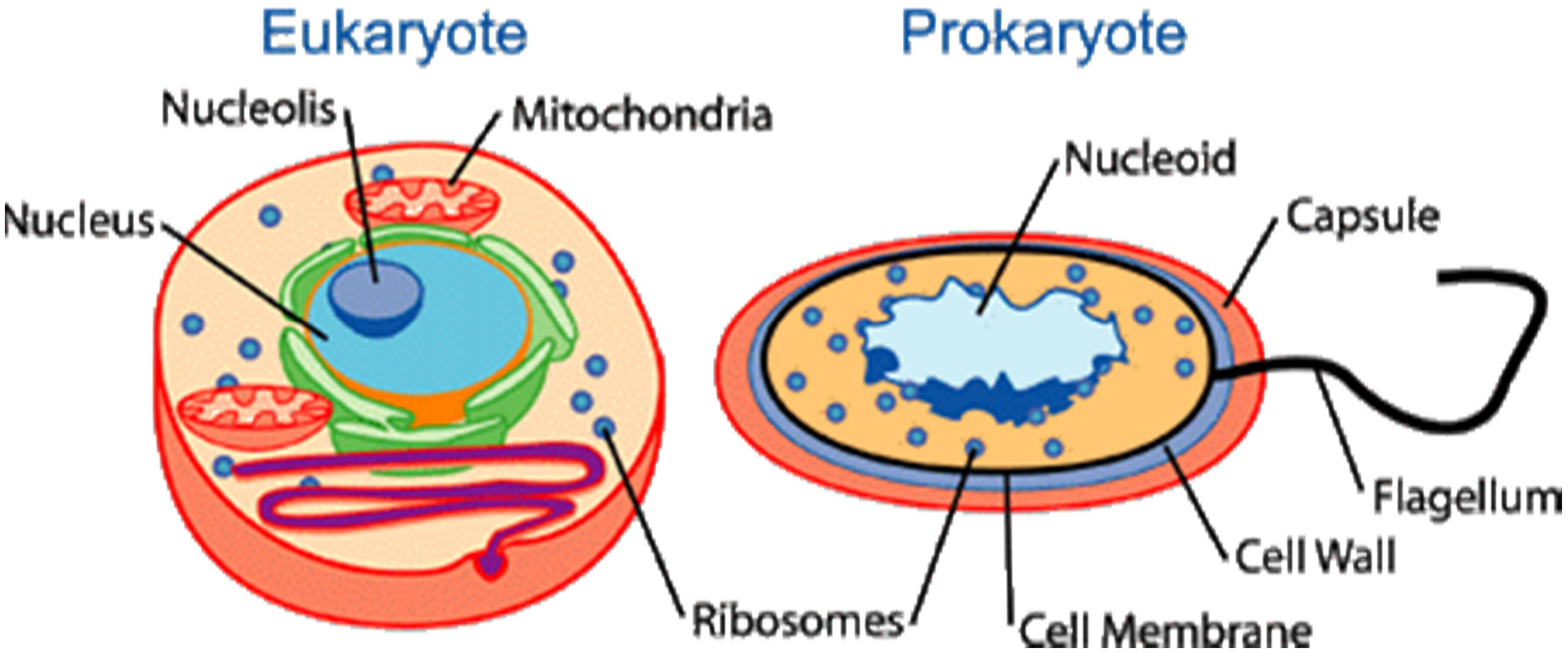
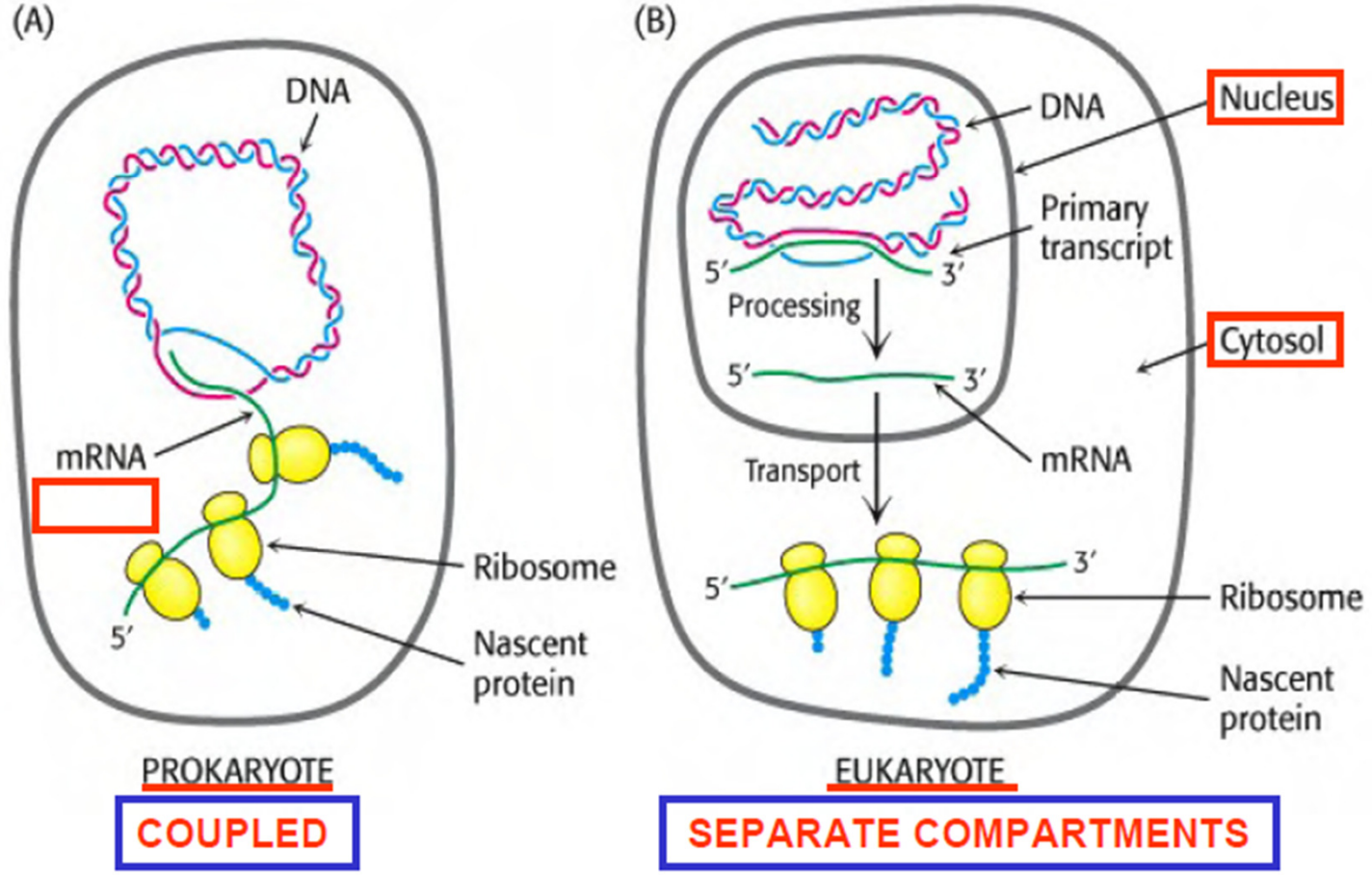
Splicing (Recomposição) do RNAm

Marcelo D Gomes


Eukaryote
Nucleolis
Mitochondria
Nucleus
Ribosomes
Prokaryote
Nucleoid
Capsule
Flagellum
Cell Wall
Cell Membrane

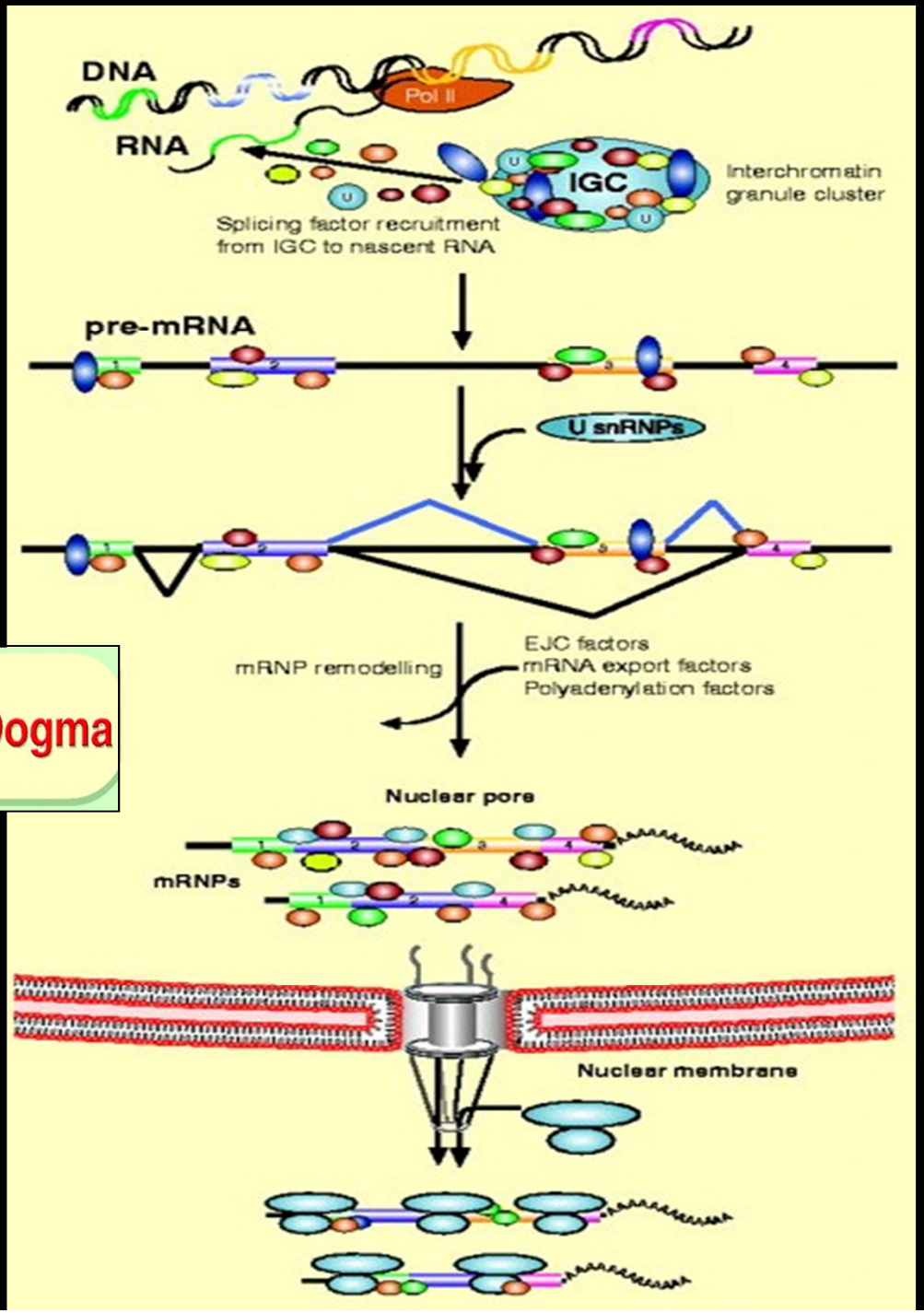
TRANSCRIPTION AND TRANSLATION:
Prokaryotic vs Eukaryotic



Pre-mRNA Splicing

Life at the Centre of the Central Dogma

Jeremy R. Sanford and Javier F. Caceres



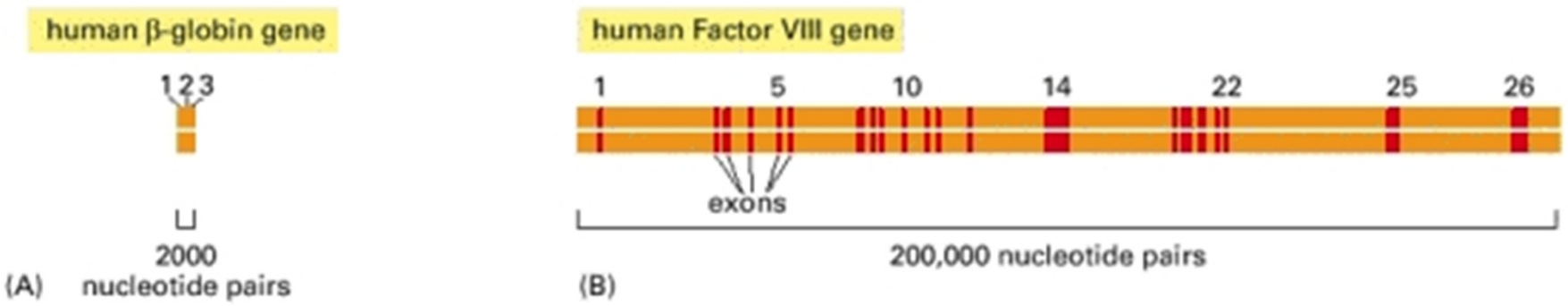


Figure 6-25 Structure of two human genes showing the arrangement of exons and introns
(A) The relatively small β-globin gene, which encodes one of the subunits of the oxygen-carrying protein hemoglobin, contains 3 exons . (B) The much larger Factor VIII gene contains 26 exons; it codes for a protein (Factor VIII) that functions in the blood-clotting pathway. Mutations in this gene are responsible for the most prevalent form of hemophilia.

Molecular Biology of the Cell. 4th edition.

Biochemistry. 5th edition.

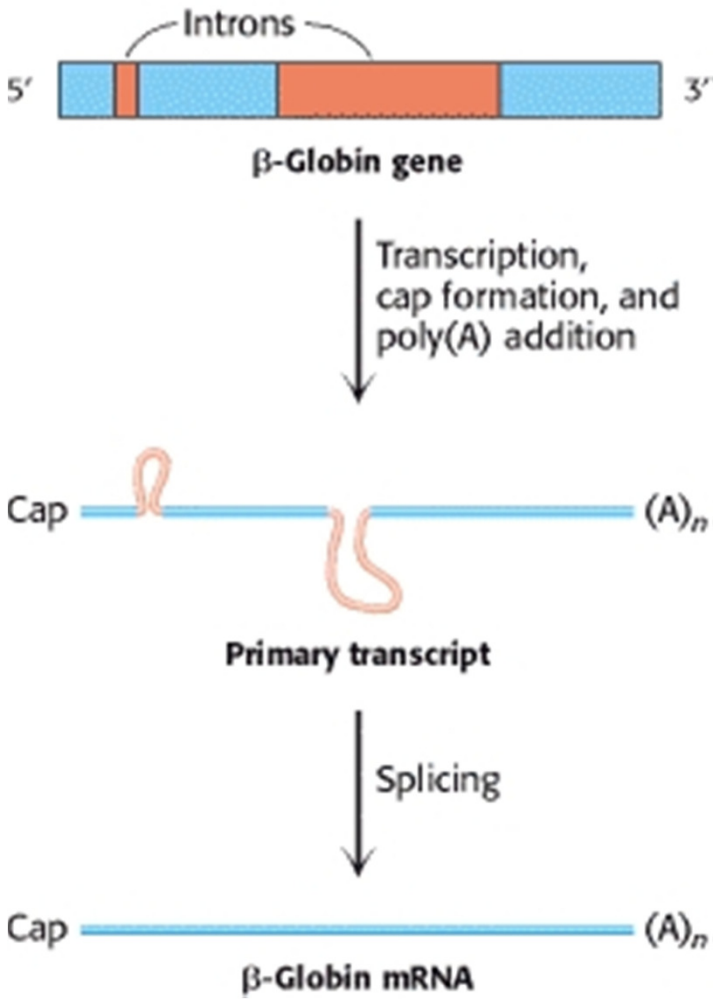


Figure 5.34 Transcription and Processing of the β -globin gene
The gene is transcribed to yield the primary transcript, which is modified by cap and poly(A) addition. The intervening sequences in the primary RNA transcript are removed to form the mRNA.

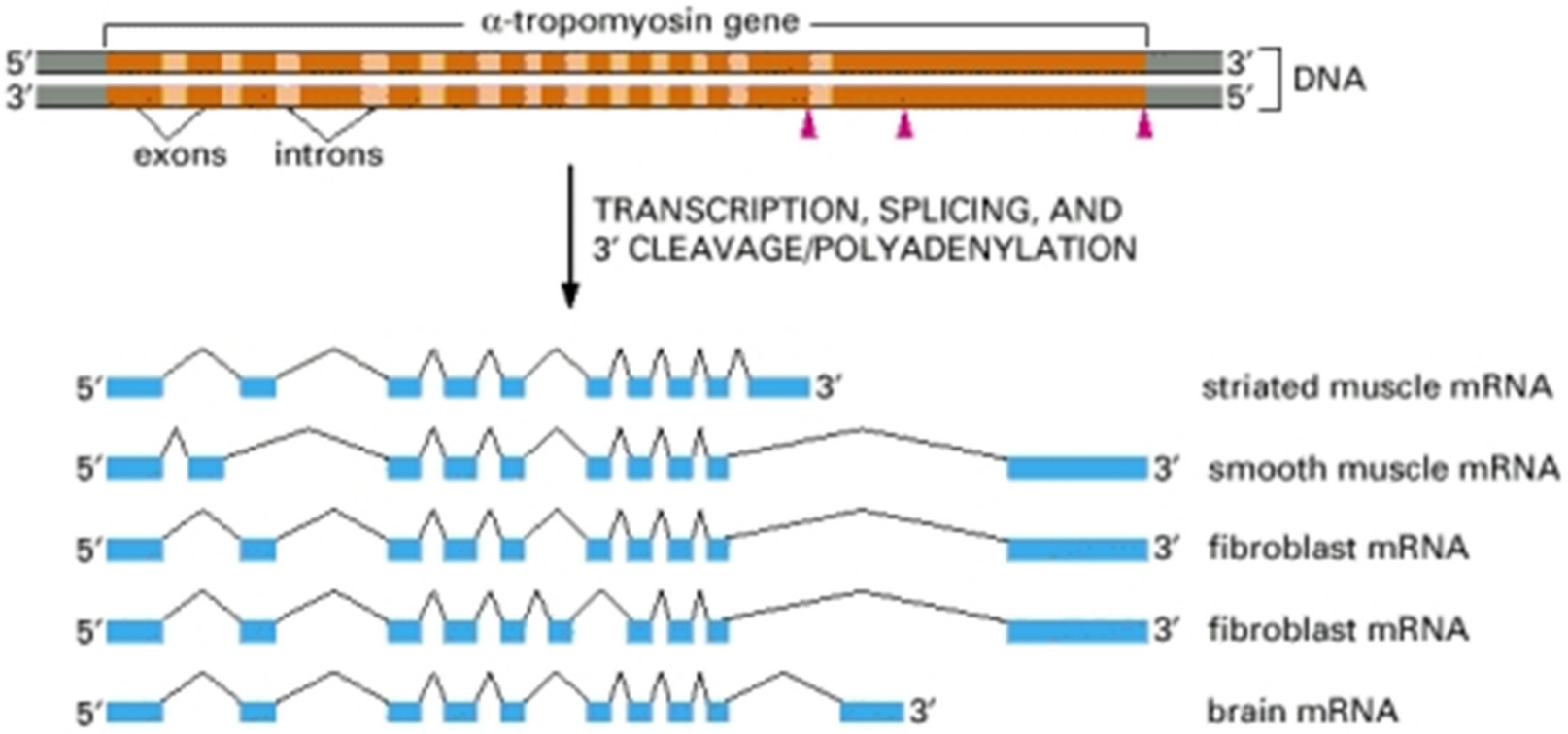
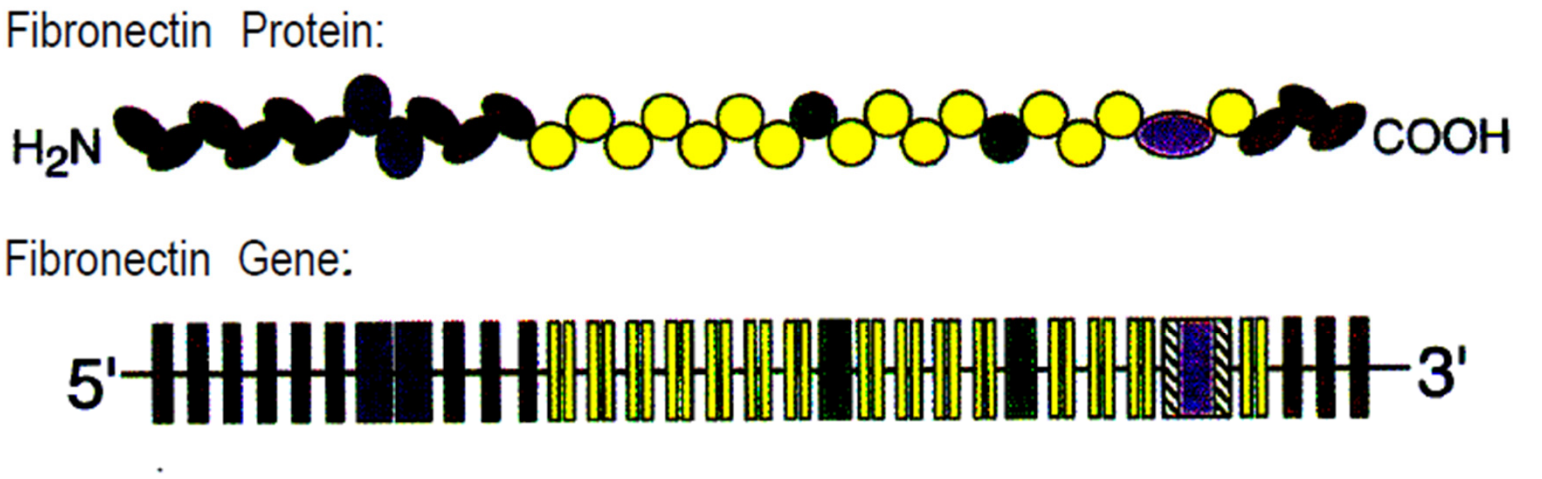


Figure 6-27 Alternative splicing of the α-tropomyosin gene from rat

α-tropomyosin is a coiled-coil protein that regulates contraction in muscle cells. The primary transcript can be spliced in different ways, as indicated in the figure, to produce distinct mRNAs, which then give rise to variant proteins. Some of the splicing patterns are specific for certain types of cells. For example, the α-tropomyosin made in striated muscle is different from that made from the same gene in smooth muscle. The arrowheads in the top part of the figure demark the sites where cleavage and poly-A addition can occur.

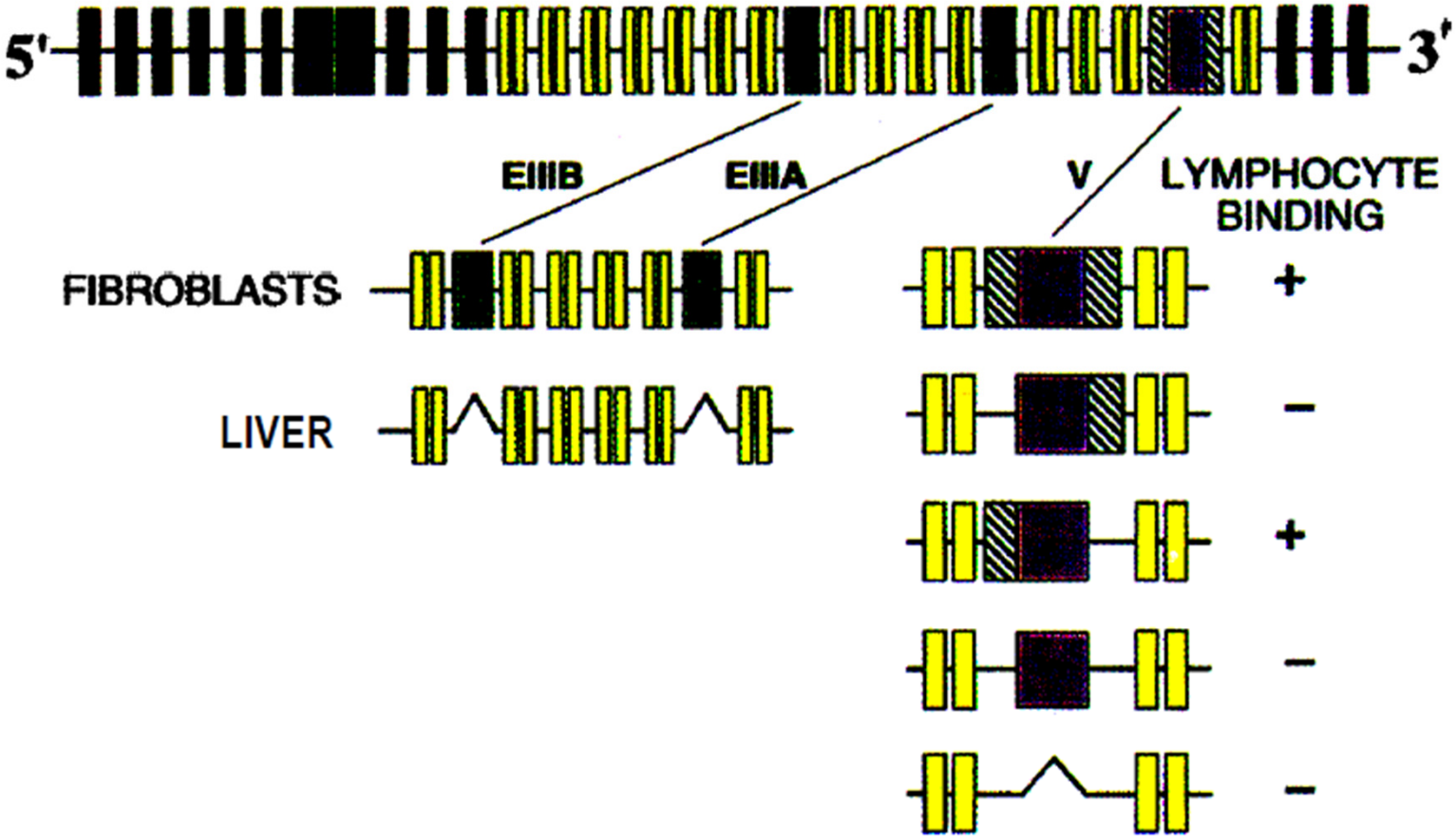
Why Split Gene and RNA splicing?

Fibronectin gene evolved by exon duplication



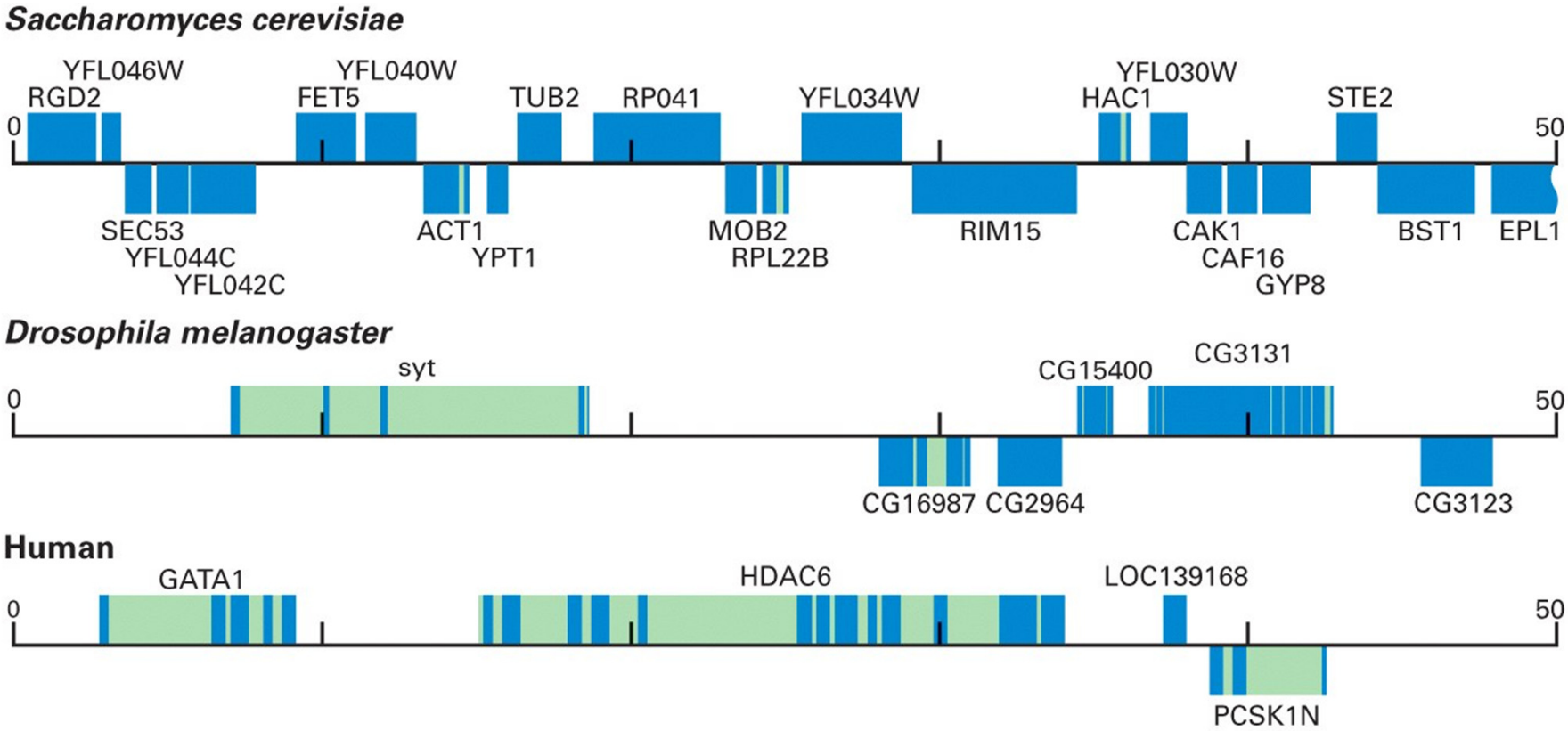
SPLIT GENES AND RNA SPLICING - Nobel Lecture, December 8, 1993 by PHILLIP A. SHARP

Alternative splicing of RNA encoding fibronectin.



SPLIT GENES AND RNA SPLICING - Nobel Lecture, December 8, 1993 by PHILLIP A. SHARP

Arrangement of Eukaryotic Genes



Yeast genes: have long exons and infrequent short introns.

Human genes: have short exons and frequent long introns.

Annu. Rev. Biochem. 2003. 72:291–336

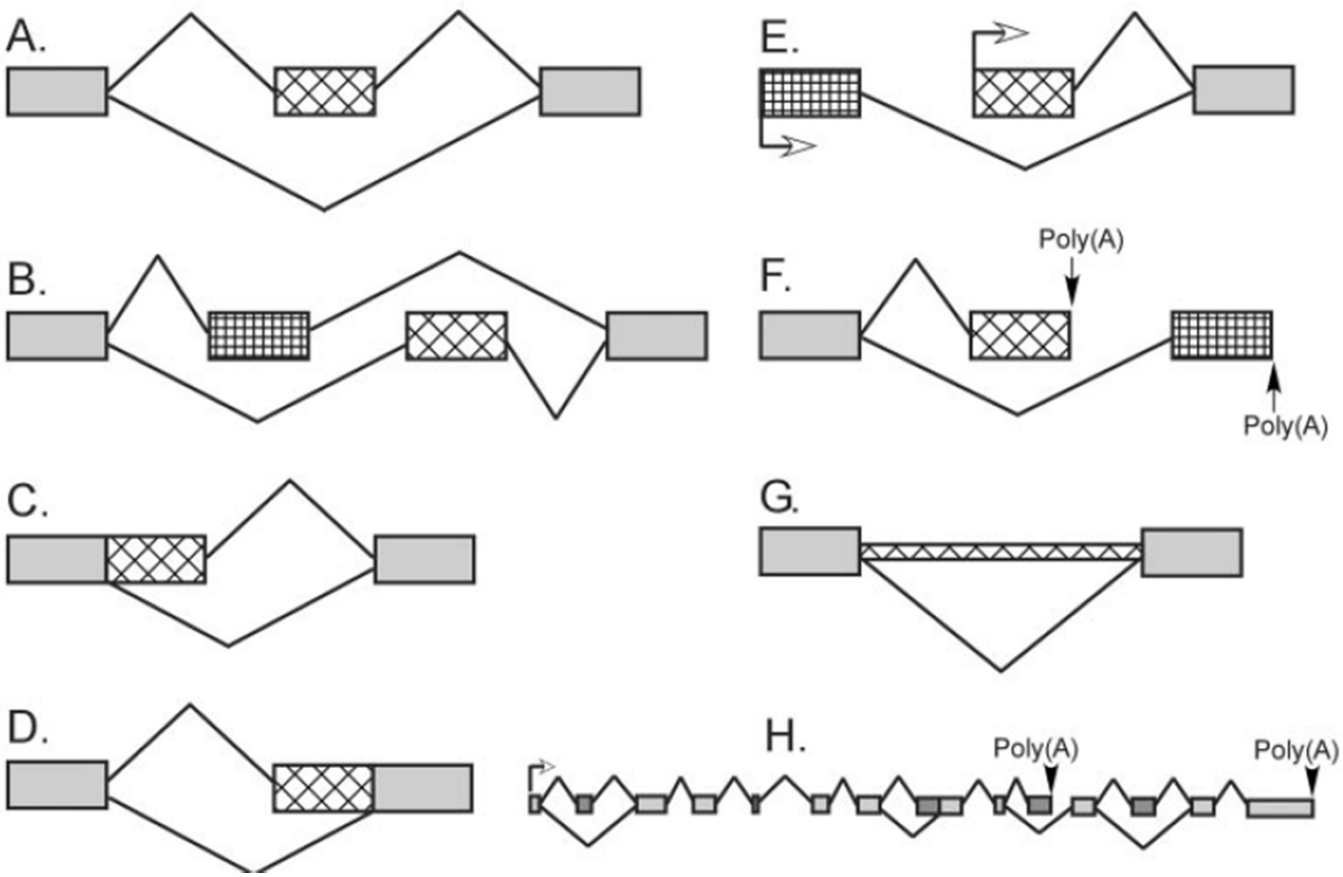


Figure 1 Patterns of alternative splicing. Constitutive sequences present in all final mRNAs are gray boxes. Alternative RNA segments that may or may not be included in the mRNA are hatched boxes. (*A*) A cassette exon can be either included in the mRNA or excluded. (*B*) Mutually exclusive exons occur when two or more adjacent cassette exons are spliced such that only one exon in the group is included at a time. (*C, D*) Alternative 5 and 3 splice sites allow the lengthening or shortening of a particular exon. (*E, F*) Alternative promoters and alternative poly(A) sites switch the 5- or 3-most exons of a transcript. (*G*) A retained intron can be excised from the pre-mRNA or can be retained in the translated mRNA. (*H*) A single pre-mRNA can exhibit multiple sites of alternative splicing using different patterns of inclusion. These are often used in a combinatorial manner to produce many different final mRNAs.

Annu. Rev. Biochem. 2003. 72:291–336

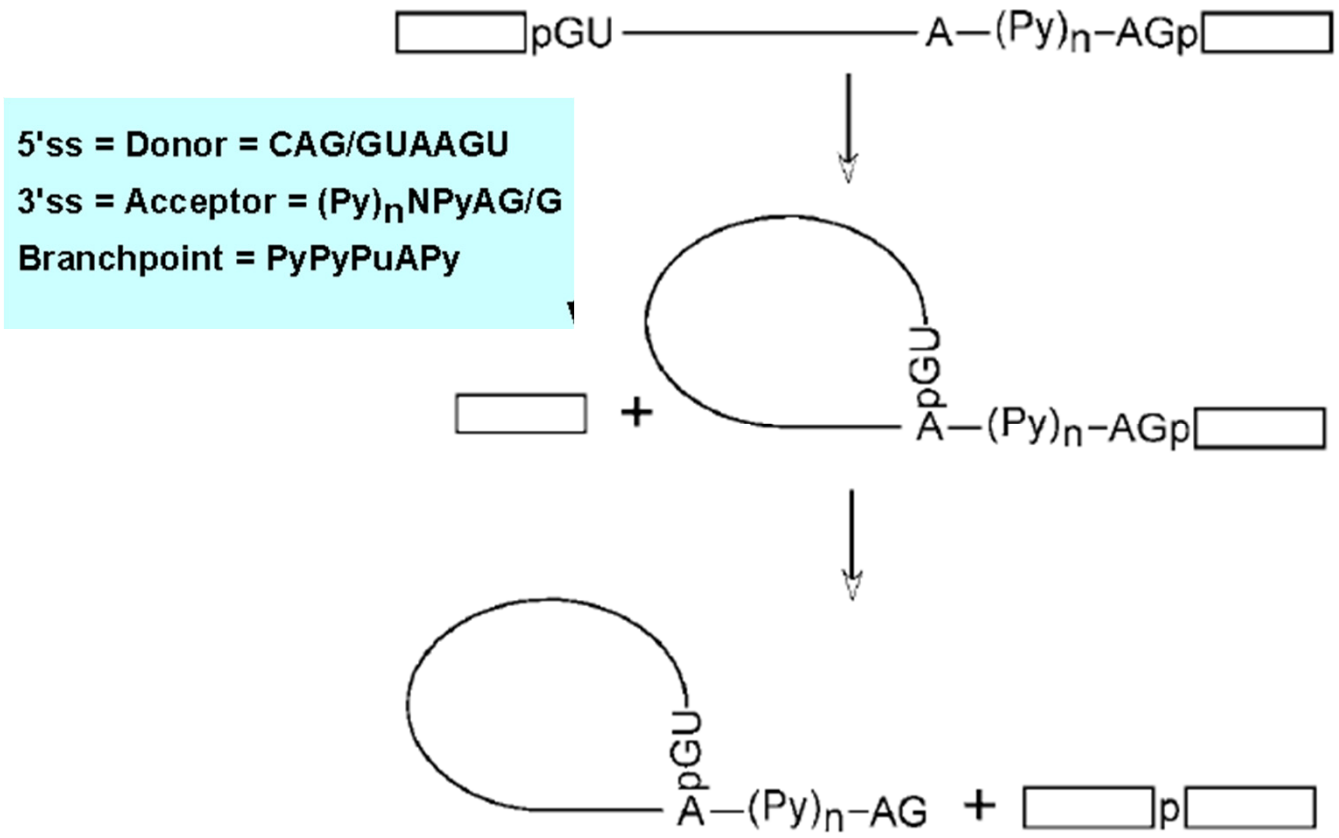


Figure 2*A* Splicing takes place in two transesterification steps. The first step results in two reaction intermediates: the detached 5 exon and an intron/3-exon fragment in a lariat structure. The second step ligates the two exons and releases the intron lariat. See text for details.


Transesterification
R1
R2
R3
(δ+)
(δ–)

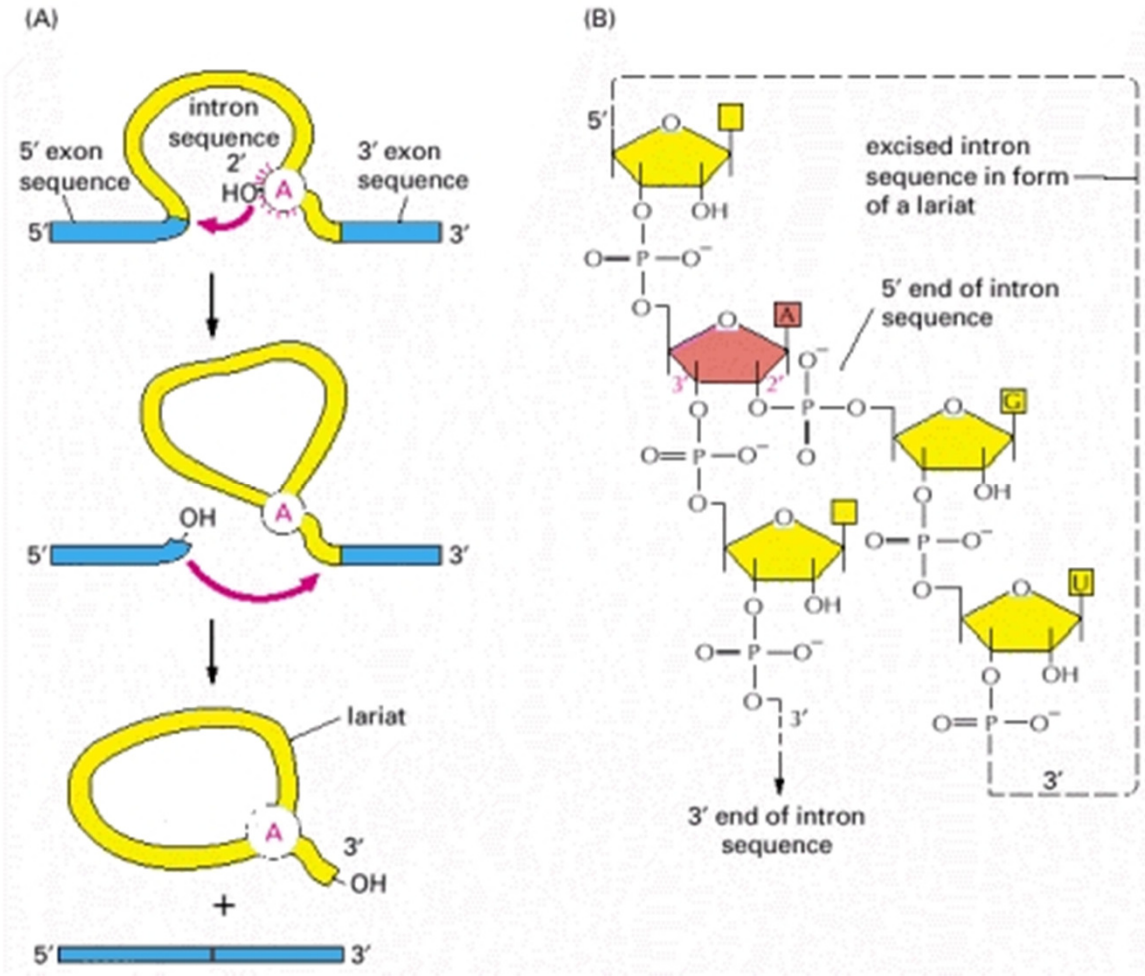
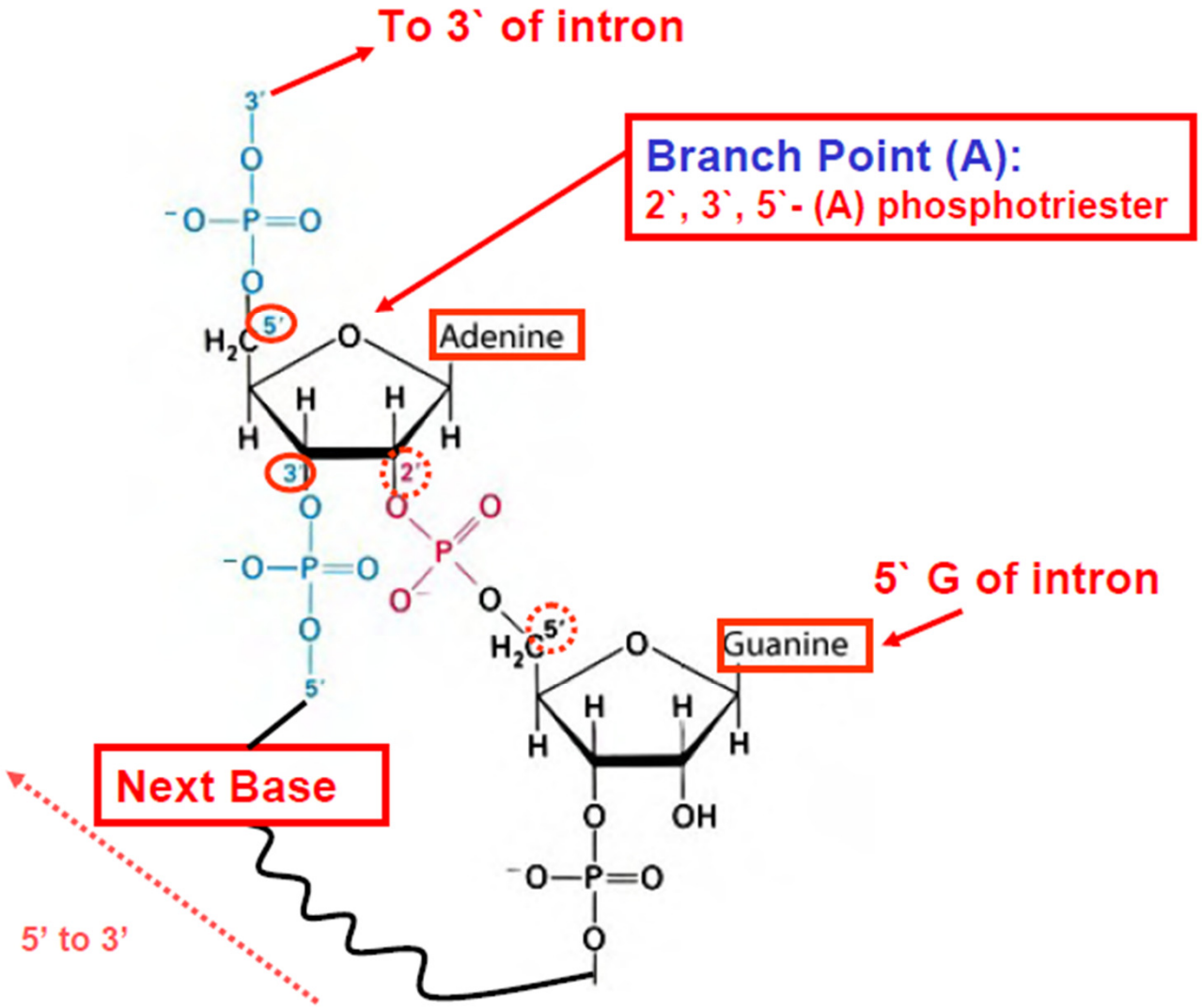


Figure 6-26The RNA splicing reaction

(A) In the first step, a specific adenine nucleotide in the intron sequence (indicated in *red)* attacks the 5′ splice site and cuts the sugar-phosphate backbone of the RNA at this point. The cut 5′ end of the intron becomes covalently linked to the adenine nucleotide, as shown in detail in (B), thereby creating a loop in the RNA molecule. The released free 3′-OH end of the exon sequence then reacts with the start of the next exon sequence, joining the two exons together and releasing the intron sequence in the shape of a *lariat.* The two exon sequences thereby become joined into a continuous coding sequence; the released intron sequence is degraded in due course.


To 3` of intron
Branch Point (A):
2`, 3`, 5`- (A) phosphotriester
Adenine
5` G of intron
Guanine
Next Base
5' to 3'

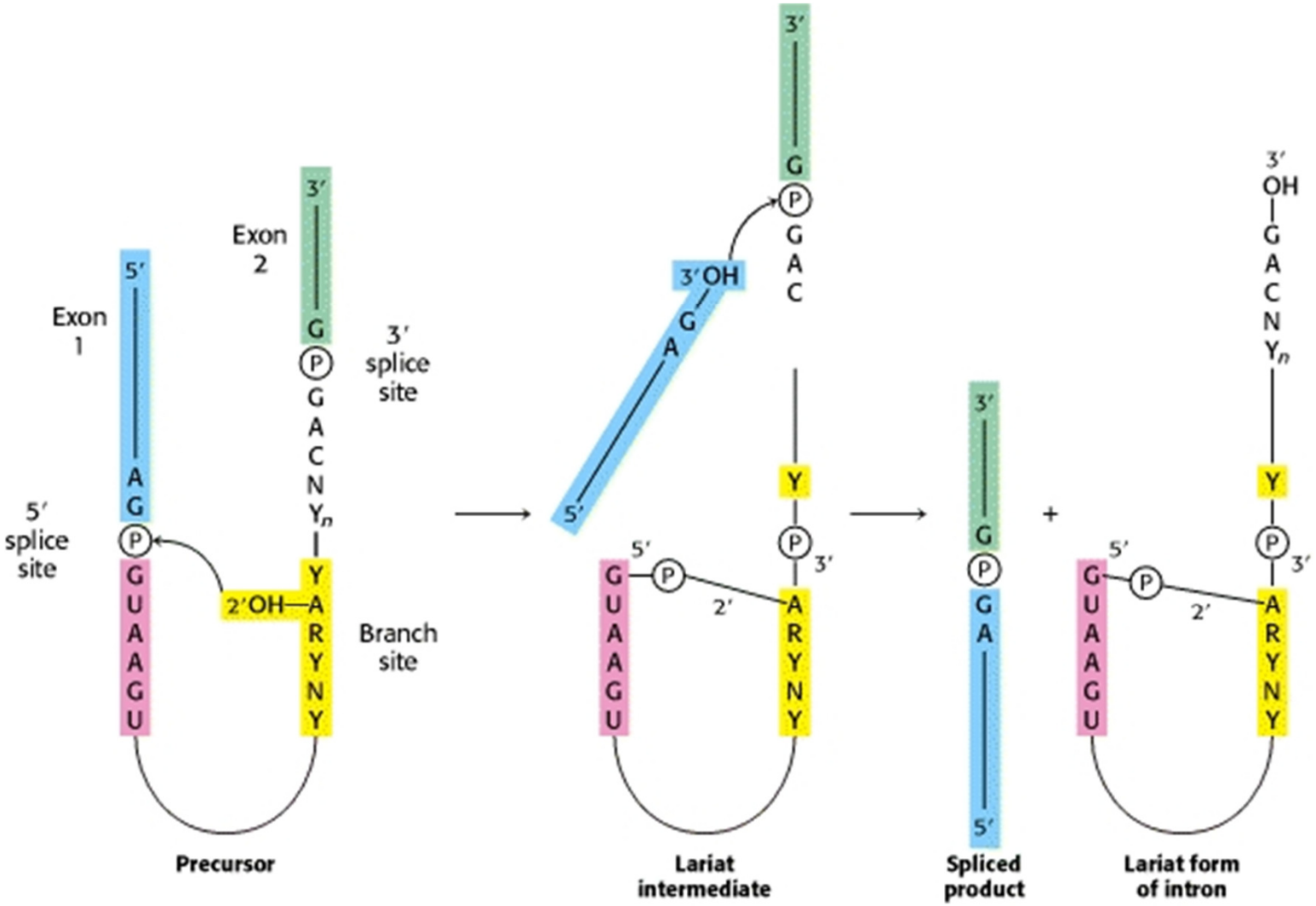


Figure 28.29Splicing Mechanism Used for mRNA Precursors

The upstream (5′) exon is shown in blue, the downstream (3′) exon in green, and the branch site in yellow. Y stands for a purine nucleotide, R for a pyrimidine nucleotide, and N for any nucleotide. The 5′ splice site is attacked by the 2′-OH group of the branch-site adenosine residue. The 3′ splice site is attacked by the newly formed 3′-OH group of the upstream exon. The exons are joined, and the intron is released in the form of a lariat.

Snurps or SnRNPs

- Small nuclear ribonuclear protein particles
- U1, U2, U4, U5 and U6 involved in nuclear RNA splicing

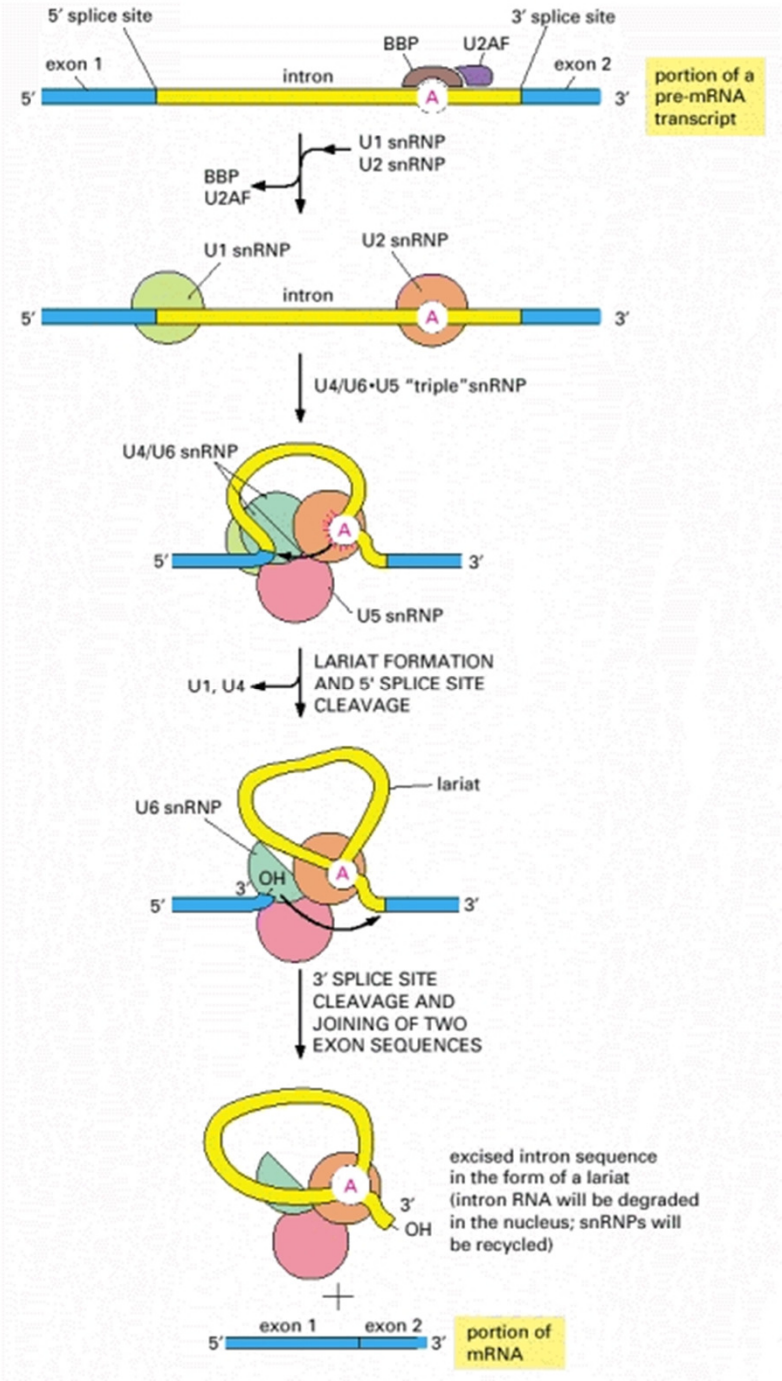
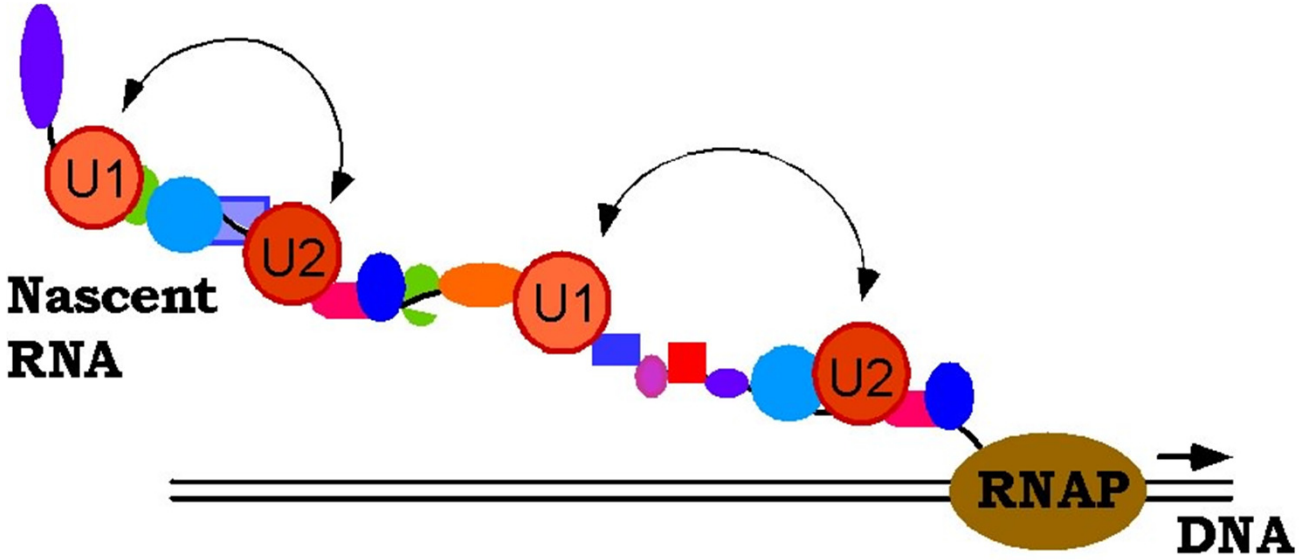


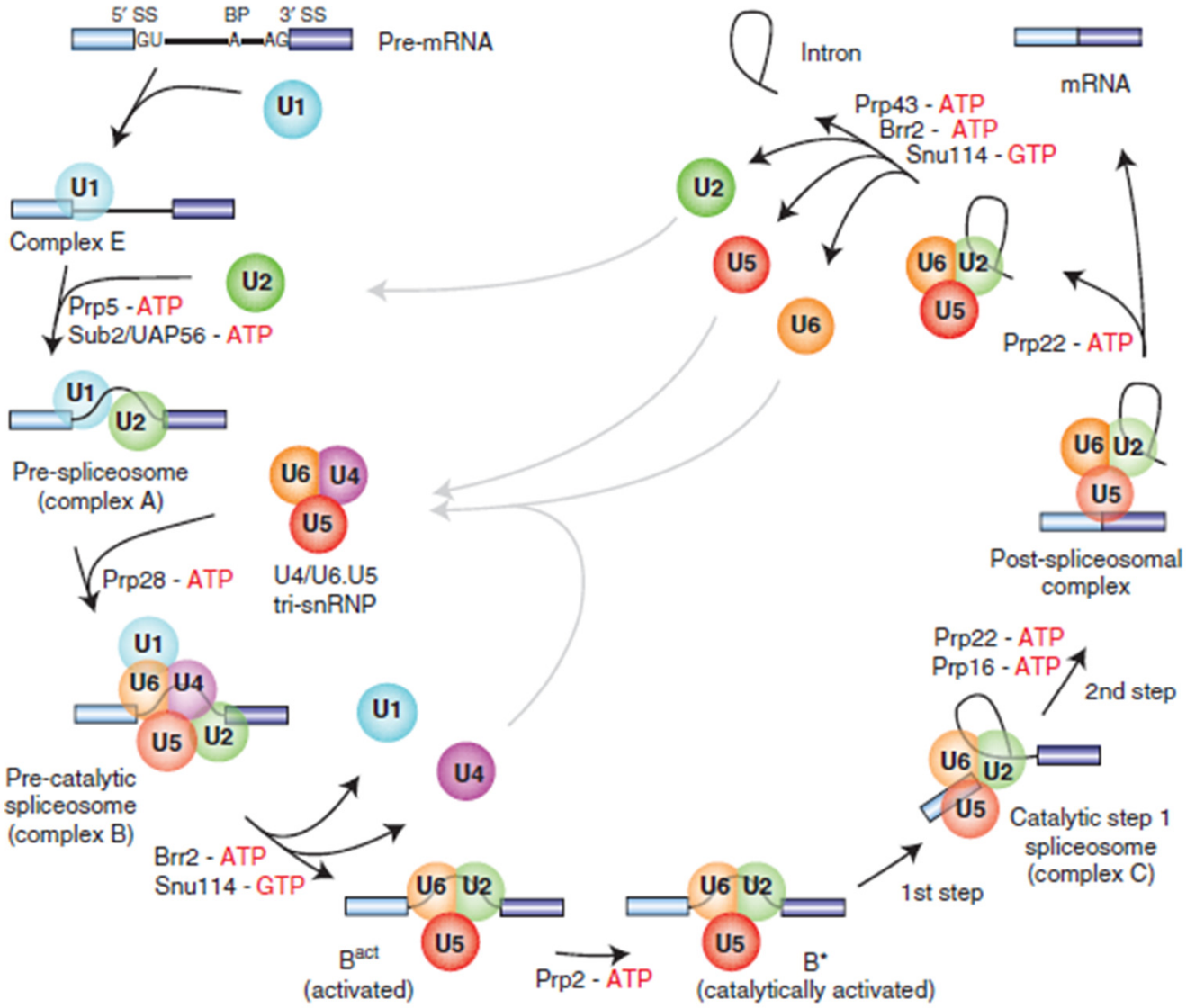
Figure 6-29The RNA splicing mechanism

RNA splicing is catalyzed by an assembly of snRNPs (shown as *colored circles)* plus other proteins (most of which are not shown), which together constitute the spliceosome. The spliceosome recognizes the splicing signals on a pre-mRNA molecule, brings the two ends of the intron together, and provides the enzymatic activity for the two reaction steps. The branch-point site is first recognized by the BBP (branch-point binding protein) and U2AF, a helper protein. In the next steps, the U2 snRNP displaces BBP and U2AF and forms base pairs with the branch-point site consensus sequence, and the U1 snRNP forms base-pairs with the 5′ splice junction. At this point, the U4/U6•U5 "triple" snRNP enters the spliceosome. In this triple snRNP, the U4 and U6 snRNAs are held firmly together by base-pair interactions and the U5 snRNP is more loosely associated. Several RNA-RNA rearrangements then occur that break apart the U4/U6 base pairs (as shown, the U4 snRNP is ejected from the splicesome before splicing is complete) and allow the U6 snRNP to displace U1 at the 5′ splice junction. Subsequent rearrangements create the active site of the spliceosome and position the appropriate portions of the pre-mRNA substrate for the splicing reaction to occur. Although not shown in the figure, each splicing event requires additional proteins, some of which hydrolyze ATP and promote the RNA-RNA rearrangements.

Spliceosomes Assemble During Pre-mRNA Synthesis



Pre-mRNAs Form Complex RNP Structures



Cold Spring Harb Perspect Biol 2011;3:a003707

Figure 1. Canonical cross-intron assembly and disassembly pathway of the U2-dependent spliceosome. For simplicity, the ordered interactions of the snRNPs (indicated by circles), but not those of non-snRNP proteins, are shown. The various spliceosomal complexes are named according to the metazoan nomenclature. Exon and intron sequences are indicated by boxes and lines, respectively. The stages at which the evolutionarily conserved DExH/D-box RNA ATPases/helicases Prp5, Sub2/UAP56, Prp28, Brr2, Prp2, Prp16, Prp22and Prp43, or the GTPase Snu114, act to facilitate conformational changes are indicated

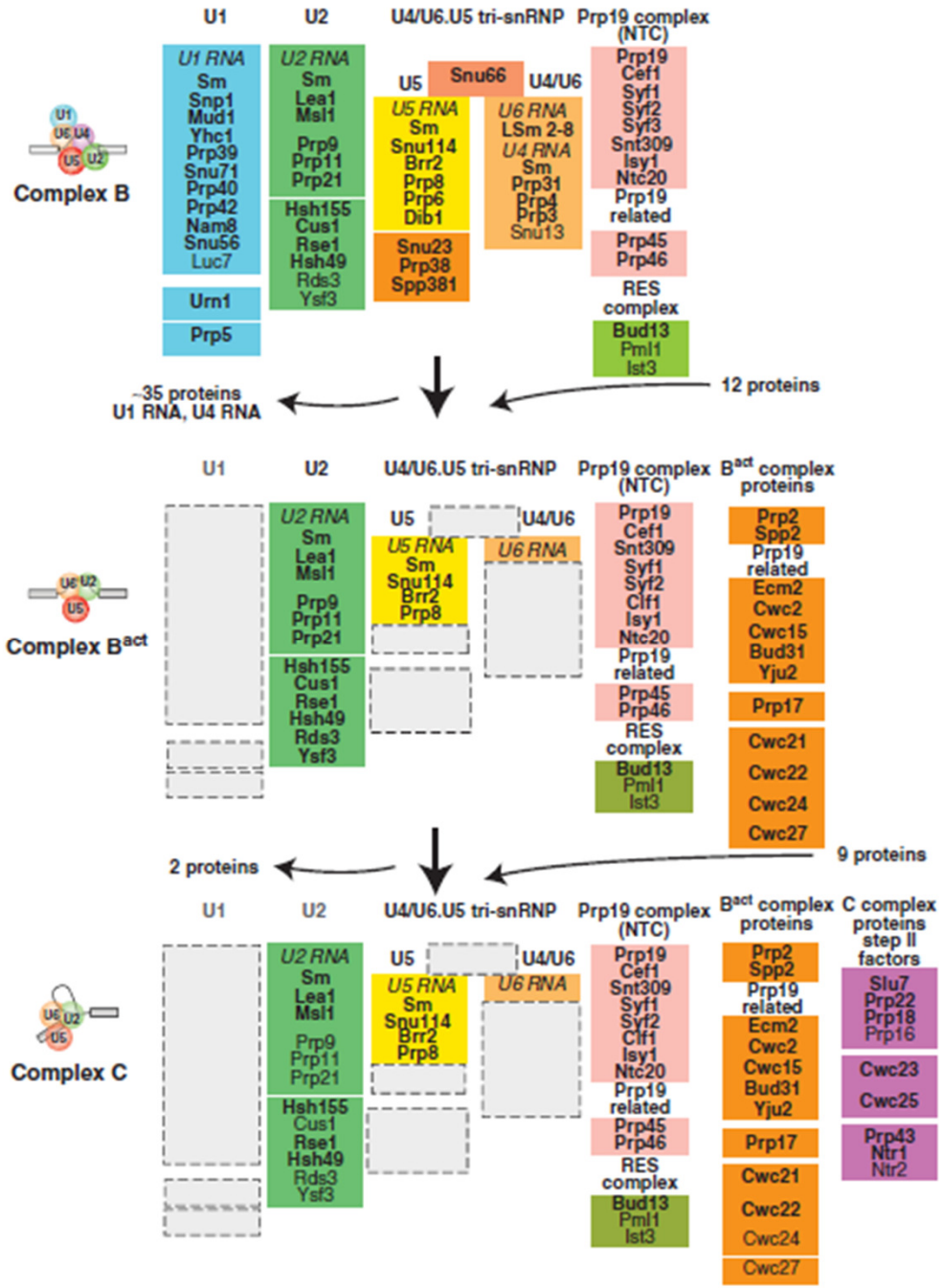


Figure 4. Compositional dynamics of the yeast spliceosome. Proteins identified by mass spectrometry in S. cerevisiae B, Bact, and C spliceosomal complexes are shown. Proteins are grouped according to their function or association with an snRNP, protein complex or spliceosomal complex. The relative abundance of the indicated proteins is indicated by light (substoichiometric) or dark (stoichiometric) lettering. (Reprinetd, with permission, from Fabrizio et al. 2009

Cold Spring Harb Perspect Biol 2011;3:a003707

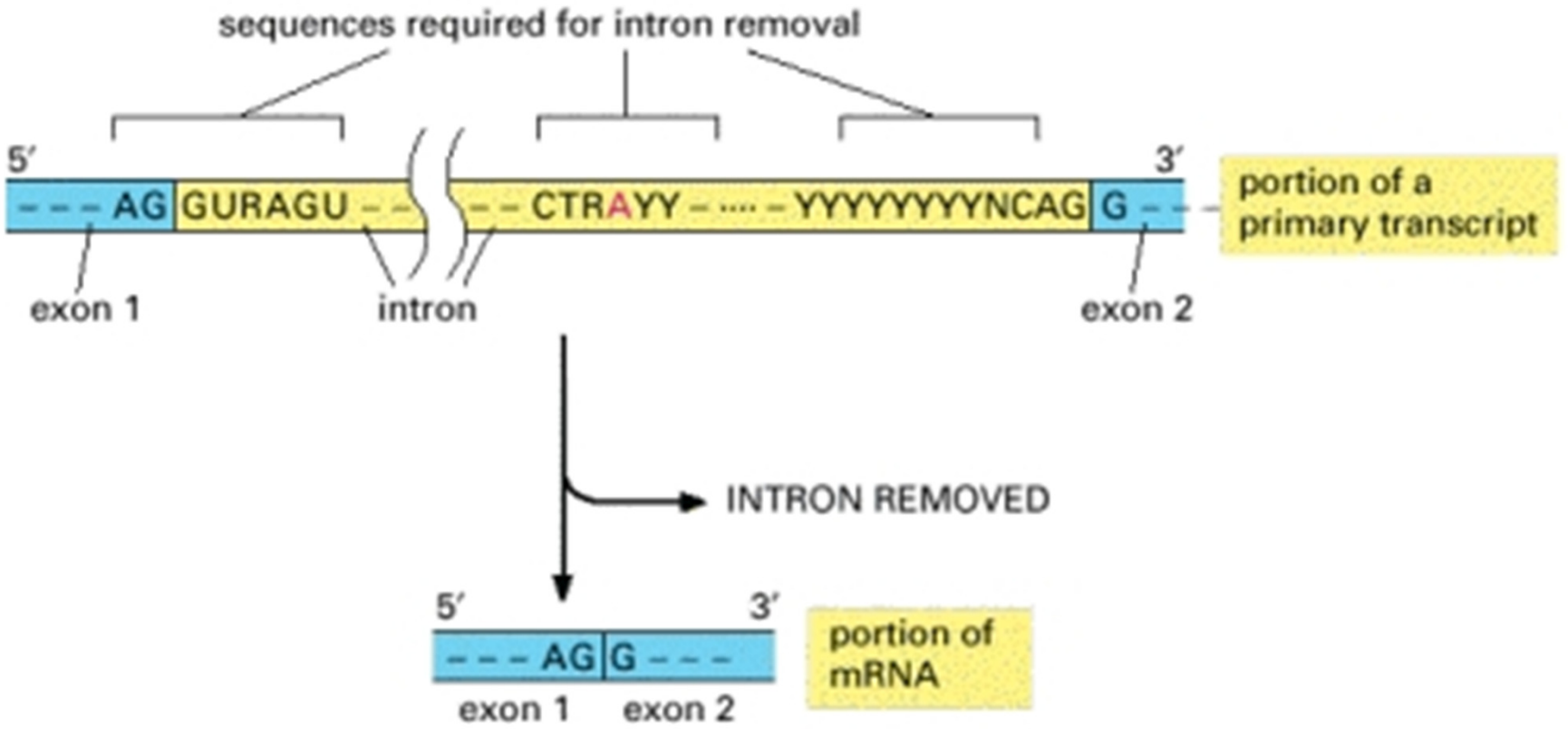


Figure 6-28The consensus nucleotide sequences in an RNA molecule that signal the beginning and the end of most introns in humans

Only the three blocks of nucleotide sequences shown are required to remove an intron sequence; the rest of the intron can be occupied by any nucleotide. Here A, G, U, and C are the standard RNA nucleotides; R stands for either A or G; Y stands for either C or U. The A highlighted in*red* forms the branch point of the lariat produced by splicing. Only the GU at the start of the intron and the AG at its end are invariant nucleotides in the splicing consensus sequences. The remaining positions (even the branch point A) can be occupied by a variety of nucleotides, although the indicated nucleotides are preferred. The distances along the RNA between the three splicing consensus sequences are highly variable; however, the distance between the branch point and 3′ splice junction is typically much shorter than that between the 5′ splice junction and the branch point.

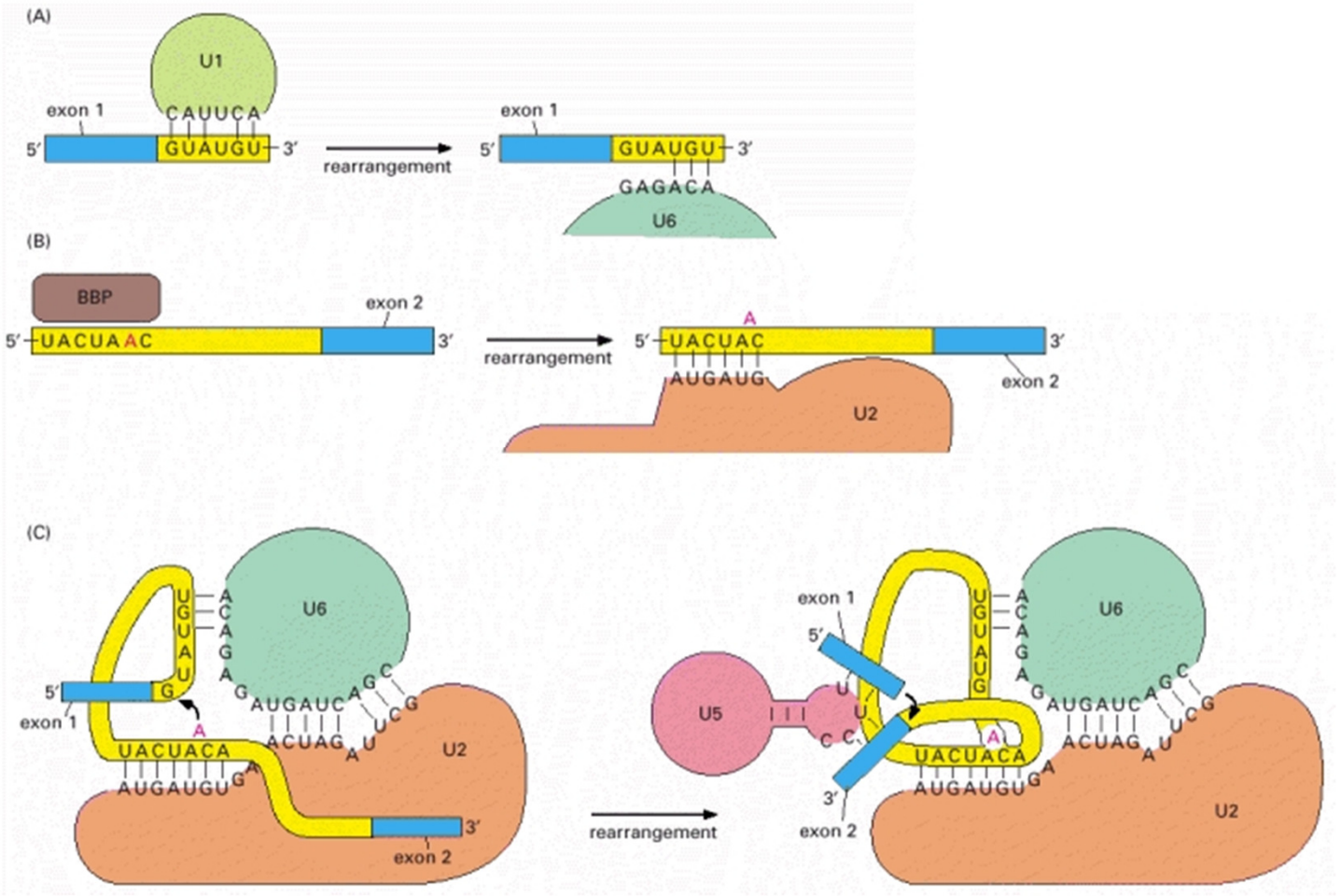


Figure 6-30 Several of the rearrangements that take place in the spliceosome during pre-mRNA splicing

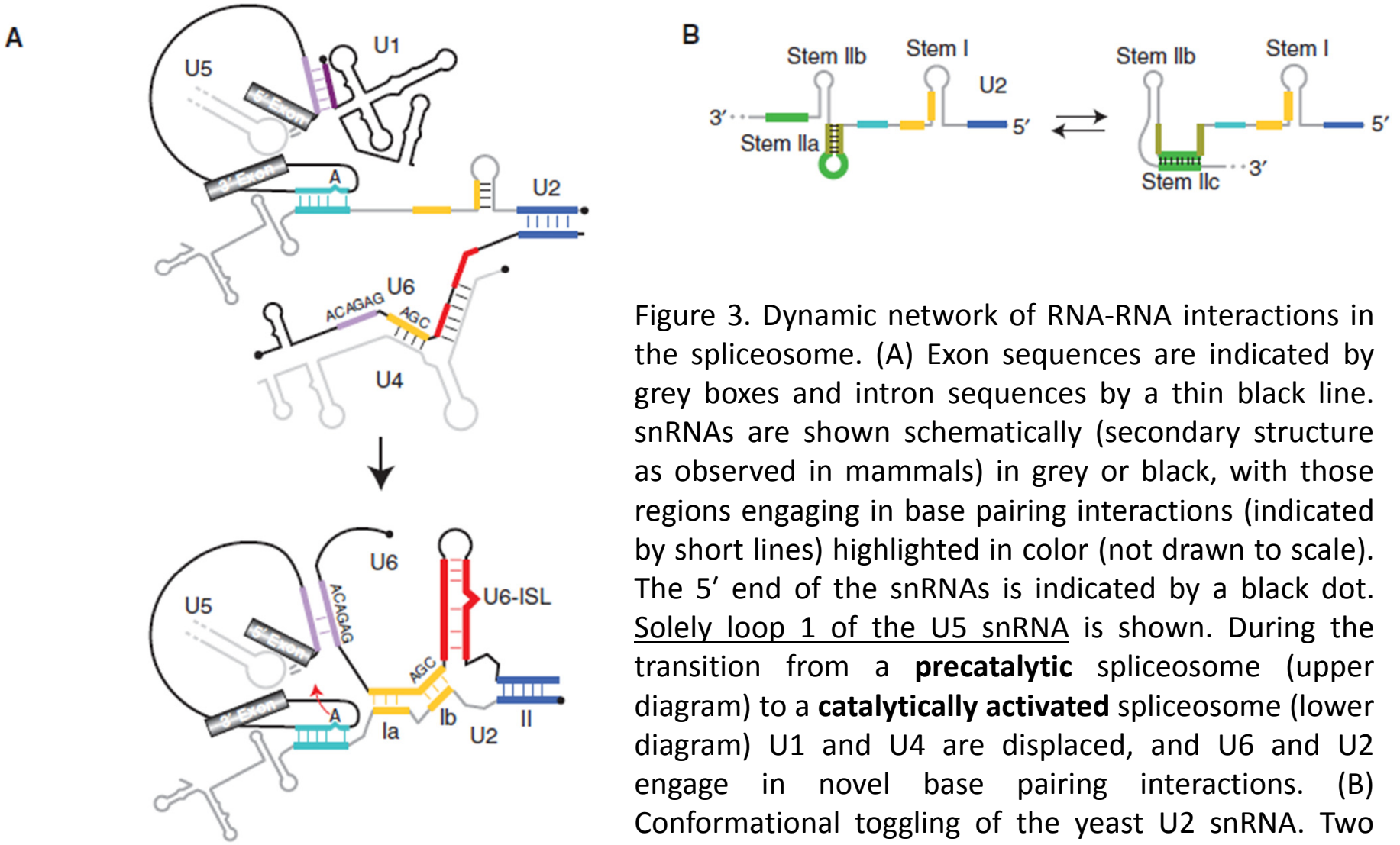


Figure 3. Dynamic network of RNA-RNA interactions in the spliceosome. (A) Exon sequences are indicated by grey boxes and intron sequences by a thin black line. snRNAs are shown schematically (secondary structure as observed in mammals) in grey or black, with those regions engaging in base pairing interactions (indicated by short lines) highlighted in color (not drawn to scale). The 5′ end of the snRNAs is indicated by a black dot. Solely loop 1 of the U5 snRNA is shown. During the transition from a **precatalytic** spliceosome (upper diagram) to a **catalytically activated** spliceosome (lower diagram) U1 and U4 are displaced, and U6 and U2 engage in novel base pairing interactions. (B) Conformational toggling of the yeast U2 snRNA. Two mutually exclusive stem structures (stem IIa and stem IIc) are thought to form within the U2 snRNA at different stages of splicing. Solely the 5′ end of the U2 snRNA is shown schematically.

Cold Spring Harb Perspect Biol 2011;3:a003707

Cold Spring Harb Perspect Biol 2011;3:a003707

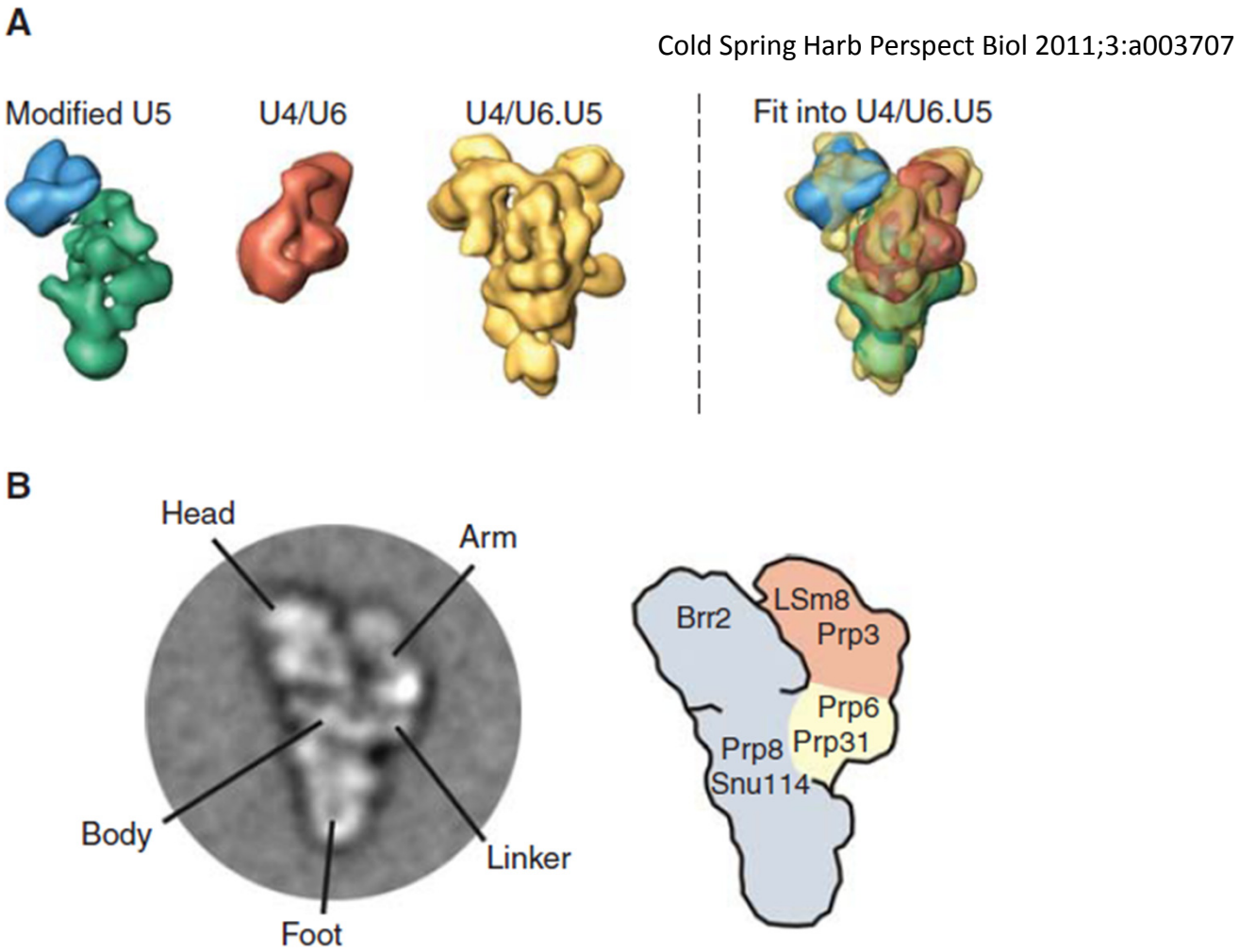


Figure 5. Three dimensional EM structure of the U4/U6.U5 tri-snRNPand localization of functionally important tri-snRNP proteins. (A) 3D reconstructions of the human U5 and U4/U6 snRNPs and tri-snRNP, and fitting of U5 and U4/U6 into the tri-snRNP 3D map. The head domain of U5 (highlighted blue) appears to be flexible and it is positioned in the U5 snRNP 3D reconstruction shown in a manner favorable for fitting into the tri-snRNP 3D map. (B) Left, representative 2D class average of the affinity purified S. cerevisiae **U4/U6.U5 tri-snRNP** as visualized by negative-stain electron microscopy after mild fixation using the Grafix protocol. The main structural domains are indicated. Right, cartoon model of the yeast tri-snRNP. Area corresponding to the U5 and U4/U6 snRNPs and the linker region are shaded grey, orange, or yellow, respectively. The position of the carboxyl terminus of several tri-snRNP proteins is indicated

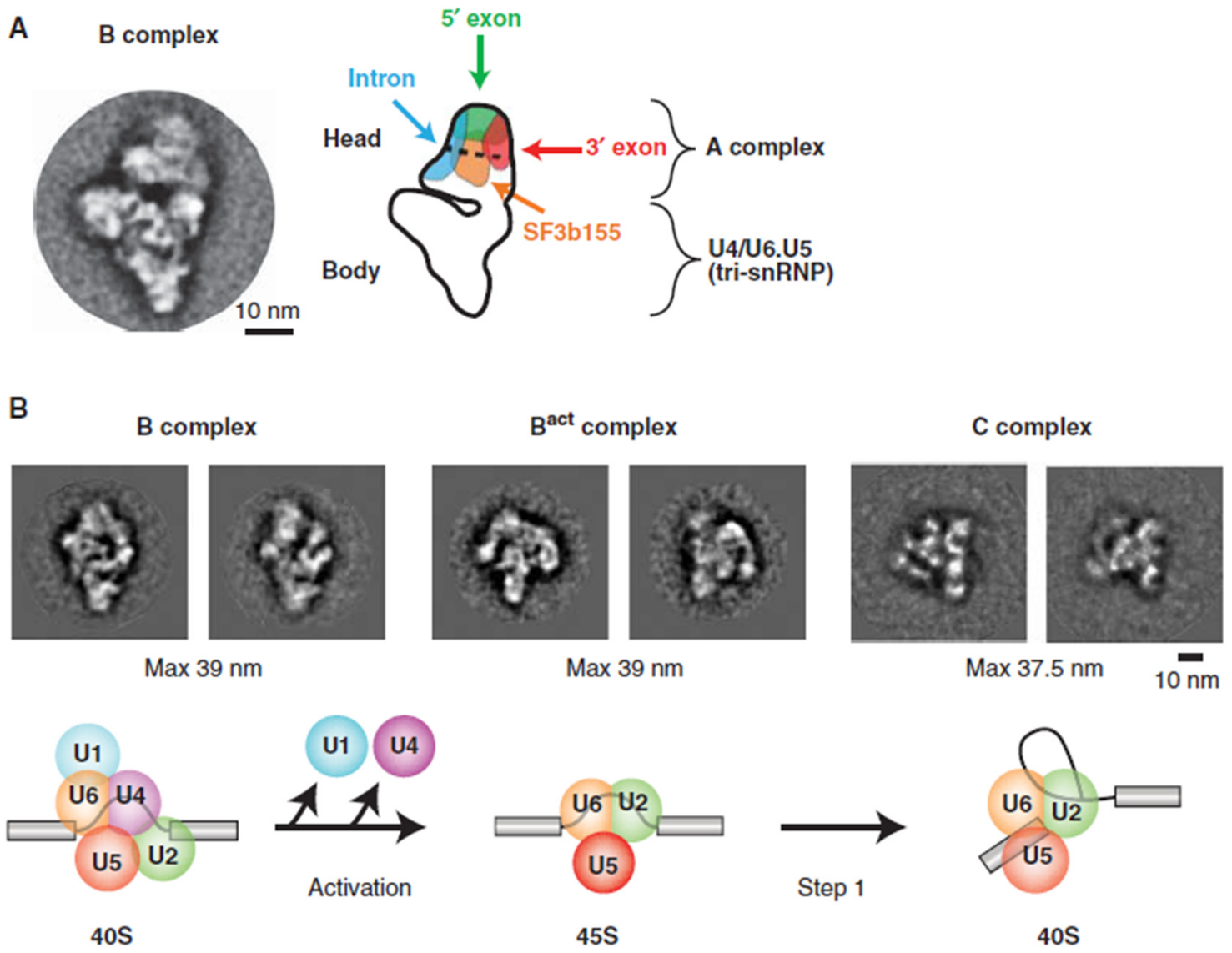


Figure 7. Structural dynamics of the yeast spliceosome as visualized by EM and localization of the pre-mRNA in the human B complex. (A) Class average of electron microscopy images of negatively stained, affinity-purified human B complexes (right). Sketch of the B complex showing regions where the 5′ exon, 3′ exon, intron and SF3b155 protein were mapped by immuno-EM, and the likely location of components of the A complex and tri-snRNP. (B) Electron microscopy of negatively-stained, affinitypurified S. cerevisiae B, Bact, and C complexes. Two prominent class averages of each complex are shown, withthe maximum dimension indicated later.

Alternative Splicing and Disease

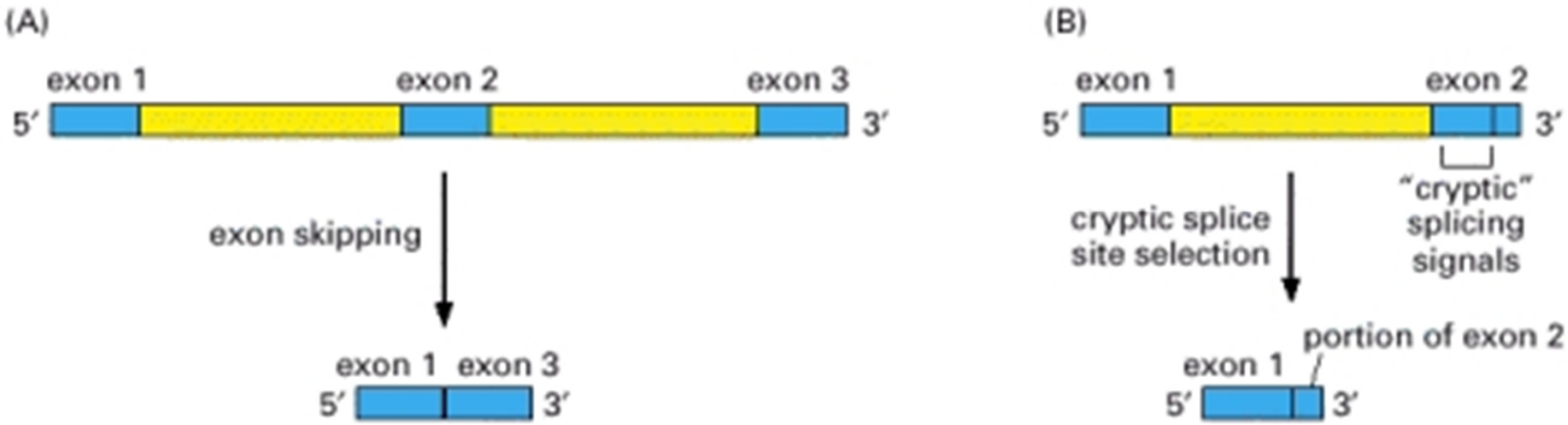
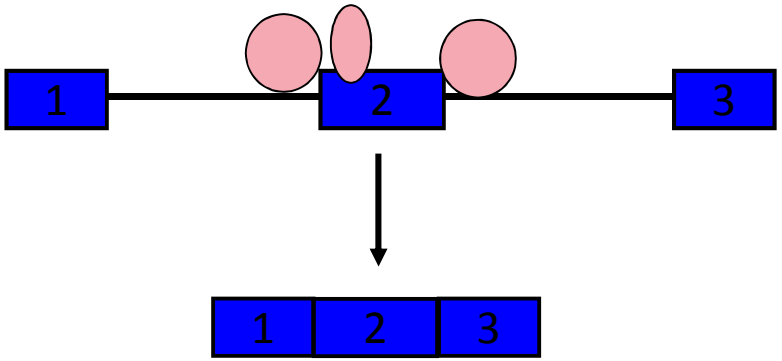


Figure 6-31 Two types of splicing errors

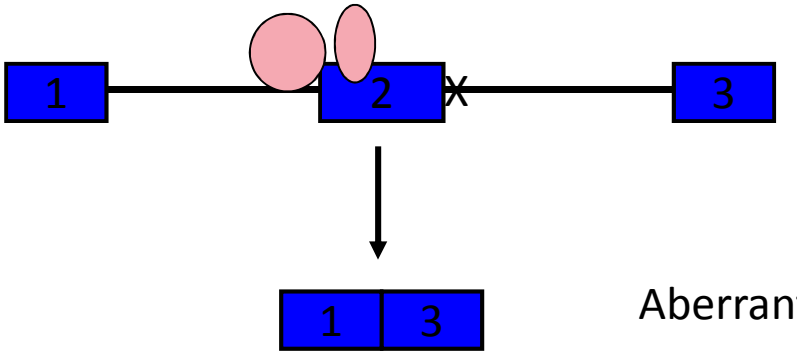
Both types might be expected to occur frequently if splice-site selection were performed by the spliceosome on a preformed, protein-free RNA molecule. “Cryptic” splicing signals are nucleotide sequences of RNA that closely resemble true splicing signals.

Because exons are recognized as units, the most common result of a mutation a splice site or ESE is skipping of the entire exon. The next most common result is activation of a nearby cryptic site. Many human diseases are caused by mutations in splice sites or splicing regulatory elements.

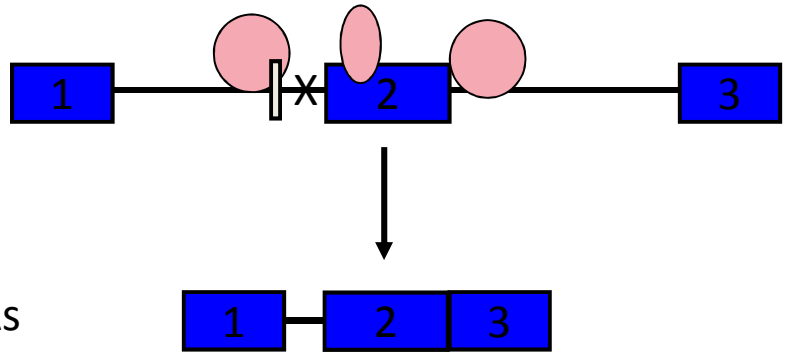


Wildtype exon

Mutant Splice site
causing exon skipping



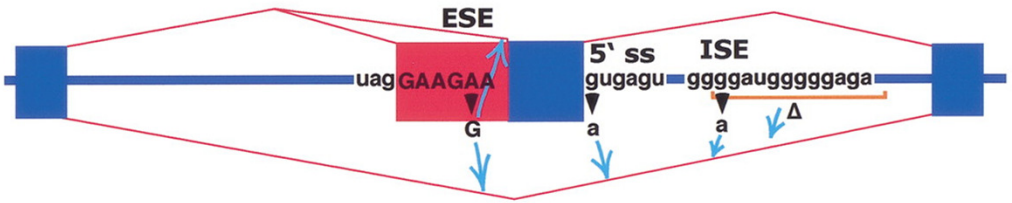
Mutant Splice site
causing activation of a
cryptic splice site



Aberrant mRNAs

Human Disease mutations that alter splicing rather than protein function are common, but can be difficult to identify.

A

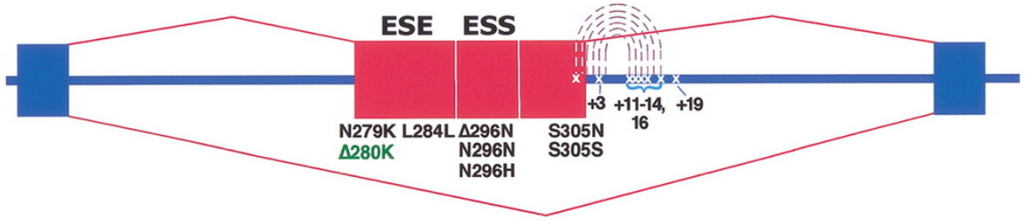


Familial Isolated Growth Hormone Deficiency Type II mutations in the GH-1 gene.

B

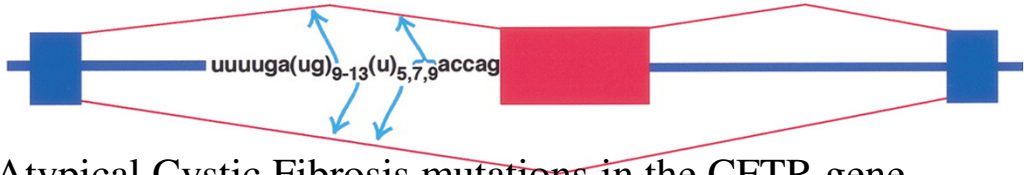
Fraser Syndrome mutations in the WT-1 gene.

C



Frontotemporal Dementia and Parkinsonism on Chr.17 (FTDP17) mutations in the Tau gene.

D



Atypical Cystic Fibrosis mutations in the CFTR gene.

Faustino and Cooper, Genes Dev. 2003

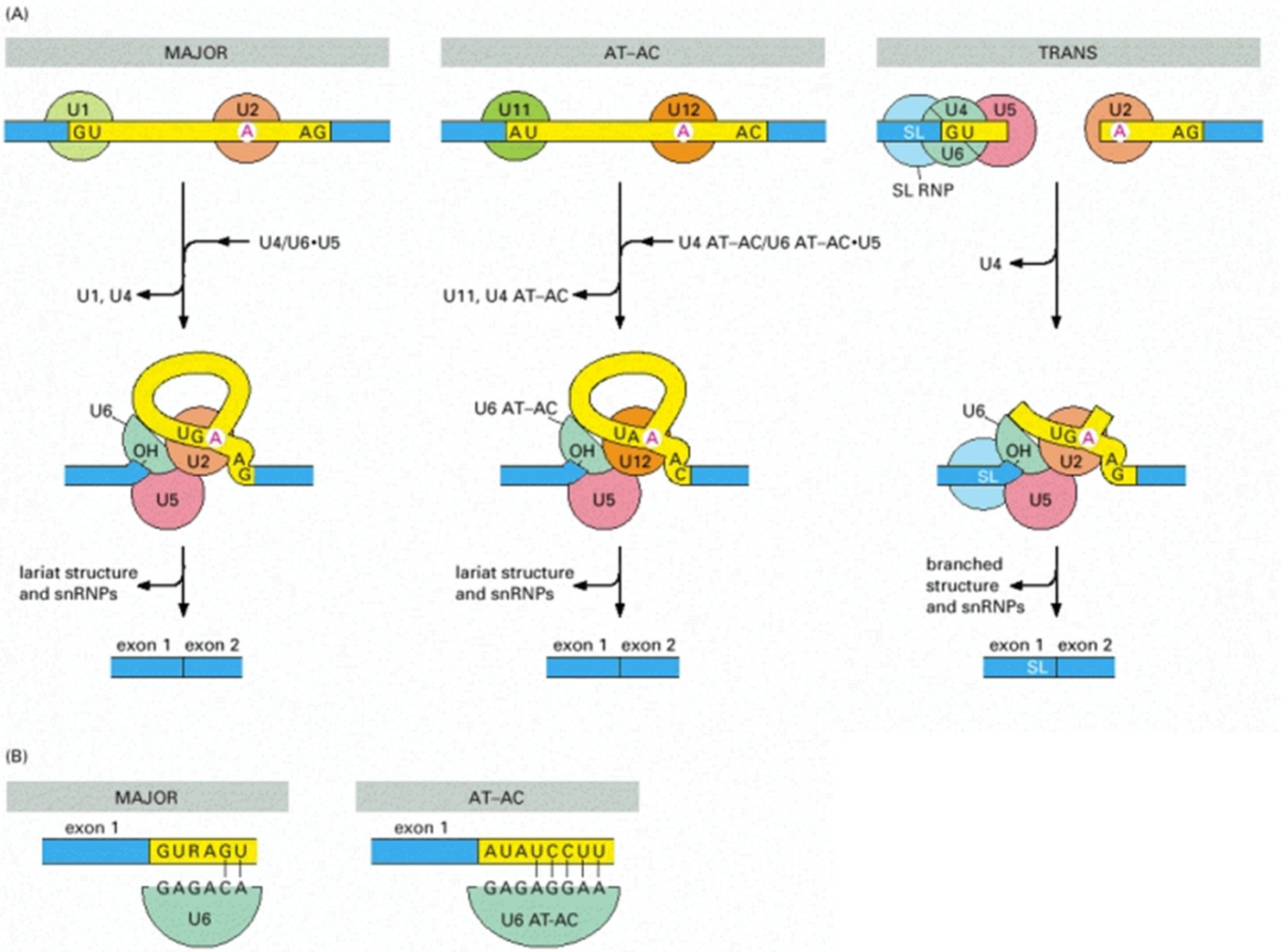


Figure 6-34 Outline of the mechanisms used for three types of RNA splicing

(A) Three types of spliceosomes. The major spliceosome *(left),* the AT-AC spliceosome (middle), and the trans-spliceosome *(right)* are each shown at two stages of assembly. The U5 snRNP is the only component that is common to all three spliceosomes. Introns removed by the AT-AC spliceosome have a different set of consensus nucleotide sequences from those removed by the major spliceosome. In humans, it is estimated that 0.1% of introns are removed by the AT-AC spliceosome. In trans-splicing, the SL snRNP is consumed in the reaction because a portion of the SL snRNA becomes the first exon of the mature mRNA. (B) The major U6 snRNP and the U6 AT-AC snRNP both recognize the 5′ splice junction, but they do so through a different set of base-pair interactions. The sequences shown are from humans. (Adapted from Y.-T. Yu et al., The RNA World, pp. 487–524. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1999.)

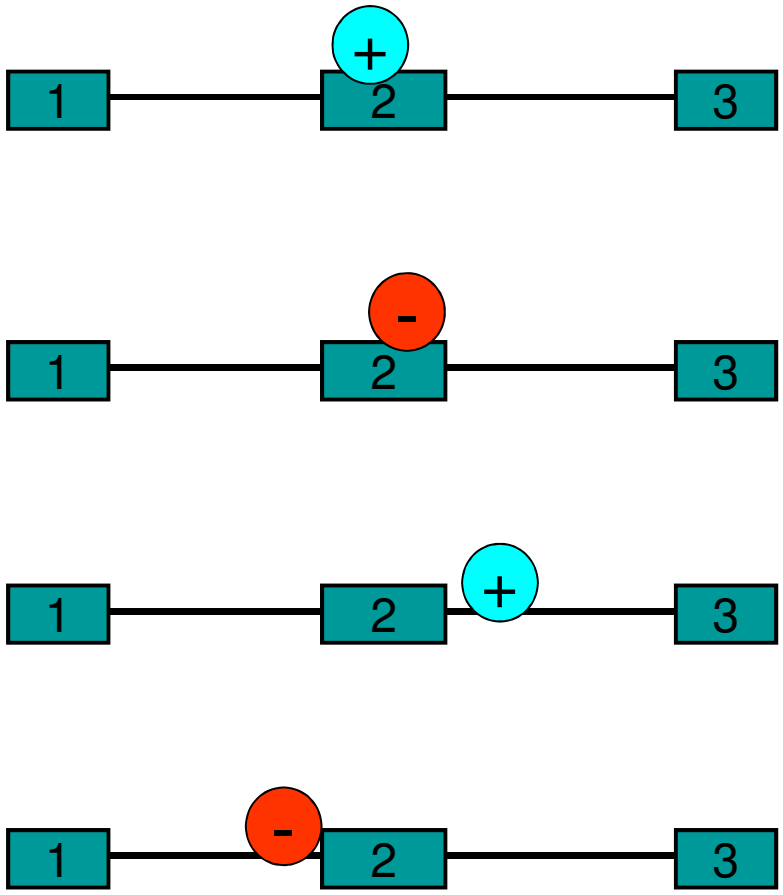
REVIEWS

POST-TRANSCRIPTIONAL CONTROL

Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches

Mo Chen and James L. Manley

Exon Definition < > Intron Definition



ESE - Exonic Splicing Enhancers

ESS - Exonic Splicing Silencers

ISE - Intronic Splicing Enhancers

ISS - Intronic Splicing Silencers

POSITIVE REGULATION FROM EXONS

Exons often contain enhancer or silencer elements that affect their ability to be spliced. There are many exonic splicing enhancers (ESEs) - The SR proteins constitute the best-studied family of splicing regulators (ASF/SF2 and SC35).

NEGATIVE REGULATION IN EXONS

Exonic silencer or repressor elements have been identified. The best characterized of these are bound by particular hnRNP proteins - The most studied of these proteins, hnRNP A1.

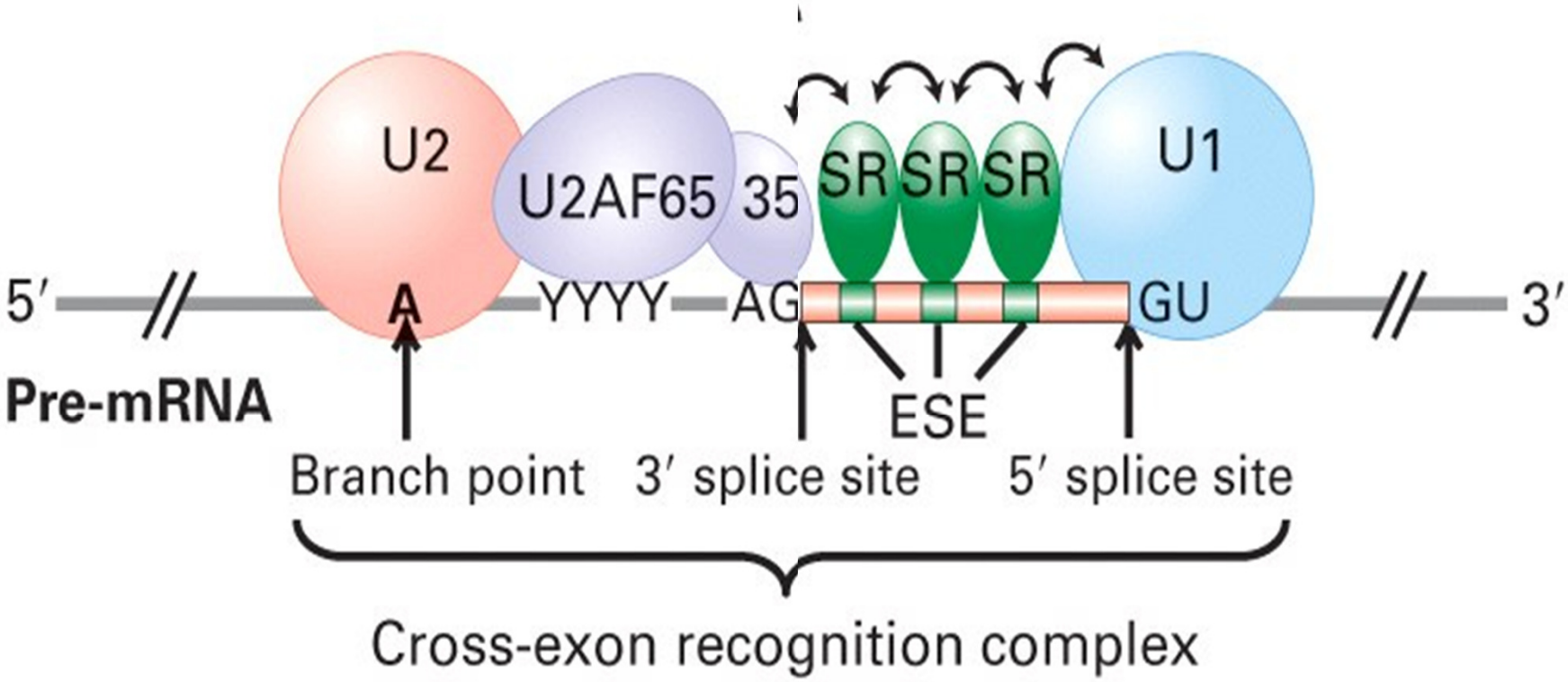
A1 could interfere directly with the assembly of spliceosomal components, it could block the exon bridging interactions that occur during exon definition, or it could block splicing activation by SR proteins binding to adjacent ESEs.

INTRONIC REGULATORY ELEMENTS

Many splicing regulatory sequences are present in introns rather than exons.
As in exonic regulation, positive- and negative-acting sequences compose intronic splicing enhancers and silencers respectively (ISEs and ISSs).

Mechanisms of Splicing Activation

Exons are recognized as units prior to assembly of the spliceosome across the long introns. This “exon definition” step involves interactions between the splice sites across the exon and special sequences in the exon called Exonic Splicing Enhancers (ESE).



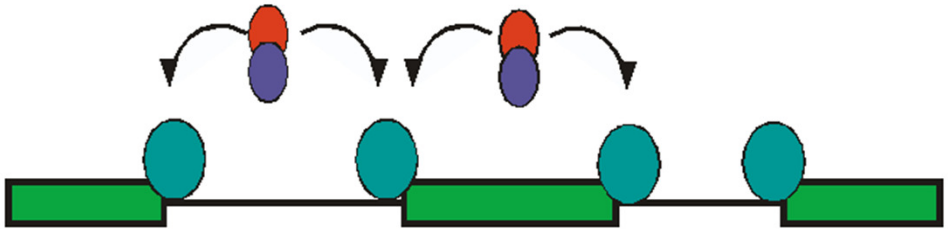
The sequences in exons are selected to not just code for particular peptide sequences, but also for binding of regulatory proteins to ESE’s.

The SR Proteins

Required splicing factors and effectors of splice site choice.

1 or 2 RNP Domains

C-terminal SR Domain containing multiple SR Dipeptides



And / Or

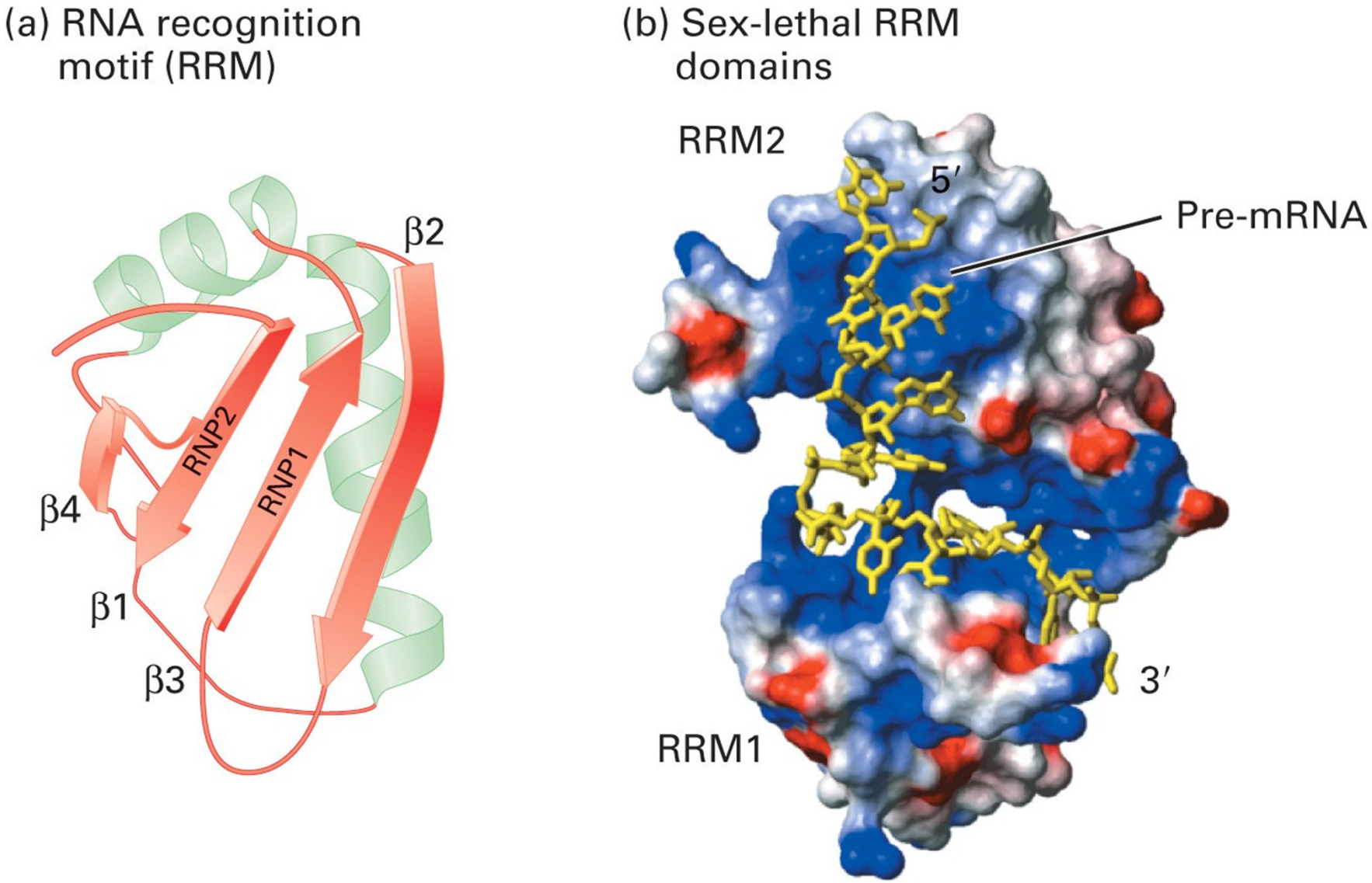
The SR Proteins are a family of proteins with a common domain structure of 1 or 2 RNP RNA binding domains (also called RRMs) and a C-terminal domain rich in SR dipeptides.

These proteins are involved in many aspects of splicing, but most significantly **they bind to Exonic Splicing Enhancers (ESEs) and stimulate spliceosome assembly at the adjacent sights.**

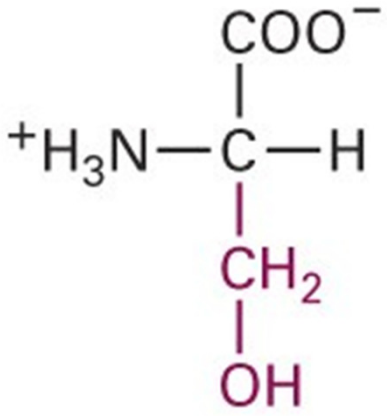
It is thought that most exons carry ESE's and require SR proteins for exon recognition.

ASF/SF2 and SC35

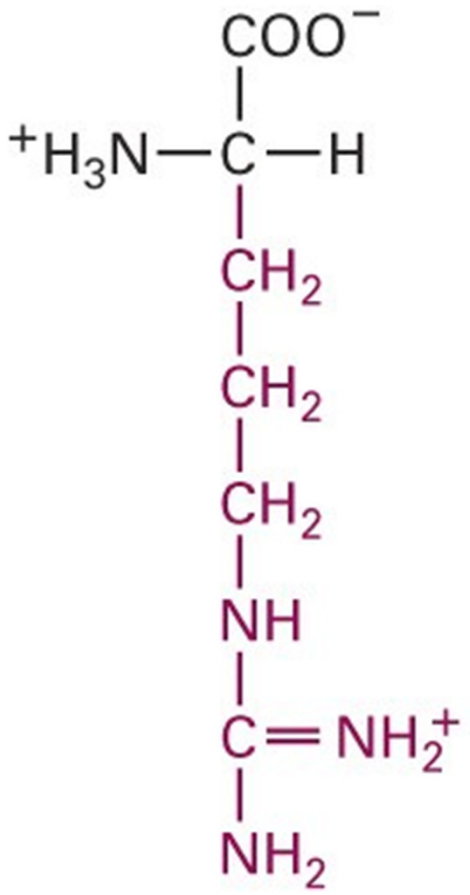
SR Proteins bind to specific RNA elements using their RNA binding domains similar to those in the Sex-Lethal protein.



The SR domain is an effector domain needed for splicing activation.
There is evidence that it interacts both with other proteins and with RNA. It can be highly phosphorylated on Serine and SR protein activity is thought to be modulated by specific kinases and phosphatases.



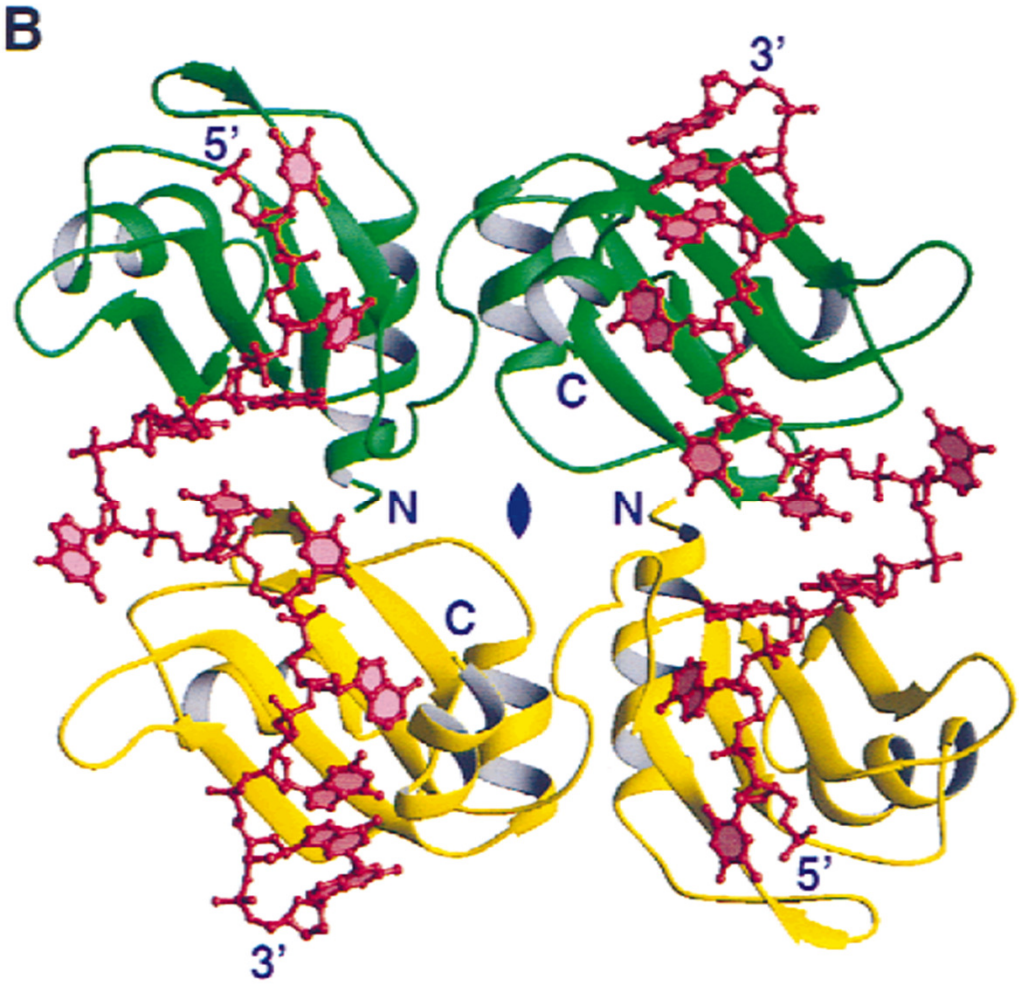
Serine
(Ser or S)



Arginine
(Arg or R)

Splicing Repression

Overall view of the UP1 (domain of human hnRNP A1) -TR2 complex. The protein molecules are shown in yellow or green as a ribbon model and the DNA molecules in red as a ball-and-stick model



hnRNP A1 was originally implicated in splicing as a factor that counteracted SR proteins

GENES & DEVELOPMENT 13:1102, 1999

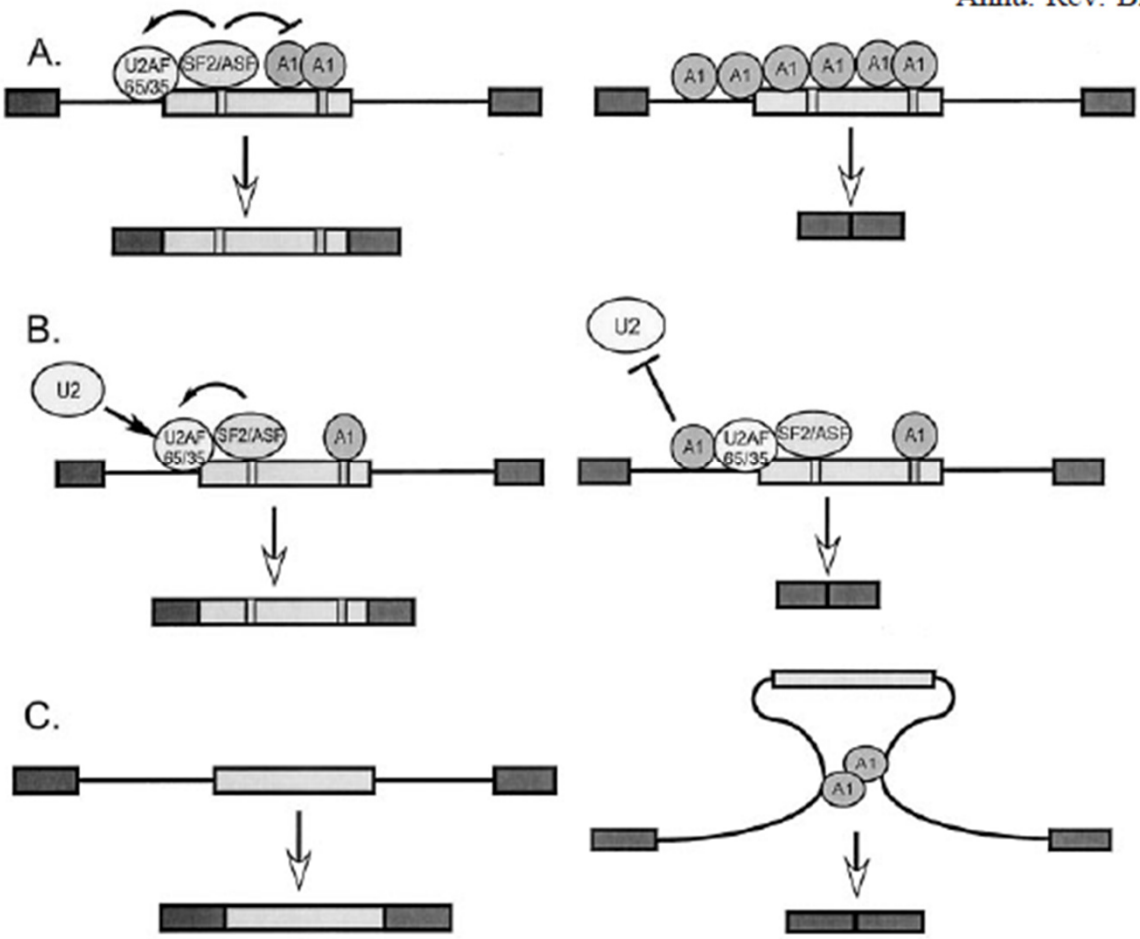
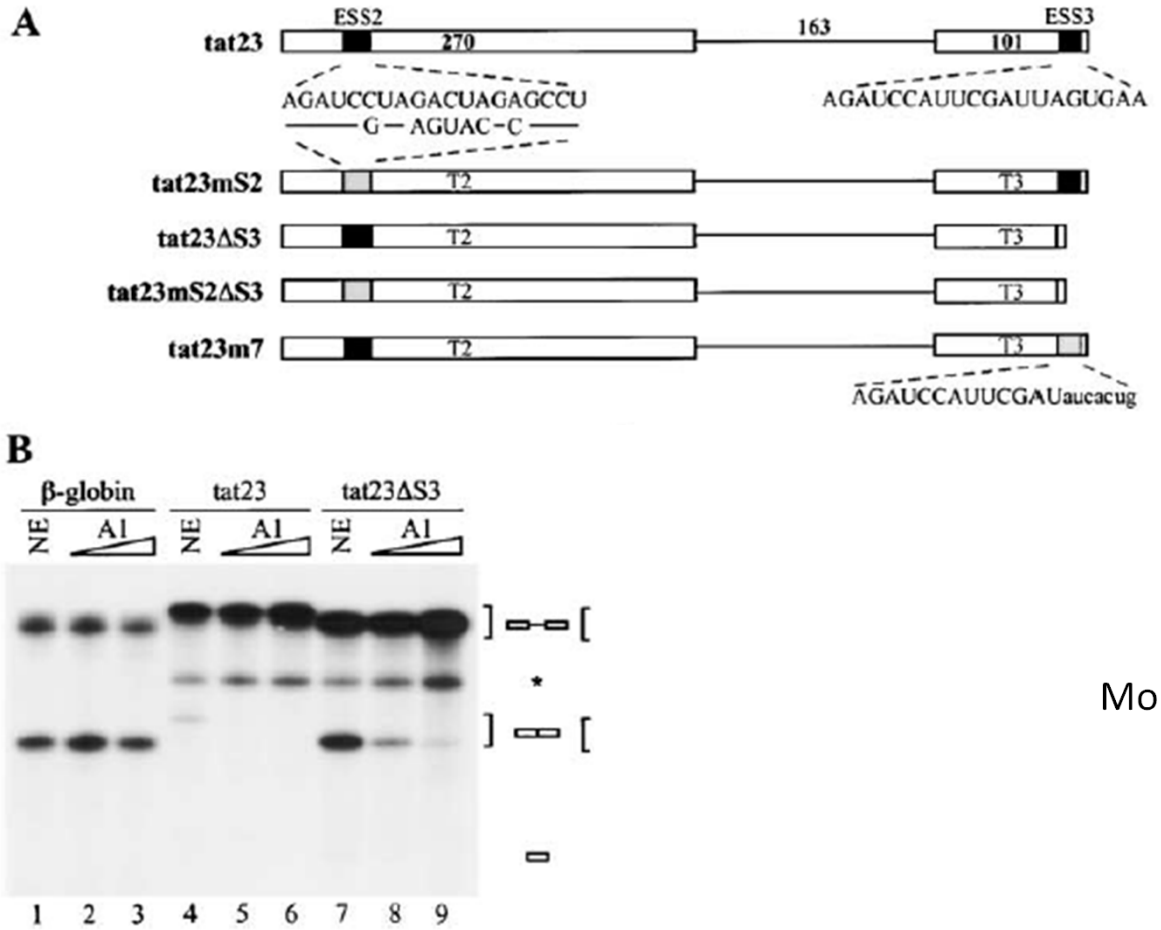


Figure 6 Models for splicing repression by hnRNP A1. (*A*) In HIV Tat exon 3, specific A1 binding to an ESS is thought to nucleate the assembly of additional A1 molecules along the RNA, creating a zone of RNA where spliceosome assembly is repressed. The A1 repression can be blocked by the strong binding of SR proteins to ESEs, which presumably also stimulates spliceosome assembly at the upstream 3 splice site, and allows exon inclusion. (*B*) There is an additional A1 binding site adjacent to the branch point for Tat exon 3 that blocks splicing in conjunction with the exonic A1 sites. A1 binding to this intronic element does not block U2AF binding to the 3 splice site but does block U2 snRNP binding to the branch point. (*C*) hnRNP A1 also represses an exon in its own transcript using intronic binding sites. A1 bound to these sites can multimerize, thus looping out the exon and causing exon skipping.

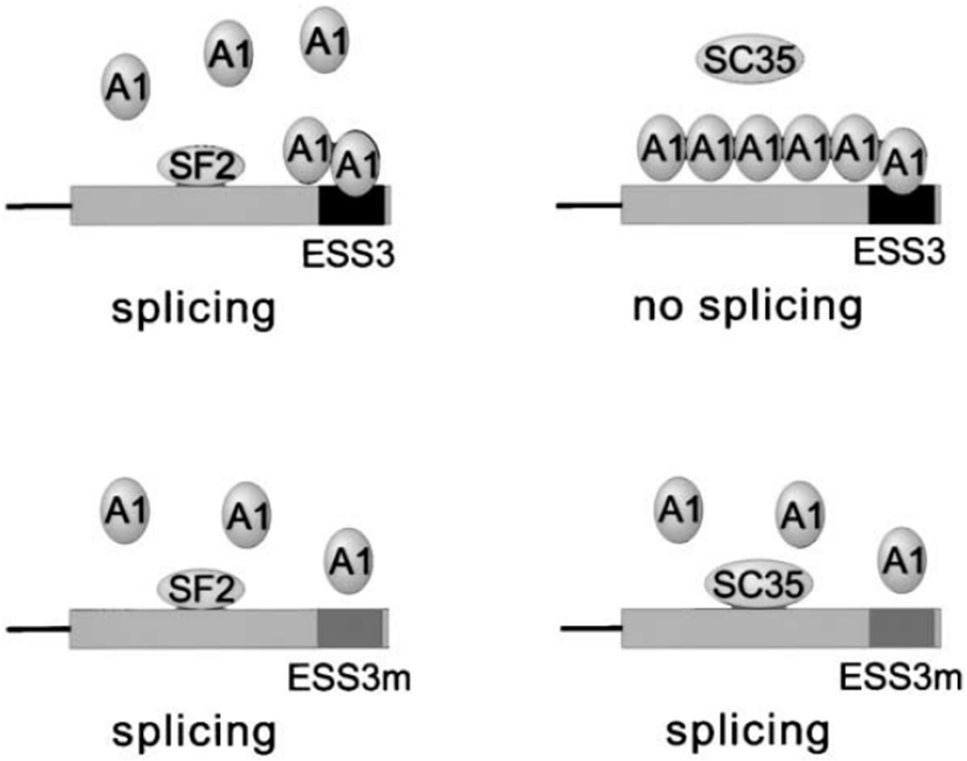
ESS - Exonic Splicing Silencers



Molecular Cell, Vol. 8, 1351, 2001

Figure 1. Effect of hnRNP A1 on Tat23 PremRNA Splicing In Vitro (A) Diagram of the pre-mRNA substrates, with mutations or deletions in ESS2 or ESS3. (B) The indicated pre-mRNAs were spliced in HeLa nuclear extract alone (lanes 1, 4, and 7) or supplemented with 3 pmol (lanes 2, 5, and 8) or 6 pmol (lanes 3, 6, and 9) of recombinant hnRNP A1. The asterisk indicates a cleavage product unrelated to splicing (Krainer et al., 1990).

Molecular Cell, Vol. 8, 1351–1361, 2001

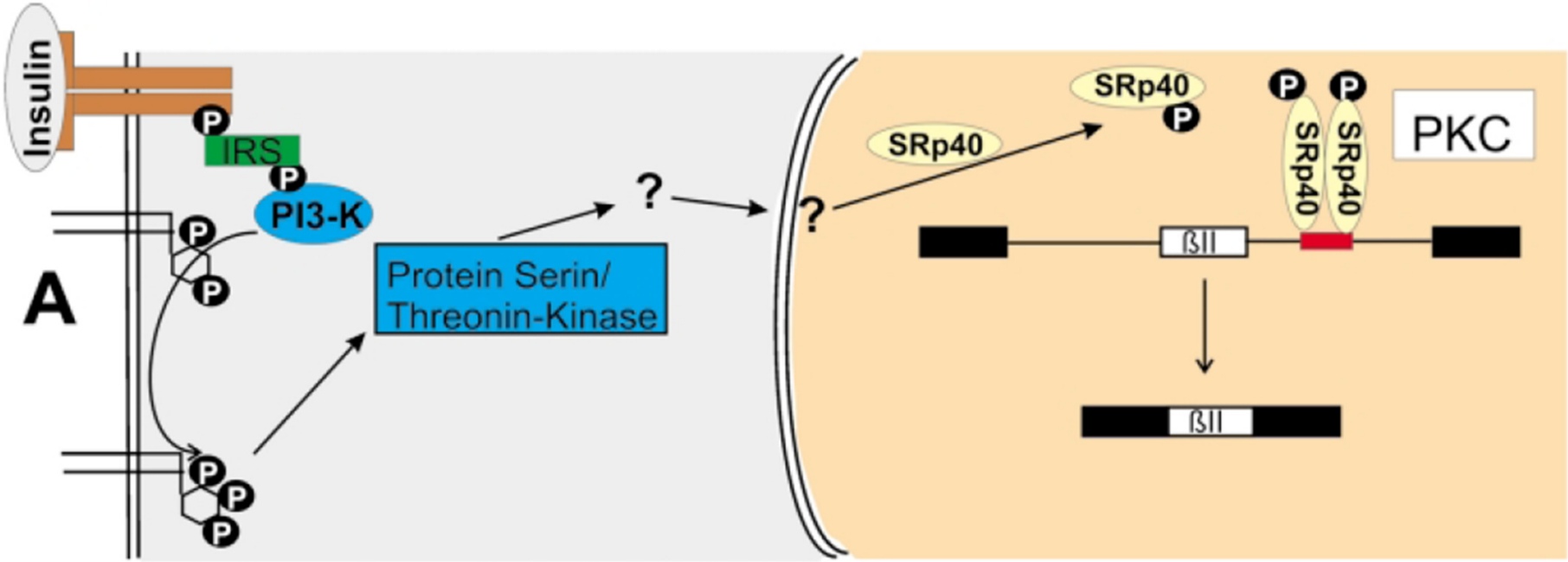


-SR proteins recognize exonic splicing enhancer (ESE) in retroviruses and promote exon use.
-hnRNP proteins bind to exonic splicing silencer (ESS) elements and block exon recognition.

How ESS3 in HIV-1 *tat* exon 3 blocks splicing promoted by one SR protein (SC35) but not another (SF2/ASF)? Only SF2/ASF prevents secondary hnRNP A1 binding by preblocking its cooperative propagation along the exon

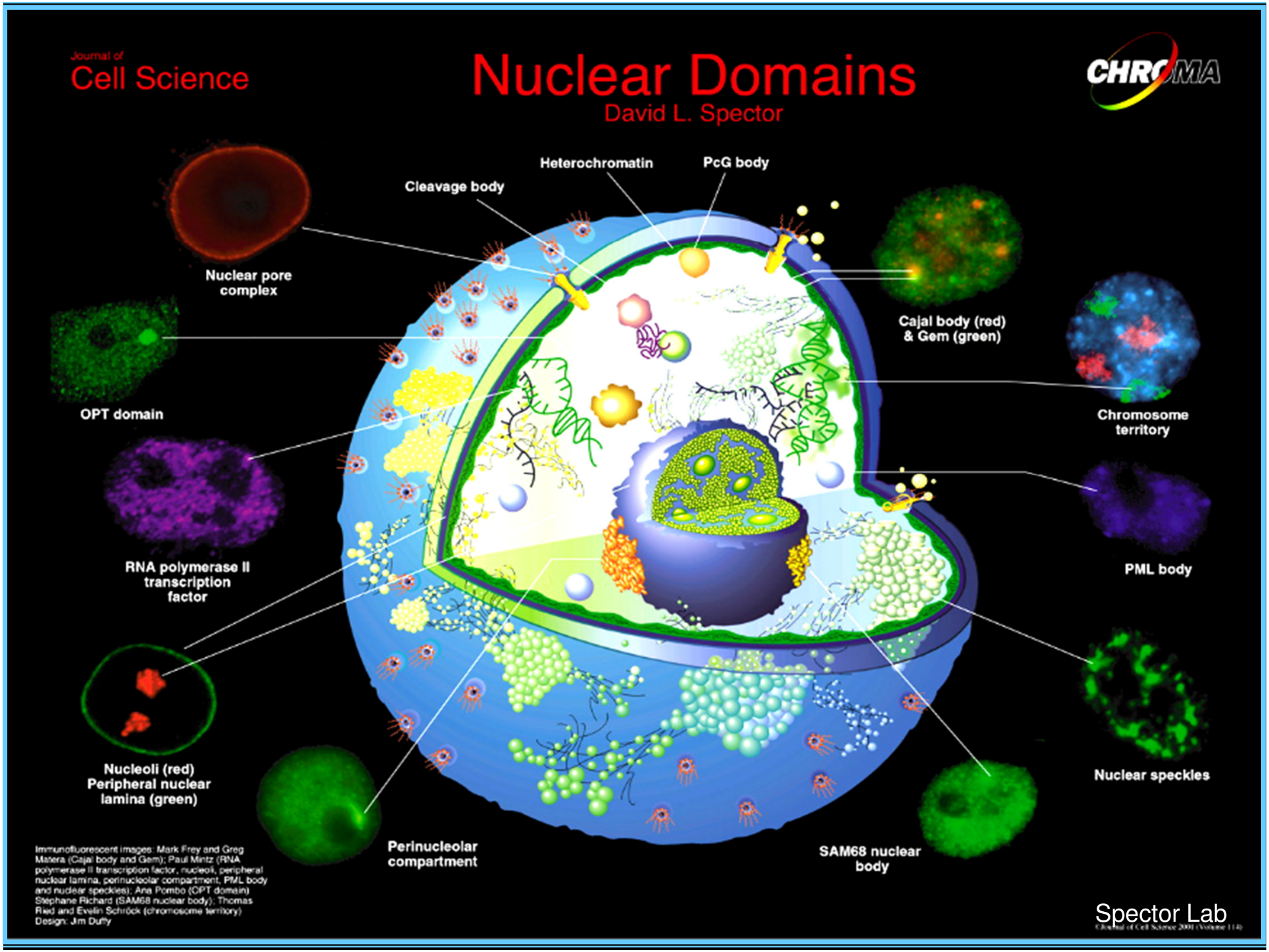
Top left: hnRNP A1 binds to ESS3, but SF2/ASF bound to the upstream ESE motifs in the exon blocks further cooperative binding and propagation of hnRNP A1 toward the 5 end; silencing is ineffective, and splicing ensues.
Top right: SC35 is unable to block the propagation of hnRNP A1 upstream from ESS3; splicing silencing ensues. Bottom, left and right: mutation of the high-affinity hnRNP A1 binding site within ESS3 blocks initial binding of hnRNP A1, and either SF2/ASF or SC35 are free to bind to their respective ESE motifs; splicing ensues.

Examples of signal transduction pathways that regulate alternative splice site selection.



Human Molecular Genetics, 2002, Vol. 11, No. 20 2409–2416

(A) Regulation of PKC splicing by insulin. Binding of insulin activates PI3-K via binding to the insulin receptor substrate (IRS). PI3-K forms phosphatidylinositol 3,4,5-trisphosphate, which activates an unknown protein serine–threonine kinase that ultimately phosphorylates SRp40 (SR Proteins), which acts on an intronic element that leads to exon βII inclusion.


Journal of
Cell Science
Nuclear Domains
David L. Spector
CHROMA
Heterochromatin
PcG body
Cleavage body
Nuclear pore complex
Cajal body (red) & Gem (green)
OPT domain
Chromosome territory
RNA polymerase II transcription factor
PML body
Nucleoli (red) Peripheral nuclear lamina (green)
Nuclear speckles
Perinucleolar compartment
SAM68 nuclear body
Immunofluorescent images: Mark Frey and Greg Matera (Cajal body and Gem); Paul Mintz (RNA polymerase II transcription factor, nucleoli, peripheral nuclear lamina, perinucleolar compartment, PML body and nuclear speckles); Ana Pombo (OPT domain) Stephane Richard (SAM68 nuclear body); Thomas Ried and Evelin Schröck (chromosome territory)
Design: Jim Duffy
Spector Lab

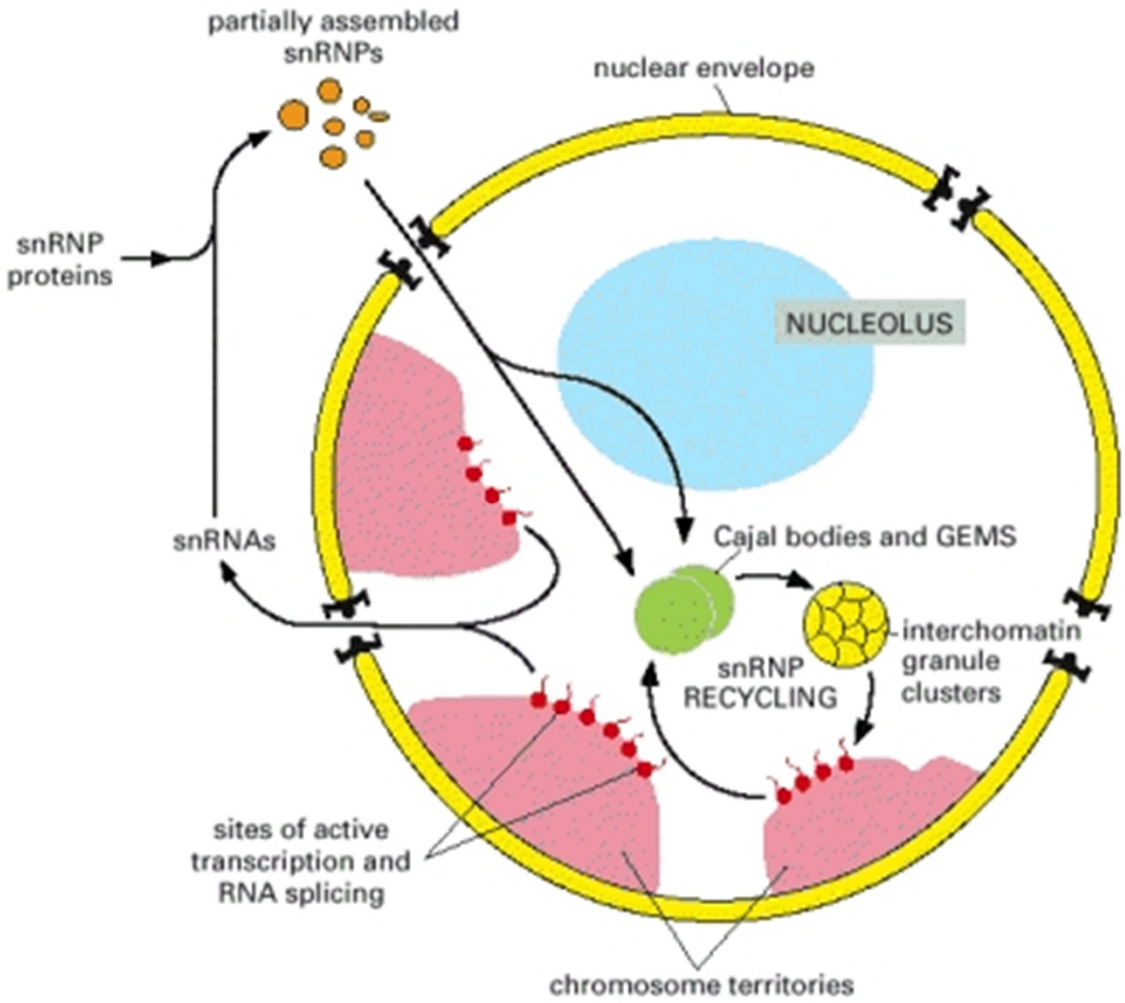
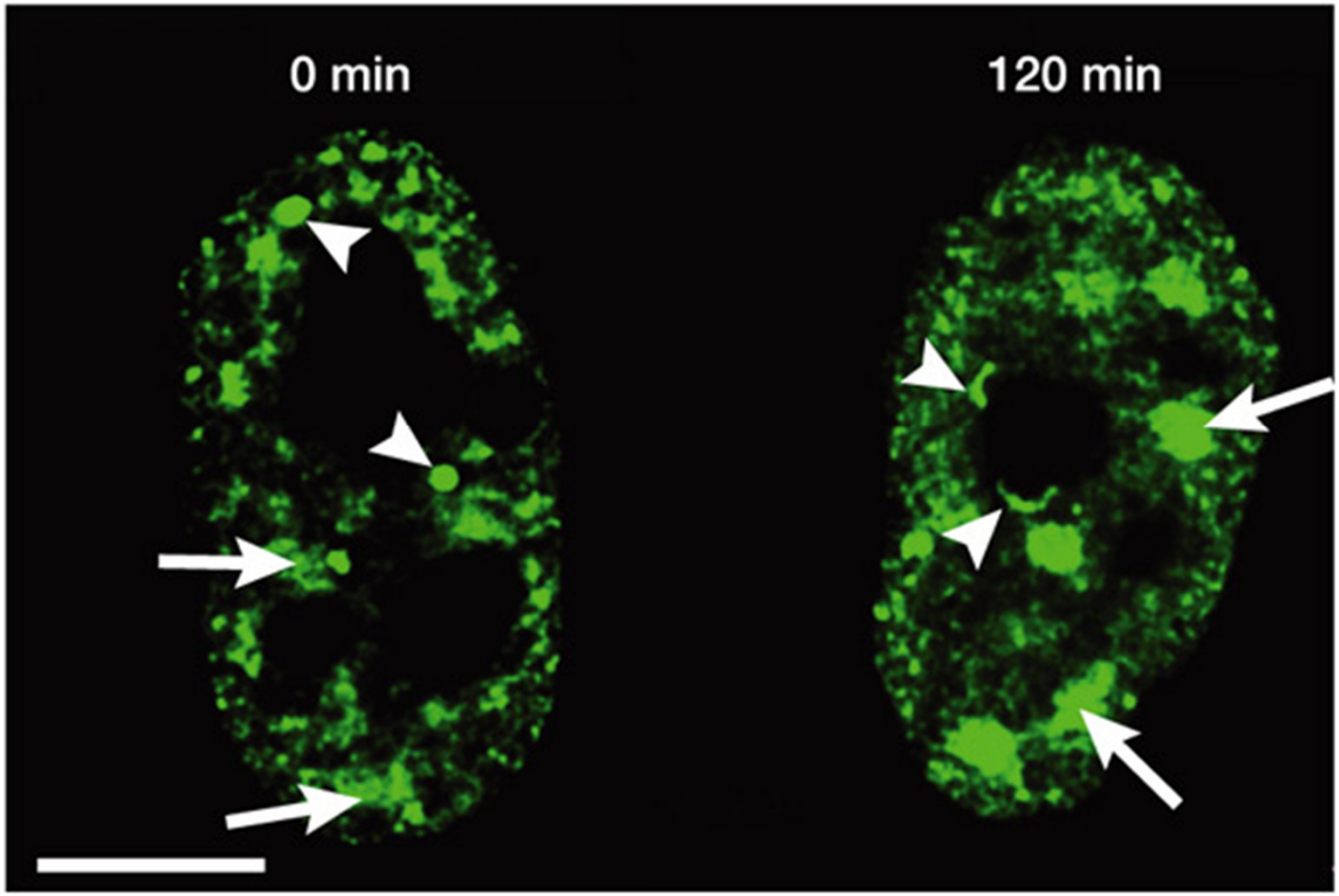


Figure 6-49 Schematic view of subnuclear structures

A typical vertebrate nucleus has several Cajal bodies, which are proposed to be the sites where snRNPs and snoRNPs undergo their final modifications. Interchromatin granule clusters are proposed to be storage sites for fully mature snRNPs. A typical vertebrate nucleus has 20–50 interchromatin granule clusters. After their initial synthesis, snRNAs are exported from the nucleus, after which they undergo 5′ and 3′ end-processing and assemble with the seven common snRNP proteins (called Sm proteins). These complexes are reimported into the nucleus and the snRNPs undergo their final modification in Cajal bodies. In addition, the U6 snRNP requires chemical modification by snoRNAs in the nucleolus. The sites of active transcription and splicing (approximately 2000–3000 sites per vertebrate nucleus) correspond to the "perichromatin fibers" seen under theelectron microscope.

Nuclear speckles: a model for nuclear organelles

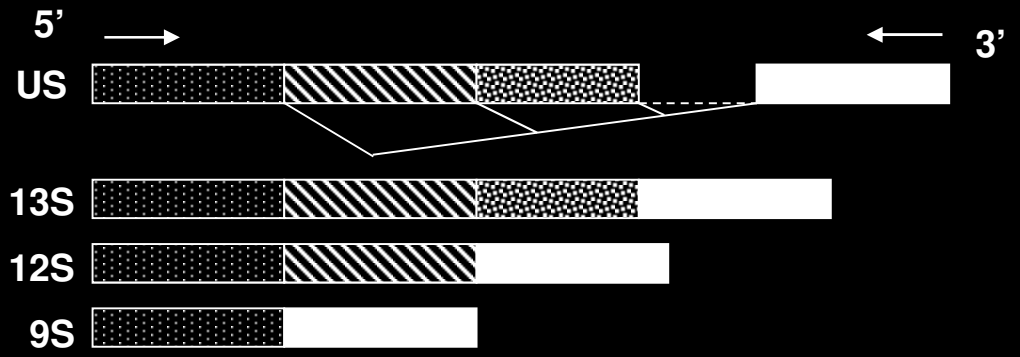


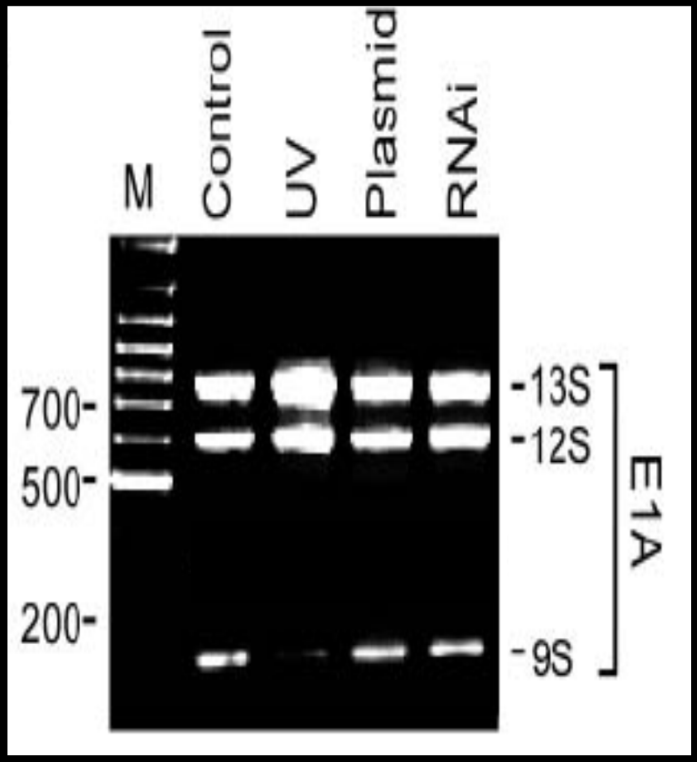
Nature Reviews Molecular Cell Biology 4, 605, 2003.

In Vivo Analysis of Alternative Splicing

Adenovirus E1A reporter gene as a model substrate to characterize the function of SR proteins

Diagram of the E1A reporter gene





Adenovirus E1A reporter gene is capable of producing multiple mRNAs (13S, 12S, and 9S) through the use of alternative splice sites (Cáceres et al., 1994).

Cell Free Sytems
In vitro splicing
In vivo splicing

They used an **S100 extract** that is depleted for all the SR proteins and further depleted it for A1. In the absence of A1, either SC35 or SF2/ASF can activate Tat exon 3 splicing. The addition of A1 specifically inhibited SC35- but not SF2/ASF-activated splicing. It was found that in addition to binding the ESS, A1 crosslinked to exonic RNA distal to the ESS in the region of the SR protein binding sites. This crosslinking occurred only if the ESS was present. It was proposed that specific A1 binding at the ESS nucleates the cooperative nonspecific binding of A1 upstream. This higher order complex of multiple A1s would create a zone of inhibition along the RNA. SF2/ASF is proposed to block the propagation of this complex, whereas SC35 does not bind tightly enough to do this.

Cross-linking immunoprecipitation (CLIP) assay