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Legends for Figures

Fig. 1. Representative splicing *cis*-elements and *trans*-factors. Tissue-specific and developmental stage-specific expressions of splicing *trans*-factors including SR proteins and hnRNP A1 enable precise regulations of alternative splicing. ISE and ISS have similar activities as ESE and ESS, but are omitted from the figure.

Fig. 2. U1 snRNA recognizes three nucleotides at the 3' end of an exon and six nucleotides at the 5' end of an intron

Fig. 3. Human consensus BPS. **(A)** Pictogram and **(B)** WebLogo presentations of BPS. Position 0 represents the branch point. **(C)** Representative sequences and positions of splicing *cis*-elements.

Fig. 4. *CHRNA1* carries a 75-nt exon P3A. Its inclusion generates a non-functional alpha subunit of the acetylcholine receptor. hnRNP H and PTB silence recognition of exon P3A and induce its skipping. The IVS3-8G>A mutation identified in a patient with congenital myasthenic syndrome weakens the binding of hnRNP H and causes inclusion of exon P3A. Tannic acid facilitates the expression of PTB and partially ameliorates aberrant splicing due to IVS3-8G>A.

Fig. 5. NASRE. Wild-type *CHRNE* generates the normally spliced transcript (a) and the exon 6-skipped transcript (b), because exon 6 carries weak splicing signals. The exon-skipped transcript carries a premature termination codon (PTC) and is degraded by NMD. A 7-nt deletion (arrowhead) in exon 7 generates a PTC in the normally

spliced transcript (c) and is degraded by NMD. The deletion resumes the open reading frame from the exon 6-skipped transcript, and the transcript escapes NMD (d).

Fig. 6. In DM1, expanded CUG repeats in the 3' UTR of DMPK sequestrate muscleblind and upregulates CUG-binding protein. Dysregulation of these splicing *trans*-factors cause aberrant splicing of their inherent target genes. Four representative target genes are indicated.

Fig. 7. Mutations on *MAPT* exon 10 cause excessive skipping (N279K and L284L) or inclusion (K280del) of exon 10.

Fig. 8. Expanded CTG on *ATXN8OS* exerts three toxic effects on the bidirectional transcripts.

Footnote for NMD in Section 4.2

Nonsense-mediated mRNA decay (NMD). NMD is a quality-assurance mechanism that degrades mRNAs harboring a premature termination codon (PTC) (Chang et al., 2007). Proteins translated from mRNAs harboring PTCs potentially have dominant-negative or deleterious activities. In pre-mRNA splicing, an exon-junction complex (EJC) is deposited 20-24 nucleotides upstream of each exon-exon junction. Ribosomes remove EJCs, but, in the presence of a PTC, EJCs stay on the transcript and trigger the NMD pathway in the cytoplasm.

Figure 1

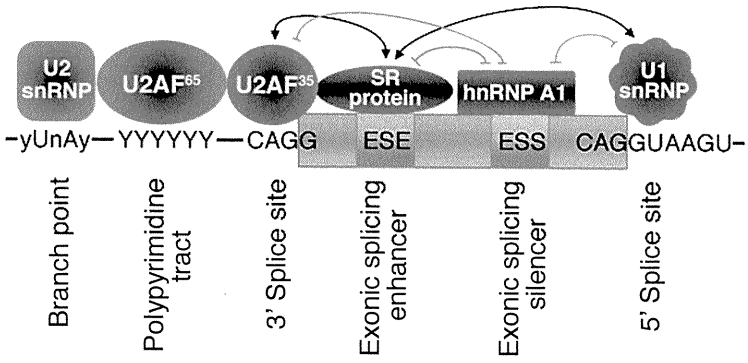

U2 snRNP
U2AF65
U2AF35
SR protein
hnRNP A1
U1 snRNP
-yUnAy—YYYYYY—CAGG
ESE
ESS
CAGGUAAGU-
Branch point
Polypyrimidine tract
3' Splice site
Exonic splicing enhancer
Exonic splicing silencer
5' Splice site

Figure 2

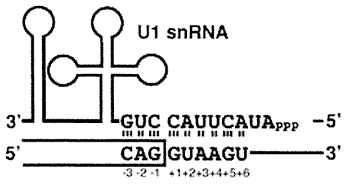


Figure 3

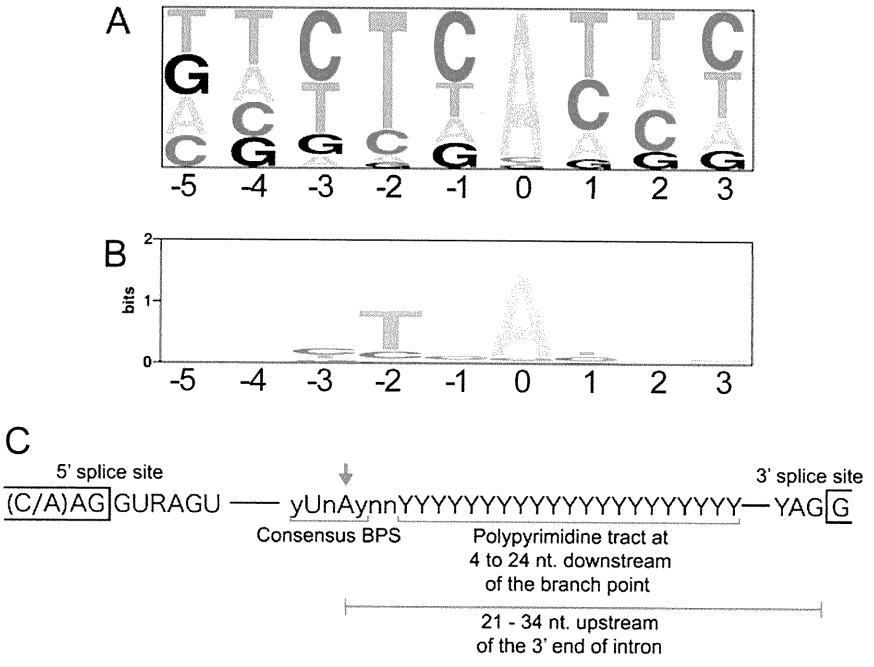

A
-5 -4 -3 -2 -1 0 1 2 3
B
bits
2
1
0
-5 -4 -3 -2 -1 0 1 2 3
C
5' splice site
(C/A)AG GURAGU
yUnAynnYYYYYYYYYYYYYYYYYYYY
YAG G
3' splice site
Consensus BPS
Polypyrimidine tract at
4 to 24 nt. downstream
of the branch point
21 - 34 nt. upstream
of the 3' end of intron

Figure 4

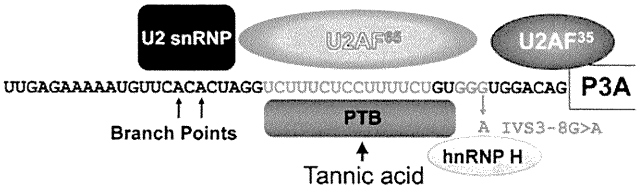

U2 snRNP
U2AF65
U2AF35
UUGAGAAAAAUGUUCACACUAGGUCUUUCUCCUUUUCUGUGGGUGGACAG
P3A
Branch Points
PTB
A IVS3-8G>A
hnRNP H
Tannic acid

Figure 5

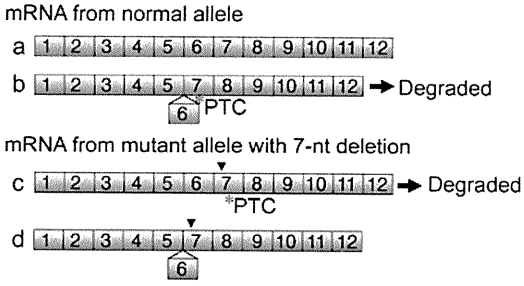

mRNA from normal allele
a
1 2 3 4 5 6 7 8 9 10 11 12
b
1 2 3 4 5 7 8 9 10 11 12
Degraded
6
PTC
mRNA from mutant allele with 7-nt deletion
c
1 2 3 4 5 6 7 8 9 10 11 12
Degraded
*PTC
d
1 2 3 4 5 7 8 9 10 11 12
6

Figure 6

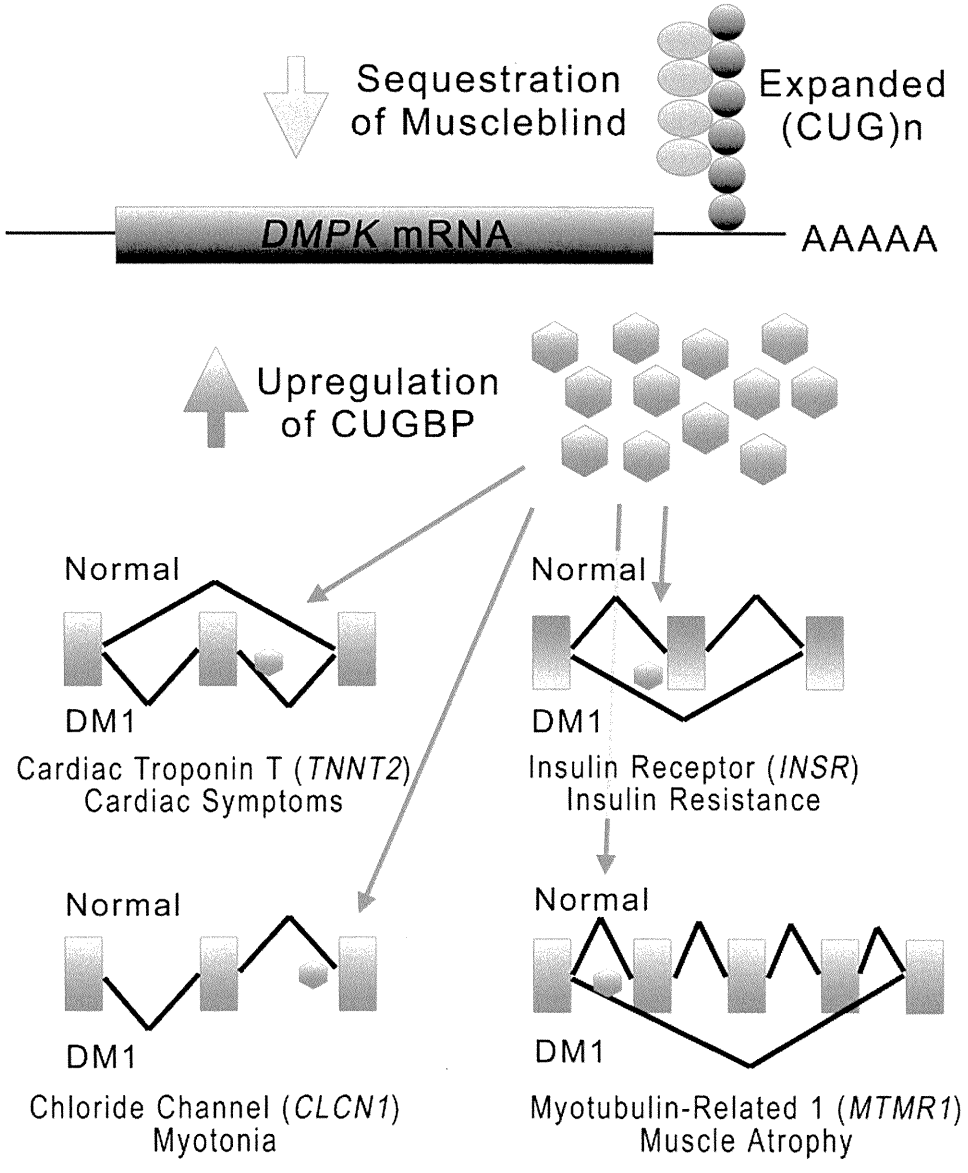

Sequestration
of Muscleblind
Expanded
(CUG)n
DMPK mRNA
AAAAA
Upregulation
of CUGBP
Normal
DM1
Cardiac Troponin T (TNNT2)
Cardiac Symptoms
Normal
DM1
Insulin Receptor (INSR)
Insulin Resistance
Normal
DM1
Chloride Channel (CLCN1)
Myotonia
Normal
DM1
Myotubulin-Related 1 (MTMR1)
Muscle Atrophy

Figure 7

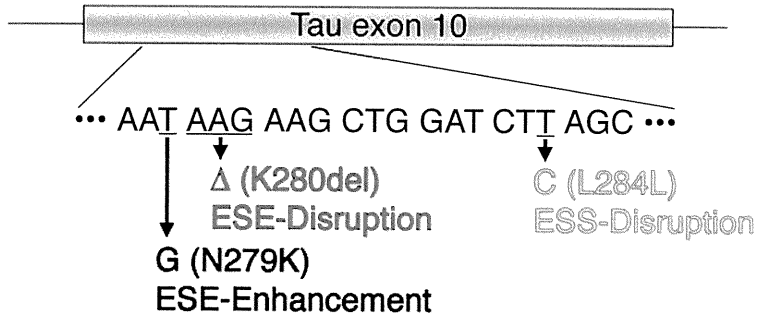
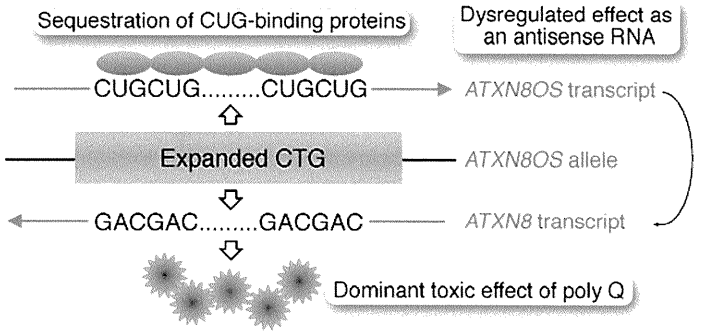

Tau exon 10
••• AAT AAG AAG CTG GAT CTT AGC •••
Δ (K280del)
ESE-Disruption
C (L284L)
ESS-Disruption
G (N279K)
ESE-Enhancement

Figure 8


Sequestration of CUG-binding proteins
Dysregulated effect as an antisense RNA
CUGCUG.....CUGCUG
ATXN8OS transcript
Expanded CTG
ATXN8OS allele
GACGAC.....GACGAC
ATXN8 transcript
Dominant toxic effect of poly Q