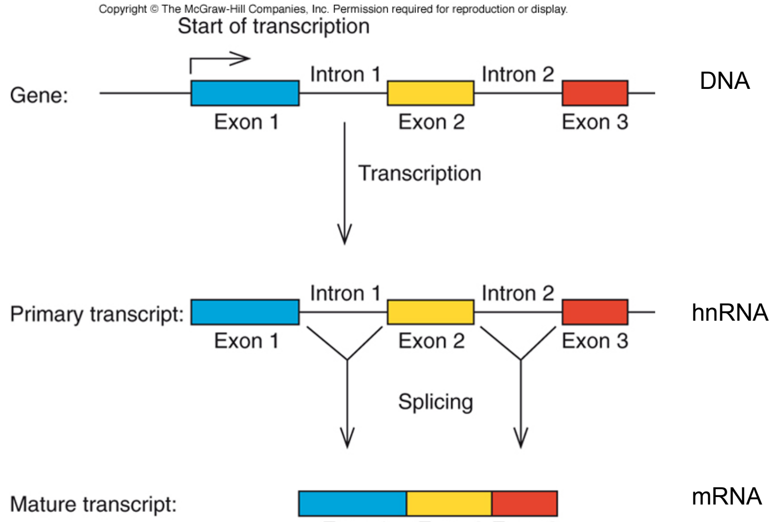
Chapter 14: Splicing

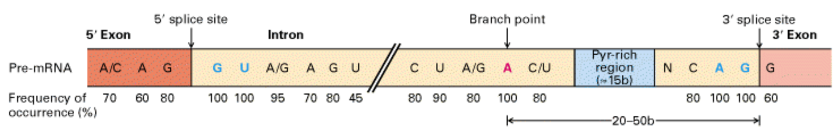
1. General Mechanism
2. Alternative Splicing
    1. Leaky
    2. Regulated
    3. Transplicing

Intron/Exon



Splice Junctions

- Three conserved regions in intron
  - 5' Splice Site – ag/GUAAGU
  - 3' Splice Site – YYYYYYYYYYNAG/g
  - Branch Site (20-50nt away from 3' splice site)
    - CURAYY



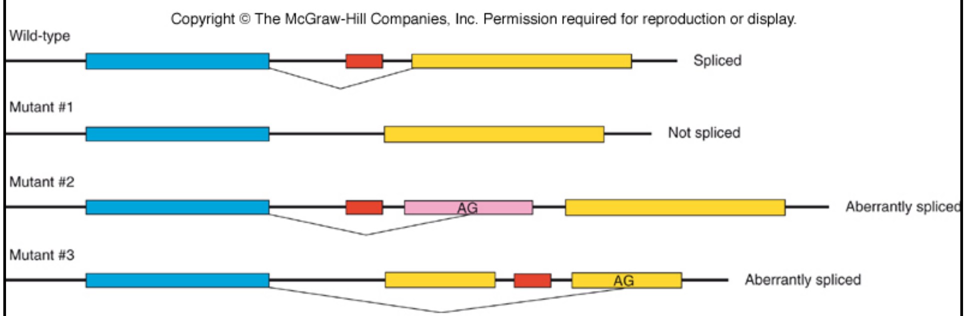
Lodish

Additional RNA sequences influence splicing

Splicing Enhancers - sequences that promote recognition and use of a splice site.
Exonic Splicing Enhancers (ESE)
Intronic Spicing Enhancers (ISE)

Splicing Silencers - sequences that reduce recognition and use of a splice site.
Exonic Splicing Silencers (ESS)
Intronic Splicing Silencers (ISS)

Importance of Branchpoint location



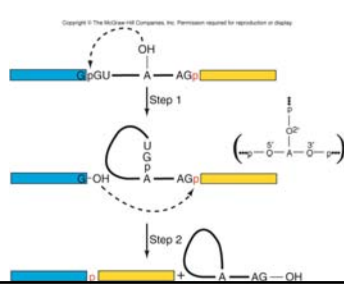
What snRNP recognizes Branch point?

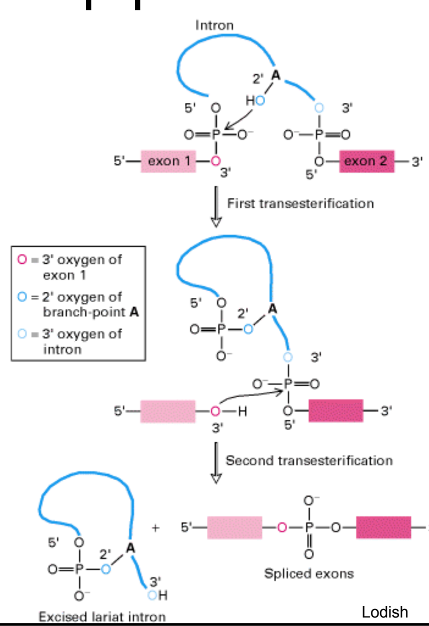
Splicing a three step process

1. First transesterfication joins 5'end of intron to branch site

2. Second transesterfication joins exon 1 to exon 2

3. Destruction of lariat

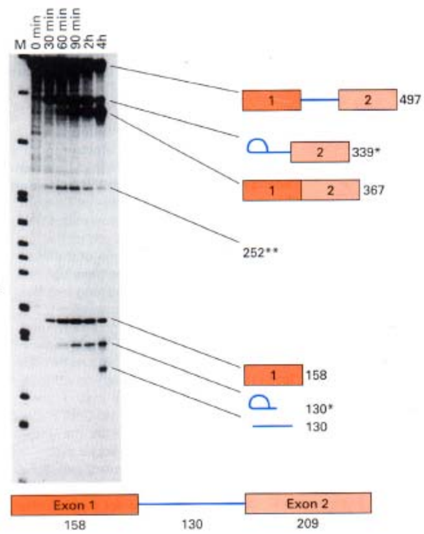




Lodish

Analysis of RNA products formed in an in vitro splicing reaction

A nuclear extract from HeLa cells was incubated with a 497-nucleotide radiolabeled RNA *(bottom)* that contained portions of two exons (orange and tan) from human β-globin mRNA separated by a 130-nucleotide intron (blue). After incubation for various times, the RNA was purified and subjected to electrophoresis and autoradiography, along with RNA markers (lane M). The number of nucleotides in the various species is indicated. Much of the slower-migrating starting RNA (497) was correctly spliced, yielding a 367-nucleotide product. The excised intron (130*) migrated slower than expected based on its molecular weight, indicating that it is not a linear molecule. Likewise, one of the reaction intermediates (339*) exhibited an anomalously slow electrophoretic mobility. Additional analysis indicated that in both cases the intron had a lariat structure resulting in the slow mobility. The 252** band, an aberrant product of the in vitro reaction, is greatly reduced in reactions in which the RNA is capped. [From B. Ruskin et al., 1984, *Cell* **38**:317; photograph courtesy of Michael R. Green. See also R. A. Padgett et al., 1984, *Science* **225**:898.]



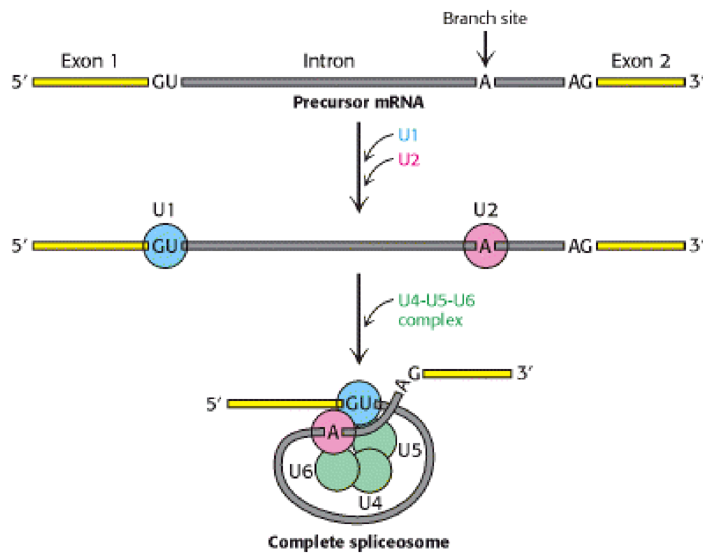
snRNP

small nuclear ribonucleoprotein

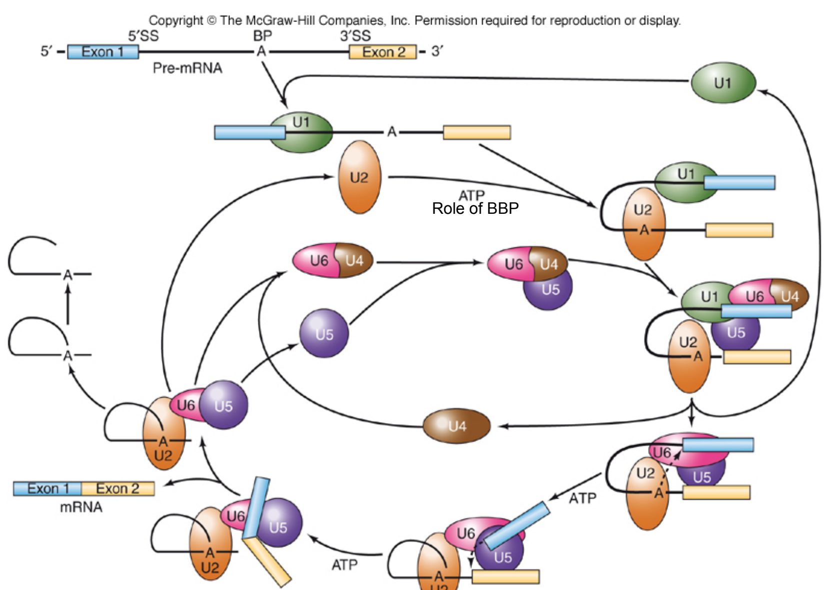
“snurps”

snRNP	Size(nt)	Role
U1	165	Binds the 5′ splice site and then the 3′ splice site
U2	185	Binds the branch site and forms part of the catalytic center
U5	116	Binds the 5′ splice site
U4	145	Masks the catalytic activity of U6
U6	106	Catalyzes splicing

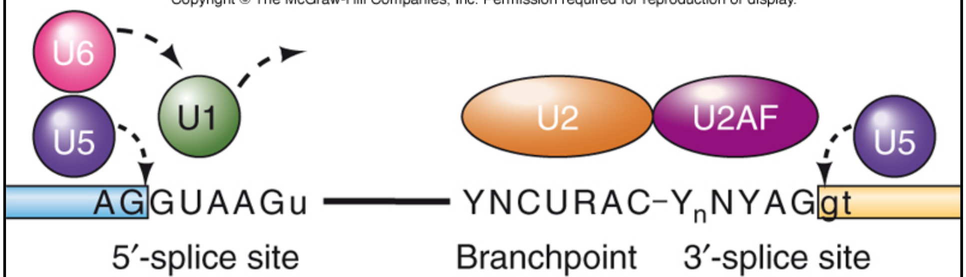
Splicosome – snRNP complex



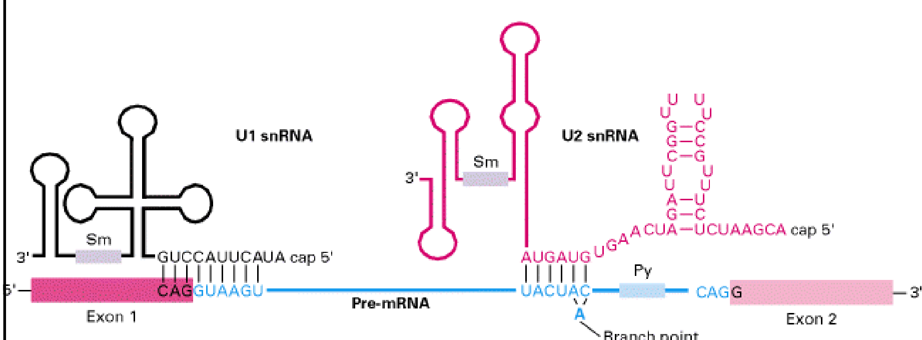
Splicosome Cycle



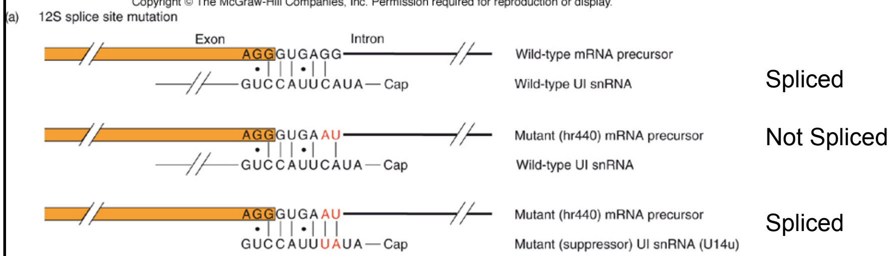
snRNP binding



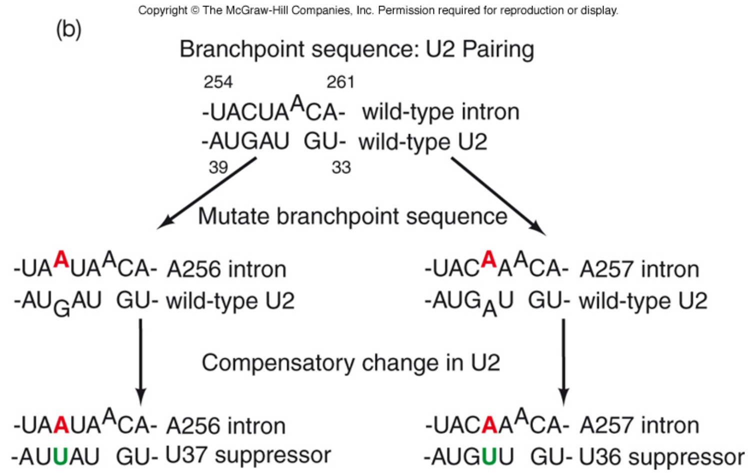
Role of snRNA in snRNP Binding



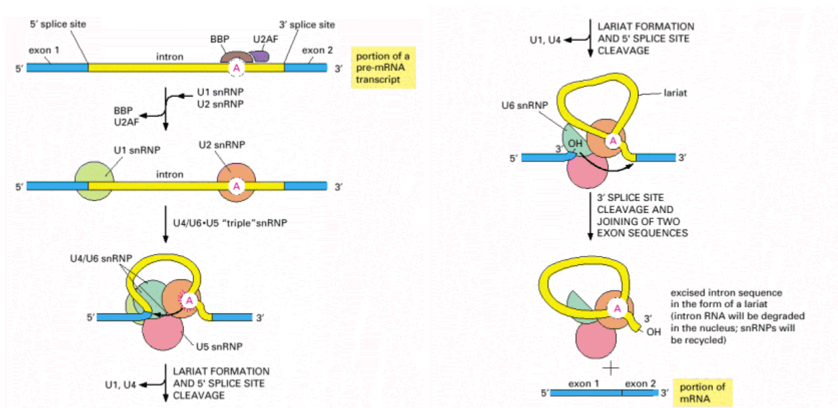
Compensating Mutations



U2 Compensating Mutations

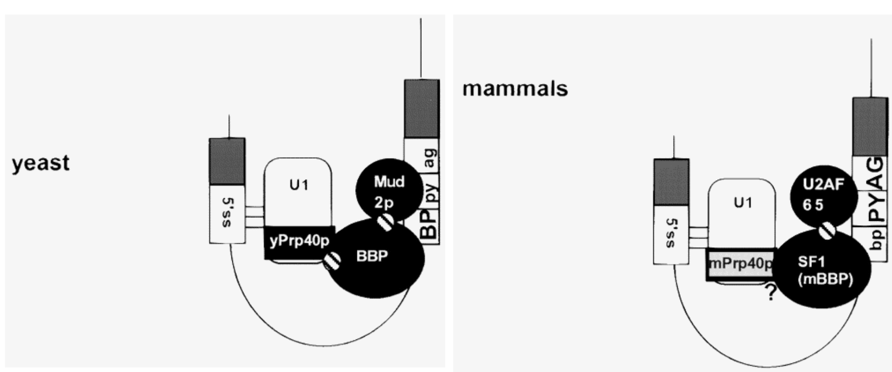


Branchpoint Bridging Protein

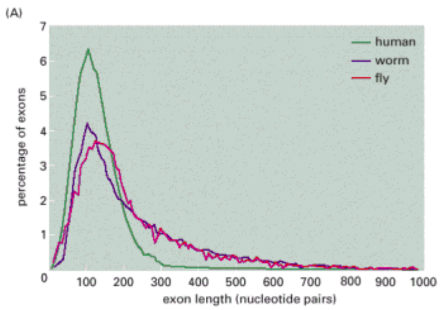


Alberts et al, 2006

Branchpoint Bridge Protein and Commitment

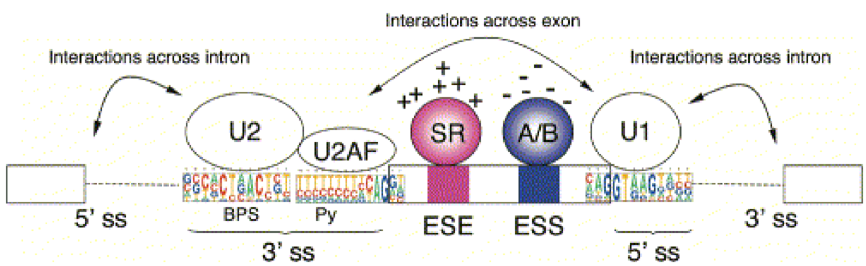


Exon vs Intron Length

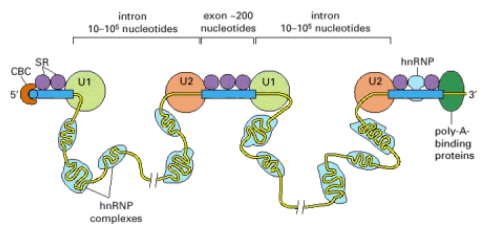


Lodish et al, 2000

Splicing Enhancers and Silencers



Exon Definition Hypothesis

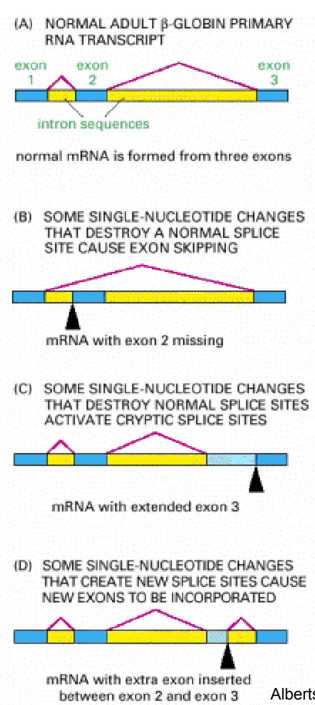


Central Role of ESE in assembly of Splicosome

Intron Definition Hypothesis?: role of intron consensus sequences

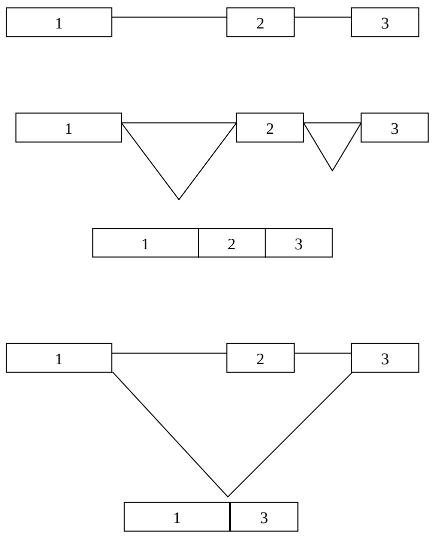
Lodish et al, 2000

Mutations can affect Spicing



Alberts et al, 2006

Alternative Splicing



Leaky Splicing

Example: a non consensus 3' splice site in intron 1 results in its inefficient use.

Regulated Splicing

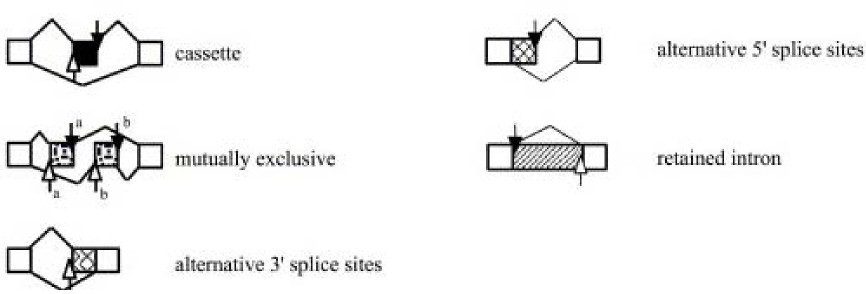
Activation of a weak splice site.

Example a non consensus 3' site in intron 1 will only be used if a protein binds ESE or ISE.

Repression of a weak splice site

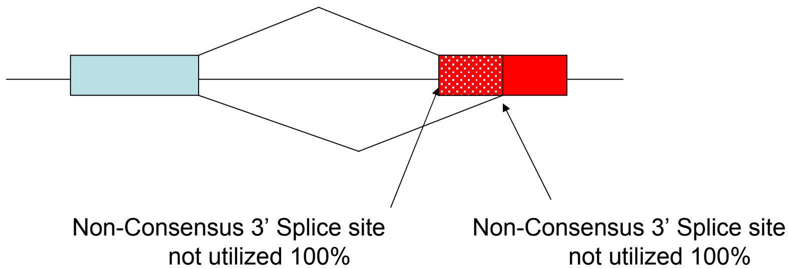
Example a strong 3' splice site in intron 1 will be repressed if a protein binds to ESS or ISS.

Types of Alternative Spicing

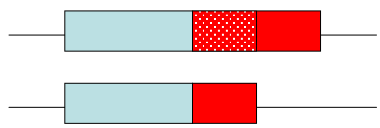


Stamm et al 2004

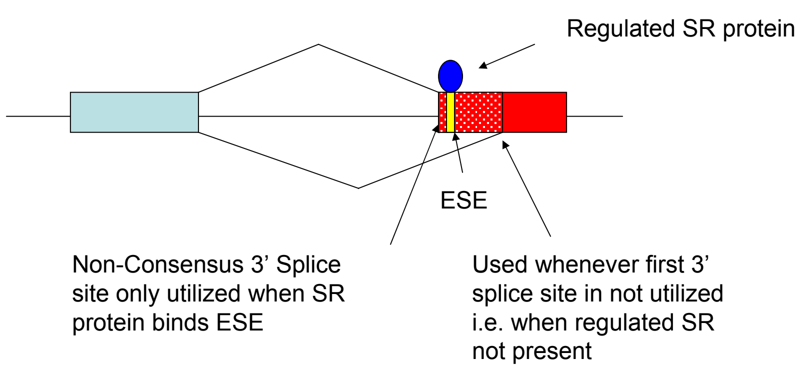
Leaky Splicing

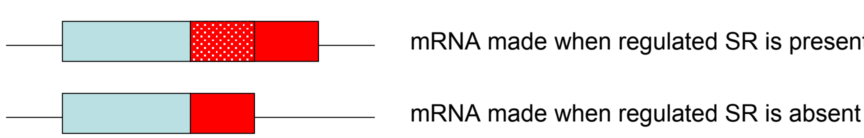


Each 3' splice site is used some of the time resulting in two distinct mRNA



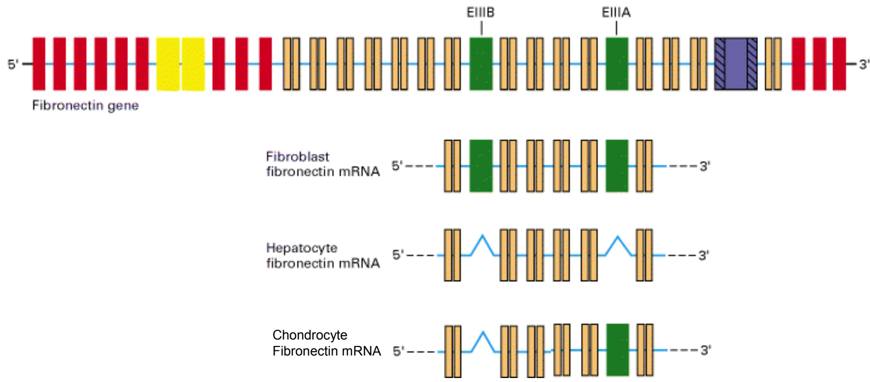
Regulated Splicing





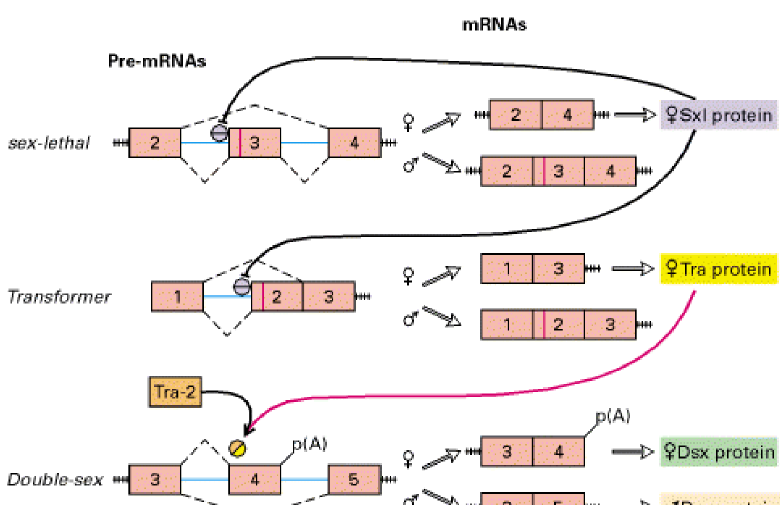
Consider possible regulation by ISS.

Regulation of Fibronectin mRNA Spicing



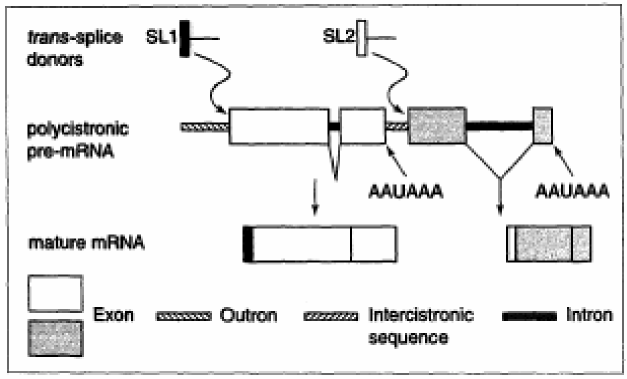
Lodish et al, 2000

Sex Determination in Drosophila



Lodish et al, 2000

Trans Spicing in *C. elegans*



Facilitates polycistronic operons in C. elegans

Spliced Leaders – 22 nt sequence facilitate translational initiation

Capping of RNA Pol1 transcripts

Transplicing Lariat