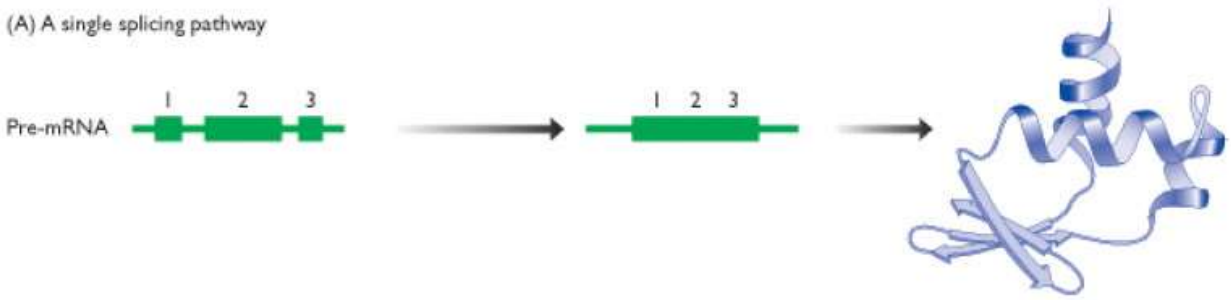
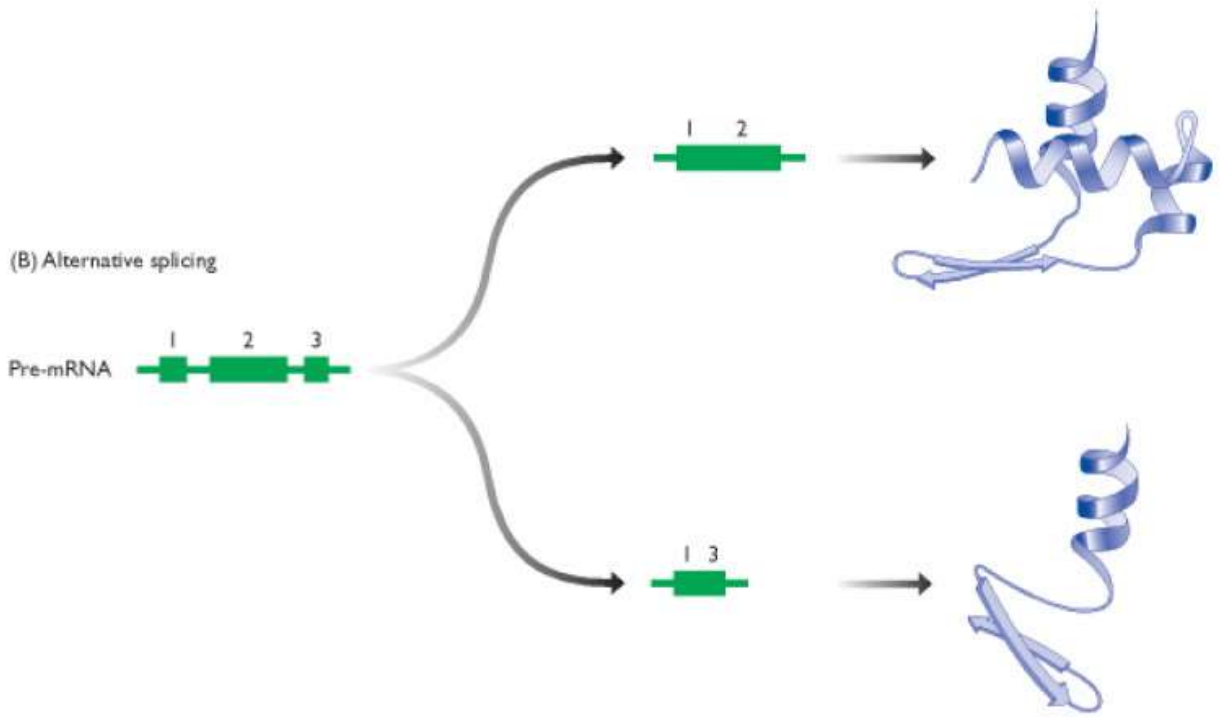
SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 201: Fundamentals of Molecular Biology (Unit IV & V)

Alternative Splicing

Splicing Mechanism(s)



- Constitutive Splicing



Alternative Pathway

Specific exons or exonic sequences may be excluded or included in the mRNA products using alternative splicing sites.

More than 90% of the genes expressed in mammals are alternatively spliced.

Alternative splicing contribute to structural and functional diversity of gene products.

Thus, alternative splicing is not just the result of mistakes made by the splicing machinery.

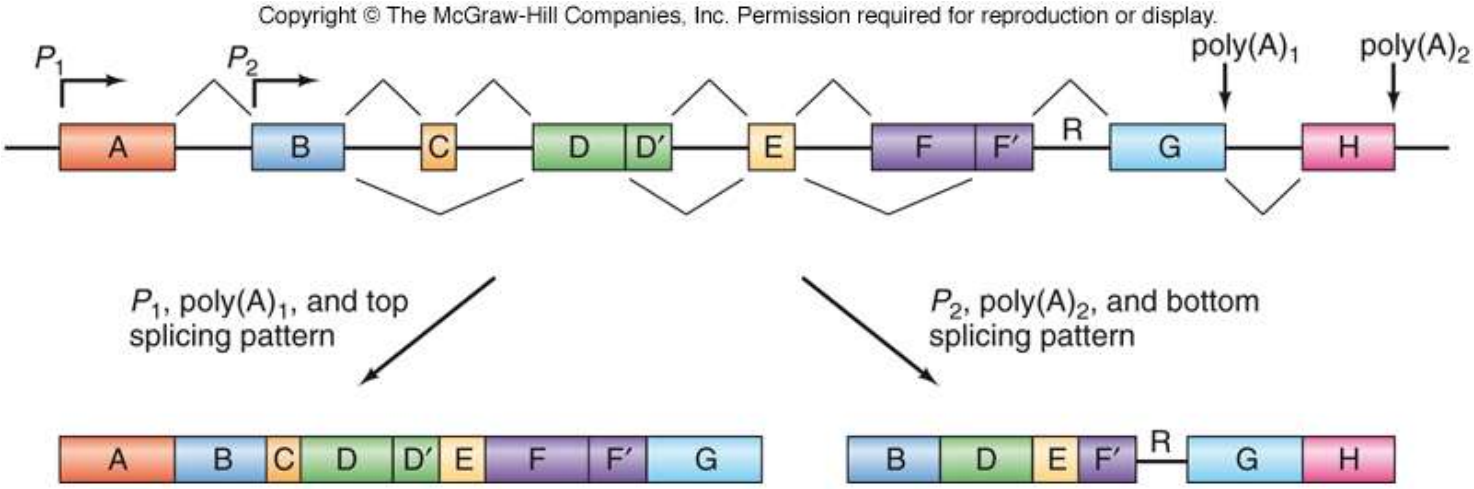
Rather, it is part of the gene expression programme.

Alternative splicing results in the formation of multiple gene products from a single gene locus.

Therefore, alternative splicing is a RULE rather than exception in multicellular eukaryotes.

Alternative Splicing Patterns

- **Alternative splicing of the same pre-mRNA gives rise to very different products**
  - **Alternative splicing patterns occur in over half of human genes**
  - **Many genes have more than 2 splicing patterns, some have thousands**

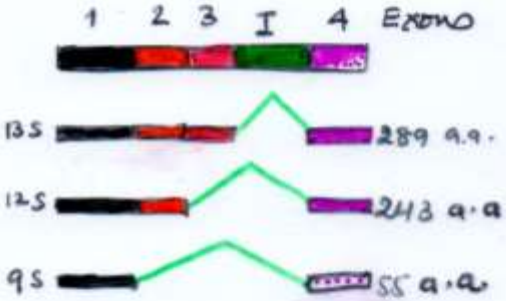


Alternative Splicing

Alternative forms of splicing may generate a variety of protein products from an individual gene. Changing the splice site may introduce termination codon (shown by asterisk) or change reading frames)

Example 1.

SV 40 T/t antigens splice two 5' sites to a common 3' site



Example 2.

Adenovirus E1A splices variable 5' sites to a common 3' site

Example 3.

Drosophila melanogaster Tra splices a 5' site to alternative 3' sites.

Q/- How many polypetides can be derived from the same pre-mRNA?

Answer

Many e.g., :

1. α-Tropomyosin	**-**	**10 different forms in a tissue specific manner**
2. Troponin T	**-**	**64 known forms**

Modes of Alternative Splicing

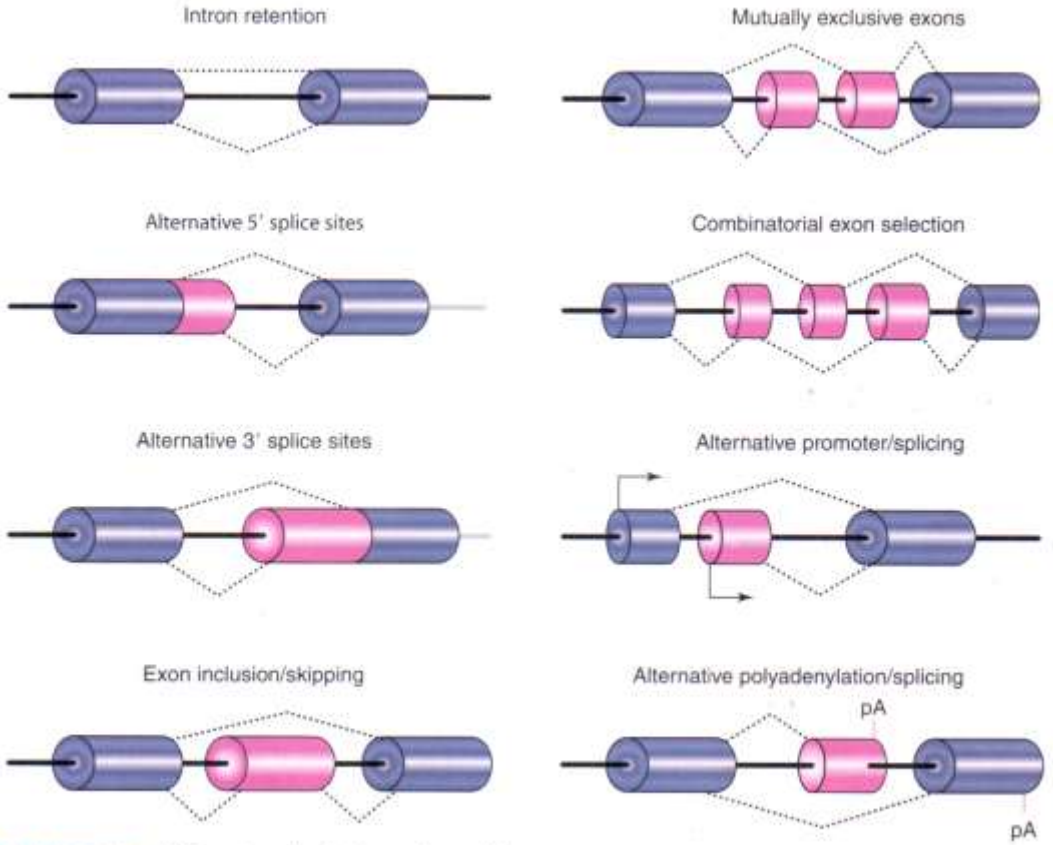


FIGURE 21.21 Different modes of alternative splicing.

A single gene primary transcript may undergo more than one mode of alternative splicing i.e.,

-Intron Retention

-Alternative 5' splice site selection

-Alternative 3' splice site selection

-Exon inclusion or skipping

-Mutually exclusive selection of the alternative exons

(may get regulated in a tissue specific manner) etc.

Q/- How alternative splicing can affect gene expression?

Answer

Through at least three following ways:

1. By creating structural diversity of gene products

- by including or omitting some coding sequences or

- by creating alternative reading frames for a portion of the gene.

(This can often modify the functional property of encoded proteins.)
(Calcium/calmodulin-dependent protein kinase type II delta, CaMKIIδ)

2. Alternative spliced products exhibit opposite functions.
(e.g., essential genes involved in the regulation of apoptosis)

3. Alternative spliced products may also affect various properties of the mRNA by including or omitting certain regulatory elements, which may significantly alter the half-life of the mRNA.

Alternative Splicing of *CaMKIIδ* Genes

(Calcium/calmodulin-dependent protein kinase type II delta)

14, 15, 16 are alternative exons

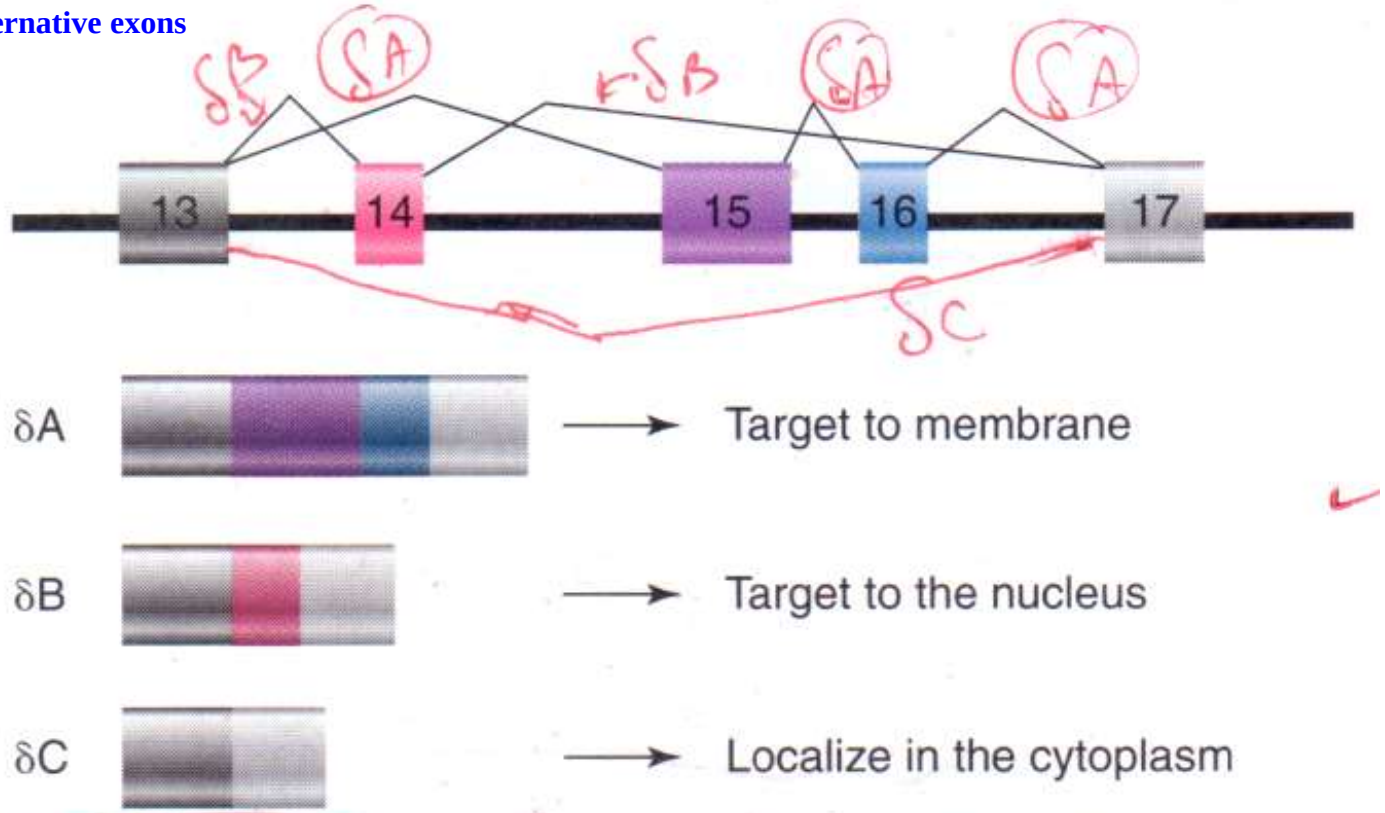


FIGURE 21.22 Alternative splicing of the *CaMKIIδ* gene: different alternative exons target the kinase to different cellular compartments.

Alternative Splicing

- Transcripts of many eukaryotic genes are subject to alternative splicing
  - This splicing can have profound effects on the protein products of a gene
  - Can make a difference between:
    - Secreted or membrane-bound protein
    - Activity and inactivity
- Products of 3 genes in sex determination pathway of the fruit fly are subject to alternative splicing

CONSEQUENCES (1)
(GOOD SIDE)

DIFFRENT PATTERN OF SPICING:

- **IN DIFFRENT TISSUES**
- **IN DIFFRENT STAGES OF DEVELOPMENT**
- **SOME OTHER (e.g. : SEX DETERMINATION IN DROSOPHILA)**

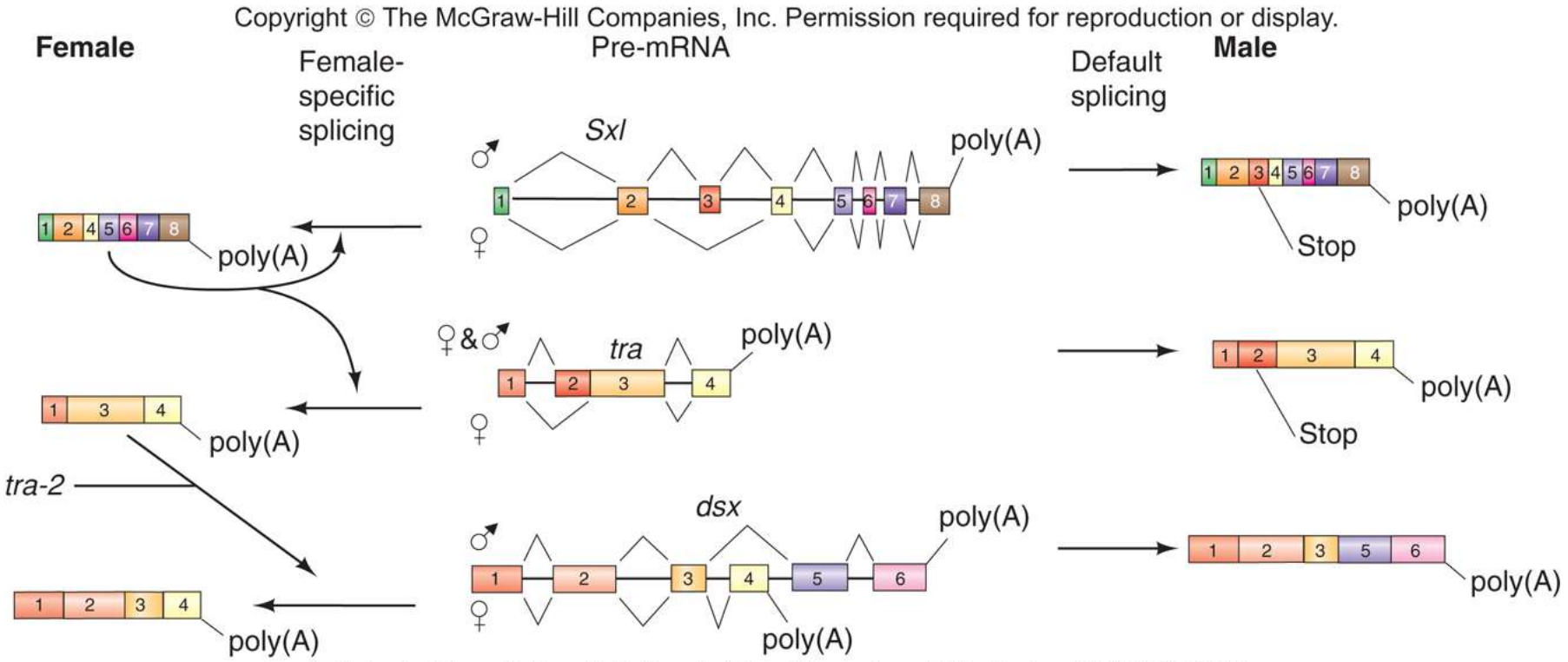
Biological Role

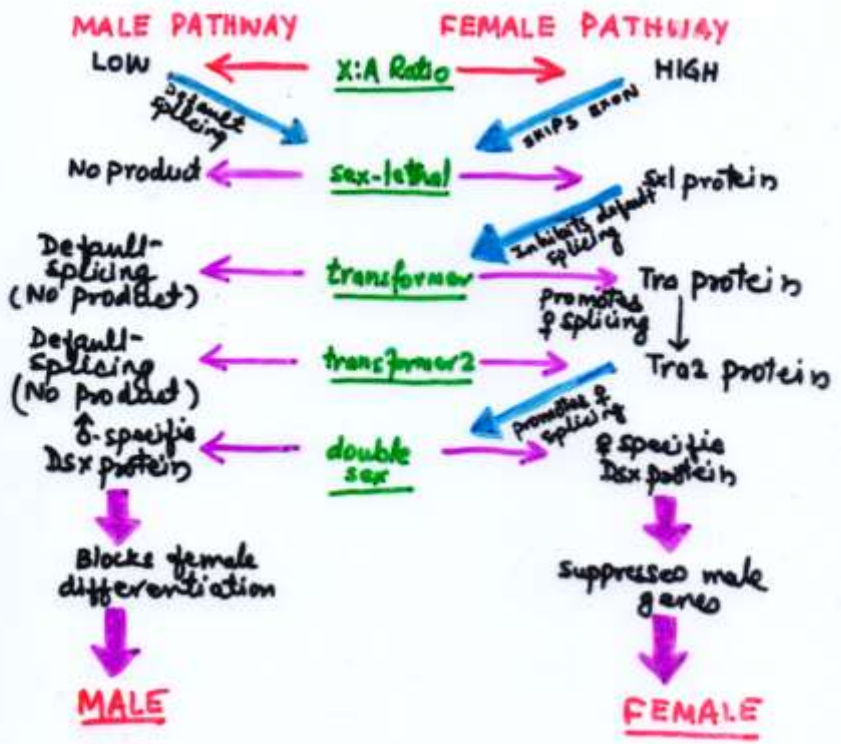
of

Alternatively Spliced Products

Sex Determination in *Drosophila*

Alternative Splicing in *Drosophila* sex determination





Sex determination in D. melanogaster involves a pathway in which different splicing events occur in females. Blocks at any stage of the pathway results in male development.

Note:-

1. Exon of sxl gene contain a termination codon that prevent synthesis of functional protein. This exon is included in ♂ but skipped in ♀ → as a result only ♀ produces sxl protein.
2. sxl protein inhibit the usage of normal 3' splice site and then the next 3' splice site is used which lead to synthesis of female specific mRNA and thus Tra is produced only in ♀
3. 5' splice site of I_3 & 3' splice site of I_4 skips stop codon & large protein is synthesized → ♂ specific Dsx
4. 5' splice site of I_3 & 3' splice site of I_2 introduces stop codon & small protein produced → ♀ specific protein.

CONSEQUENCES (2)
(BAD SIDE)

- **MUTATIONS IN SEQUENCES INVOLVED IN REMOVING OF INTRONS MAY CAUSE UNPHYSIOLOGICAL SPLICING AND THEREFORE DISEASES**

- **MAINLY NEURODEGENERATIVE DISEASES (MYOTROIC DYSTROPHY, SPIRAL MUSCULAR ATROPY ETC.)**

REGULATION

of

ALTERNATIVE SPLICING

Splicing can be regulated by Exonic and Intronic Splicing Enhancers and Silencers

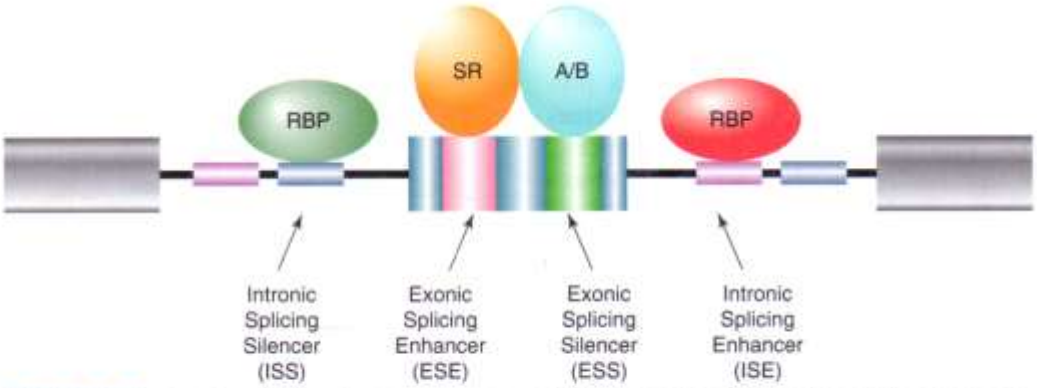


FIGURE 21.24 Exonic and intronic sequences can modulate the splice site selection by functioning as splicing enhancers or silencers. In general, SR proteins bind to exonic splicing enhancers and the hnRNP proteins (such as the A and B families of RNA binding proteins) bind to exonic silencers. Other RNA binding proteins (RBP) can function as splicing regulators by binding to intronic splicing enhancers or silencers.

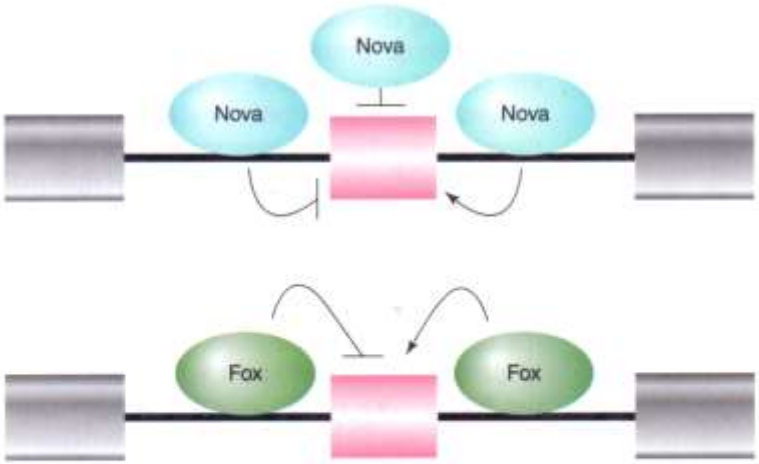


FIGURE 21.25 The Nova and Fox families of RNA binding proteins can promote or suppress splice site selection in a context dependent fashion. Binding of Nova to exons and flanking upstream introns inhibits the inclusion of the alternative exon while Nova binding to the downstream flanking intronic sequences promotes the inclusion of the alternative exon. Fox binding to the upstream intronic sequence inhibits the inclusion of the alternative exon whereas binding of Fox to the downstream intronic sequence promotes the inclusion of the alternative exon.

Alternative Splicing Summary

- Alternative splicing is very common in higher eukaryotes
- It represents a way to get more than one protein product out of the same gene and a way to control gene expression in cells
- Such control is exerted by splicing factors that bind to splice sites and a branch point, and also by proteins that interact with ESEs, ESSs and intronic splicing elements