはない。またDM患者生検筋は、患者からインフォームドコンセントを取得し、国立精神・神経センター倫理委員会で承認を受けたものを用いたもので、西野一三部長との共同研究である。

C．研究結果と考察

1）DM筋でのスプライシング異常とRNA結合タンパク質の発現量の検討

今回、初めてリアルタイムPCRを用いてRNA結合タンパク質の発現量を、DM患者21例と疾患対象者12例の骨格筋で調べた。筋にはMBNL3の発現がほとんど見られないため、MBNL1とMBNL2、CELF1とCELF2を定量した。その結果、どのmRNAの発現も患者と対照との間で有意差は認められなかった。

in vitro スプライシングアッセイ系にMBNLやCELF cDNAを導入し、mini-geneのスプライシングパターンが変化するかどうかを調べた。その結果、マウス塩素チャネルを用いた実験では、MBNLのどれもエキソン7Aのスキップによる機能的タン

パク質の合成方向に働くのに対し、CELF3-6 ではエキソン 7A を含むようにスプライスし、不活性なタンパク質を作らせることがわかった。

2）酵母 two-hybrid による RNA 結合タンパク質の相互作用

酵母 two-hybrid 法による MBNL1 結合タンパク質のスクリーニングの結果、YB-1 という RNA 結合タンパク質が MBNL1 と相互作用することが共免疫沈降法によって確認された。実際に YB-1 は、αアクチニンのスプライシングを MBNL1 と協同的に調節することがわかった。

興味深いことに、MBNL1 と YB-1 は細胞質中にある stress granules (SGs)に共局在することがわかった。

3）線虫モデル作製

線虫から K02H8.1 遺伝子 (CeMBL と命名) をクローニングし、それがヒト MBNL1 のホモログ (37%の相同性) であることを発見した。しかし、ヒトのように 4 つの RNA 結合モチーフは持たず、N 末端に 2 つ存在するのみであった。CeMBL はヒト MBNL1 同様、CUG や CCUG リピートに結合することが、酵母 three-hybrid 法によって明らかになった。また、CeMBL のエキソン 1 を含む 511 塩基の欠失を持つ変異体 *Tm1563* を分離した。この変異体の寿命は 14 日と短かった。

4）スプライシングを変える薬剤の検討

マウス塩素チャネルのスプライシングを指標に、DM 患者で認められる酸化ストレスに対して防御的効果のあるビタミン E、N アセチルシステインの効果を見た。ビタミン E 添加については、5μM で効果が認められたが、それ以上の濃度では有意差が認められなかった。N アセチルシステインでは、100μM まで効果が認められなかった。この他に、抗生物質のネオマイシンも効果がなかった。次に、二糖類であるトレハロースの効果を調べた。その結果、100mM 以上の濃度で、塩素チャネルの正常型スプライシングを促進することが明らかになった。

5）筋分化促進物質の探索

DM 筋は、未熟であることがいわれており、分化を促進させれば症状を改善する可能性がある。そこで各種薬剤を細胞に添加し、分化を促進する因子のスクリーニングを行った。その過程で、マイオチューブラリン関連タンパク質 1 の発現が分化依存性であることが判明した。多くの化合物を C2C12 筋細胞培溶液に添加してみたが、C2C12 を用いたアッセイによって、はっきりと筋分化を促進させる因子は現在のところ見つかっていない。特に、カテキン、アスタキサンチンなどの分子の効果は認められなかった。

D．結論

筋強直性ジストロフィーの示す全身症状のほとんどが、塩素チャネル（ミオトニア）、インスリン受容体(糖耐能の異常)、トロポニン T(心筋異常)など種々の遺伝子のスプライシング異常に起因することが明らかになってきた。例えば、マウス塩素チャネル遺伝子では、エキソン 7A が入るスプライシングが起こると停止コドンが入り、短いタンパク質が作られて機能が消失するが、エキソン 7A がスキップされると正常機能型の塩素チャネルになる。前者は胎児筋で優位であり、後者は成熟筋で優位となる。患者筋では胎児型になっていることから、胎児型を正常型に変える薬剤があれば治療として有用である。

そのために本研究では、まずスプライシング調節因子である MBNL や CELF の量を測定し、患者と正常とでは変化がないことを

確認した後に、薬剤を用いてスクリーニングを行った。その結果、トレハロースという候補が見つかった。今後は、モデル生物でのスプライシング調節が可能かどうかについて検討していきたい。

E．健康危険情報

なし

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G．知的財産権の出願・登録状況

なし

別紙４

研究成果の刊行に関する一覧表レイアウト

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Research Signpost
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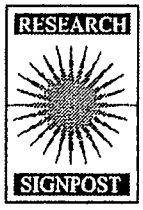

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2

Molecular basis of myotonic dystrophy: A new molecular mechanism called RNA gain-of-function

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Abstract

Myotonic dystrophy (dystrophia myotonica, DM) is one of the most common human muscular dystrophies, occurring with a frequency of about one in 8,000. Clinical features in DM include myotonia, cataracts, insulin abnormality, dementia, and frontal baldness. DM is an autosomal dominant condition and is classified into two types, DM1 and DM2, each caused by a different gene mutation. The gene responsible for DM1 is DM protein kinase (DMPK).

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Surprisingly, the DMPK mutation in DM1 patients is not in the coding region but is an increased number of CTG triplet repeats in the 3'-untranslated region of the gene. The CTG repeat number in DM1 patients is hundreds to thousands, whereas normal controls have only five to 30 CTG repeats. The gene responsible for DM2 is ZNF9. Similar to the DMPK mutation in DM1, the ZNF9 mutation causing DM2 is an expanded CCTG quadruplet repeat in the intron of the gene, with thousands of repeats reported in DM2 patients. Neither DM1 nor DM2 has any mutation in the coding region of the transcribed mRNA, and normal proteins are translated in DM patients. The molecular basis of DM1 and DM2 appears to lie in the RNA transcription stage, and the same mechanism could cause DM symptoms in both DM1 and DM2 patients. The transcribed CUG or CCUG repeat RNA forms a hairpin structure, an abnormal secondary structure observed only in DM patients. A specific RNA-binding protein recognizes and binds to the expanded number of repeats, trapping the RNA in this abnormal hairpin form and disturbing normal RNA-binding protein functions such as splicing and/or editing of other genes. As a result, splicing abnormalities could occur in many genes, leading to the variety of symptoms seen in DM patients.

Introduction

Myotonic dystrophy (dystrophia myotonica, DM) is a major muscular dystrophy, occurring with a frequency of one in 8,000 to 10,000. Its main symptom is myotonia (a tension in distal skeletal muscles) and muscle weakness. Multisystemic symptoms, such as cataracts, insulin abnormality, dementia, and frontal baldness, have also been described in DM patients [1]. In 1992, reverse genetics and positional cloning studies revealed a gene responsible for DM and its mutation in DM patients. An expanded number of CTG triplet repeats was observed on chromosome 19q13.3 in DM patients. These patients had hundreds to thousands of CTG repeats, whereas normal controls had only five to 30 repeats [2-4] (Fig. 1). It was reported that the greater the number of CTG repeats, the more severe the symptoms and the earlier the age of DM onset (called anticipation) [2, 5]. DM is categorized as a triplet repeat disease, as are Huntington's disease and Fragile X syndrome. However, unlike the other triplet repeat diseases, the unique feature in DM is that the gene mutation in the responsible gene is located in the 3'-untranslated region (3'-UTR) of the mRNA, in spite that DM is inherited in an autosomal dominant manner. Generally, autosomal dominant hereditary diseases are caused by gene mutations in coding regions, which produce amino acid conversions leading to translated proteins with altered activities. DM apparently does not follow this general rule.

In 2001, a different gene mutation was identified in a group of DM patients who did not exhibit the CTG repeat expansion. Instead, these patients

had an expanded CCTG quadruplet repeat region in the intron of a gene named *ZNF9*, located on chromosome 3q21 [6] (Fig. 1). In these DM patients, 5000 quadruplet repeats were found, whereas normal controls had up to 100 repeats. Since the discovery of a second non-coding repeat expansion, the major form of DM attributable to the CTG repeat expansion has been designated DM type 1 (DM1), and the type caused by the CCTG repeat expansion has been named DM type 2 (DM2). Both DM1 and DM2 have gene mutations in a non-coding region, indicating that no abnormal proteins are translated.

Here, we describe the results of recent studies on DM, which suggest a new point of view on the molecular mechanisms of some genetic diseases; alterations in the RNA sequence itself may be a key factor in some exceptional genetic diseases.

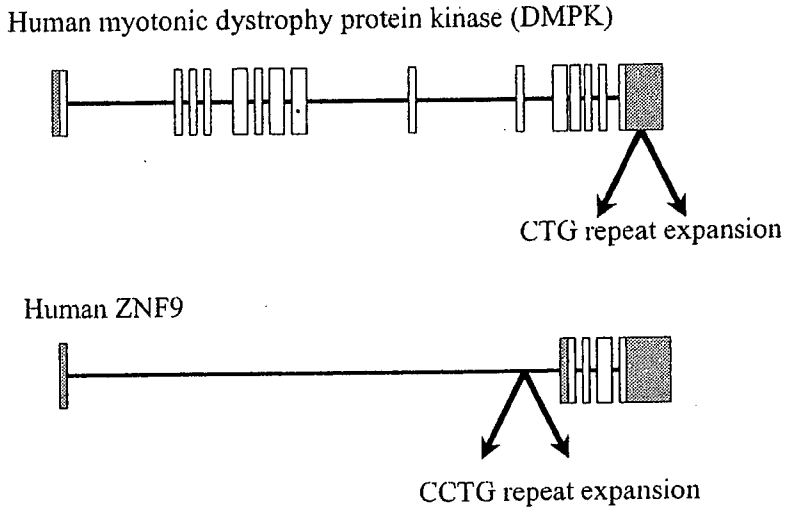

Figure 1. Diagram showing the gene structures of *DMPK* and *ZNF9*, the genes responsible for DM. The boxes represent exons. Open boxes are coding regions, and gray boxes are non-coding regions. The lines represent introns. The CTG triplet repeat is located in the 3'-UTR of *DMPK*, and the CCTG quadruplet repeat is located in the intron of *ZNF9*.

Gene responsible for myotonic dystrophy type 1

The gene responsible for DM1 encodes a serine/threonine protein kinase called dystrophia myotonica protein kinase (DMPK) [5, 7]. DMPK protein is distributed in skeletal muscle, cardiac muscle, brain, and stomach in humans [7-11]. The full-length human DMPK protein has a leucine-rich domain at the N-terminus, a serine/threonine kinase domain in the middle, and a hydrophobic region at the C-terminus. Six major spliced forms of human DMPK have been reported [12] (Fig. 2), and these are classified into two main subgroups, the DMPK-A and -B group and the DMPK-C and -D group. All of the isoforms

have the same kinase domain; the primary differences lie in the C-terminus. Both groups have hydrophobic C-termini, but they share no homology. The DMPK-A and -B group has higher hydrophobic amino acid content than the DMPK-C and -D group. Although the relationship between the expression of DMPK isoforms and the symptoms of DM is unclear, the C-terminus of each DMPK isoform might have an important physiological function, as the C-terminus of DMPK is reported to regulate its localization [13]. DMPK-A and -C are reported to be localized in mitochondria and endoplasmic reticulum [14], whereas an immunoelectron microscopy study revealed that DMPK-C or D is found mainly in the terminal cisternae of the sarcoplasmic reticulum [15].

DMPK is a member of the serine/threonine protein kinase family, and several homologs/orthologs have been reported from the mouse, rat, *Caenorhabditis elegans*, and *Drosophila melanogaster*. These genes are thought to constitute a myotonic dystrophy family of protein kinases (MDFPK) [16, 17]. Several MDFPK members have been shown to interact with small GTPases. The over-expression of DMPK protein in cultured cells, fission yeast, and budding yeast has indicated that DMPK functions as a regulator of cytoskeletal reorganization and/or cell division [18-20]. The physiological substrates for DMPK are not clear, although it can phosphorylate dihydropyridine receptor [21], myosin phosphatase I [13, 22], phospholemman [23], and phospholamban [24] *in vitro*. We have also confirmed that recombinant DMPK exhibits autophosphorylation [19].

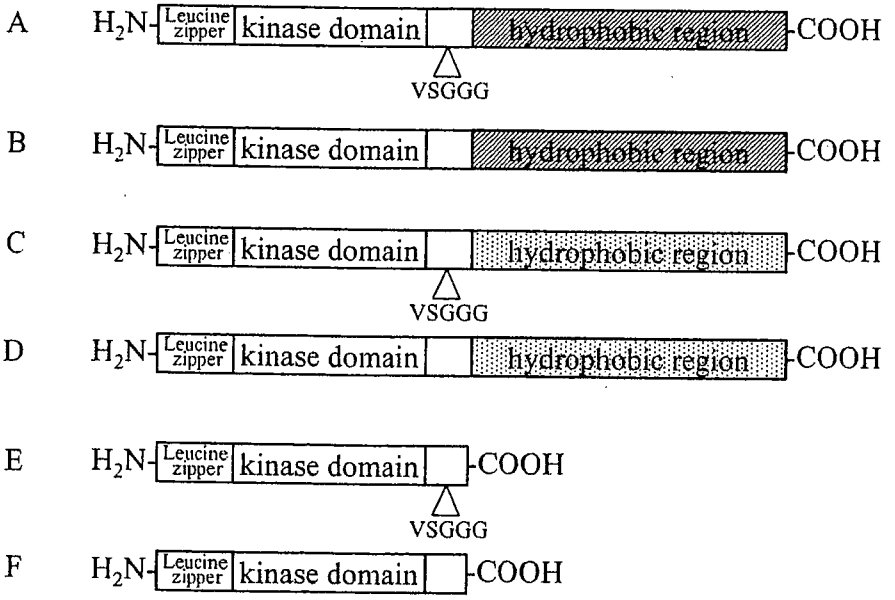

Figure 2. The six major splicing isoforms of human and murine DMPK. The isoforms are distinguished by an insertion of five amino acids (VSGGG) and differences in their hydrophobic C-terminal regions.

Gene responsible for myotonic dystrophy type 2

The gene responsible for DM2 encodes a zinc-finger protein named ZNF9 [25], also called cellular nucleic acid-binding protein (CNBP). ZNF9 has seven tandem zinc fingers and is thought to bind to chromosomal DNA and to function as a transcription factor. The ZNF9 amino acid sequence is highly conserved among *Xenopus laevis*, chickens, mice, and humans. ZNF9 is reported to be involved in the early development of the forebrain formation in mice [26].

Gene-manipulated model mice for DMPK and ZNF9

DMPK knockout and transgenic mice have been established and used to study not only the physiological function of DMPK but also the dose effect of DMPK on the DM phenotype. Given that the gene mutation (CTG repeat expansion) expressed in DMPK mRNA is not located in the coding region but in the 3'-untranslated region, the CTG repeat expansion might exert a *cis* effect on the expression level of DMPK, and the altered DMPK expression level might cause DM1 symptoms through a haplo-insufficiency mechanism. Two research groups independently reported the establishment of DMPK knockout mice, both showing a myopathy [27, 28]. DMPK deficiency resulted in sodium current abnormalities in skeletal muscle [29] and cardiac muscle [30]. DMPK knockout mice showed a decrease in the decremental potentiation, with a duration of 30 to 180 min, that accompanies long-term synaptic potentiation [31]. Cultured skeletal muscle cells from DMPK knockout mice showed smaller and slower calcium responses [32]. There are contradictory reports of decreased [33] and increased [34] calcium uptake by the sarcoplasmic reticulum in DMPK knockout mice, but both studies showed abnormal calcium cycling in DMPK-deficient cells.

The DMPK transgenic mice, expressing human *DMPK* with 20 CTG repeats, are reported to represent only myotonic myopathy [28], hypertrophic cardiomyopathy, and hypotension traits [35]. As a result, these knockout and transgenic mice showed only mild phenotypes and lacked other clear phenotypes characteristic of DM patients, such as myotonia and cataracts. Considering that knockout mice do not express DMPK but DM patients have a certain amount of DMPK protein, the phenotypic changes in the mice were much less than expected. Although it is still possible that abnormal DMPK expression may cause some of the DM phenotype, lower or higher DMPK expression levels do not appear to be able to explain the entire DM phenotype.

ZNF9 knockout mice show developmental abnormalities, especially in forebrain induction and specification [26]. Currently, there is little evidence that these phenotypical changes contribute to DM symptoms. Thus, both *DMPK* and *ZNF9* gene-targeting experiments have indicated that the genes responsible for DM are not responsible for, or have only little effect on, DM symptoms.

Models for the over-expression of CUG repeat RNA

A mouse model that over-expresses the CTG repeat has been reported. The transgenic mouse expressing the untranslated CUG repeat driven by the human skeletal actin promoter exhibited myotonia and myopathy, which are typical symptoms in DM patients [36]. Transgenic mice carrying the human genomic sequence for the DMPK region (~45 kb) with a long CTG repeat region showed histological muscle abnormalities and myotonia [37].

Primary human DM muscle cells show delayed differentiation [38], and the over-expression of the 3'-UTR of DMPK inhibited myoblast differentiation in cultured mouse C2C12 cells [39]. Moreover, the over-expression of DMPK cDNA with a long CTG repeat region the in 3'-UTR also inhibited myogenic differentiation in cultured C2C12 cells compared to the over-expression of DMPK cDNA with a normal repeat [40, 41]. The inhibition of differentiation required not only the CTG repeat but also the other part of the DMPK 3'-UTR [41], and the MyoD pathway was involved in the inhibition [42]. It was also reported that C2C12 cells transfected with human DMPK cDNA with a long CTG repeat in the 3'-UTR show higher sensitivity to oxidative stress [43, 44].

These results in a mouse model and in cultured cells strongly suggest that the long CUG repeat RNA, which is transcribed from the long CTG repeat in the chromosome, has an important physiological function, despite the fact that its location in the 3'-UTR means that it is not translated into amino acids.

RNA-binding proteins contributing to DM pathogenesis

In the late 1990s, a new proposal for DM pathogenesis was that the long CTG repeat in DM patients had a function at the RNA transcription stage. The CUG repeat of the RNA transcript can form a hairpin structure. If this abnormal structure were to have a physiological function in cells, it might disturb the regulation of cellular gene expression, perhaps in response to certain signals (RNA gain-of-function). The presence of a protein that binds to the CUG repeat RNA would make this hypothesis feasible.

In 1996, a protein that binds to eight repeats of the CUG triplet was reported. This protein, named CUGBP1, was the first molecule shown to bind CUG repeat RNA [45], making it a candidate protein for the molecular pathway of DM pathogenesis. Mice over-expressing CUGBP1 exhibited developmental delay of skeletal muscle [46]. CUGBP1 transgenic mice were established and shown to have histological changes of skeletal muscle and abnormalities of splicing for several genes [47].

However, an electron microscopic study revealed that the binding of CUGBP1 to the CUG repeat was not proportional to the CUG repeat length

[48], which did not correlate with the observation that longer repeat lengths were associated with more severe symptoms in DM patients. Moreover, CUGBP1 preferentially bound the UG dinucleotide repeat and showed only weak binding to the CUG repeat [49]. Creating further uncertainty about the role of CUGBP1 are two inconsistent reports: one indicating that CUGBP1 accumulates in the nucleus in DM patients [50] and another showing that CUGBP1 localizes similarly in fibroblasts of both normal and DM patients [48]. Although evidence exists that CUGBP1 and its homolog family proteins (CUGBP1-like and ETR-3-like factors, CELF) have an important role in DM, it is ambiguous whether CUGBP1 and CELF family proteins are the prime factors in DM pathogenesis.

A second candidate protein that binds CUG repeat RNA has been reported. This molecule, called muscleblind-like 1 (MBNL1), was first shown to bind CUG repeat RNA in an UV cross-linking experiment [51]. MBNL1 is an ortholog of *Drosophila* muscleblind, which belongs to a family of zinc-finger proteins and is required for eye development [52]. The binding of MBNL1 is dependent upon the repeat length [51], which is consistent with the repeat-length dependence of the severity of DM symptoms. Moreover, MBNL1 was reported to bind not only to CUG repeat RNA but also to CCUG repeat RNA [53]. Thus, MBNL1 is a more likely candidate than CUGBP1 for directly binding CUG/CCUG repeat RNA. MBNL1 knockout mice exhibited cataracts as well as electrophysiological and histological abnormalities in skeletal muscle, which are commonly observed in DM patients [54]. These indicate that MBNL1 might play an important role in DM pathogenesis.

The co-expression of the expanded CUG repeat and MBNL1 protein creates foci in the nucleus [55, 56], but the foci do not include CUGBP1, further supporting a key role for MBNL1 in DM pathogenesis. The formation of these foci suggests that MBNL1 might be trapped by the CUG repeat RNA, consequently exhausting the supply of functional MBNL1 protein. However, it has also been suggested that the co-localization of MBNL1 and the CUG repeat RNA in nuclear foci is separable from the molecular pathogenesis of DM [57]. Even if MBNL1 were the molecule responsible for DM symptoms, the mechanism of its action would remain to be elucidated.

Splicing abnormalities in a DM mouse model and in DM cells

Although the relationship between the expanded CUG/CCUG repeat and RNA-binding proteins such as CUGBP1 and MBNL1 needs further investigation, many studies have reported that the splicing of several genes is disturbed in a DM mouse model and in DM cells. The splicing pattern of cardiac troponin T (cTNT) was altered in DM patients, a finding confirmed by

mini-gene assays using fibroblasts from DM patients [58]. The expression of insulin receptors was altered to predominantly the non-muscle type in DM patients, and a contribution by CUGBP1 was indicated in this insulin receptor abnormality [59]. The function of the chloride channel was reduced in mice over-expressing the CTG repeat, and this was attributed to the abnormal splicing of the chloride channel premature mRNA confirmed in these mice and DM1 patients [60]. The skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum calcium-ATPase are also candidate genes for altered splicing in mice over-expressing the CTG repeat and in DM1 patients [61]. CUGBP1 has been reported to bind at the intron of the chloride channel gene [62]. Myotubularin-related 1 was found to change its splicing patterns in DM1 muscle cells [63]. The expression pattern of tau protein was altered in transgenic mice carrying the DMPK region of the human genome containing a long CTG repeat [37], and abnormal tau RNA maturation was observed in DM1 patients [64]. Splicing abnormalities were observed for NMDA and amyloid beta precursor protein in DM1 patients, and decreased expression of MBNL1 was also detected in the same samples [65]. These data lead us to conclude that the splicing patterns of many genes change in the presence the expressed long CTG triplet repeat and that DM could be categorized as a "splicing syndrome."

Concluding remarks

Recent evidence has suggested an unexpected mechanism of DM pathogenesis (Fig. 3). The expanded CTG triplet repeat expressed in DM patients can itself have a physiological function that leads to abnormal gene expression through its binding to RNA-binding proteins, resulting in the depletion of these proteins. This fact strongly indicates that the purpose of RNA molecules is not only to mediate the transfer of DNA information into protein but also to serve as physiologically functioning molecules in the cell. Recently, the functions of many small RNAs have been reported, revealing that such RNAs work ubiquitously in cellular gene systems. Investigations on DM pathogenesis have also been able to uncover new possibilities for RNA functions.

There are still many questions to be answered. The normal functions of RNA-binding proteins such as CUGBP1 (or CELF family proteins) and MBNL1 are unclear, as are their target RNAs. CUGBP1 does not seem to be a key factor in binding long CUG hairpin RNA, although some evidence implicates CUGBP1 in DM pathogenesis. MBNL1, however, does appear to directly bind the CUG repeat hairpin RNA, but the pathway involving MBNL1 has not been elucidated. It is possible that CUGBP1 (and CELF family proteins) and MBNL1 work together, and the possibility remains that another

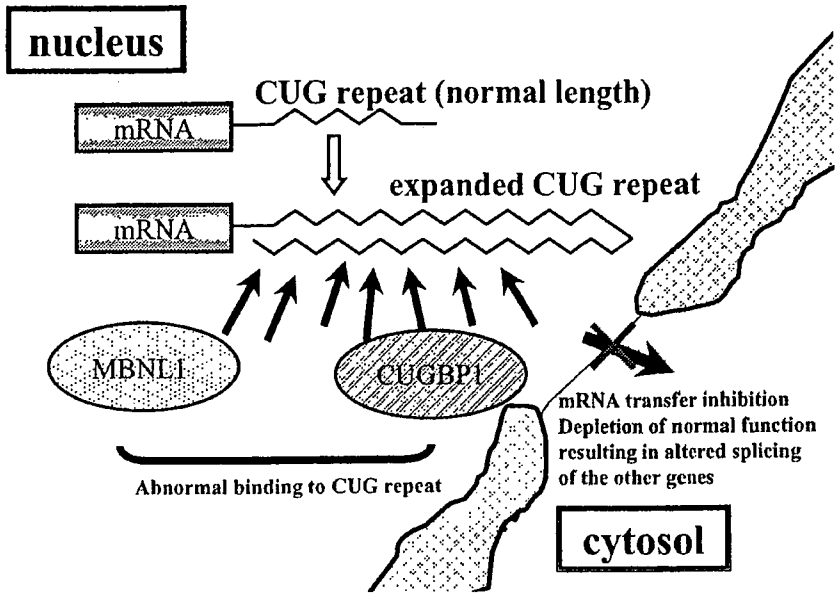

Figure 3. Scheme for the molecular pathogenesis of myotonic dystrophy.

new candidate RNA-binding protein is involved in DM pathogenesis. These investigations and future studies on the molecular basis of myotonic dystrophy will help to establish new methods for DM therapy.

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