Splicing Factors Induce Cystic Fibrosis Transmembrane Regulator Exon 9 Skipping through a Nonevolutionary Conserved Intronic Element*

Franco Pagani‡, Emanuele Buratti‡, Cristina Stuani‡, Maurizio Romano‡, Elisabetta Zuccato‡, Martina Nkisic‡, Luisella Giglio§, Dino Faraguna§, and Francisco E. Baralle‡

From the §International Centre for Genetic Engineering and Biotechnology, Padriciano 99 and ¶IRCCS, Burlo Garofolo, via dell’Istria 65/1, Trieste, TS 34012 Italy

In monosymptomatic forms of cystic fibrosis such as congenital bilateral absence of vas deferens, variations in the TG₉ and Tₚ polymorphic repeats at the 3’ end of intron 8 of the cystic fibrosis transmembrane regulator (CFTR) gene are associated with the alternative splicing of exon 9, which results in a nonfunctional CFTR protein. Using a minigene model system, we have previously shown a direct relationship between the TG₉ₚ polymorphism and exon 9 splicing. We have now evaluated the role of splicing factors in the regulation of the alternative splicing of this exon. Serine-arginine-rich proteins and the heterogeneous nuclear ribonucleoprotein A1 induced exon skipping in the human gene but not in its mouse counterpart. The effect of these proteins on exon 9 exclusion was strictly dependent on the composition of the TG₉ and Tₚ polymorphic repeats. The comparative and functional analysis of the human and mouse CFTR genes showed that a region of about 150 nucleotides, present only in the human intron 9, mediates the exon 9 splicing inhibition in association with exonic regulatory elements. This region, defined as the CFTR exon 9 intronic splicing silencer, is a target for serine-arginine-rich protein interactions. Thus, the non-evolutionary conserved CFTR exon 9 alternative splicing is modulated by the TG₉ and Tₚ polymorphism at the 3’ splice region, enhancer and silencer exonic elements, and the intronic splicing silencer in the proximal 5’ intronic region. Tissue levels and individual variability of splicing factors would determine the penetrance of the TG₉ₚ locus in monosymptomatic forms of cystic fibrosis.

Cystic fibrosis (CF),¹ the most common life-shortening auto-

* This work was supported by Friuli-Venezia Giulia Region Grant 199/EC.FIN, by the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Burlo Garofolo Grant Progetto finalizzato 1327, and by Telethon Onlus Foundation Grant E1038. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: A.S.S.2 Ospedale di Gorizia, Gorizia, Italy.

¹ To whom correspondence should be addressed. Tel.: 39-040-3757397; Fax: 39-040-3757361; E-mail: baralle@icgeb.trieste.it.

The abbreviations used are: CF, cystic fibrosis; CFTR, transmembrane regulator; CBAVD, congenital bilateral absence of vas deference; SR, serine-arginine-rich; hnRNP, heterogeneous nuclear ribonucleoprotein; bp, base pairs; PCR, polymerase chain reaction; RT, reverse transcription; h-int, human intron; m-int, mouse intron; ISS, intronic splicing silencer; ESS, exonic splicing silencer; βTM, β-tropomyosin; TNT, troponin T; mAb, monoclonal antibody; HIV, human immunodeficiency virus.
analysis of the phenotypic variability in patients with monomorphous forms of CF. To this end, we have developed an in vivo model system consisting of a reporter minigene by means of which the effect of the different CFTR alleles can be experimentally analyzed. With this system we have previously shown that the TG<sub><i>α</i></sub> and T<sub><i>α</i></sub> repetitions are directly involved and cooperate in exon 9 skipping and that intron 9 sequences can modulate the alternative splicing. In the present study we have carried out a functional analysis of the role of the regulatory trans-acting factors hnRNP1 and members of the SR family on the alternative splicing of CFTR exon 9.

**EXPERIMENTAL PROCEDURES**

**Hybrid Minigene Constructs**—Human genomic DNA was amplified with hcfIVS8dir 5′-tttttcattggccgctgctagctgtagttgaagcaaatatta-3′ and hcfIVS9rev 5′-ccctgtgcagtcgctgtagttagagc-3′ to generate a fragment that contains exon 9 along with part of the flanking introns (154 bp for intron 8, 183 bp for exon 9, and 209 bp for intron 9). This fragment, which contains two additional NdeI sites at the ends, was subcloned in SmaI-digested pBluescript plasmid. Mouse genomic regions containing exon 9 (154, 183, and 209 bp for intron 8, exon 9, and intron 9, respectively) were amplified from mouse genomic DNA with mCF8idir 5′-tttttcattggccgctgctagctgtagttgaagcaaatatta-3′ and mCF9rev 5′-ccctgtgcagtcgctgtagttagagc-3′, which contain additional NdeI sites and subcloned in pBluescript plasmid. In both mouse and human constructs, at position +15 of exon 9, an EcoRI site was introduced by PCR-mediated site directed mutagenesis (A→C) to facilitate subsequent cloning procedures. By PCR-mediated site directed mutagenesis different T<sub><i>α</i></sub> and TG<sub><i>α</i></sub> alleles were introduced at the 3′ end of intron 8 in the human construct with the use of antisense primers 5′-aagaacccgcaactctgtgtaaacaatttaa-3′ and 5′-aagaacccgcaactctgtgtaaacaatttaa-3′ (19). The construct hmCF was prepared cloning the human EcoRI/XbaI cassette into the corresponding sites of the mouse construct. hm intron 9 plasmid was created by amplification of the mouse construct with M2CF9dir 5′-gtgattgctgctgctagttagagc-3′ and an external primer in the pBS vector. The resulting fragment was digested with BamHI/KpnI and subcloned in hcf TG11-T5. hm intron 9 3′ was created by two-step PCR overlap extension with CFPS8dir 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′ and CFPS9rev 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′. The CFTR intron 8-exon 9-intron 9 cassette between introns (from bronectin EDB minigene, the EDB exon along with part of the flanking regions containing exon 9 (154, 183, and 209 bp for intron 8, exon 9, and intron 9) was introduced by PCR-mediated site directed mutagenesis (A→C) (16) or different amounts of mCF8idir 5′-taggatccggtcaccaggaagttggt-3′ and B2 5′-caacttcaagctcctaagccactgc-3′ and B2 5′-caacttcaagctcctaagccactgc-3′. For quantitation of the PCR reactions, [32P]dCTP was included in the PCR reaction mixture, and the products were loaded on 10% polyacrylamide gels and quantitated by using a PhosphorImager. Data are expressed as percentages of exon inclusion and are the means of at least three independent experiments.

**Fig. 1.** SR proteins and hnRNP1 negatively regulate human CFTR exon 9 splicing. A, schematic representation of the hybrid CFTR exon 9 minigenes. α-Globin, fibronectin EDB, human CFTR exons are indicated in black, shaded, and white boxes, respectively. The gray circle indicates the polymorphic locus. The transcription of the minigenes is driven by a minimal α-globin promoter and SV40 enhancer (small arrow at 3′ end). The primers used in the RT-PCR assay are indicated by the superimposed arrows. Relevant restriction sites are indicated. The EcoRI site, marked with an asterisk, was created by site-directed mutagenesis. The length of the relevant CFTR fragments (intron 8, exon 9, and intron 9) is indicated. B, expression of the human CFTR exon 9 minigene variant with eleven TG and five T repeats in the presence of different splicing factors. The minigene (3 μg) was transfected in Hep3B cells along with 500 ng of the empty vector pCG (control) or the indicated splicing factor plasmids. RNA splicing variants were detected by RT-PCR and analyzed on a 1.5% agarose gel. Exon 9 positive (+) and negative (−) mRNAs are indicated. C, histogram showing the quantification of exon 9 inclusion. The RNA splicing variants, detected by radioactive PCR, were resolved on 6% native polyacrylamide gels and quantitated by using a PhosphorImager. Data are expressed as percentages of exon inclusion and are the means of at least three independent experiments.

should not be taken as absolute values occurring in vivo in the whole organism.

**UV Cross-linking Assay**—To generate the human intron 9 competitor RNAs, human CFTR gene was amplified with the direct primer int/A 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′ and each of the following reverse oligonucleotides: h-int 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′, h-int117 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′, h-int176 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′, and h-int 77 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′. Mouse CFTR gene was amplified with int/A and m-int 5′-gtgattgctgctgagctctcagttatcagtttcatct-3′. The PCR products were digested with BamHI/KpnI and subcloned in the same restriction sites of pBS plasmid. The UV cross-linking assay was performed by adding [α-32P]UTP-labeled RNA probes (1 × 10⁶ cpm/incubation) for 15 min at
The alternative splicing of many pre-mRNAs is affected by the intracellular concentrations of antagonistic splicing factors of the SR family and hnRNPA1. To evaluate the role of these factors in the regulation of human CFTR exon 9 alternative splicing, we have prepared hybrid minigenes containing this exon as well as part of the flanking introns, including the different polymorphic TG\textsubscript{m}T\textsubscript{n} alleles (Fig. 1A). These variants were inserted in the well characterized \textalpha-globin/fibronectin reporter system (17). Different cDNA plasmids coding for SF2/ASF, hnRNPA1, SRp20, SRp30c, SRp40, SRp55, SRp75, and SC35 were transiently expressed in Hep3B cells simultaneously with the transfection of reporter CFTR constructs. The expression of these regulatory proteins on the splicing of CFTR exon 9 was analyzed by RT-PCR amplification using specific primers. This procedure generates two bands of 239 and 422 bp that correspond to the exclusion or inclusion of exon 9, respectively. In the absence of overexpressed splicing factors, the construct containing TG\textsubscript{11} and T\textsubscript{5} repeats at the 3’ end of intron 8 produced about 65% of exon 9 inclusion. Overexpression of different splicing factors caused an increase in human CFTR exon 9 skipping (Fig. 1, B and C). SF2/ASF, SRp40, SRp55, and SRp75 inhibited the most, resulting in only \textasciitilde25% of mRNA containing the exon 9 (Fig. 1, B and C). These results provided the first evidence that different SR proteins and hnRNPA1 are important inducers of aberrant human CFTR exon 9 skipping in vitro.

The TG\textsubscript{m}T\textsubscript{n} Polymorphic Variants Modulate the SR Protein-mediated Splicing Inhibition—To analyze the effect of the polymorphic locus at the 3’ end of intron 8 on the negative role of splicing factors, minigenes variants with different numbers of TG and T repeats were cotransfected along with the splicing factor SF2/ASF. To evaluate the relative sensitivity to inhibition by SF2/ASF of different alleles, we conducted dose-response studies (Fig. 2). Increasing amounts of SF2/ASF plasmid transfected resulted in a greater amount of CFTR mRNA without exon 9 in all cases. However, the proportion of exon 9 exclusion was strictly dependent on the composition of the polymorphic locus. In fact, the number of TG and T repeats affected independently both basal and splicing factor-induced levels of exon 9 skipping (Fig. 2). For instance, the TG\textsubscript{11}-T\textsubscript{9} construct produced 88% exon 9 inclusion with the addition of 500 ng of SF2/ASF plasmid, which was only 50 and 24% in the case of TG\textsubscript{11}-T\textsubscript{7} and TG\textsubscript{11}-T\textsubscript{5}, respectively (Fig. 2, A and C). On a T5 background, an increasing number of TG repeats further reduced exon 9 inclusion (from 24% for TG\textsubscript{11} to 3% for TG\textsubscript{13}) (Fig. 2, B and C). Similar dose-response curves were obtained with SRp55 and SRp75, whereas a lower efficiency of
inhibition was observed for hnRNPA1 (data not shown), suggesting a different mechanism of splicing inhibition by this ribonucleoprotein as recently reported (26). As a control, we cotransfected a hybrid minigene containing the fibronectin EDA exon along with the CFTR and ASF/SF2 constructs. The analysis of the splicing pattern of the EDA exon showed, as previously shown (21), that SF2/ASF produced a dose-dependent increase of exon inclusion, whereas the splicing of the CFTR exon was affected in the opposite way, as described above (Fig. 2A). This indicates that the exon skipping of the human CFTR minigene induced by SR proteins is specific to the sequence of exon 9 and/or of its flanking introns and is modulated by the polymorphic locus.

**Negative Regulation by Splicing Factors Is Mediated by Sequences Present in Intron 9**—To identify the RNA elements in human CFTR mediating the negative regulation induced by the splicing factors, we have compared the human and mouse CFTR genes. As previously reported, the mouse gene is significantly different from the human one within the flanking introns of exon 9. Both TG and T repeats are absent at the intron 8-exon 9 junction, and in addition there are substantial sequence differences in the intron 9 (19). The mouse CFTR exon 9 and its flanking sequences were cloned in the same minigene construct used for the human exon and transfected along with increasing amounts of SF2/ASF splicing factor. Contrary to the human counterpart, the mouse CFTR exon 9 was not significantly skipped in the presence of SF2/ASF overexpression (Fig. 3, A and B, mCF). This result is consistent with the observed lack of alternative splicing of this exon (10). We then prepared hybrid minigene constructs with parts of the human homologue inserted in the mouse context. These mouse-human hybrids were transiently transfected along with increasing amounts of SF2/ASF. The human intron 8 with the polymorphic repeats did not confer an SR protein-mediated inhibitory effect to the mouse exon, indicating that the human polymorphic tract is not by itself the target of this splicing factor (Fig. 3, A and B, hmCF). When intron 9 of the human construct was substituted with the corresponding intron from mouse, overexpression of SF2/ASF did not induce exon 9 skipping (Fig. 3, hm int9 3'). Similar results were obtained with SRp55 and SRp75 (data not shown). These data indicate that the inhibitory splicing effect of SR proteins is mediated by sequences present in the human but not in the mouse intron 9.

**Functional Analysis of Intronic and Exonic Splicing Regulatory Elements Mediating the SF2/ASF Splicing Inhibition**—To evaluate the functional significance of the human intron 9 in mediating the splicing inhibition the intronic sequences were progressively deleted and the corresponding hybrid minigenes.
transiently transfected in Hep3B cells. The partial or complete deletion (up to 77 bp from the 5' splice site) of the intronic sequences leads to the complete disappearance of the CFTR exon 9 minus form, indicating that this regions behave like an intronic splicing silencer (ISS). When increased concentration of SF2/ASF plasmid were cotransfected, the CFTR exon 9 minus form was induced at a significant lower level than the amount present in the minigene, which contains the entire intronic element (Fig. 4, minus lanes for Δint1 and Δint2). The splicing inhibition was related to the length of the deletion of the intron, suggesting the presence of multiple regulatory elements with inhibitory properties. These data are consistent with the role of the ISS in mediating the inhibitory activity, although the data also indicate that this is not the only element involved. Exonic regulatory elements have been found in different alternative spliced genes; hence we evaluated the presence of such elements in the CFTR exon 9 and their putative role in splicing inhibition. Deletion analysis was carried out on exon 9 selected chosen taking into account the RNA secondary structure of this region (19), which in some cases have been found to be of critical importance (17). Cotransfection experiments in Fig. 4 identified two key exonic regulatory sequences behaving like an exonic splicing enhancer (hCFD1) and an exonic splicing silencer (hCFD2), respectively. Both elements modulate the response to SF2/ASF splicing inhibition. In fact the splicing inhibition mediated by SF2/ASF was completely prevented when both the exon (ESS) and the intron (ISS) silencers were deleted. Similar results were obtained with SRp55 and SRp 75 (data not shown). These data suggest that the two silencer elements are necessary for the splicing inhibition mediated by SR proteins.

The Intronic Splicing Silencer Element Binds to SR Proteins—The human and mouse intron 9 sequences have a strikingly different behavior regarding the SR proteins inhibitory splicing effect. We have tested the ability of the these two intronic sequences to interact with nuclear proteins using an UV cross-linking assay with specific constructs (Fig. 5A). Fig. 5B shows that human and mouse intron 9 have a different pattern of protein binding in the UV cross-linking assay (Fig. 5A). In particular three bands in the range of 35–44 kDa and to a lesser degree a band of ~75 kDa do not interact with the mouse homologue (Fig. 5B, bands a–d). Competition experiments using truncated human intron 9 RNA sequences show that the binding of one of the proteins in the 35–44 kDa range (bands d) requires a ~150-bp region located between 117 and 264 bases downstream of the 5' splice site that we named CFTR exon 9 ISS (Fig. 5B, C). The band of ~75 kDa (band a) was only partially competed by h-int 176 and h-int. The molecular masses of these proteins are consistent with those of some of the SR proteins that have an effect in the functional assay and in particular the d band, which molecular weight is similar to SF2/ASF and/or SC35 when cross-linked to RNA (see above). In an attempt to characterize them, we have used competitor RNA sequences derived from β-tropomyosin (βTM) and TNT RNAs, which are known to bind specifically to SF2/ASF, SC35 and SRp75 (23, 24). The UV cross-linked d band in the 35–44-kDa range was specifically and completely competed by the βTM and TNT SR binding sequences (Fig. 5C, lanes βTM and TNT).

To address more directly the interaction of cellular factors with the ISS element, we have performed gel shift assay with both nuclear extracts and purified SR proteins (Fig. 5D). The ISS RNA transcript formed stable complexes with both nuclear extract and SR proteins that were disrupted by the coincubation with the anti-SR antibody mAb104, indicating that SR proteins contributed to the shift of the ISS element. The addition of a nonspecific antibody had no effect on the migration of the complex. This effect mediated by mAb104 has been previously observed in the NCAM E17 exon (27). These data, together with the cotransfection experiments, indicate that the interaction of SR proteins with the ISS element contribute to the exon 9 splicing inhibition.

DISCUSSION

Our results demonstrate that the alternative splicing of human CFTR exon 9 is negatively regulated by the intracellular concentration of different splicing factors and modulated by a number of cis-acting elements, the number of polymorphic TG and T repeats, the exonic splicing regulatory regions (exonic splicing enhancer (ESE) and ESS), and the ISS. The splicing factors affecting CFTR exon 9 alternative splicing belong to two different groups of RNA binding proteins, the SR proteins and hnRNP. SR proteins in general are activators of splicing (12–
17), although they can act as repressors depending on their position of binding to the pre-mRNA (18, 28). For example, adenoviral IIIa splicing is repressed by an SR protein binding to an intronic repressor element located immediately upstream of the 3' splice site (18). Nonproductive interactions of SF2/ASF and the small nuclear ribonucleoproteins U1, U2, and U11 at the negative regulator of splicing element are responsible for splicing inhibition in Rous sarcoma virus (28–30). We show here that a protein complex containing SR proteins binds to the ISS element in the CFTR intron 9 (Fig. 5) and represses splicing. SR proteins-ISS complex could interfere with the recruitment of essential splicing factors at the adjacent 5' splice site of intron 9, resulting in the observed splicing inhibition. Alternatively, SR proteins binding to the ISS element could modulate pre-mRNA conformation by interacting with other regulatory elements bound to the region of the TG and T repeats at the 3' end on intron 8 or in the exon. Regarding the latter further studies are needed to characterize the enhancer and to determine whether the CFTR exon 9 ESS acts as a target for SR-RNA interactions or whether, as is the case of the fibronectin EDA exon, the ESS only modulates RNA conformation enhancing SR binding on distant target sequences (17). The other splicing factor analyzed, hnRNPA1, has a general inhibitory effect on splicing (14, 16), and this is also the case for the

![Figure 5](http://www.jbc.org/)

**FIG. 5.** The CFTR exon 9 ISS element of human intron 9 binds SR proteins. A, schematic representation of human CFTR exon 9 showing the location of the RNAs used in the UV cross-linking experiments and in gel shift assay. The ISS element in the intron 9 of the human CFTR gene is shown as a black box. B, UV cross-linking assay of HeLa nuclear extracts with human and mouse intron RNAs (h-int and m-int, respectively) challenged with the indicated competitors. Four UV cross-linked bands, one at ~75 kDa (a) and three in the 35–44-kDa range (b–d) bind to the human (h-int) and not to the mouse (m-int) intron 9. The bottom band (e) binds to both the mouse and human introns. Specific binding to the human intron is shown for the a and c proteins as they can be competed by the human cold RNA only (B, lane + h-int) but not by the cold mouse RNA (lane + m-int). C, competitor RNAs h-int 77, h-int 117, and h-int 176 correspond to truncated human intron 9 RNA sequences extending 77, 117, and 176 bases from the 5' splice site on exon 9 (whereas the total length of h-int is 269 bases). The d band in the 35–44-kDa range and the a band of 75 kDa are entirely competed by the complete intron h-int and by h-int 176, partially competed by h-int 117 and not competed at all by h-int 77 or m-int. βTM and TNT are RNAs containing well characterized strong SR binding sites (23) (24). These RNAs specifically compete the d band in the 35–44-kDa range whose absence is indicated by an asterisk. D, gel shift assay with nuclear extracts (NE) and purified SR proteins from HeLa cells using h-int3' labeled RNA. The position of free and bound complexes are shown. α-SR is a monoclonal antibody that recognize phosphorylated SR proteins (mAb104), whereas α-An is an aspecific monoclonal antibody.
human CFTR exon 9 (Fig. 1). It has been proposed that the mechanism of action of this splicing factor involves changes in mRNA secondary structure (26). In our case, we have made the unexpected observation that both splicing factors, which frequently have antagonistic effects (12–17), inhibit CFTR exon 9 splicing.

In patients with atypical CF, some of the phenotypic variability can be due to an aberrant regulation of CFTR exon 9 alternative splicing mediated by tissue-specific and/or developmentally controlled changes in the concentration of splicing factors. In somatotyposomatic forms of CF, like CBAVD and disseminated bronchiectasies, the partial penetrance of the well studied T5 allele at the polymorphic locus can be modulated not only by the TG repeats upstream, as previously suggested (3), but also by a variability in the individual tissue concentration of splicing factors (12, 31–36). Relatively higher amounts of both SR proteins and hnRNP A1 are expected to negatively affect the recognition of CFTR exon 9 and result in itsSkipping with the subsequent development of a tissue-specific CFTR defect. This would be particularly apparent with low T and high TG repeat numbers at the polymorphic intron 8-exon 9 junction. In fact, overexpression of only one splicing factor could produce up to 97% of exon 9 skipping (Fig. 2). Splicing factors could induce exon 9 skipping during organ development in tissues where CFTR is functionally important and not necessarily during the adult age, as in the case of CBAVD.

Aberrant regulation of CFTR exon 9 alternative splicing mediated by splicing factors could represent a new mechanism causing disease in humans. Recently, aberrant splicing in the EAAT2 glutamate transporter mRNA. The presence of a specific CFTR defect. This would be particularly apparent with low T and high TG repeat numbers at the polymorphic intron 8-exon 9 junction. In fact, overexpression of only one splicing factor could produce up to 97% of exon 9 skipping (Fig. 2). Splicing factors could induce exon 9 skipping during organ development in tissues where CFTR is functionally important and not necessarily during the adult age, as in the case of CBAVD.

The inhibitory effect of the splicing factors in the human CFTR exon 9 mediated by the nonselevelopment conserved ISS intronic element could be the result of the activity of some ancient transposable elements in the human lineage (39). In fact, in mammalian genomes are abundant traces of recombinant events via retrotransposons or retroviruses that resulted in substantial changes in specific regions of the genome (40). In the case of CFTR, scars of a retrotransposon interaction can be seen in the larger size of the introns flanking human exon 9, the peculiar repetitive TG and T sequences, the ISS element, and the amplification of exon 9 sequences found in different chromosomes throughout the genome (39). It is noteworthy that SF2/ASF has been implicated in the negative regulation of retroviral splicing in Rous sarcoma virus and HIV-1 intronic sequences (30, 41, 42). A fascinating hypothesis would be to consider the human CFTR exon 9 ISS as a reminiscence of functionally similar retroviral sequences accidentally left by retrotransposition and amplification events in the human genome. The results presented in this paper indicate for the first time that SR proteins can interact with an intronic element and modulate the penetrance of a disease-causing mutation.

Acknowledgments—We are grateful to Rodolfo Garcia for the monoclonal antibody α-An and helpful discussion. We thank J. Caceres for the plasmids expressing SR proteins and hnRNP A1 and useful suggestion. mAb104 was a kind gift from Christopher W. Smith.

REFERENCES

1. Welsh, M. J., Tsui, L. C., Boat, T. F., and Beaudet, A. L. (1995) The Metabolic and Molecular Basis of Inherited Diseases, (Sriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 3799–3876 McGraw-Hill, New York, NY
2. Larriba, S., Bassea, L., Gimenez, J., Ramon, M. D., Segura, A., Nunes, V., Estivill, X., and Casals, T. (1998) Hum. Mol. Genet. 7, 1739–1743
3. Cuppen, H., Lit, W., Jaspers, M., Costes, B., Teng, H., Vankeerberghen, A., Jorissen, M., Droogmans, T., Reynaert, I., Goossens, M., Nilius, B., and Cassiman, J. J. (1998) J. Clin. Invest. 101, 487–496
4. Chillon, M., Casals, T., Merrier, B., Bassea, L., Lissens, W., Silber, S., Romey, M. C., Ruiz Romero, J., Verlingue, C., Clauset, M., Nunes, V., Ferec, C., and Estivill, X. (1995) N. Engl. J. Med. 332, 1475–1480
5. Costes, B., Girardon, E., Ghanem, N., Flori, E., Jardin, A., Soufr, J. C., and Goossens, M. (1995) Eur. J. Hum. Genet. 3, 285–293
6. Pignatti, P. F., Bonblier, C., Benetazzo, M., Casartelli, A., Trabetti, E., Gile, L. S., Martinati, L. C., Boner, A. L., and Lusigi, M. (1996) Am. J. Hum. Genet. 58, 889–892
7. Tomkiewicz, J., Jorissen, M., Van Poppel, H., Legius, E., Cassiman, J. J., and Cuppens, H. (1997) Hum. Mol. Genet. 6, 85–90
8. Rave-Harel, N., Keren, E., Nissim-Rafinia, M., Madjar, I., Goshen, R., Augarten, A., Rahat, A., Hurwitz, A., Darvasi, A., and Keren, B. (1997) Am. J. Hum. Genet. 60, 87–89.
Splicing Factors Induce Cystic Fibrosis Transmembrane Regulator Exon 9 Skipping through a Nonevolutionary Conserved Intronic Element
Franco Pagani, Emanuele Buratti, Cristiana Stuani, Maurizio Romano, Elisabetta Zuccato, Martina Niksic, Luisella Giglio, Dino Faraguna and Francisco E. Baralle

J. Biol. Chem. 2000, 275:21041-21047.
doi: 10.1074/jbc.M910165199 originally published online April 13, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M910165199

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 42 references, 17 of which can be accessed free at http://www.jbc.org/content/275/28/21041.full.html#ref-list-1