Enhanced Cleavage of an Atypical Intron of Dopamine D₃-Receptor Pre-mRNA in Chronic Schizophrenia

Claudia Schmauss

Department of Psychiatry and Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, New York, New York 10029

The D₂-class of dopamine receptors (D₂, D₃, and D₄) is a target for typical and atypical neuroleptic drugs. They have been considered, therefore, as factors that may contribute to the pathophysiology of psychotic disorders. Interestingly, in cortical brain tissues obtained postmortem from patients with chronic schizophrenia D₃ mRNA was found to be significantly lower than in the corresponding anatomic regions of controls. Because the expression of a truncated D₃-like mRNA (named D₃nf) appeared to be unaffected in schizophrenic brains, these findings suggest the possibility that the loss of D₃ mRNA results from an abnormal splicing of D₃ pre-mRNA in schizophrenia that is accompanied by an increased accumulation of the truncated D₃nf mRNA. To test this, three approaches were taken. (1) Substrate D₃ pre-mRNA was spliced in vitro in HeLa nuclear extracts. Results from these experiments show that D₃nf mRNA results from the alternative removal of a short spliceosomal intron in D₃ pre-mRNA that has a noncanonical 3' splice site. (2) Substrate D₃ pre-mRNA was spliced in vivo in stably transfected rat GH3 cells. Despite the atypical 3’ cleavage that is necessary to generate D₃nf mRNA, D₃ and D₃nf mRNA were found to be processed at similar amounts. (3) The relative D₃/D₃nf splicing efficiencies were then determined in the anterior cingulate cortex of postmortem brains obtained from controls and from patients with chronic schizophrenia. Significant differences were found between the relative levels of D₃ and D₃nf mRNA, suggesting that an enhanced D₃nf-specific splicing of D₃ pre-mRNA in schizophrenia leads to a decreased expression of D₃ mRNA.

Key words: D₃ pre-mRNA; in vitro splicing; in vivo splicing; primer extension; S1 nuclease protection; postmortem brain RNA

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Correspondence should be addressed to Dr. Claudia Schmauss, Mount Sinai School of Medicine, Box 1229, One Gustave L. Levy Place, New York, NY 10029.

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(Giros et al., 1991; Snyder et al., 1991; Nagai et al., 1993; Schmauss et al., 1993). Their function is unknown. The longest of these mRNAs, named D₃nf, results from a deletion of 98 nt that constitute the C-terminal region of the putative third cytoplasmic domain of the D₃ receptor and, therefore, encodes a D₃-like protein with a different C terminus (Schmauss et al., 1993; Liu et al., 1994). In human brain, D₃nf mRNA was shown to be abundantly expressed and also translated into protein (Liu et al., 1994), suggesting that D₃nf mRNA does not result from an RNA processing error. Another interesting observation is that D₃nf mRNA was expressed in certain neocortical regions of brains obtained from patients with chronic schizophrenia, regions in which the expression of D₃ mRNA was found to be lost (Schmauss et al., 1993).

The human genome contains a single, intron-containing D₃-encoded gene in which the 98 nt that are deleted in D₃nf are embedded within a large continuous exon (Liu et al., 1994). Thus, to generate D₃nf mRNA via alternative splicing, these 98 nt must be recognized by the splicing machinery as an alternative intron. Removal of this intron, however, would require cleavage of a rare (and nonconforming) 3' splice site sequence that lacks the penultimate AG dinucleotides. However, these dinucleotides are known to be the most highly conserved dinucleotides of 3' splice sites (Reed, 1989) and have been found to be essential for both lariat formation (Reed and Maniatis, 1985) and snRNP binding (Charbot and Steitz, 1987).

The present study shows that D₃nf mRNA is indeed generated via atypical alternative splicing and, therefore, suggests a mechanism by which our previous results in schizophrenic brains would be explained: that an increased splicing of D₃ pre-mRNA leads to...
a decrease of D₃ mRNA and an increased accumulation of D₃nf mRNA.

MATERIALS AND METHODS

Splicing of substrate D₃ pre-mRNA in vitro. The sequence schematically diagrammed in Figure 1 (top), which codes for the 3' part of the putative third cytoplasmic domain of the D₃ receptor and the 5' region of the putative 6th transmembrane-spanning domain, was cloned into the plasmid vector pCR II (Invitrogen, San Diego, CA). A 5'-capped 2.3 kb pre-mRNA transcript (see Fig. 1, top) of this recombinant plasmid was synthesized in vitro using 10 U of Sp6 RNA polymerase (Stratagene, La Jolla, CA). This D₂-encoded substrate pre-mRNA (20 fmol) was added to 50 μl of HeLa nuclear extract [prepared as described by Dignam et al. (1983) and supplemented with MgCl₂, ATP, and creatine phosphate as described by Kraemer et al. (1984)]. Aliquots (10.5 μl) were incubated at 30°C for 1–4 hr. At the end of each incubation, the splicing reaction was treated with protease K, extracted with phenol/chloroform, and precipitated. First-strand cDNA was synthesized with 200 U of Molehele murine leukemia virus (M-MLV) reverse transcriptase (United States Biochemical, Cleveland, OH) and the primer sequence—5'-GTATTGGAACATGGGT-3'—which is complementary to the most 5' sequences of exon I (see Fig. 1)—were used for the PCR amplification to selectively amplify the ligated exons of the in vitro splicing reaction. PCR products were separated on a 15% agarose/TBE gel, transferred to membrane, and subjected to Southern blot analysis using a 32P-radiolabeled random-primed cDNA encoding the human D₃ receptor as a probe. D₃- and D₃nf-specific PCR products were cloned into the plasmid pCR II (Invitrogen) and subjected to nucleotide sequence analysis of both strands.

Primer extension analysis of intermediates and products of in vitro-spliced wild-type and mutant substrate D₃ pre-mRNA. For these experiments, a 5'-capped 223 nt T7 RNA polymerase (Stratagene) transcript of the wild-type or mutant exon I sequences (see Fig. 1) was spliced in vitro in HeLa nuclear extracts as described above. 3' splice site mutants were generated in vitro using the Chameleon Double-Stranded, Site-Directed Mutagenesis kit (Stratagene). Briefly, exon I was cloned into the plasmid pCR II, which served as the target plasmid DNA to which two oligonucleotide primers were used:

Wild-type sequence: 5'-TTGCAACACTCCTCGGGAGATGTCACACTTCGGGAAGA3'

Mutant 1: 5'-CTGCAACACTCCTCGGGAGATGGCACAATCTCGGGAGA3'

Mutant 2: 5'-CTGCAACACTCCTCGGGAGATGGCACAATCTCGGGAGA3'

Mutant 3: 5'-CCTGCAACACTCCTCGGGAGATGTCACACTTCGGGAAGA3'

Extended and digested plasmid DNA was then transformed into the repair-deficient Escherichia coli strain XL1-Blue. Competent cells. The correctness of each mutation was verified by nucleotide sequencing of the entire exon I.

In vitro-spliced wild-type or mutant RNA was precipitated and annealed to 5 × 10⁵ cpm of a 32P-end-labeled oligonucleotide sequence (5'-CAAGCACAAATGGCACC-3') that is complementary to the most 3' sequence of exons I and II (see Fig. 1) and is identical to the 5' region of the putative third cytoplasmic domain and the 5' region of the putative transmembrane domain.

RT-PCR analysis of D₃ and D₃nf mRNA expression in D₃ minigene-expressing GH3 cells and in human cingulate cortex. First-strand cDNA was generated from 10 μg of total cytoplasmic RNA extracted from transfected GH3 cells using an oligo-dT₁₅ primer in conjunction with 200 U of M-MLV reverse transcriptase. The cDNA was then amplified by PCR using a 5' primer (5'-GTATTGGAACATGGGT-3') that is identical to the most 3' sequence of exon I (see Fig. 1) and a 5' primer (5'-ATCCGGCTAGGGAGA3') that is identical to the 5' sequence of exon II (see Fig. 1). The resulting PCR products were separated on a 7 M urea/6% acrylamide gel. RT-PCR amplification of D₃ and D₃nf specific mRNAs from the primary transcript of the transfected plasmid pCEP4/D3mg (the underlined sequence is derived from the transcribed vector sequence immediately upstream of the 5' sequence of exon I; see Fig. 1). This primer pair specifically enables the amplification of cRNA that is derived from the primary transcript of the transfected plasmid pCEP4/D3mg. PCR products were separated on 1.5% agarose/TBE gels, transferred to membrane, and subjected to Southern blotting using a 32P-end-labeled oligonucleotide probe (5'-CAAGCACAAATGGCACC-3') that is complementary to the most 3' sequence of exon I (see Fig. 1).

For amplification of D₃ and D₃nf mRNAs expressed in the anterior cingulate cortex of control and schizophrenic brains, first-strand cDNA (generated as described above) was amplified by PCR using the primer pair D3S5'–5'-ATTGGAACATGGGT-3' and D3S3': 5'-ATTGGAACATGGGT-3'. This pair of primers allows the simultaneous amplification of the C-terminal halves of both D₃ and D₃nf cDNAs. Details of this PCR amplification were described previously (Schmauss et al., 1993). PCR products were separated on ethidium bromide-stained 1% agarose gels. The relative quantities of D₃- and D₃nf-specific amplification products were compared to the quantities of ethidium bromide-stained amplification products that resulted from parallel experiments with plasmid DNA templates consisting of a mixture of equal amounts of the plasmids pRC/CMV/D3 and pRC/CMV/D3nf (see Schmauss et al., 1993) ranging from 0.5 fg to 0.5 ng.

RNA extraction and SI nuclease protection assays. RNA was extracted from stably transfected GH3 cells (10⁷ cells per clone) or from 0.5 gm of each postmortem tissue using the guanidine isothiocyanate/CSCl ultracentrifugation methods as described previously (Schmauss et al., 1993). A 20 μg sample of RNA was hybridized to 5 × 10⁵ cpm of a 32P-end-labeled 39-mer antisense oligonucleotide at 50°C overnight. The nucleotide sequences of these oligonu-
RESULTS

To clarify whether D_3nf mRNA is indeed generated via atypical splicing of D_3 pre-mRNA, in vitro splicing experiments were done. A 2.3 kb in vitro-synthesized RNA transcript of the D_3-encoded genomic locus (shown in Fig. 1) was incubated with HeLa nuclear extracts, and the products and intermediates of this substrate pre-mRNA-spliced in vitro were analyzed.

In the first set of experiments, the spliced RNA was precipitated and used as a template for first-strand cDNA synthesis that was primed with a 17-mer oligonucleotide sequence complementary to the most 3' sequence of the distal exon of the substrate pre-mRNA (exon II; see Fig. 1). The cDNA was then amplified by PCR using the same 3' primer in conjunction with a 5' primer whose sequence is identical to the most 5' sequence of the proximal exon of the pre-mRNA (exon I; Fig. 1). A Southern blot of the PCR products is shown in Figure 2A. The lengths of the three hybridizing products correspond to the lengths of the unreacted pre-mRNA (2.3 kb), the D_3-specific ligated exons (226 nt), and the D_3nf-specific ligated exons (128 nt). The nucleotide sequences of the latter two products were found to be identical to D_3- and D_3nf-specific sequences expressed in vivo (10) (Fig. 2B). Thus, in vitro splicing of the substrate D_3 pre-mRNA resulted in the removal of the 2.1 kb intron to allow reigation of the proximal exon (I) and distal exon (II) to generate D_3 mRNA (constitutive splicing; see Figs. 1, 2). In addition, the proximal exon (exon I) of some of the pre-mRNA is further cleaved to remove the 98-nt-long splicingosomal intron [resulting in the consecutive reigation of the two short proximal exons (exons Ia and Ib) and the distal exon II] to yield D_3nf mRNA (alternative splicing; see Figs. 1, 2). These results suggest that the sequence UGA:GU in exon I is recognized as a (typical) 5' splice site, whereas the sequence GGA:GU serves as an AG-independent 3' splice site. Because such a 3' splice site sequence is indeed atypical, it should be stressed that the possibility of a sequencing compression at the boldfaced G of the 3' splice site (GA:GT), which would make canonical splicing quite feasible (AG:GT), has been excluded by analyzing multiple sequencing reactions of both strands using 7-deazaGTP and dITP.

For mammalian introns, AG-independent 3' splice sites are
extremely rare. Thus, to verify further the cleavage of the non-canonical 3' splice site GA:GU, primer extension experiments were performed with wild-type substrate pre-mRNA corresponding to exon I (see Fig. 1) and various mutants thereof with altered or lacking 3' splice site sequences. These substrate pre-mRNAs were spliced in vitro in HeLa nuclear extracts at 30°C for 2 hr, precipitated, and subjected to primer extension analysis using a primer sequence that is complementary to the most 3' 17 nt of exon I (see Fig. 1). Primer extension products were expected to correspond in size to the unspliced substrate (D3) RNA (238 nt; the 5' end of exon I is lacking 3' exon Ib). Primer extension products were separated on 7 M urea/6% polyacrylamide gels. Gels were exposed to film for 15 hr.

In conclusion, the analysis of the intermediates and products of D3 pre-mRNA splicing in vitro revealed clearly that D3nf mRNA is derived from D3 pre-mRNA via removal of a short alternative spliceosomal intron that has a noncanonical 3' splice site. However, these experiments did not directly address the relative D3/ D3nf splicing efficiencies. Therefore, additional in vivo splicing experiments were performed with transfected rat GH3 cells that stably express the D3 minigene that is schematically diagrammed in vivo.

Figure 3. Determination of the length of the splicing intermediates and products of in vitro-spliced wild-type and mutant substrate pre-mRNAs by primer extension. A T7 RNA polymerase transcript of exon I (see Fig. 1) was spliced in vitro in HeLa nuclear extracts for 2 hr at 30°C. The 238 nt extension product corresponds in size to the unspliced D3 mRNA, and the 140 nt extension product corresponds to the spliced and religated D3nf-specific exons Ia and Ib (see lane 1). The 81 nt extension product resulted from the extension of the splicing intermediate exon Ib/branched intron (see Results). Primer–extension products were separated on 7 M urea/6% polyacrylamide gels. Gels were exposed to film for 15 hr.
In Figure 4 (top), to test whether transcripts of the transfected D3 minigene are processed in GH3 cells to yield cytoplasmic D3- and D3nf-specific mRNA sequences, RNA was extracted from stable transfectants and analyzed by RT-PCR analysis. PCR primers were used that specifically enable the amplification of the expression vector pCEP4/D3mg-derived transcripts (see Materials and Methods). These amplification products are expected to be 235 nt (D3 mRNA) and 137 nt (D3nf mRNA) in length. Figure 4A shows a Southern blot of the PCR-amplified products. In 2 of 4 hygromycin-resistant GH3 cell clones, D3- and D3nf-specific amplification products were obtained; the other two clones did not express the minigene. Nucleotide sequence analysis (data not shown) revealed that the sequences of these two PCR products are identical to those shown in Figure 2B. Thus, as in HeLa nuclear extracts, in the neuroendocrine cell-derived GH3 cells D3 pre-mRNA is also processed constitutively and alternatively to yield D3 and D3nf mRNA.

To analyze further the relative D3/D3nf splicing efficiencies, S1 nuclease protection assays were performed with RNAs extracted from the two D3 minigene-expressing cell clones (see Fig. 4A). For these experiments, antisense oligonucleotides that are either complementary to or D3nf-specific mRNA sequences are extracted from stable transfectants and analyzed by RT-PCR analysis. PCR primers were used that specifically enable the amplification of the expression vector pCEP4/D3mg-derived transcripts (see Materials and Methods). These amplification products are expected to be 235 nt (D3 mRNA) and 137 nt (D3nf mRNA) in length. Figure 4A shows a Southern blot of the PCR-amplified products. In 2 of 4 hygromycin-resistant GH3 cell clones, D3- and D3nf-specific amplification products were obtained; the other two clones did not express the minigene. Nucleotide sequence analysis (data not shown) revealed that the sequences of these two PCR products are identical to those shown in Figure 2B. Thus, as in HeLa nuclear extracts, in the neuroendocrine cell-derived GH3 cells D3 pre-mRNA is also processed constitutively and alternatively to yield D3 and D3nf mRNA.

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between both diagnostic groups. A sense N-encoded riboprobe (a T7 RNA polymerase transcript of the HindIII-linearized plasmid pCRII-N) did not protect RNA from S1 nuclease digestion.

A second series of experiments, performed on the same RNAs, involved the simultaneous protection of D₃ and D₃nf mRNA via hybridization of a 39-mer antisense oligonucleotide (D₃ALL; see Materials and Methods) that is complementary to a sequence that codes for the N terminus of the putative third cytoplasmic domain of D₃. This probe, therefore, protects both D₃ and D₃nf mRNAs from S1 nuclease digestion. As shown in Figure 5A, the signals obtained for the sum of D₃ and D₃nf mRNAs were also found to be similar in the control and schizophrenic tissues. The sense sequence of the oligo-D₃ALL did not protect these mRNAs from S1 nuclease digestion.
It is noted that the variability of the sum of D₃ mRNA expression between samples is substantially less than that of N-encoded mRNA. This is perhaps because of differences in the turnover rates (stabilities) between both mRNAs. The abundance of the 1.6 kb N-encoded mRNA has been shown to be similar to that of the very abundant actin-encoded mRNA (Schmauss and Lerner, 1990). In contrast, D₃ mRNA (8.3 kb) is known to be expressed at very low levels (Sokoloff et al., 1990). It is possible that an mRNA with a high transcription rate also has a relatively short turnover rate and that differences in the postmortem intervals (PMIs) between samples (the SDs of the PMIs of the samples analyzed here are ~3 hr), therefore, result in the detection of different levels of N mRNA. This is supported by the observation that higher levels of N-encoded mRNA were detected in samples with shorter PMIs. Thus, if D₃ mRNA transcripts are substantially more stable than N mRNA, the magnitude of the differences between the individual PMIs of the samples analyzed here may not be large enough to result in the detection of similarly different levels of D₃ mRNA.

A third series of experiments targeted D₃ and D₃ₙf sequences separately, using D₃- and D₃ₙf-specific antisense 39-mer oligonucleotides. As shown in Figure 5B, in 5 of 8 cingulate cortices obtained postmortem from normal controls D₃ₙf mRNA was more abundant than D₃ mRNA, although D₃ mRNA was clearly detected in all 8 individuals. In contrast, no D₃ mRNA could be detected in cingulate cortical tissues obtained from 8 long-term hospitalized patients with chronic schizophrenia, whereas in all 8 tissues D₃ₙf mRNA was found to be expressed at levels that, in most cases, are higher than the corresponding levels found in control tissues.

Differences in the expression of D₃ mRNA in control and schizophrenic brains are also apparent in RT-PCR experiments in which D₃ and D₃ₙf cDNAs are simultaneously amplified. This is demonstrated in Figure 5C. PCR experiments on the five control samples that have a higher ratio of D₃ₙf/D₃ mRNA (see Fig. 5B) also indicate that the amplification of D₃ cDNA is 5- to 10-fold less than that of D₃ₙf if one compares ethidium bromide-stained signals of these PCR products to the corresponding ones obtained from PCR amplifications, performed in parallel, of a mixture of equal amounts of D₃- and D₃ₙf-encoded plasmid DNA templates that range from 0.5 to 0.5 ng (Fig. 5C; see Materials and Methods). Both D₃- and D₃ₙf-specific amplifications are also obtained from five randomly selected schizophrenic samples, indicating that not all of the D₃ pre-mRNA is spliced in a D₃ₙf-specific manner. However, for the schizophrenic samples the difference in the amounts of D₃ₙf and D₃ amplification products is at least 100-fold (Fig. 5C).

In summary, the analysis of the relative abundance of D₃ and D₃ₙf mRNA by RNase protection as well as by RT-PCR revealed a significant decrease of D₃ mRNA in the cingulate cortex of brains of patients with chronic psychosis. In the same anatomical region, however, D₃ₙf mRNA is abundantly expressed and this can explain why the sum of D₃ and D₃ₙf mRNAs (see Fig. 3A) is similar between control and schizophrenic brains. Altogether, these data support the suggestion that the loss of D₃ mRNA in some neocortical regions obtained postmortem from patients with chronic schizophrenia (Schmauss et al., 1993), and the decrease of D₃ mRNA in the cingulate cortex found in this study, results from an enhanced D₃ₙf-specific splicing of the D₃-encoded primary transcript.

DISCUSSION

The results of in vitro and in vivo splicing experiments shown here demonstrate that the truncated D₃-like mRNA (D₃ₙf) results from D₃ pre-mRNA via removal of a short alternative spliceosomal intron that is retained in D₃ mRNA. This intron is flanked by a noncanonical 3' splice site sequence (GA:GU).

Although in general one would predict that the in vivo splicing efficiency of such a rare and nonconforming alternative 3' splice site is significantly lower than the cleavage of constitutive and/or canonical 3' splice sites, results from in vitro splicing experiments with GH3 cells that stably express RNA transcripts of a transfected D₃ minigene revealed that both D₃ (constitutive)- and D₃ₙf (alternative)-specific splicing events occur with similar frequency. Furthermore, in human brain the expression levels of D₃ₙf mRNA are either equal to or higher than the corresponding levels of the constitutively spliced D₃ mRNA (Liu et al., 1994) (this study). Interestingly, substitution of the penultimate GA (GA:GU) dinucleotides of the D₃ₙf-specific 3' splice site of substrate D₃ pre-mRNA with the consensus AG (AG:GU) dinucleotides does not increase the D₃ₙf-specific alternative splicing efficiency. What unexpectedly, substitution of the boldfaced G (GA:GU) of the wild-type 3' splice site with a uridine (UA:GU) also does not affect the 3' cleavage (see Fig. 3). Thus, the recognition signals for cleavage of the noncanonical 3' splice site of the D₃ₙf-specific intron are obviously not determined by the sequence of the 3' splice site (GA:GU) alone.

Because the frequency of D₃ₙf-specific splicing of D₃ pre-mRNA ultimately determines the level of D₃ mRNA, this alternative splicing could function as an important regulator of D₃-receptor expression. It is of interest, therefore, to note that different relative ratios of D₃/D₃ₙf mRNA were observed in the anterior cingulate cortex of controls and chronic schizophrenics. Whereas D₃ mRNA levels are lower in schizophrenics compared to controls, D₃ₙf mRNA levels are increased. This suggests that the D₃ₙf-specific splicing activity is higher in brains of the chronic schizophrenic population studied here (and in Schmauss et al., 1993).

It is unlikely that the results of the S1 nuclease protection assays and RT-PCR experiments shown here reflect different stabilities of D₃ and D₃ₙf mRNA in schizophrenic and normal brains (which could obscure estimates of the relative D₃/D₃ₙf-specific splicing activities). First, in schizophrenia, D₃ and D₃ₙf mRNAs change in opposite directions and the level of the sum of both mRNAs is not different from the corresponding level found in control brains (see Fig. 5). Second, in stably and tetracycline-regulated D₃- and D₃ₙf-expressing GH3 cells (see Howe et al., 1995) the stabilities of both cDNA-derived RNAs (determined with transcription inhibition experiments) were identical (t₁/₂ = 80 min; C. Schmauss, unpublished observation). Although this result does not exclude the possibility that other destabilizing or stabilizing factors operate on full-length D₃ and D₃ₙf mRNAs, it clearly shows that the absence or presence of the alternative 98 nt intron has no significant effect on the stability of either RNA.

In summary, the results reported here demonstrate that it is possible to reconstitute both in vitro and in vivo an alternative splicing pathway of D₃ pre-mRNA that involves cleavage of a noncanonical 3' splice site to generate the truncated D₃ₙf mRNA. A comparison between the relative levels of the products of this splicing (D₃ and D₃ₙf mRNA) in the anterior cingulate cortex of schizophrenics and controls suggests an abnormal post-transcriptional processing of D₃ pre-mRNA in schizophrenia. This result may implicate altered activities of post-transcriptional regulators of gene expression in this disease. Clearly, it will now be of interest to search for other introns with a 3' splice site sequence specificity similar to the one described here and to analyze the corresponding splicing pattern in schizophrenics and controls. It is
possible that the $D_{nat}$-specific alternative intron is a member of a novel class of minor introns. In this respect, it is of interest to note that recent studies on another novel group of minor introns, called the AU–AC introns (which have atypical, but evolutionarily highly conserved, 5' and 3' splice sites), have shown that a deviation of consensus-sequence splice sites of major-class introns is accompanied by the assembly of different snRNPs (Hall and Paggett, 1996; Tarn and Steitz, 1996). Four pre-mRNAs are presently known that have such AU–AC introns. Importantly, these introns are spliced by a U11/U12 and U5 snRNP-containing spliceosome known that has such AU–AC introns. Importantly, these introns are spliced by a U11/U12 and U5 snRNP-containing spliceosome that is completely different from the U1, U2, and U6 snRNP-containing spliceosome known to be essential for cleavage of all major-class introns (GU–AG introns). It is possible, therefore, that the cleavage of the alternative $D_{nat}$-specific intron requires the presence of unique splicing factors and that an altered expression of such splicing factors may underlie the observed abnormality in the $D_3$ pre-mRNA processing. If unique splicing factors indeed mediate the cleavage of atypical introns like the one described here, they are likely to be found in the common nuclear repertoire of snRNPs. This assumption is supported by the observation that, in addition to neuronal cells, $D_3$ pre-mRNA is also spliced both constitutively and alternatively in two completely different cell types, human HeLa cells and rat GH3 cells.

It is also important to note that the patient population studied here and previously (Schmauss et al., 1993) represents a rather unique group of schizophrenic patients that were chronically and severely ill, therapy-resistant, and hospitalized long-term. Future studies will show whether a similar alteration of $D_3$ and $D_{nat}$ mRNA expression can be detected in brains of schizophrenic patients with a less severe course of the disease. Another unresolved issue is whether the altered splicing of $D_3$ pre-mRNA is an outcome of neuroleptic treatment, which is the most common therapeutic strategy for schizophrenia. It is impossible, at present, to address this issue with studies on tissues obtained from drug-naive schizophrenic patients because of an extremely limited availability of such brains. However, the reconstitution of $D_3$ pre-mRNA splicing in vivo in transfected cells that express $D_2$ and/or $D_3$-like receptors ($D_2$, $D_3$) will now allow us to test directly whether different pharmacological manipulations of these receptors have effects on the splicing of $D_3$ pre-mRNA. If neuroleptic drugs can alter the splicing pattern of $D_3$ pre-mRNA, this would not necessarily imply that another side effect of such drugs has been discovered. It could, in fact, be a mechanism by which neuroleptics mediate their antipsychotic effect.

REFERENCES


