Foci of Trinucleotide Repeat Transcripts in Nuclei of Myotonic Dystrophy Cells and Tissues

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Abstract. We have analyzed the intracellular localization of transcripts from the myotonin protein kinase (Mt-PK) gene in fibroblasts and muscle biopsies from myotonic dystrophy patients and normal controls. In affected individuals, a trinucleotide expansion in the gene results in the phenotype, the severity of which is proportional to the repeat length. A fluorochrome-conjugated probe (10 repeats of CAG) hybridized specifically to this expanded repeat. Mt-PK transcripts containing CTG repeat expansions were detected in the nucleus as bright foci in DM patient fibroblasts and muscle biopsies, but not from normal individuals. These foci represented transcripts from the Mt-PK gene since they simultaneously hybridized to fluorochrome-conjugated probes to the 5'-end of the Mt-PK mRNA. A single oligonucleotide probe to the repeat and the sense strand each conjugated to different fluorochromes revealed the gene and the transcripts simultaneously, and indicated that these focal concentrations (up to 13 per nucleus) represented predominantly posttranscriptional RNA since only a single focus contained both the DNA and the RNA. This concentration of nuclear transcripts was diagnostic of the affected state, and may represent aberrant processing of the RNA.

Myotonic dystrophy (DM) is an autosomal dominant neuromuscular inherited human genetic disease with multisystem effects (Harper, 1989). The underlying basis of DM is an unstable trinucleotide repeat sequence (CTG)n in a gene encoding a protein kinase (Mt-PK) (Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992). The severity of the disease, which can increase during transmission from one generation to the next is roughly proportional to the extent of expansion of (CTG)n repeat sequences (Harley et al., 1992; Tsilifidis et al., 1992; Caskey et al., 1992; Richards and Sutherland, 1992; Howeler et al., 1989). Normal individuals carry alleles containing between 5 and 30 CTG repeats. DM patients that are mildly affected have at least 50 copies, while the more severely affected individuals can have up to several thousand (Brook et al., 1992). The expansion of a trinucleotide repeat sequence has been found to be the basis of an increasing number of human diseases, including fragile x-syndrome, FraX (CGG repeat in 5'-untranslated region), in spinal and bulbar muscular atrophy, SBMA (CAG repeat in coding region), and more recently in Huntington disease (CAG repeat in coding region) (Morell, 1993). In DM, the CTG repeat is located in the 3' UTR of the mRNA, 500 bp upstream of the poly(A) signal, and it is expressed in many tissues (Brook et al., 1992). The mRNA containing the CTG repeat encodes a protein that has homology with the protein kinase gene family. The mechanism by which the expansion of the trinucleotide repeat results in a pathological phenotype is not understood.

Our hypothesis that addresses this question is that expanded trinucleotide repeat sequences may act by causing an alteration in the subcellular (cytoplasmic or nuclear) distribution of Mt-PK transcripts leading to their aberrant function. To address this issue, we have used fluorochrome- or digoxigenin-conjugated oligonucleotide probes to localize the sites of Mt-PK transcripts both in the nucleus and the cytoplasm of fibroblasts derived from DM patients and normal individuals. To visualize the transcript of the allele with the expanded trinucleotide separately, we used an antisense probe to the expanded trinucleotide repeat sequences. The cytoplasmic distribution appeared to be identical between normal and patient-derived fibroblasts. However, our results indicated a striking difference in the nuclear distribution of the Mt-PK transcripts, a difference that was verified in a muscle biopsy from an affected individual.

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1. Abbreviations used in this paper: DAPI, 4,6 diamidino-2-phenylindole; DM, myotonic dystrophy; Mt-PK, myotonin protein kinase.

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Materials and Methods

Preparation of Oligonucleotide Probes

The 30-base-long oligonucleotide probes to the repeat sequence CAG-30 (antisense) and CTG-30 (sense) were synthesized on a DNA synthesizer with two amino-modified 5' terminal at either end (Applied Biosystems, Foster City, CA). In addition, 13 oligonucleotide probes (40-45 bases long), but to the 5' end of the transcript (DM-1 to DM-13), were synthesized with five amino-modified 5' terminals bases apart (Kislauskis et al., 1993). Sequences were obtained from GenBank (accession No. L00027; Caskey et al., 1992) and Housman, D., and M. McCurrach (unpublished results). After depurination, probes were purified by gel electrophoresis. The purified probes were labeled with fluorescein isothiocyanate or the red dye CY3 (Biological Detection Systems, Pittsburgh, PA) by using 0.1 M NaHCO3/Na2CO3, pH 9.0, overnight at room temperature in the dark (Agrawal et al., 1986). Reaction products were passed twice through Sephadex G-50 (using a 25 ml disposable pipette). Fractions were combined, lyophilized, and further purified on a 10% polyacrylamide gel. Purified probe was then extracted from the gel by soaking overnight in 1 M triethyl ammonium bicarbonate (TEAB). The probes were 5'-end labeled with digoxigenin using dig-11-dUTP and terminal deoxy transferase (Boehringer Mannheim Biologicals, Indianapolis, IN).

Oligonucleotide sequences used in this work were:

5'-5CTGCTGCTGCTGCTG5CTGCTGCTGCTGCTG-3'
5'-5GACAATC5CCGCCAGGSAGAAGCGCGCCATC5CGGCCGGAASC-3'
5'-5TCATGATC5TCATGGCA5ACACCTGGCCCG5CTGCTTCATCST-3'
5'-ASGCATGGAGAA5CTCAG5CACACTG5CACCCCAASAAA-3'
5'-5GGCCACAGCGG5CCAGCAGGATG5TGTCGGGTTTGATG5C-3'
5'-C5CCCTCCT5CCAGGGCCCSTCAGAACCCSTCAGTGCTAG5G-3'
5'-C5GTCCCTGGC5GTCCCCC5GGGCCTCTCSGCCACTTCTC5C-3'
5'-C5GGCAGCCCC5GTCCAGGCCC5GGAGCCC5GGCTGCAGG5C-3'
5'-5CTGATCGTCT5CTGCTG5CA5ACACTGGCGCG5CTGCTTCATCST-3'
5'-5TCAGTATGCT5CTGATCG5AC5AGCTGCGGCG5CTGCTTCATCST-3'
5'-5GCCAGG5GCC5CCGCG5CAGACTCAGG5ACGAGAGS-3'
5'-5SGACAATC5CCGCCAGGSAGAAGCGCGCCATC5CGGCCGGAASC-3'
5'-5GGCCACAGCGG5CCAGCAGGATG5TGTCGGGTTTGATG5C-3'
5'-5CCCAACG5CCGAG5AACGATCG5CTGCGGGGGAC5ACTC5GCGG5-3'
5'-5CGS5GAAATCCGG5GGAGGGG5CTGCGCA5AGAACATC5T-3'
5'-5CGAGACGACGACGACGACGACGACGACGACG-3'

In Situ Hybridization

Primary skin fibroblast cells derived from two DM patients (3132 and 3755), and normal human diploid fibroblast cells were grown in dishes containing gelatin-coated coverslips at 106 cells/100-mm dish. Cells on the coverslip were washed with HBSS and terminal deoxy transferase (Boehringer Mannheim Biologicals, Indianapolis, IN). Cells from patients were incubated in culture with 0.015 ng/ml colcemid for 45 min. After incubation, cells were trypsinized with 1% trypsin-EDTA in HBSS. Fresh medium was added to stop the trypsin reaction. The cells were centrifuged, and the cell pellet was resuspended in 5 ml of 0.075 M KCl, incubated 17 min at 37°C, and again centrifuged. Freshly prepared 3:1 methanol/acetic acid (10 ml) was added drop by drop to the cell pellet, with mixing at 25°C for 10 min and centrifuged. About 10 ml of methanol/acetic acid (3:1) was added to the cell pellet and incubated for 10 min at 25°C. The cells were centrifuged, the supernatant was removed, and the cell pellet was resuspended in 1 ml of methanol/acetic acid. Cells were dropped onto ethanol-washed slides from a distance of 2 ft and dried in air overnight. The slides were incubated at 65°C for 10 min and stored at -80°C (Johnson et al., 1991). The slides were then hybridized, as described with oligonucleotide probe. This preparation also resulted in chro- mosome spreads.

Digital Imaging Microscopy

The methods used have been described in detail previously (Taneja et al., 1992; Carrington et al., 1990; Fay et al., 1989). Briefly, images of the distribution of fluorescence were obtained by a conventional inverted microscope but modified to capture images at various planes of 0.25 µm within the cell. Images at each focal plane were acquired with a thermoelectrically cooled CCD (Photometrics Inc., Tucson, AZ). Images were restored to remove out-of-focus light. 0.20-µm diameter latex beads (Molecular Probes, Inc., Eugene, OR), into which the appropriate fluorophors were embedded, were used as fiduciary markers to align the two images captured at each wavelength. Those voxels containing both red and green signals are turned white. Images were displayed on a Silicon Graphics (Mountain View, CA) workstation, and were inspected using graphics software developed by CSPS, Inc. (Billerica, MA) and photographed using a digital-to-analogue film recorder (Matrix Instruments, Orangeburg, NY).

Results

13 oligonucleotide probes (DM-13) from the 5'-end seven exons of Mt-PK RNA (there are 14 exons total) were labeled with digoxigenin and used as a mixture for in situ hybridization (Fig. 1). Probes from the 5'-end of Mt-PK RNA should detect the transcripts from both the normal and DM allele. After in situ hybridization, the signal of Mt-PK mRNA was significant difference in the cytoplasmic location of the Mt-
Figure 2. Hybridization of digoxigenin-labeled 5'-end and triplet repeat probes to RNA transcripts within the cytoplasm of normal and patient fibroblasts. The hybridized probe was detected with antidigoxigenin-alkaline phosphatase. (A) Myotonic dystrophy patient (3132) cells hybridized with the antisense triplet repeat probe (CAG-30). (B) Same as in A, but the 5'-end probe mixture (DMI-13) was used. (C) Normal human fibroblasts, probed as in B with the 5'-end probe. (D) Normal human fibroblasts probed as in A with the triplet repeat probe (CAG-30).
Figure 3. Hybridization of sense and antisense triplet repeat probes to nuclear transcripts within normal and patient fibroblasts. Probes were directly conjugated to the fluorescent dye CY3. Cells were counterstained by DAPI. (A) DM fibroblasts (3132) hybridized in situ with CY3-labeled antisense repeat probe (CAG-30). (B) Same as in A, but CY3-labeled sense repeat probe (CTG-30) was used. (C) Nuclear preparation from DM fibroblasts hybridized in situ with CY3-labeled antisense probe. (D) Normal human diploid fibroblast cells hybridized in situ with CY3-labeled antisense repeat probe. (E and F) Diagnostic detection of repeat transcripts in patient biopsy material. Histological sections from a 49-yr-old female DM patient (H680) and a normal individual were hybridized to a fluorescein-conjugated antisense repeat probe. Tissues were counterstained with DAPI. (E) Tissue from the DM patient. The nuclei containing foci are designated by number and enlarged below the figure. (F) Tissue from a normal individual. No signal was seen in the nucleus.
PK mRNA was observed in cells between DM patients and normal individuals. Probes for the 5' end of the Mt-PK mRNA could not distinguish between the transcripts of the normal and the expanded allele. To achieve this goal, we constructed a probe complementary to the CTG repeat. Since the transcript of the allele containing the expansion should have considerable repeat target sequence, unlike the transcript from the normal allele, we expected that this probe would give a DM-specific signal. The sites of subcellular localization of transcripts with the expansion could thus be distinguished. Digoxigenin-labeled oligonucleotide probes to the repeat sequence and the sense control (CAG-30 and CTG-30) were hybridized to the fibroblast cells from normal and DM patients, and the hybridized probe was detected with antidigoxigenin alkaline phosphatase conjugate. It was found that the CTG repeat sequence was present in the DM fibroblasts and distributed perinuclearly within the cytoplasm (Fig. 2 A). In contrast, signal from the repeat was absent in the cytoplasm of normal fibroblasts (Fig. 2 D). We then confirmed this observation in diseased muscle by investigating the distribution of the CTG expansion in the mRNA from muscle biopsies from a DM patient and a normal control. The signal of CTG repeat in the sarcoplasm was predominantly at the periphery of DM myofibers and was not found in the normal tissue (data not shown). These results confirmed that the CAG-30 probe detected the presence of cytoplasmic mRNA of the expanded repeat allele of the Mt-PK gene. In the affected fibroblasts, the mRNA with the expansion was localized in the cytoplasm apparently identical to the localization of the mRNA from the normal allele, as determined by using the 5' probes on normal or affected fibroblasts. Both the normal and the expanded mRNA remained after nonionic detergent extraction, indicating that both mRNAs were attached to the cytoskeleton (data not shown). Since there was no detectable abnormality in the spatial distribution or cytoskeletal association of the Mt-PK mRNA observed in affected cells, evidence does not support the hypothesis that DM pathology is caused by mislocalization of the Mt-PK mRNA in the cytoplasm.

However, during the course of these studies, we observed a striking distribution of the in situ hybridization signal in the nuclei of both fibroblasts and muscle cells of DM patients: the Mt-PK transcripts were present as foci of nuclear aggregations. As shown in Fig. 3 A, the antisense probe revealed a number of bright foci in nuclei of intact fibroblasts from DM patients. Foci of hybridization were absent when the sense probe was hybridized to the nuclei (Fig. 3 B) of the myotonic dystrophy patient cells or when the probe was hybridized to nuclei from normal human fibroblasts (Fig. 3 D). Individual cells showed a strong signal represented by many discrete foci that were scattered throughout the nucleus in apparently random positions. This punctate hybridization pattern suggested that the subnuclear localization of the Mt-PK transcripts may have a functional significance.

To visualize these foci more clearly, nuclei were isolated to eliminate cytoplasmic contamination, and also showed the signal with antisense probe as a number of discrete foci (Fig. 3 C). No signal was detected with the sense probe in these nuclei. Skin fibroblast cells from one myotonic dystrophy patient (3132) were analyzed for the number of foci in each nucleus. Nuclei contained a mean of five foci, but some were found with 13. Cells from another myotonic dystrophy pa-
tient (3755) were very similar. To determine that these foci were characteristic of the disease, and that they were not an artifact of cell culture, tissues with the primary lesion were investigated. Histological preparations of muscle biopsies from DM and normal patients were hybridized to fluoresceinated CAG-30 probe and counterstained with DAPI. It was found that the nuclei of DM tissue contained one to three intense foci (Fig. 3, E and bottom) that were not detected in normal tissue (Fig. 3 F).

These foci may have resulted from repeated sequences not related to the myotonic dystrophy allele. To determine whether the repeat probe was detecting Mt-PK transcripts specifically, we cohybridized with the mixture of the CAG-30 probe (green) and the 13-oligonucleotide probes from the 5' end of Mt-PK transcripts (red) to nuclei from myotonic dystrophy patient samples, and we analyzed the distribution of each probe simultaneously. The 5'-end probes (DMI-13) were labeled such that each probe contained five red fluorochromes, hence, a total of 65 fluorochromes would hybridize to the 5'-end of each transcript. The antisense probe (representing 10 repeats) contained two fluorescein molecules. Since there are ~2,000 repeats (Brook et al., 1992; McCurrach, M., unpublished data), the total number of fluorochromes conjugated to the probe that repetitively hybridized to the 3'-end would be as many as 400, generating a greater signal to noise ratio than the 5'-end of the transcript. This was observed (Fig. 4) when the 5'-end (DMI-13) and CAG-30 were mixed together in equimolar ratios and hybridized to preparations of DM fibroblast nuclei; the intensity of the foci revealed by the 5' (red) probes contained less signal than the 3' (green) probe when compared to their respective background levels. Reversal of the fluorochromes on the 5'-end and repeat probes gave the same results (data not shown). These results confirmed that the CAG-30 probe hybridized to transcripts arising from the DM allele. To evaluate the extent to which the 5'-end probes colocalized with the CAG repeat probe, we used digital imaging microscopy to provide an assessment of spatial congruence of the two labels. Optical sections at each wavelength were taken on a CCD camera and restored mathematically to remove fluorescent light not contributing to the specific section (Carrington et al., 1990). Two images from the same Z plane were superimposed using fiduciary markers. It was found that the green foci always colocalized exactly with red foci, indicating that foci containing the expanded CTG repeat sequences were present only in the Mt-PK transcript (Fig. 4 C). However, one and sometimes two red (5'-end) foci did not colocalize with any green (i.e., contained no repeat hybridization). These would be expected to represent the transcription sites of the normal allele. Therefore, the large number of foci obtained using the repeat probe were in excess of the number of transcription sites, and must represent released transcripts. Additionally, all of these supernumerary foci contained the repeat expansion, indicating that they have resulted from transcription of the affected allele, but not the normal allele.

To characterize the nascent transcripts from the released transcripts unequivocally, we hybridized differently labeled probes to the DNA and RNA specifically. The sense probe hybridized to only one, and occasionally two, of these foci when DNA in the interphase nucleus was denatured (Fig. 5 A), whereas the antisense probe hybridized to the DNA and
Figure 4. Colocalization of nuclear foci obtained from in situ hybridization using the 5'-end probe mixture to Mt-PK mRNA (red) and the antisense (CAG-30) probe to trinucleotide repeat sequences (green). Probes were hybridized in situ to DM fibroblasts, and each color was independently detected by a CCD camera and a series of optical planes digitized. Restored images from the same Z-plane were superimposed using a fiduciary marker bead that can be seen outside the nucleus for the exact alignment of each image (Carrittton, et al., 1990). (A) Hybridization of the antisense (CAG-30) probe to triplet repeats in patient (3132) DM fibroblasts (green). (B) Hybridization of the probe mixture (DMI-13) to the 5'-end of Mt-PK mRNA (red). (C) Superimposition of A and B. Where red and green spots superimpose are indicated by white spots. A single red spot (arrow) remains after superimposition. This indicates that no repeat probe has hybridized to this focus and, therefore, this is the transcription site of the normal allele.

Figure 5. Colocalization of the gene and its transcripts. Antisense probe (CAG-30) conjugated to CY3 and the sense probe (CTG-30) conjugated to fluorescein were simultaneously hybridized to DM fibroblasts (3132) after denaturing the nuclear DNA, and the triplet repeat was detected. The antisense probe detects both the gene and its transcripts, whereas the sense probe detects only the DNA. Superimposition of the green image from the sense (A) and the red image from the antisense (B) probes show only a single colocalized white spot (C) that must represent the site of the gene and transcripts from this gene, presumed to be the nascent chains (arrow). The red foci that do not colocalize must represent posttranscriptional RNA.

In the nuclei of cells derived from normal individuals, the 5'-end probes (DMI-13) showed at least two foci of signal (data not shown), consistent with previous observations that these are the sites of transcription of both alleles (Zhang et al., 1994). These were considerably dimmer than the foci containing the repeat seen in the DM cells using the same probe. Therefore, it appeared that the Mt-PK transcript in normal cells, and presumably the unaffected transcript in DM cells, in contrast to the affected transcript, was efficiently processed and transported to the cytoplasm. In DM cells, the buildup of foci of the repeat-containing transcript in the nucleus may be the consequence of a rate limiting step in RNA processing such as splicing, polyadenylation, or transport to the cytoplasm. To investigate whether the expanded CTG repeat was compartmentalized in the nucleus similar to a non-snRNP splicing factor, SC35 ("speckles," Fu and Maniatis, 1990) or similar to concentrations of poly(A), ("patches,"
Xing et al., 1993; Carter et al., 1991); foci containing the expanded CTG repeat were colocalized with regions of SC-35, or poly(A) distribution. These compartments are possible regions where excess nuclear factors accumulate (Rosbash and Singer, 1993), and the posttranscriptional mRNA containing the repeats might be expected to accumulate there as well. Splicing factor, SC-35 (Fig. 6 A, green areas) detected by indirect immunofluorescence or, alternatively, in situ hybridization using a fluoresceinated poly dT probe (Fig. 6 B, green areas) were each colocalized with the foci detected by the antisense triplet repeat probe (red). High resolution optical sections were taken using digital imaging microscopy and superimposed. Foci representing the CTG repeat sequences did not exclusively superimpose with the high concentrations of SC-35 or poly(A). The pattern of the colocalization that did occur could just as easily be explained by random coincidence. In Fig. 6 A, three or four foci out of 14 appeared to colocalize with the SC-35 "speckles," and in Fig. 6 B, approximately two out of seven foci colocalized with poly(A). We have determined the SC-35 concentrations to occupy 31 ± 7% of the nucleoplasmic area in HeLa cells (Zhang et al., 1994) and, therefore, the observed colocalization is consistent with random coincidence. The poly(A) was similar in its distribution. This suggests that the repeat transcripts do not share the same nuclear compartment with the "speckles" or "patches" (Rosbash and Singer, 1993) in any way that may be argued to be significant.

**Discussion**

The results presented here demonstrate that focal accumulations of posttranscriptional RNA are a characteristic of the expanded repeat sequences, (CTG)n, from the affected allele of the Mt-PK gene, the gene that has been shown to be responsible for the myotonic dystrophy phenotype. No other examples exist which show RNA accumulated in foci subsequent to its transcription; the only foci identified to date are the sites of nascent chain transcription (Lawrence et al., 1989; Shermoen and O'Farrell, 1991). The fact that this observation occurs only in the nuclei of affected cells, and only with transcripts from the affected allele, suggests that these foci represent the primary events of the disease lesion. Furthermore, because the repeat transcripts appear to "build up" in the nuclei, these results suggest that some aspect of nuclear RNA metabolism may be responsible for the foci we observe. The rate-limiting step in this metabolism is not evident from the type of analysis done here, and no unusual disposition of these foci was seen that could be indicative of sequestration of the transcripts to nuclear compartments different from normally processed and transported transcripts. For instance, the lack of an exclusive colocalization of triplet repeat transcripts with the splicing factor SC-35 domains were not unlike the association of the neurotensin transcripts (Xing et al., 1993) or sites of adenovirus or actin transcription (Zhang et al., 1994). Similarly, poly(A) "patches" that were suggested to be associated with new transcripts (Carter et al., 1991) showed no exclusive colocalization with the foci or lack of it. Since the functional significance of these compartments is not understood, their relevance to the distribution of the foci is not enlightening. However, a high degree of colocalization of triplet repeat transcripts to any nuclear structure or component could potentially give insights into their role in RNA processing. Such an approach (for instance, the screening for nuclear antigens that codistribute with the foci) may reveal functionally significant relationships by first establishing spatially significant relationships. This approach is proceeding with potentially informative antigens (e.g., coilin).

Nuclear export from the affected allele does not appear to be significantly suppressed in the steady state, since RNA containing the repeat is evident in the cytoplasm. However, the export kinetics of the RNA from the nucleus may be affected by the expansion. Transcripts from the normal allele show only one focus, at the site of transcription. Therefore, the absence of posttranscriptional foci from this allele suggests that the same sequences lacking the repeat do not "build up" and may be efficiently exported to the cytoplasm. An interesting feature of the disease is that it is dominant; possibly, the posttranscriptional foci may inhibit other nuclear functions indirectly. Further investigation of differentiated
muscle, where this gene is upregulated, may reveal the mechanism whereby these transcripts eventually induce the pathology. In the muscle biopsy investigated, however, it appeared that fewer foci were evident in these nuclei than in fibroblast nuclei. This may be caused by several factors. First, the tissue represents sections, and thus all the nuclei may not be present. Second, all nuclei are in Go, and hence have half the DNA of many of the cells in a cycling population. Finally, the preservation of the material over several months may have resulted in RNA degradation. Notwithstanding these possibilities, the differences between the number of foci in nuclei of primary fibroblasts compared to adult muscle may reveal a significant difference.

Recent evidence has implicated the 3' untranslated region in the posttranscriptional regulation of mRNA expression (Jackson, 1993), and in particular, the differentiation of muscle (Rastinjad and Blau, 1993). We have shown the 3' untranslated region to be important in the localization of actin mRNA in the cytoplasm (Kislauskis et al., 1994). It is not ruled out that in the milieu of the sarcoplasm, the distribution of the Mt-PK mRNA is affected, resulting in the aberrant localization of the protein kinase and consequent disruption in sites of intracellular phosphorylation.

There is some controversy concerning the expression of the Mt-PK mRNA containing the repeat, with some arguing that it is either increased (Sabouri et al., 1993), decreased (Fu et al., 1993), or entirely absent (Carango et al., 1993). Recently, it has been suggested that the DNA repeat stabilizes nucleosome structure, which represses the transcription of the gene (Wang et al., 1994). We have shown the latter two cases to be unlikely, as we detect abundant transcripts in foci. Why these foci remain in the nuclei and how they might affect the cytoplasmic levels of transcripts from both alleles remains to be determined.

We suggest that these foci may have functional significance in the pathogenesis of myotonic dystrophy and merit further investigation. The consistent appearance of nuclear foci in DM fibroblasts and their absence in normal fibroblasts suggests a simple diagnostic approach for DM that could be performed on skin biopsies in a rapid and sensitive manner. Of particular interest would be the potential to carry out this procedure on amniocytes or chorionic villus samples in the context of prenatal diagnosis. Since the severity of the disease is proportional to the length of the repeat, this approach also provides a means of prognosis; the amount of fluorescence resulting from the hybridization is an indication of the degree of expansion that allows a prediction as to the fate of the affected individual.

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