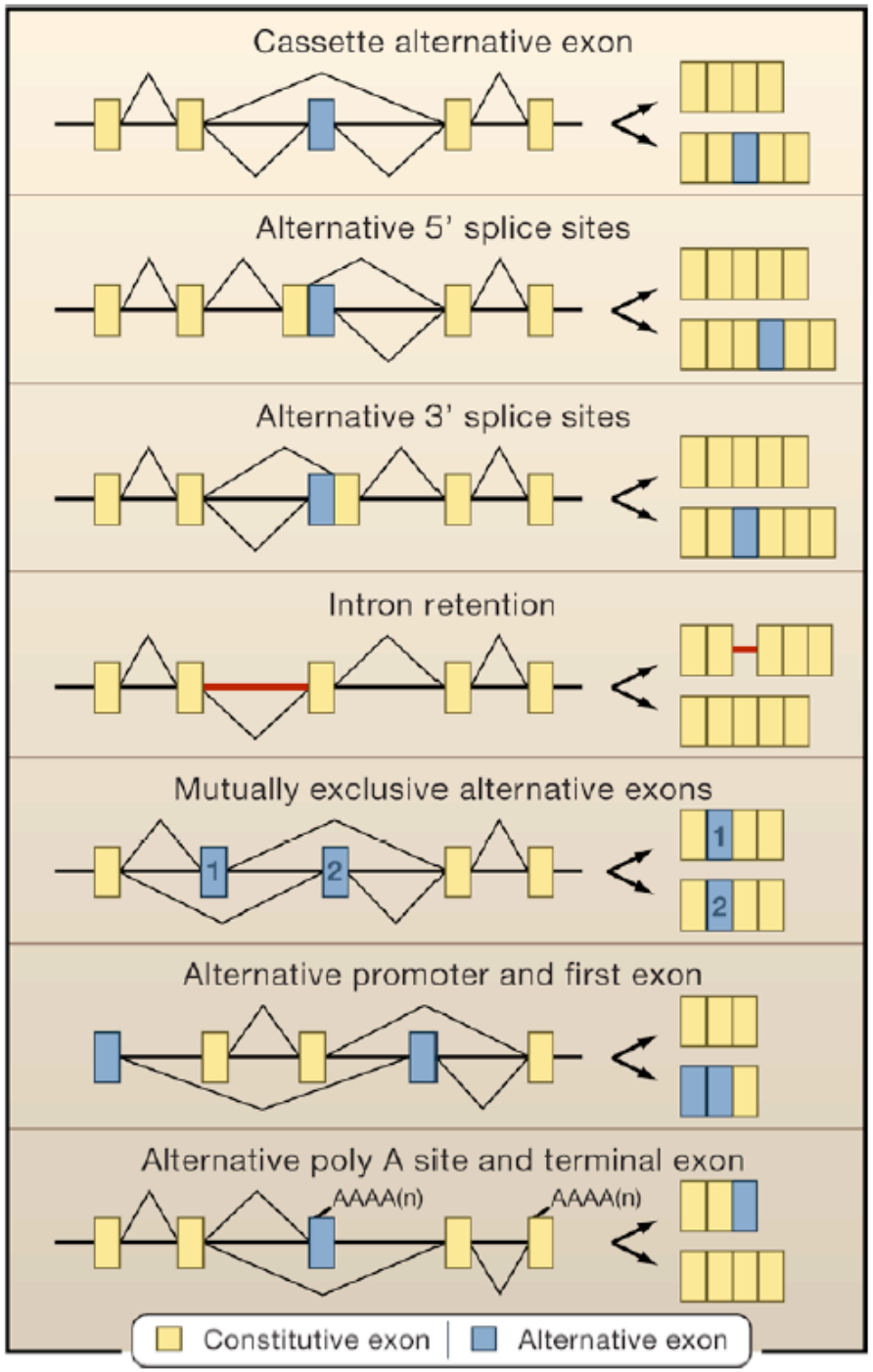
Alternative splicing

Types of Alternative Splicing



38%

18%

8%

3%

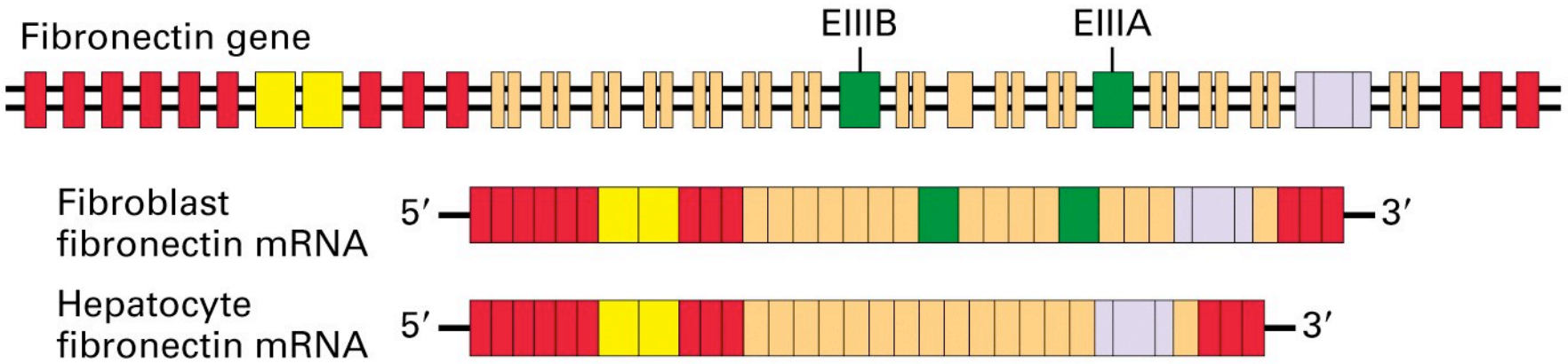
Remaining 33%

Cell 126:37 (2006)
Nature Reviews: Genetics 5:773 (2004)

Cell type-specific splicing of fibronectin pre-mRNA in fibroblasts and hepatocytes: concept of alternative splicing

•The presence of multiple introns in many eukaryotic genes permits expression of multiple, related proteins from a single gene by means of alternative splicing, an important mechanism for the production of different forms of proteins, called isoforms, by different types of cells.

•Nearly 60% of all human genes are expressed as alternatively spliced mRNAs, leading to an expansion of the coding capacity of our genome.

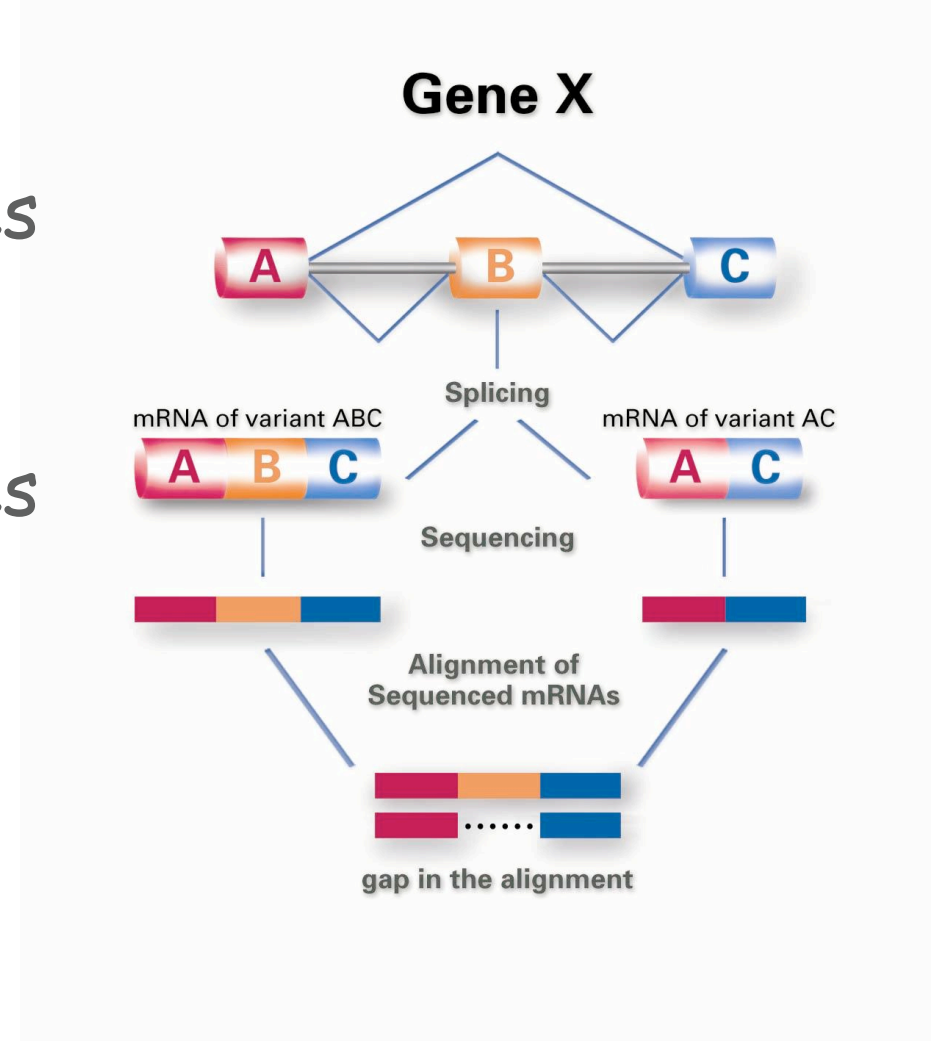


•Fibroblasts produce fibronection with exons EIIIA and EIIIB, which allow the protein to adhere to proteins in the fibroblast plasma membranes and enable fibroblasts to stick to the extracellular matrix.

•Hepatocytes produce fibronection without EIIIA and EIIIB, which circulates in the serum and is important during the formation of blood clots.

Finding Alternatively Spliced Exons

- Compare cDNA & genomic DNA sequences
- Compare ESTs & genomic DNA sequences
- Compare protein & genomic sequences



How Prevalent is Alternative Splicing?

EST Database estimates between 35 - 60% of protein coding gene have alternative mRNAs

Caveat - These databases contain sequences derived from aberrant, as well as, alternative splicing, they are typically 3' and 5' end biased, and have insufficient number to infer frequency

Therefore, database mining may overestimate the rate of alternative splicing

Array-Based Numbers

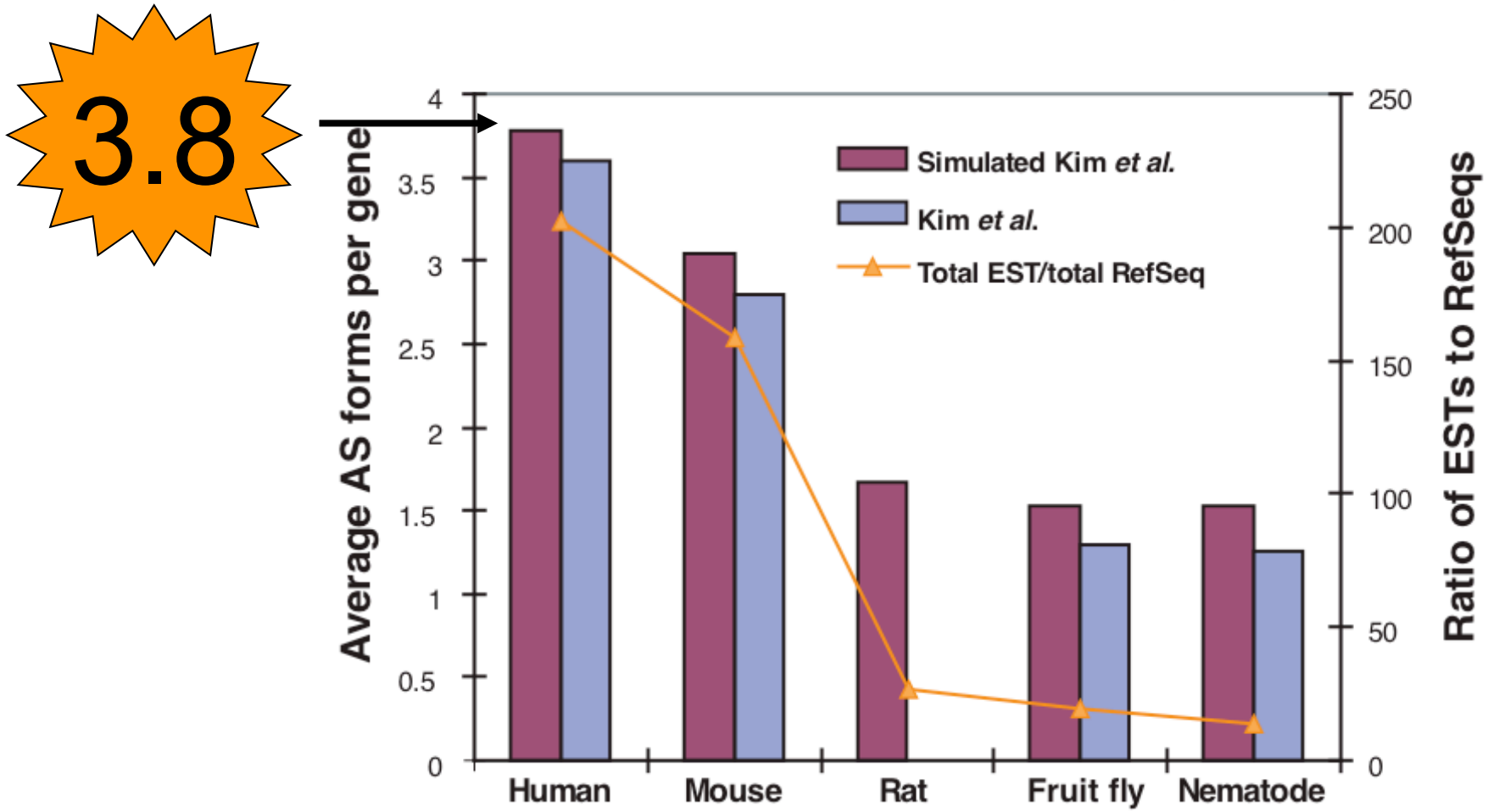
Genome-Wide Survey of Human Alternative Pre-mRNA Splicing with Exon Junction Microarrays

Jason M. Johnson,* John Castle, Philip Garrett-Engele, Zhengyan Kan, Patrick M. Loerch, Christopher D. Armour, Ralph Santos, Eric E. Schadt, Roland Stoughton, Daniel D. Shoemaker*

Alternative pre–messenger RNA (pre-mRNA) splicing plays important roles in development, physiology, and disease, and more than half of human genes are alternatively spliced. To understand the biological roles and regulation of alternative splicing across different tissues and stages of development, systematic methods are needed. Here, we demonstrate the use of microarrays to monitor splicing at every exon-exon junction in more than 10,000 multi-exon human genes in 52 tissues and cell lines. These genome-wide data provide experimental evidence and tissue distributions for thousands of known and novel alternative splicing events. Adding to previous studies, the results indicate that at least 74% of human multi-exon genes are alternatively spliced.

Science 302, 2141-44 (2003)

Number of Splicing Isoforms per Gene by EST Comparison



Harrington et al. Nature Genetics 36:916 (2004)

Alternative splicing: functional consequences

Protein - Substitution/deletion of domains, change of reading frame, termination of reading frame

- Altered localization
- Antagonistic isoforms
- Modulation of function
- Unrelated proteins
- On/off switch for gene expression

Developmental -

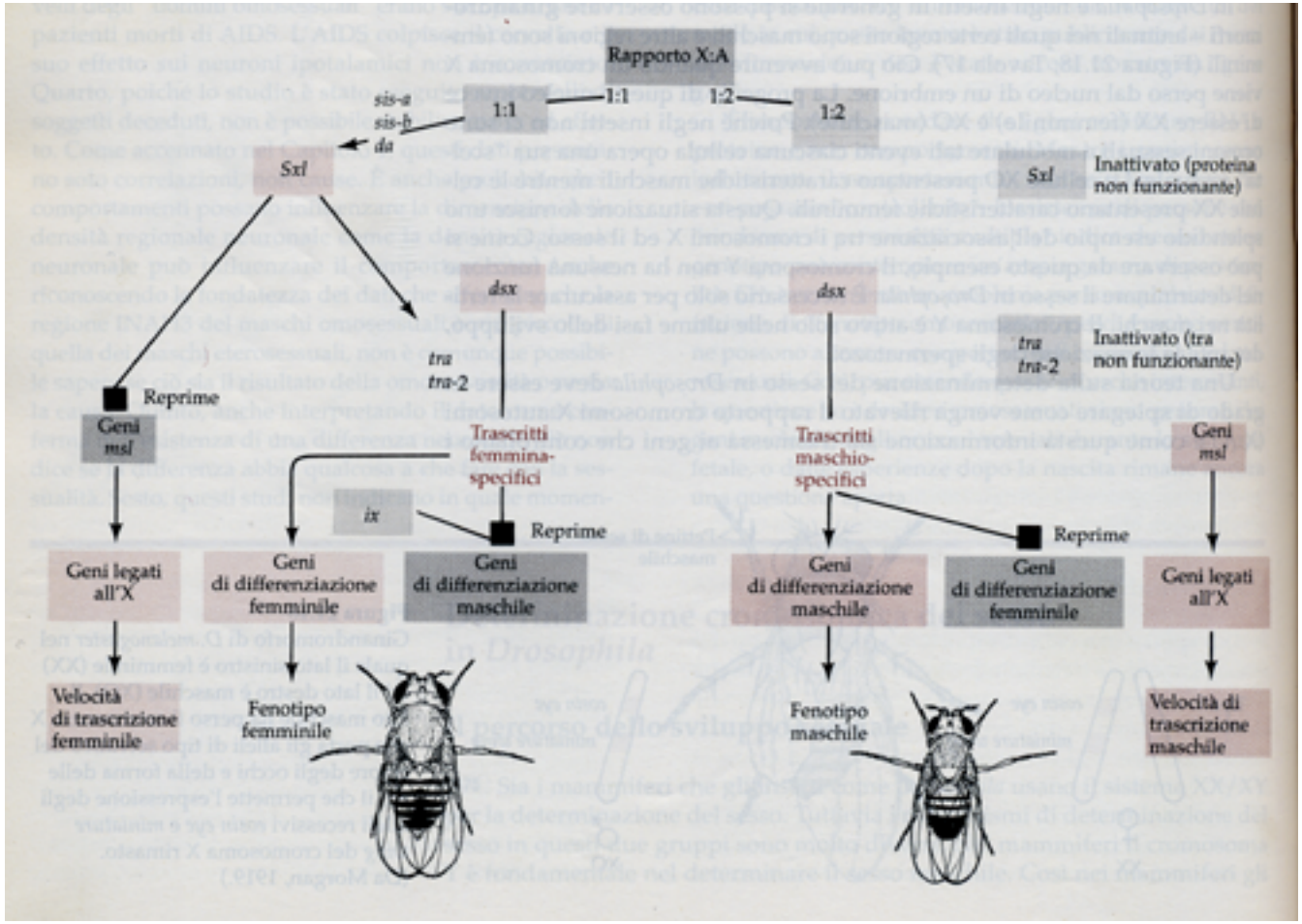
- Sex determination in *Drosophila*
- Apoptosis

Pathology - misregulation of alternative splicing and disease.

- Cystic fibrosis
- Spinal Muscular Atrophy
- Myotonic Dystrophy
- FTDP1 Fronto-temporal dementia
- WT-1 Wilms tumors

Alternative Splicing Is a Rule, Rather Than an Exception, in Multicellular Eukaryotes

- Sex determination in *Drosophila* involves a series of alternative splicing events in genes coding for successive products of a pathway.


Rapporto X:A
1:1
1:1
1:2
1:2
sis-a
sis-b
da
Sxl
Sxl
Inattivato (proteina non funzionante)
dsx
dsx
tra
tra-2
tra
tra-2
Inattivato (tra non funzionante)
Reprime
Geni msl
Trascritti femmina-specifici
Trascritti maschio-specifici
Geni msl
ix
Reprime
Reprime
Geni legati all'X
Geni di differenziazione femminile
Geni di differenziazione maschile
Geni di differenziazione maschile
Geni di differenziazione femminile
Geni legati all'X
Velocità di trascrizione femminile
Fenotipo femminile
Fenotipo maschile
Velocità di trascrizione maschile

Regulation of/by Alternative Splicing

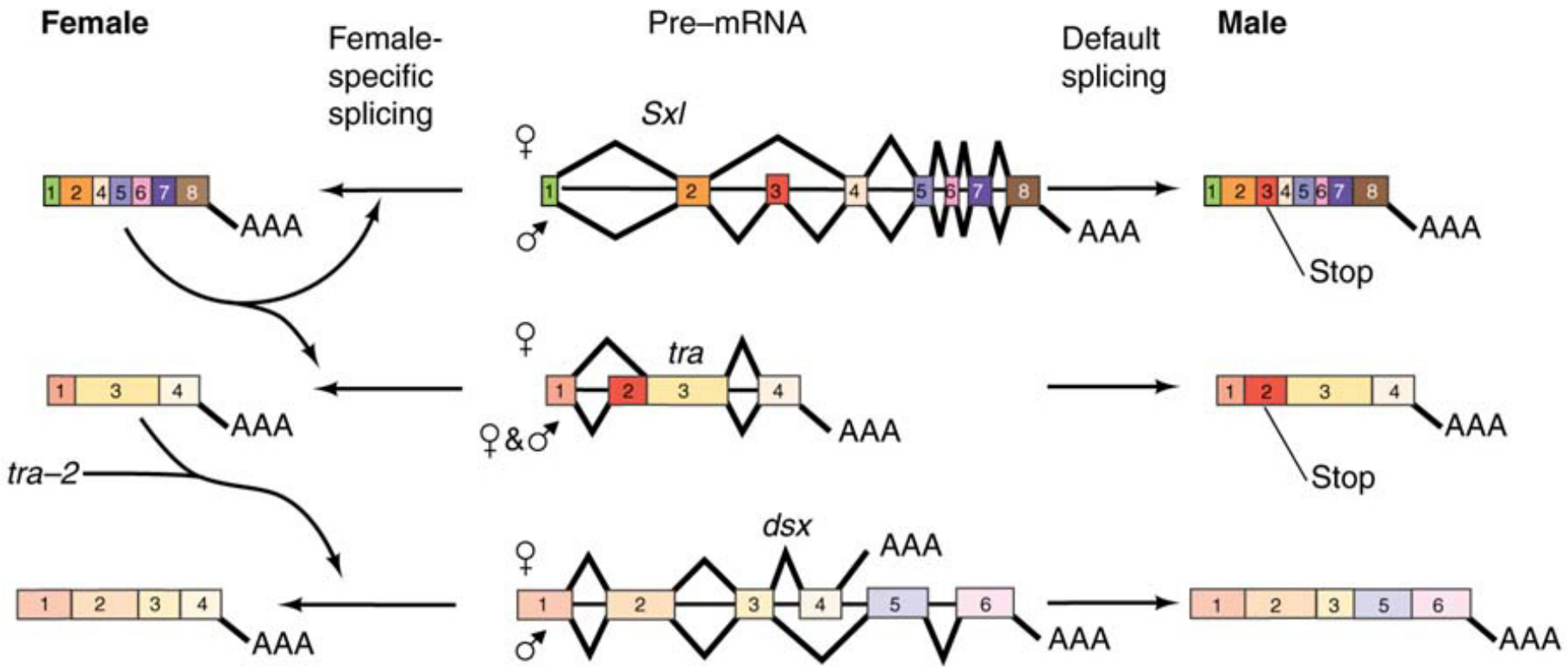
Sex determination in *Drosophila* involves 3 regulatory genes that are differentially spliced in females versus males; 2 of them affect alternative splicing

1. ***Sxl*** (sex-lethal) - promotes alternative splicing of *tra* (alternative 3'ss selection) and of its own (exon 3 is skipped) pre-mRNA

2. ***Tra*** – promotes alternative splicing *of dsx* (last 2 exons are excluded)

3. ***Dsx*** (double-sex) - Alternatively spliced form of *dsx* needed to maintain female state (Tra promotes exon 4 inclusion)

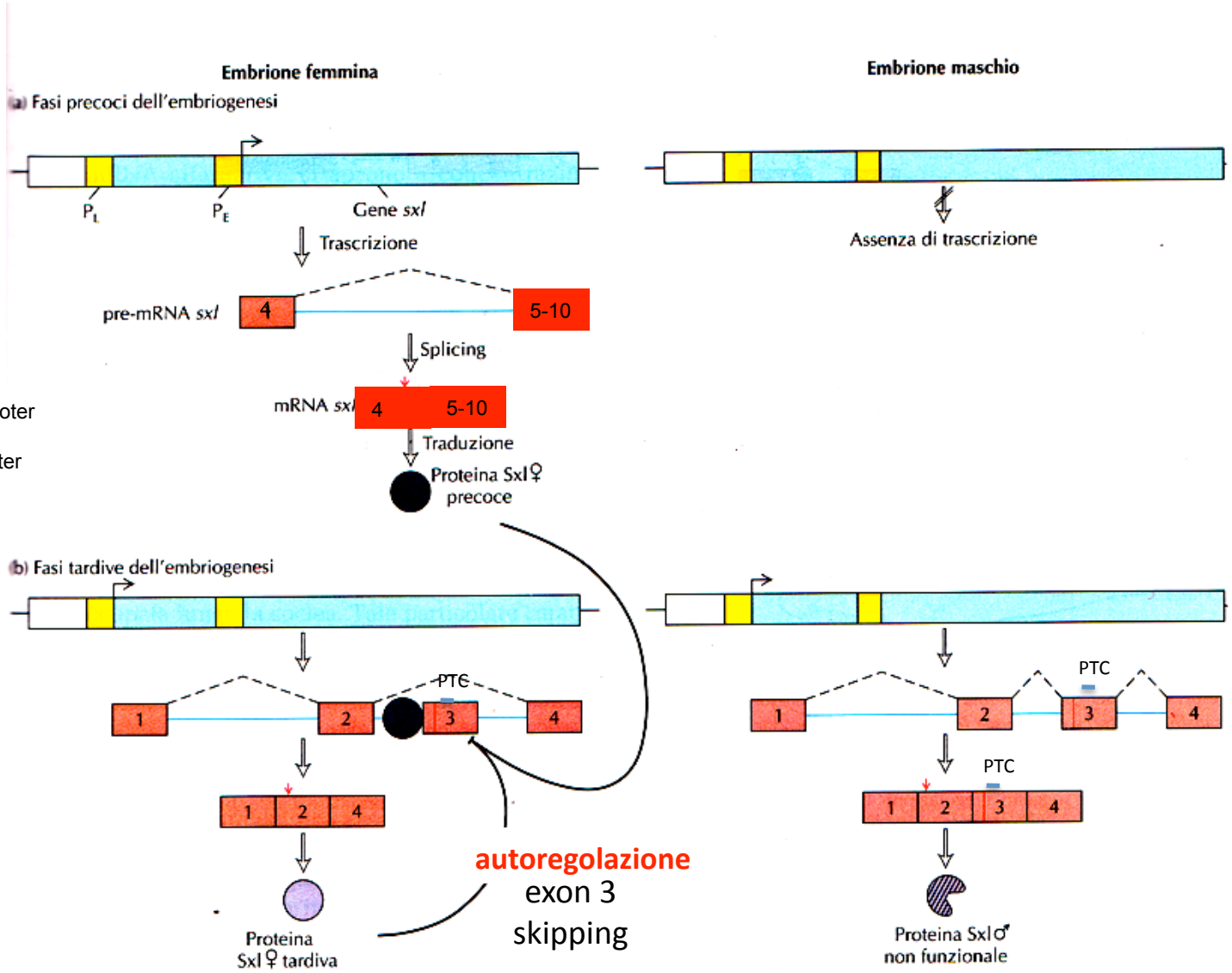
Alternative splicing in Drosophila maintains the female state

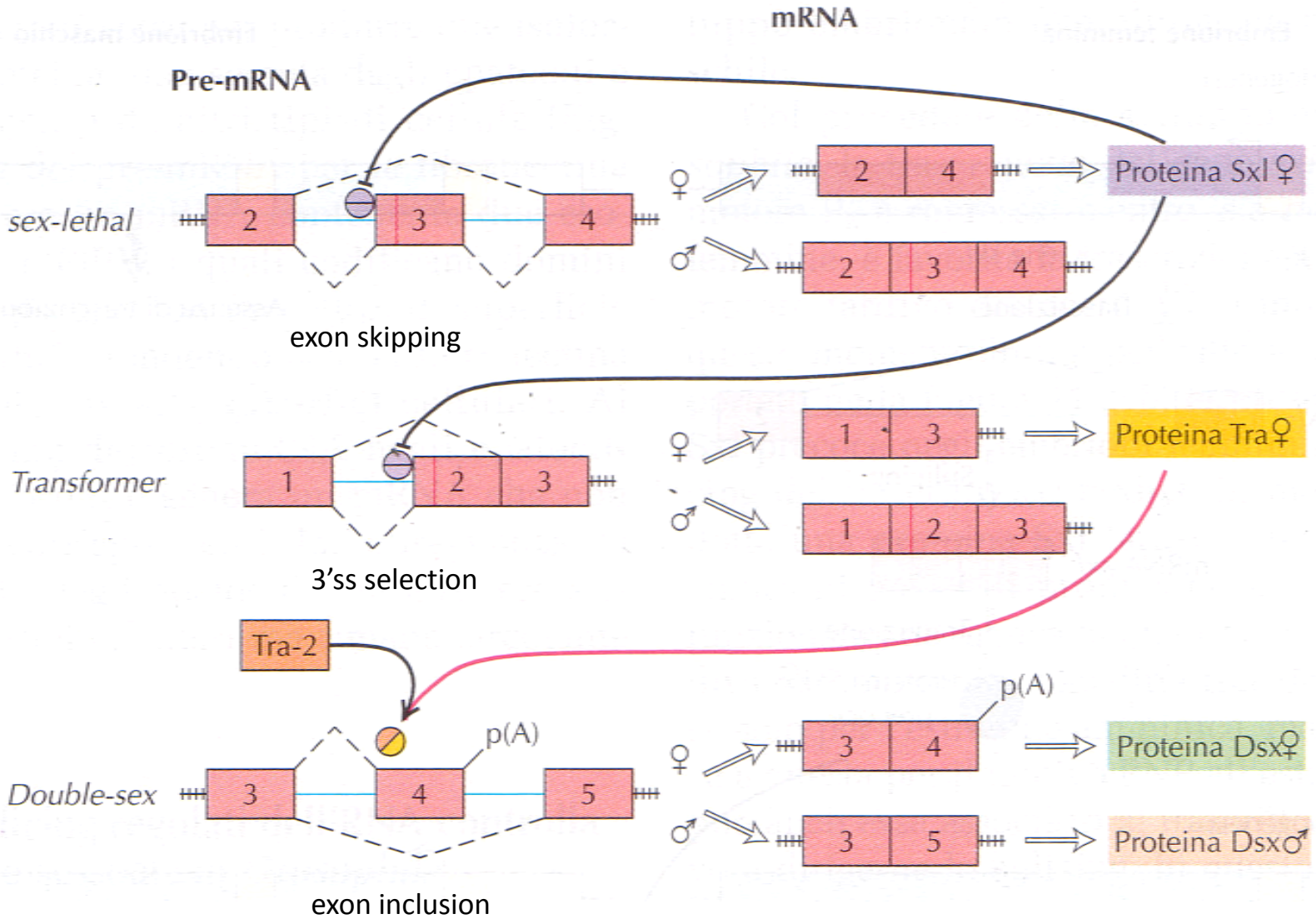


Sxl and Tra are SR proteins
Tra binds exon 4 in *dsx* mRNA causing it to be retained in mature mRNA
dsx is a transcriptional factor.

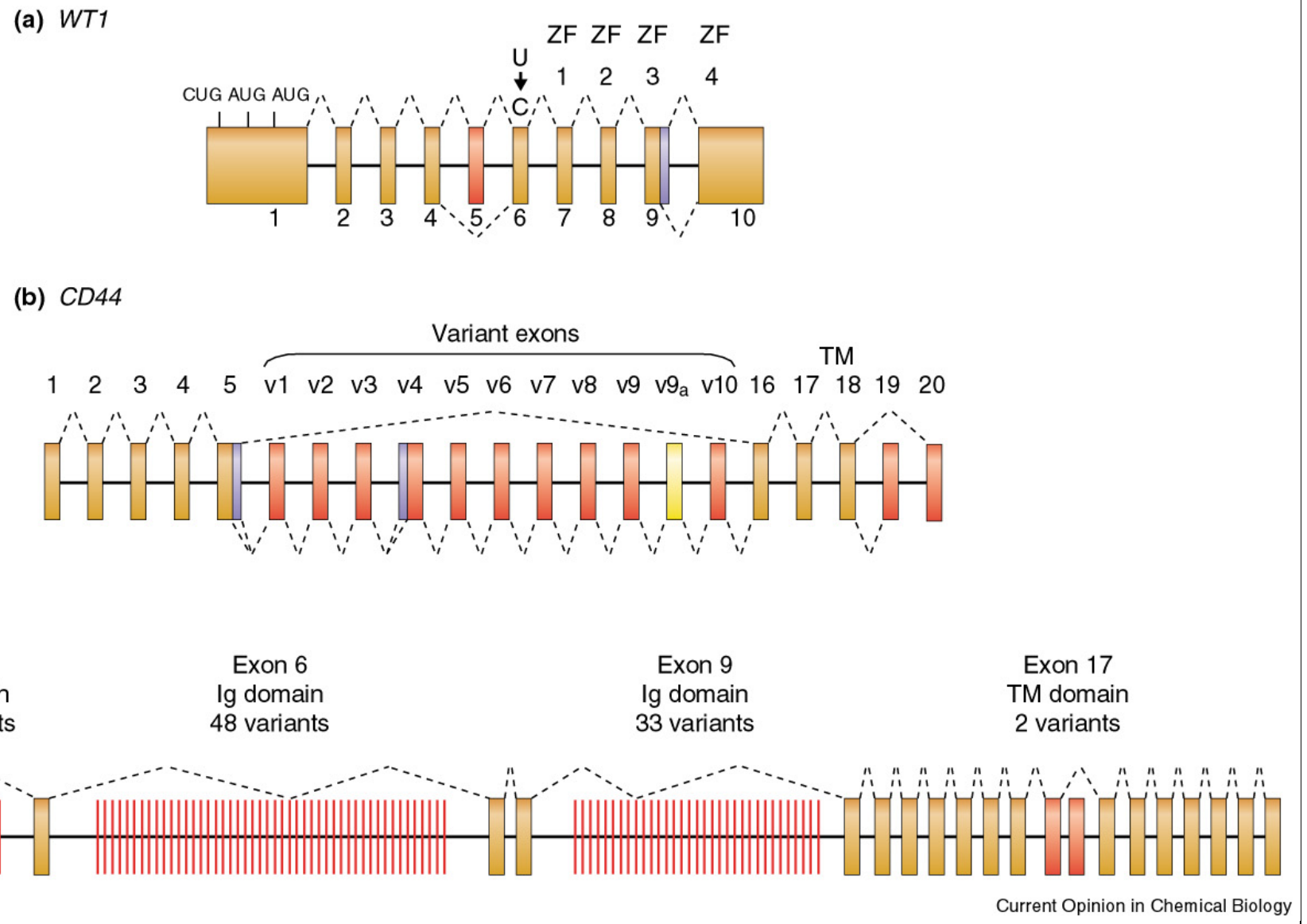
P_E = early promoter

P_L = late promoter

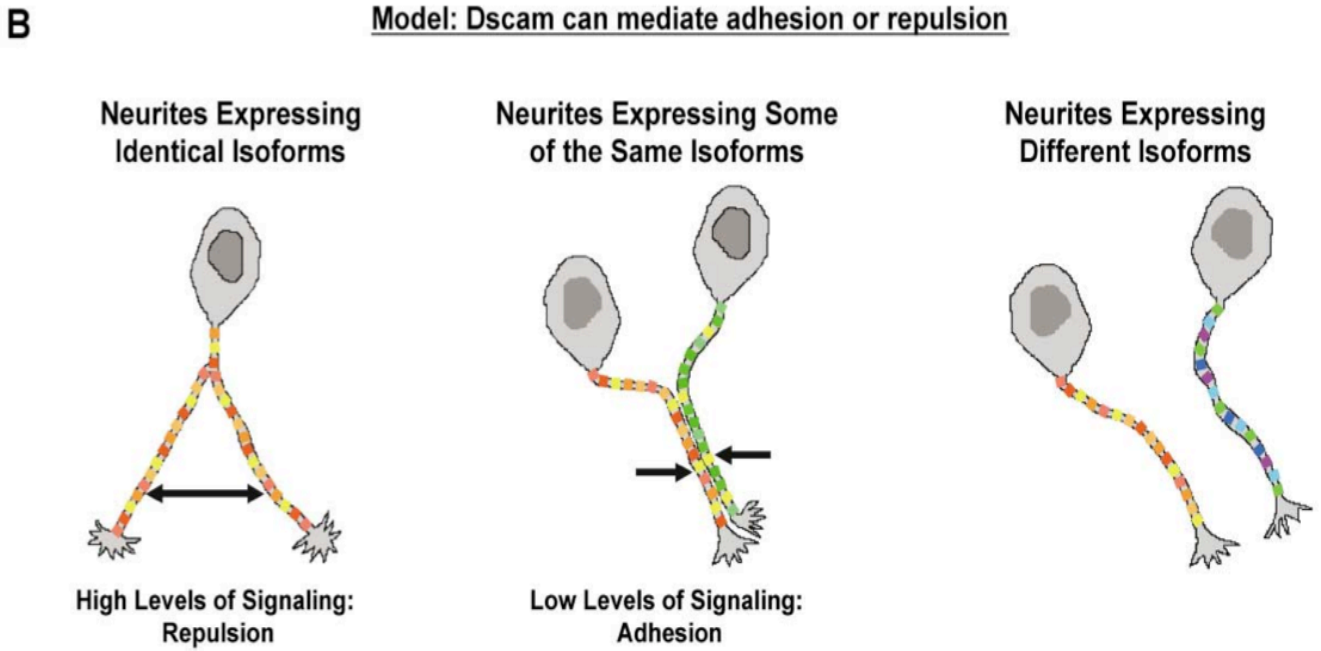



mRNA
Pre-mRNA
sex-lethal
2
3
4
♀
♂
2
4
2
3
4
Proteina Sxl♀
exon skipping
Transformer
1
2
3
♀
♂
1
3
1
2
3
Proteina Tra♀
3'ss selection
Tra-2
p(A)
Double-sex
3
4
5
♀
♂
p(A)
3
4
3
5
Proteina Dsx♀
Proteina Dsx♂
exon inclusion

One gene: thousands of polypeptides



Alternative splicing can potentially generate more than 38,000 Down syndrome cell adhesion molecule isoforms. This molecular diversity may contribute to the specificity of neuronal connectivity.



(B) Schematic representation of Dscam-mediated interactions between neurites. We propose that differences in levels of Dscam signaling influence the nature of the interactions between neurites. High levels of signaling between neurites expressing the same array of Dscam isoforms result in contact-dependent repulsion (left panel), while low levels of signaling between neurites expressing some of the same isoforms or isoforms that bind weakly to each other result in adhesion (middle panel). Some neurites may express isoforms of Dscam that do not interact (right panel). Dscam-mediated contact-dependent repulsion and adhesion may regulate interactions between neurites during axon guidance, targeting, or synapse formation.

Cell, Vol. 118, 619–633, 2004,

35% of all alternatively spliced human transcripts undergo Nonsense Mediated Decay