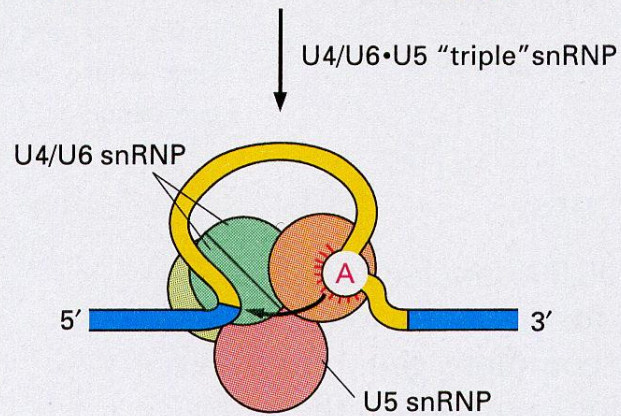
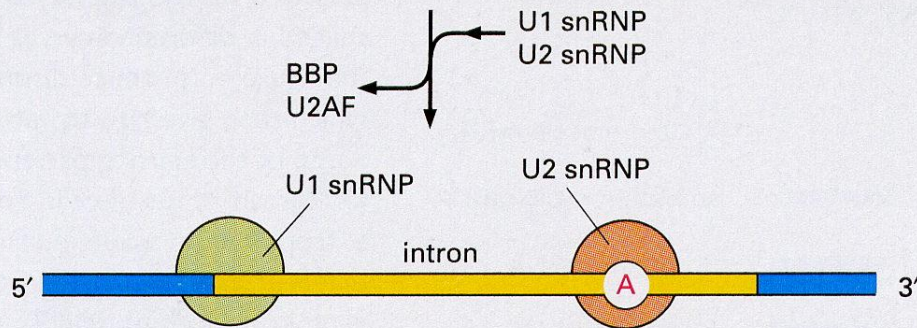
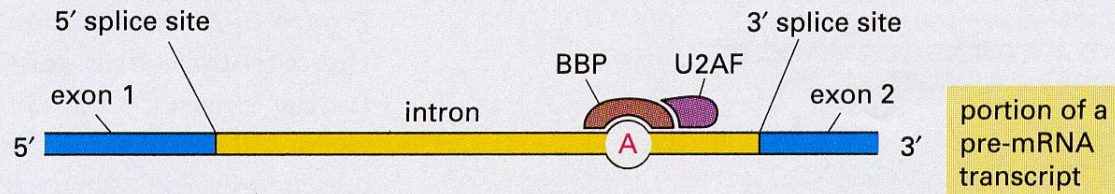


Splicing alternativo e malattie genetiche dell'uomo

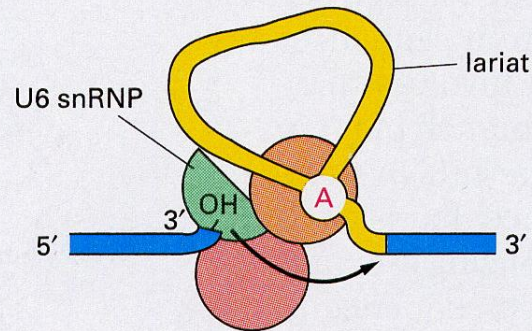
Caceres JF, Kornblihtt AR (2002)

Alternative splicing: multiple control mechanisms
and involvement in human disease.

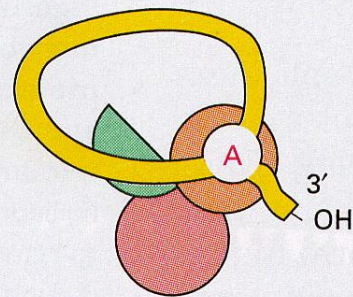
Trends in Genetics 18, 186-193.



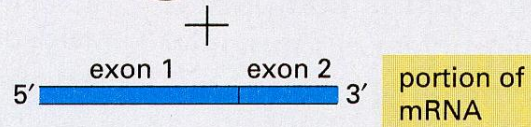
U1, U4 → LARIAT FORMATION
AND 5' SPLICE SITE
CLEAVAGE



↓ 3' SPLICE SITE
CLEAVAGE AND
JOINING OF TWO
EXON SEQUENCES

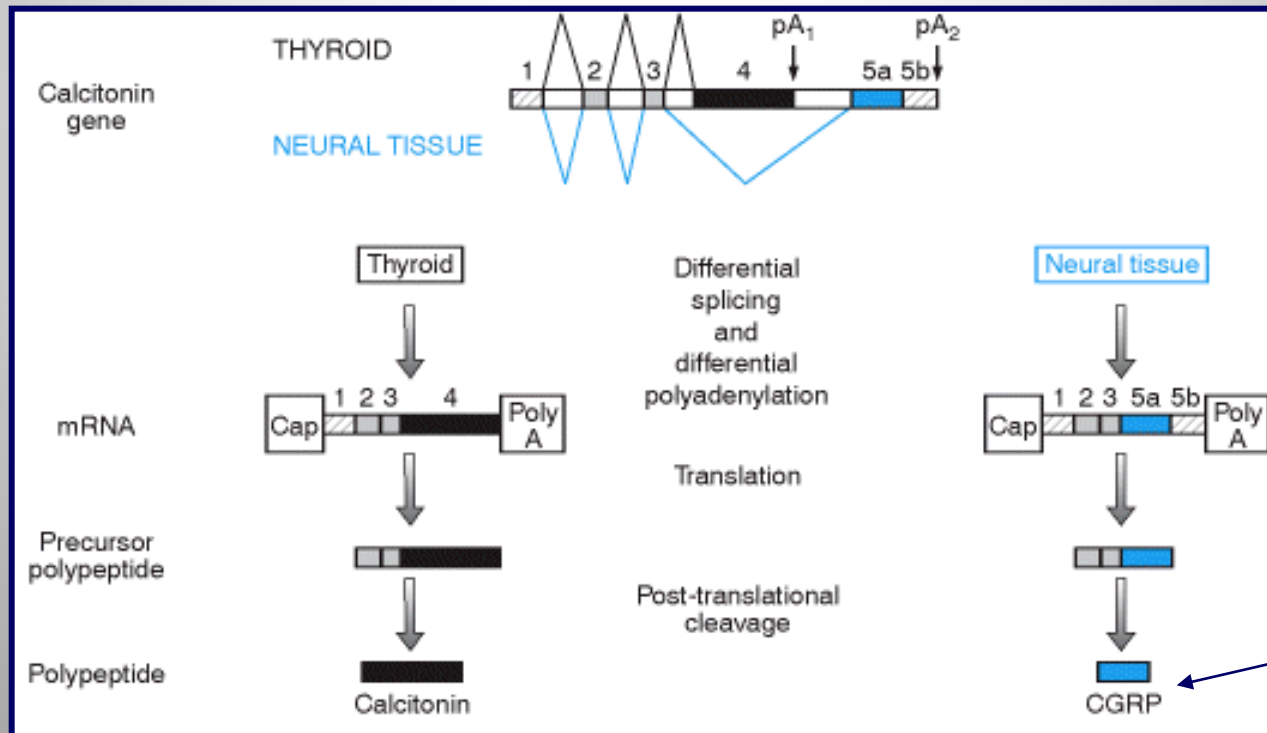


excised intron sequence
in the form of a lariat
(intron RNA will be degraded
in the nucleus; snRNPs will
be recycled)



Splicing alternativo

Molti geni umani vengono sottoposti a splicing alternativo per produrre sequenze differenti di mRNA che codificano isoforme proteiche provenienti dallo stesso gene e che possono essere tessuto specifiche

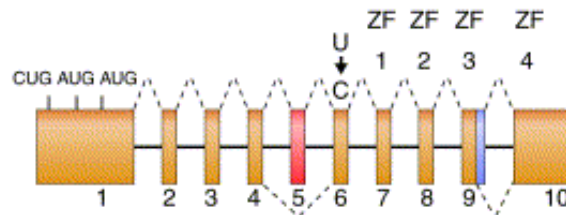


calcitonin
gene-related
peptide

Splicing alternativo

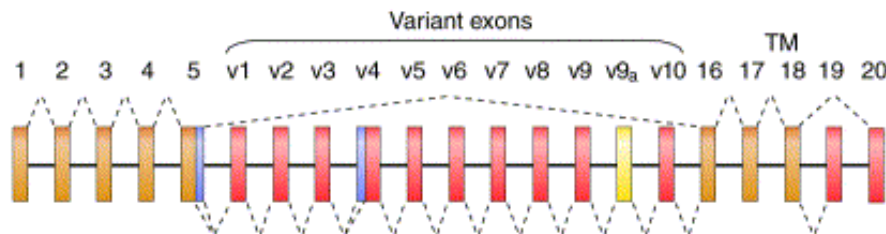
Le isoforme prodotte, secondo un calcolo combinatoriale, potrebbero essere migliaia

(a) WT1



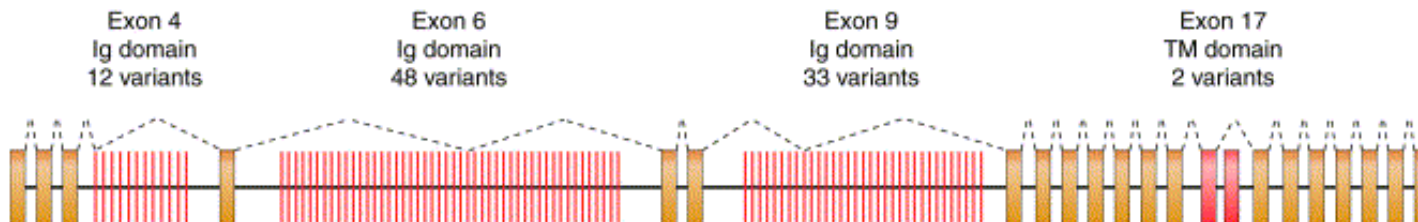
a) Wilms
Tumor gene:
25 isoforme

(b) CD44



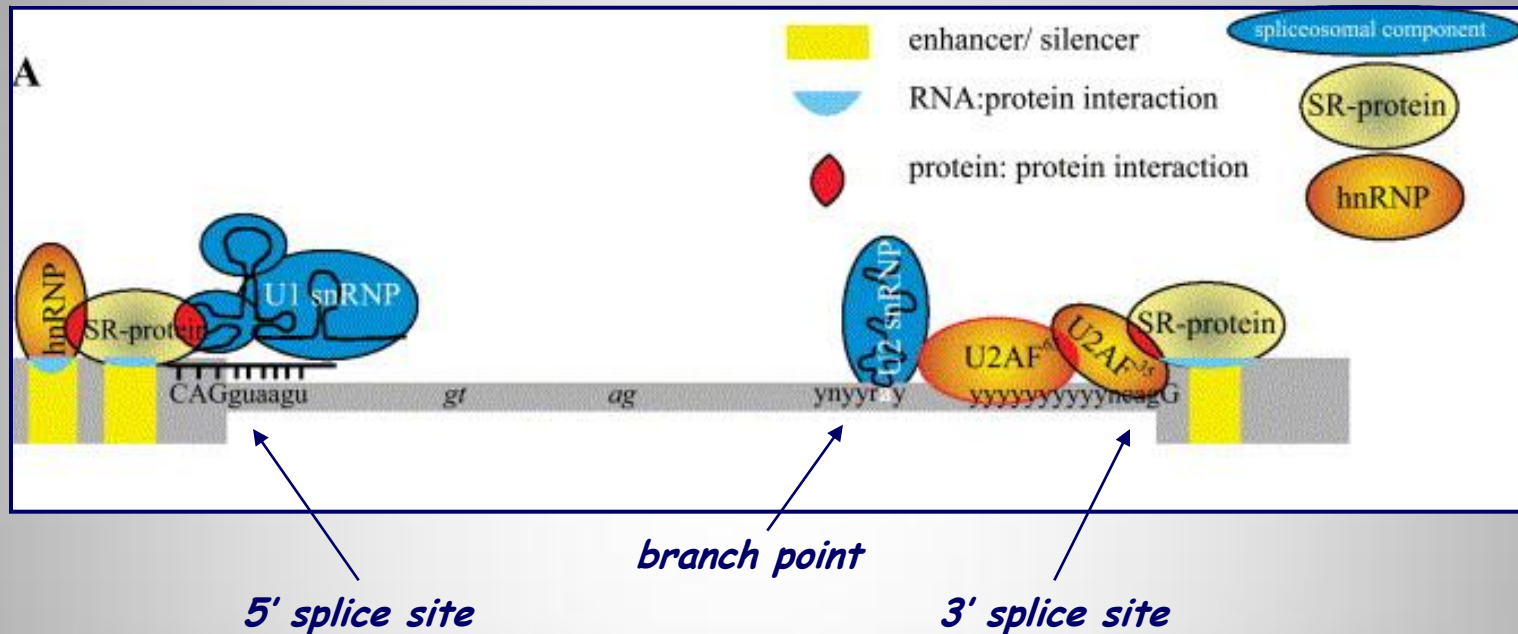
b) CD44
cell-surface
glycoprotein
gene: 1024
isoforme

(c) Dscam



c) Dscam di
Drosophila:
38.016
isoforme

Splicing alternativo

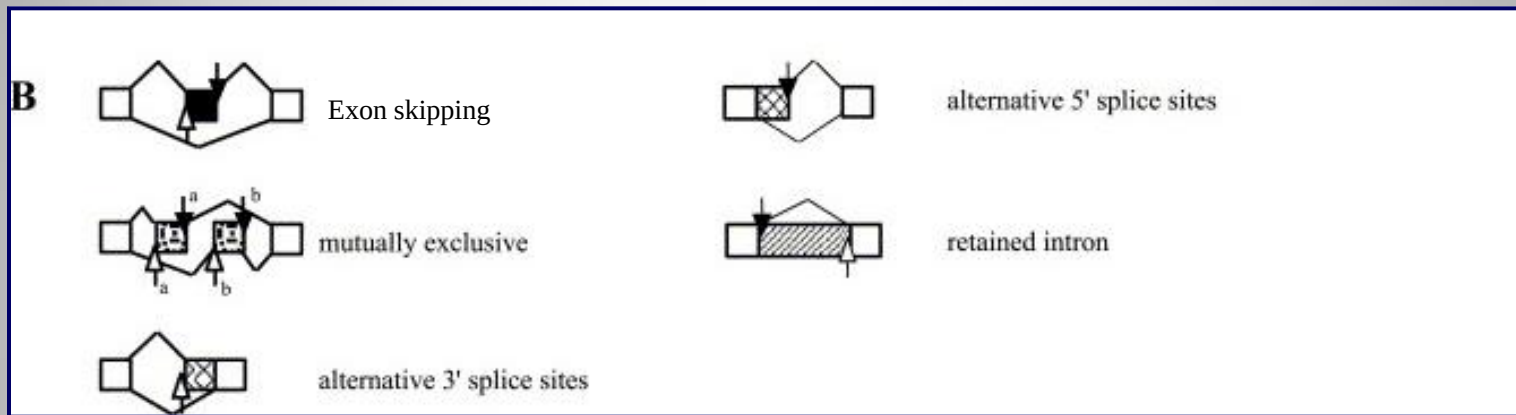


snRNP = small nuclear ribonucleoproteins. Sono 4: U1, U2, U4/U6 e U5

SR proteins = proteine altamente conservate che contengono 1 o 2 *RNA-recognition motif* (RRM) e un dominio C-terminale ricco di residui alternati Serina/aRginina (*RS domain*)

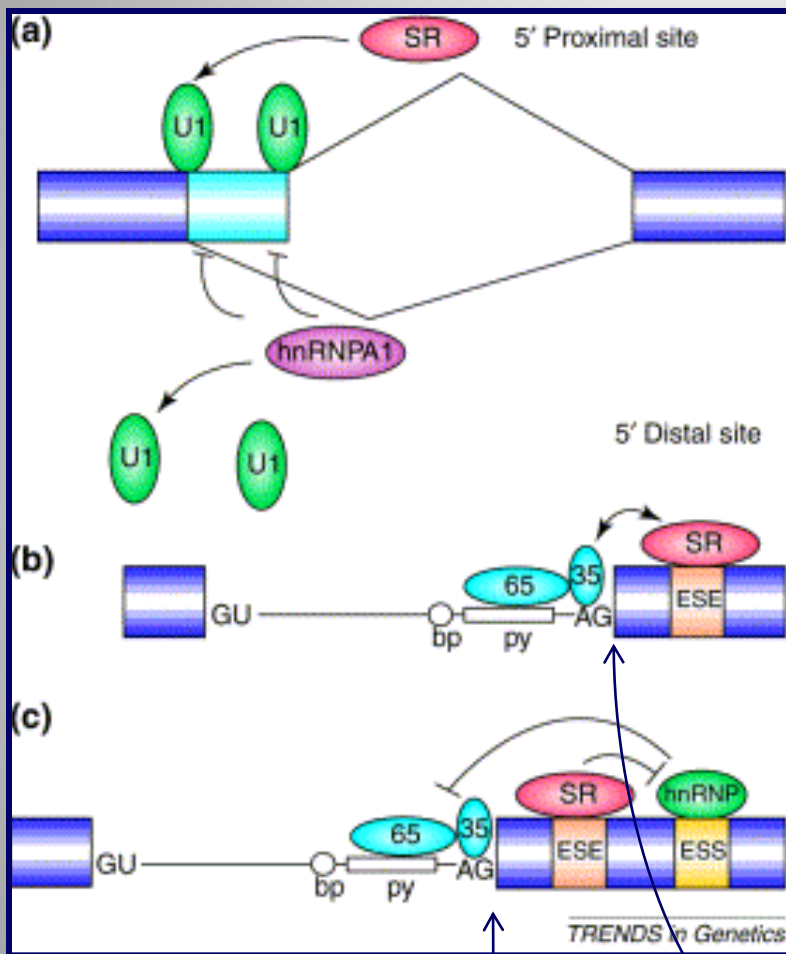
hnRNP = *heterogeneous nuclear ribonucleoproteins*. Circa 50-100 fattori che si legano alla nascente molecola pre-mRNA e influenzano (negativamente) lo *splicing*.

Splicing alternativo



Tipi di eventi di *splicing* alternativo

Splicing alternativo



La selezione del sito di splicing si basa sulla competizione nel legare il pre-RNA

a) SR interferisce con hnRNP aumentando il legame di U1. Questo favorisce la scelta del sito 5' più vicino.

b) U2AF recruitment model for weak 3' splice site

c) inhibitor model

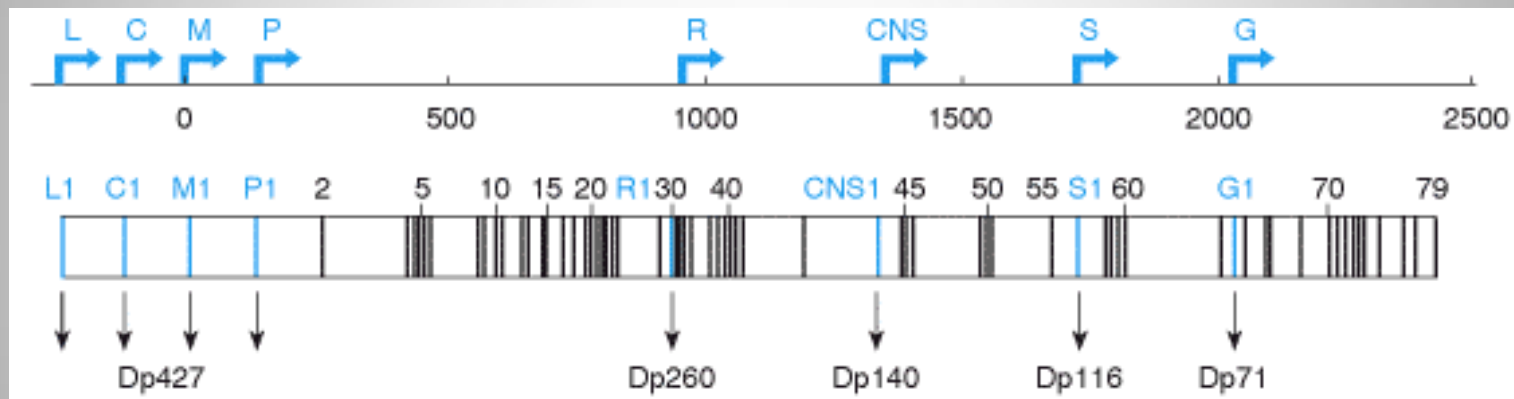
U2AF = 65

U2AF³⁵ = 35

Sito 3' di splicing debole

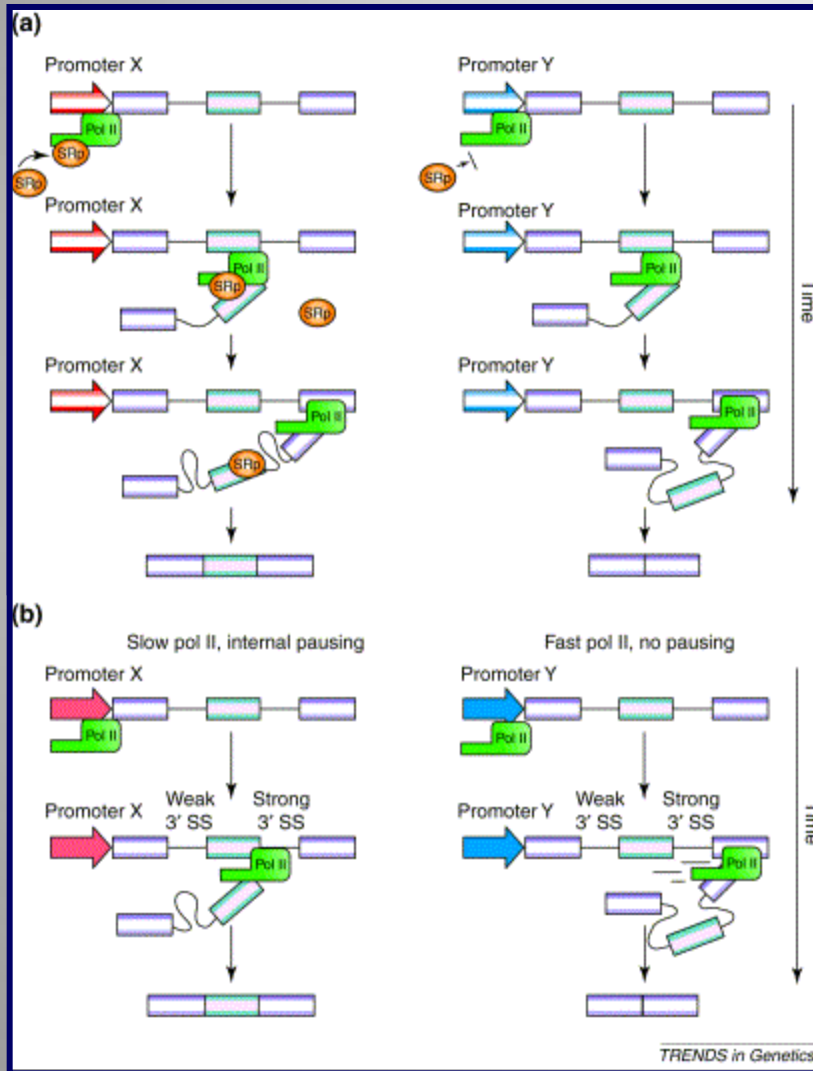
Splicing alternativo

lo splicing alternativo può riguardare anche la scelta del promotore



Gli otto promotori del gene che codifica per la *distrofina*

Splicing alternativo



a) = SR recruitment model

b) = elongation model

Splicing alternativo

Il risultato dello splicing alternativo può consistere in una alterazione (fisiologica o patologica) della proteina codificata, in cui mancano, o vi si aggiungono, domini funzionali.

Altre volte le varie isoforme differiscono nelle loro parti non codificanti (5'UTR o 3'UTR).

- le isoforme al 5'UTR sono comuni e potrebbero coinvolgere la regolazione dell'espressione dei geni attraverso l'uso di promotori alternativi;
- le isoforme al 3'UTR potrebbero controllare la localizzazione subcellulare dei prodotti, la stabilità dell'RNA e l'efficienza di traduzione.

Splicing alternativo

Le mutazioni localizzate nelle regioni non codificanti, come quelle che costituiscono i siti di *splicing* 5' e 3', i segnali di poliadenilazione o i segnali di scelta per il promotore, sono causa frequente di malattie genetiche

Circa il 15% delle mutazioni che influenzano il corretto *splicing* del pre mRNA sono la causa di malattie.

Spesso le mutazioni sinonime, ma anche quelle nonsense e missense, in regioni codificanti si sono rivelate mutazioni che influiscono sulla maturazione dell'mRNA.

Queste infatti possono provocare:

- eliminazione o creare *exon splicing enhancers* e *silencer*

- creare nuovi siti di *splicing*

- rafforzare siti di *splicing* criptici

- provocare cambiamenti del processo di polimerizzazione che può alterare la scelta dei siti di *splicing*

Splicing alternativo

Disorder		Gene	Missense	Nonsense	Translationally silent	Refs
Acute intermittent porphyria		Porphobilinogen deaminase			R28R (C→G, 3)	[69]
Breast and ovarian cancer		<i>BRCA1</i>		E139K (G→T, 18)		[70]
Carbohydrate deficient glycoprotein syndrome type 1a		<i>PMM2</i>		E139K (G→A, 5)		[71]
Cerbroretinoid xanthomatosis		Sterol-27-hydroxylase			G112G (G→T, 2)	[72]
Cystic fibrosis		<i>CFTR</i>		E60X (G→T, 3); R75X (C→T, 3); R553X (C→T, 11); W1228X (G→A, 20)		[65]
Ehlers–Danlos syndrome type VI		Lysyl hydroxylase		Y511X (C→A, 14)		[73]
Fanconi anemia		FANCG		Q356X (C→T, 8)		[74]
Frontotemporal dementia (FTDP-17)		Tau	S305N (G→A, 10); N297K (T→G, 10)		L284L (T→C, 10); S305S (T→C, 10)	[75,76]
Hemophilia A		Factor VIII		E1987X (G→T, 19); R2116X (C→T, 22)		[65]
HIPRT deficiency		Hypoxanthine phosphoribosyl transferase	G40V (G→T, 2); R48H (G→A, 3); A161E (C→A, 6); P184L (C→T, 8); D194Y (G→T, 8); E197K (G→A, 8); E197V (A→T, 8)		F199F (C→T, 8)	[65]
Leigh's cephalomyelopathy	en-	Pyruvate dehydrogenase E1α			G185G (A→G, 6)	[77]
Marfan syndrome		Fibrillin-1			I2118I (C→T, 51)	[78]
Metachromatic leukodystrophy (juvenile form)		Arylsulfatase A	T409I (C→T, 8)			[79]
Neurofibromatosis type 1		<i>NF1</i>		R304X (C→T, 7); Q756X (C→T, 14); Y2264X (C→A, 37); L304F (G→T, 9)		[80]
OCT deficiency		Ornithine carbamoyltransferase				[81]
Porphyria cutanea tarda		Uroporphyrinogen decarboxylase			E314E (G→A, 9)	[82]
Sandhoff disease		Hexosaminidase	P404L (C→T, 11)			[83]
Severe combined immunodeficiency		Adenosine deaminase	R142Q (G→A, 5)	R142X (C→T, 5)		[84]
Spinal muscle atrophy		SMN1		W102X (G→A, 3)		[85]
Spinal muscle atrophy		SMN2			F280F (C→T, 7)	[86]
Tyrosinemia type I		Fumaryl acetoacetate hydrolase	Q279R (A→G, 8)		N232N (C→T, 8)	[87,88]

Table 7
List of database resources on alternative splicing

Database	Entry mode and notes	Species covered	Reference	URL
<i>(I) Databases on alternative splicing</i>				
AEDB—Alternative exon database	Manually created from literature	All animals	(Stamm et al., 2000; Thanaraj et al., 2004)	http://www.ebi.ac.uk/asd/aedb/index.html
ASD—AltsplICE (and AltExtron)	Computationally generated (delineating from gene-EST/mRNA alignments)	Human and other model species	(Clark and Thanaraj, 2002; Thanaraj et al., 2004)	http://www.ebi.ac.uk/asd/
AltRefSeq	Computationally generated (RefSeq genes with detected alternative splicing patterns)	Human	(Kan et al., 2001)	http://sapiens.wustl.edu/~zkan/TAP/ALTSEQ.html
ASDB: database of alternatively spliced genes	Computationally generated (from SwissProt protein and EMBL nucleotide sequence databases)	All animals	(Gelfand et al., 1999; Dralyuk et al., 2000)	http://cbcg.nersc.gov/asdb
EASED	Computationally generated (by examining high-scoring ESTs to mRNA alignments)	Human and other species	(Pospisil et al., 2004)	http://eased.bioinf.mdc-berlin.de/
AsMAMDB	Computationally generated (by examining EST/mRNA alignments with gene sequences)	Mammalian	(Ji et al., 2001)	http://166.111.30.65:100/
ASAP	Computationally generated (through mapping of unigene clusters of EST sequences to genes)	Human	(Lee et al., 2003)	http://www.bioinformatics.ucla.edu/ASAP/
MouSDB-Splice variants in mouse transcriptome	Computationally generated (through mapping of RIKEN's full length mouse cDNA sequences and dbEST mouse sequences to genes)	Mouse	(Zavolan et al., 2003)	http://genomes.rockefeller.edu/MouSDB/
Intronator-Alt-Splicing Catalog	Computationally generated (through mapping of EST/cDNAs with genes)	<i>C. elegans</i>	(Kent and Zahler, 2000)	http://www.cse.ucsc.edu/~kent/intronator/altsplice.html
Ensembl	Computationally generated (data presented as alternate transcripts identified through a series of computational tools)	Human and other species	(Bimney et al., 2004)	http://www.ensembl.org/
VARSPLICE records in Swiss-Prot	Manual (through submissions by experimentalists) and Computational. (through similarity searches)	Human and other species	(Bimney et al., 2004)	http://www.ebi.ac.uk/swissprot/
EMBL-BANK nucleotide sequence database	Manual (through submissions by experimentalists), Annotation of Alternative exons or Alternative splicing feature lines in	Human and other species	(Kulikova et al., 2004)	http://www.ebi.ac.uk/embl/
<i>(II) Databases that are used often in the derivation of data on alternative splicing</i>				
SANBI STACK	EST clusters and consensus sequences	Human	(Christoffels et al., 2001)	http://www.sanbi.ac.za/Dbases.html
TIGR gene indices	EST clusters and contigs	Human and other species	(Quackenbush et al., 2001)	http://www.tigr.org/tldb/tgi/
SANBI's eVOC	Set of detailed human terms/vocabularies that describe the sample source of human experimental material such as cDNA and SAGE libraries	Human	(Kelso et al., 2003)	http://www.sanbi.ac.za/evoc/
<i>(III) Data sets on splice regulatory proteins, sequences and diseases</i>				
Human splicing factor database for	Manually created-collection of splice variants from various resources. Presentation of data for array oligo design	Human	(Stamm et al., unpublished)	To be made available soon at http://www.ebi.ac.uk/asd
<i>Drosophila</i> splicing protein database	Manually created. Information on gene sequences and comparisons with other species	<i>Drosophila</i>	(Mount and Salz, 2000)	http://www.wam.umd.edu/~smount/DmRNAfactors/table.html
RRM-containing proteins	Computer generated-collection of proteins containing RNA recognition motifs	Metazoan splicing factors	(Bateman et al., 2004)	http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF00076
Splice-site mutations	Manual	Human	(Nakai and Sakamoto, 1994)	http://www.hgc.ims.u-tokyo.ac.jp/~knakai/