Procesamiento de mRNA - Splicing.

•Concepto de "split genes" ⟶

•En evolución:

-pérdida de intrones (¿evidencias?)

-rápida acumulación de mutaciones en intrones.

-conservación de sitios de empalme: mecanismo universal

•¿Porqué persisten?

- Mayor diversidad con igual número de genes (ribotipo)

-Ventaja evolutiva, nuevas combinaciones de exones producen nuevas proteínas

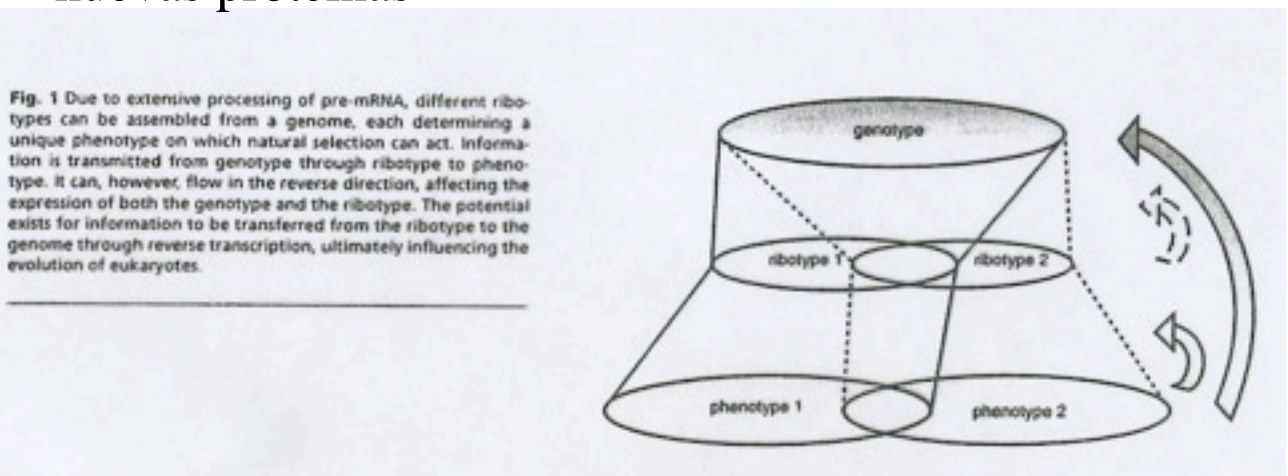
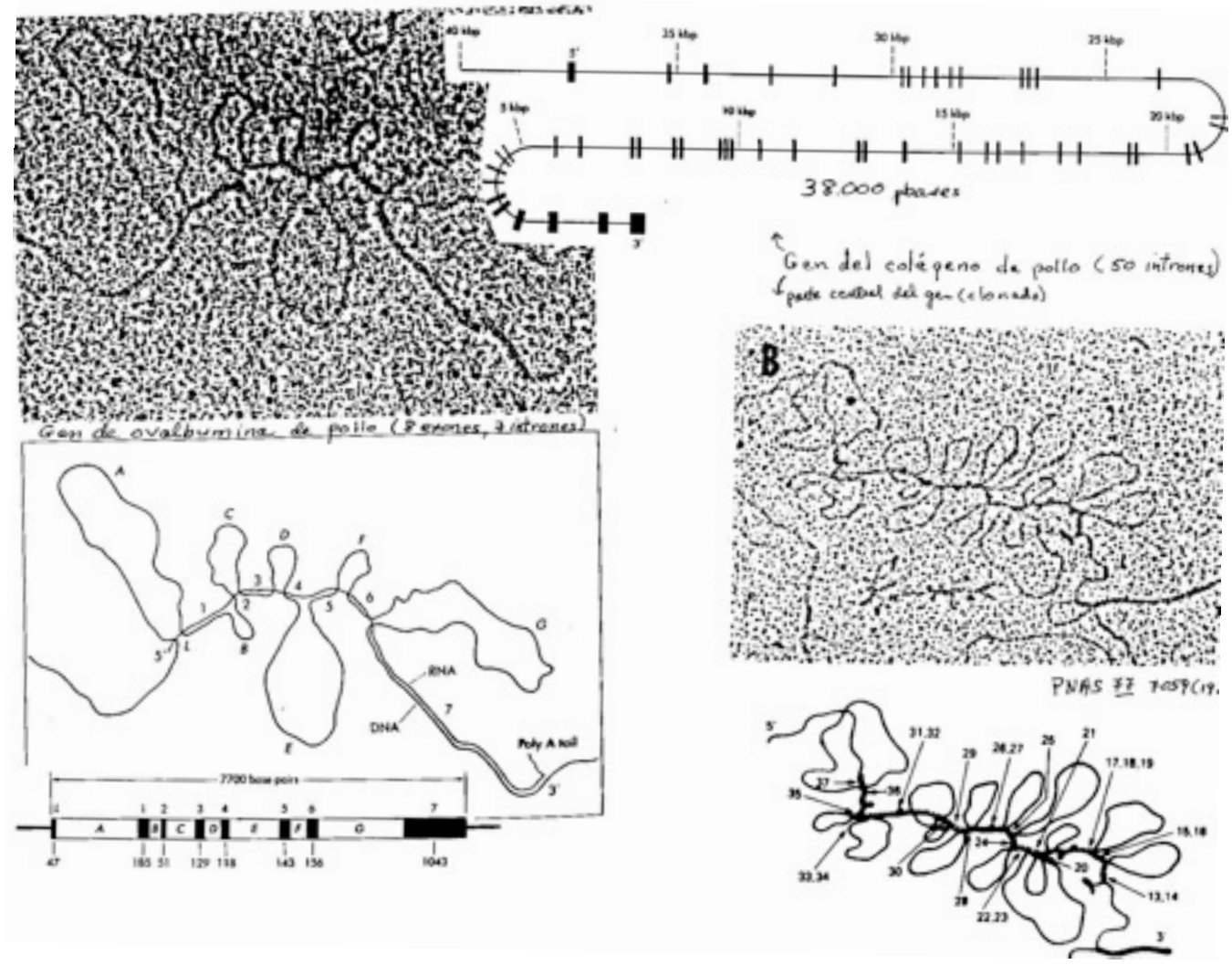
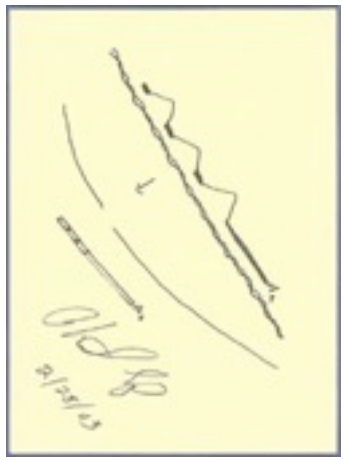


Fig. 1 Due to extensive processing of pre-mRNA, different ribotypes can be assembled from a genome, each determining a unique phenotype on which natural selection can act. Information is transmitted from genotype through ribotype to phenotype. It can, however, flow in the reverse direction, affecting the expression of both the genotype and the ribotype. The potential exists for information to be transferred from the ribotype to the genome through reverse transcription, ultimately influencing the evolution of eukaryotes.

RNA processing and the evolution of eukaryotes. Herbert and Rich. 1999. Nature Genet. 21: 265.

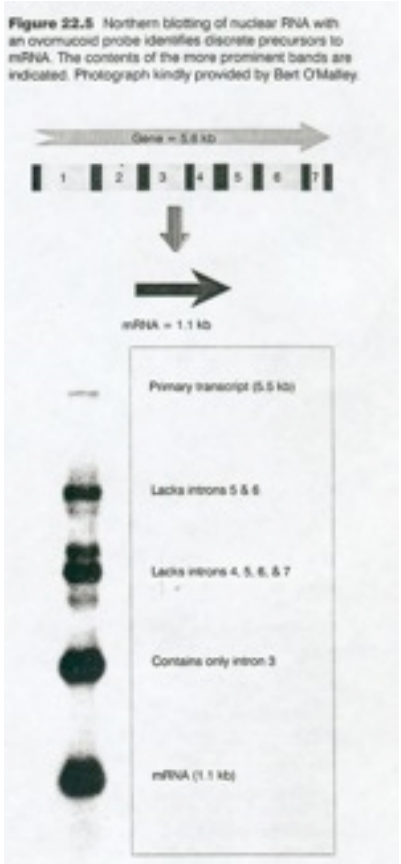




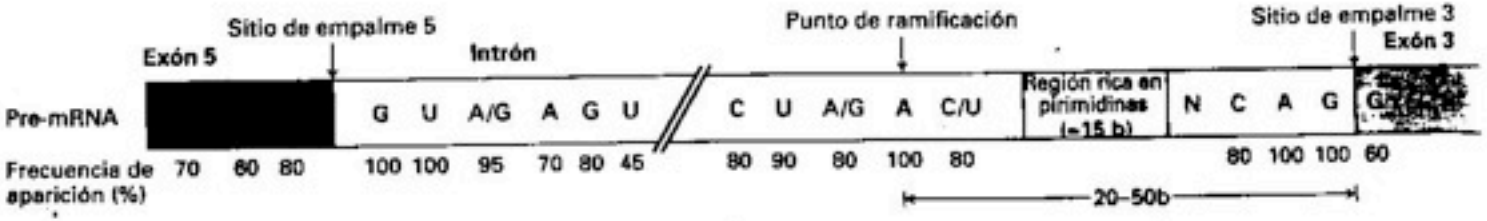
Phillip Sharp 2003

Splicing ocurre simultaneamente con la Tx
Otros procesamientos: CAP y PoliA.

Remoción de intrones sigue un órden.

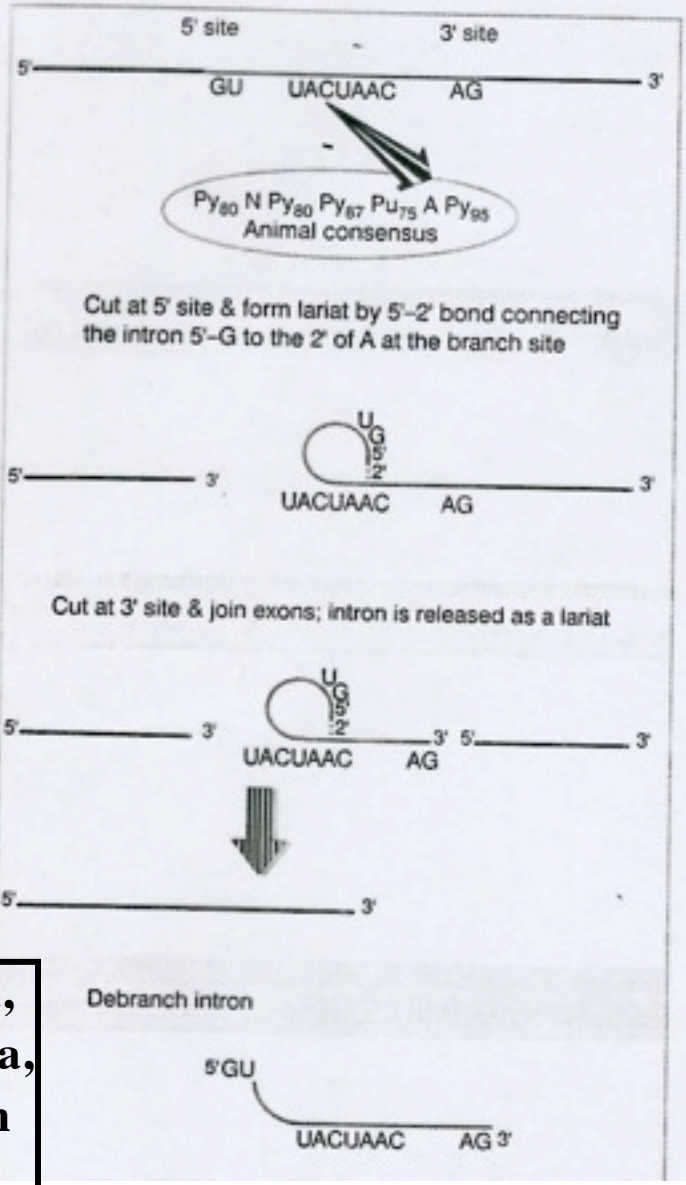


Secuencias consenso en sitios de unión y en el intrón (menos conservado)



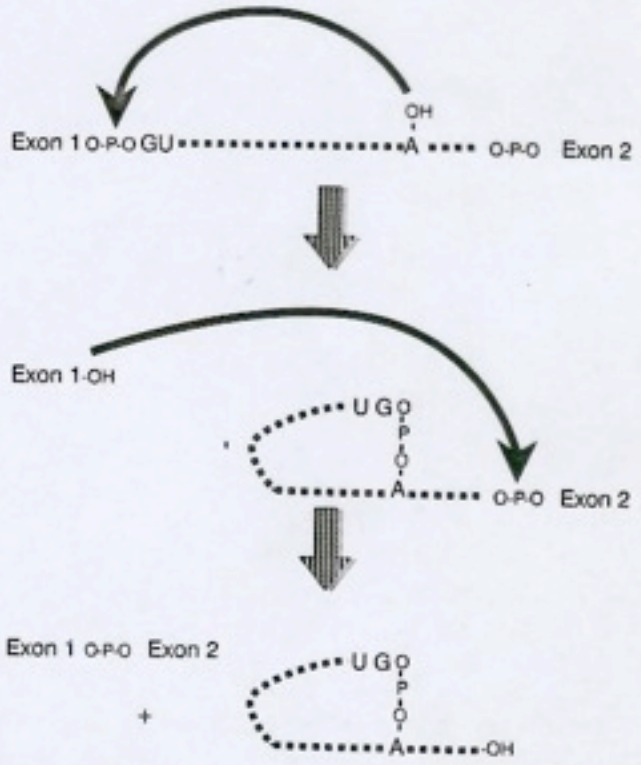
▲ **Fig. 11-14. Secuencias consenso alrededor de los sitios de empalme 5′ y 3′ en los pre-mRNA de vertebrados.** Las únicas bases casi invariables son las GU (5′) y AG (3′) del intrón, aunque las bases flanqueantes indicadas se encuentran con frecuencias mayores que las esperadas en una distribución aleatoria. En la mayoría de los casos se encuentra una región rica en pirimidinas (en celeste) cerca del extremo 3′ del intrón. La adenosina del punto de ramificación, también invariable, suele estar a 20-50 bases del sitio de empalme 3′. La región central del intrón, cuya longitud oscilaría entre 40 bases y 50 kilobases, en general no es necesaria para que el empalme tenga lugar. (Véase R.A. Padgett, y col, *Ann Rev Biochem* 1986;55:1119; E.B. Keller y W.A. Noon, *Proc Nat'l Acad Sci USA* 1984;81:7417.)

Figure 22.6 Splicing occurs in two stages, in which the 5' exon is separated and then is joined to the 3' exon.



The branch site lies 18–40 nucleotides upstream of the 3′ splice site. Mutations or deletions of the branch site in yeast prevent splicing. In higher eukaryotes, the relaxed constraints in its sequence result in the ability to use related sequences in the vicinity when the authentic branch is deleted. Proximity to the 3′ splice site appears to be important, since the cryptic site is always close to the authentic site. When a cryptic branch sequence is used in this manner, splicing otherwise appears to be normal; and the exons give the same

Figure 22.7 Nuclear splicing occurs by two transesterification reactions in which a free OH end attacks a phosphodiester bond.



La separación física, aunque momentánea, sugiere la formación de un complejo.

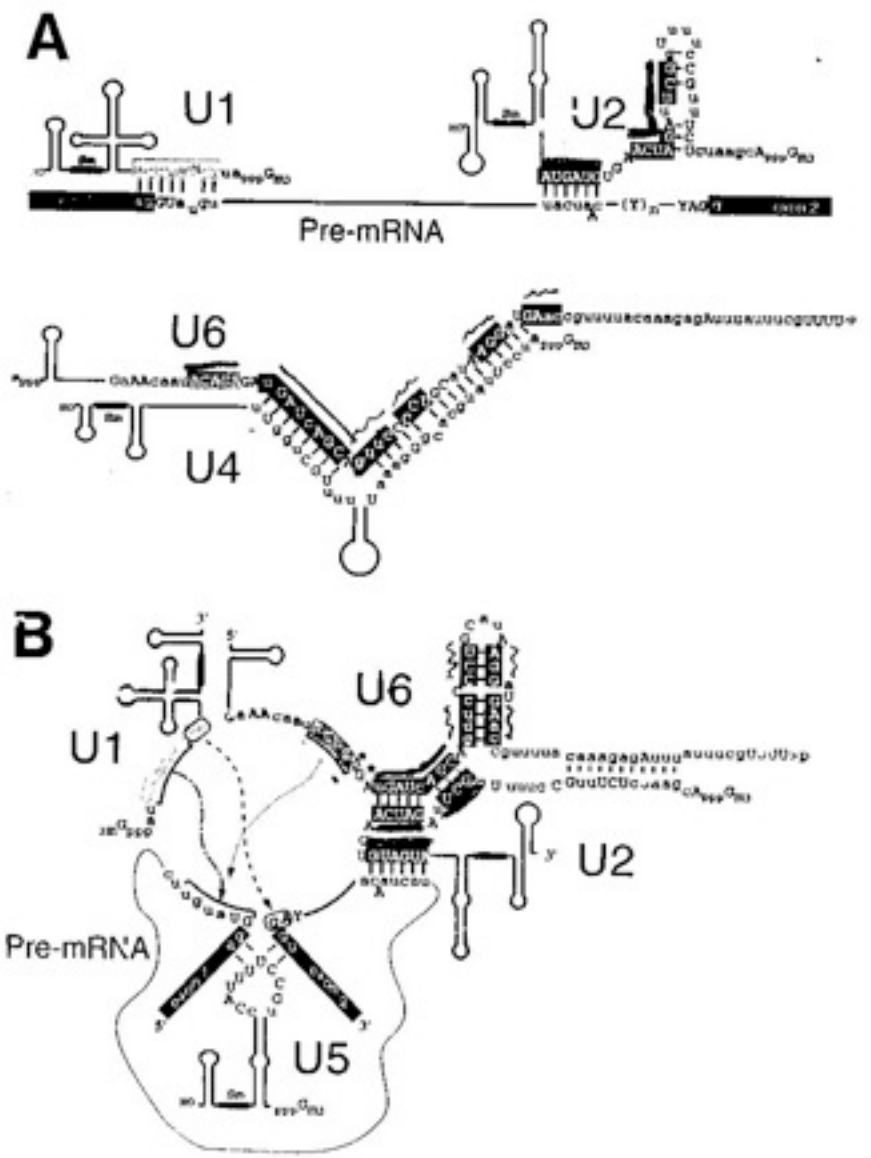


Figure 5. RNA Interactions between Spliceosomal snRNAs and Pre-mRNA Substrates

(A) (Top) Base pairing interactions between U1 and U2 snRNAs and pre-mRNA are indicated on the left and right of the intron, respectively. (Bottom) Extensive base pairing between U4 and U6 snRNAs.

(B) Interactions between U1, U2, U5, and U6 snRNAs and pre-mRNA.

In both (A) and (B), pre-mRNA consensus sequences and snRNA sequences are those of Saccharomyces cerevisiae; uppercase nucleotides are highly conserved between S. cerevisiae and all known sequences of other organisms (excluding trypanosomes, which do not have a GUAGUA sequence in U2 snRNA). The different colored areas highlight sequences in U2 and U6 snRNAs that change base pairing partners during the spliceosome cycle. Internal snRNA secondary structures that do not change between (A) and (B) are shown as stylized stems and loops. Asterisks indicate snRNA positions at which mutations specifically block the second step of splicing.

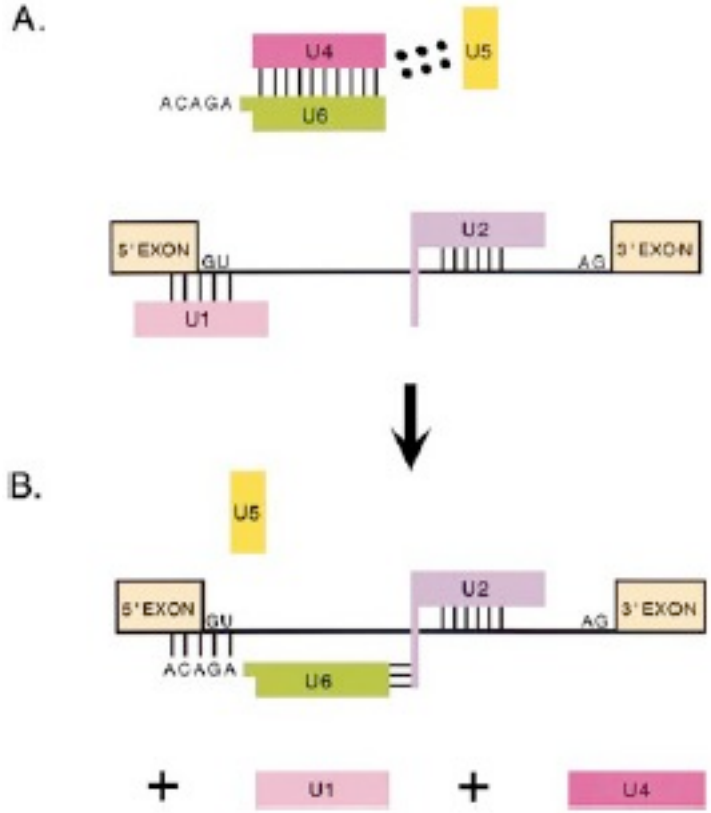


Figure 1. Comparison of the Inactive and the Active Spliceosome

(A) The assembled but inactive spliceosome. Although the spliceosome is known to harbor up to 50 protein components, for simplicity only the pre-mRNA (i.e., the substrate) and the five snRNAs are shown. The substrate consists of the intron (black line) flanked by the 5′ and 3′ exons (rectangles). Two of the snRNAs (U1 and U2) are bound to the substrate by base pairing. Another two snRNAs (U4 and U6) are paired with one another. The "dots" indicate that U5 is in close proximity to U4 and U6.

(B) The active spliceosome. In the active spliceosome U2 is paired to U6, and U6 is paired with the 5′ splice site. Finally, U1 and U4, which are not required for catalysis, have left the spliceosome.

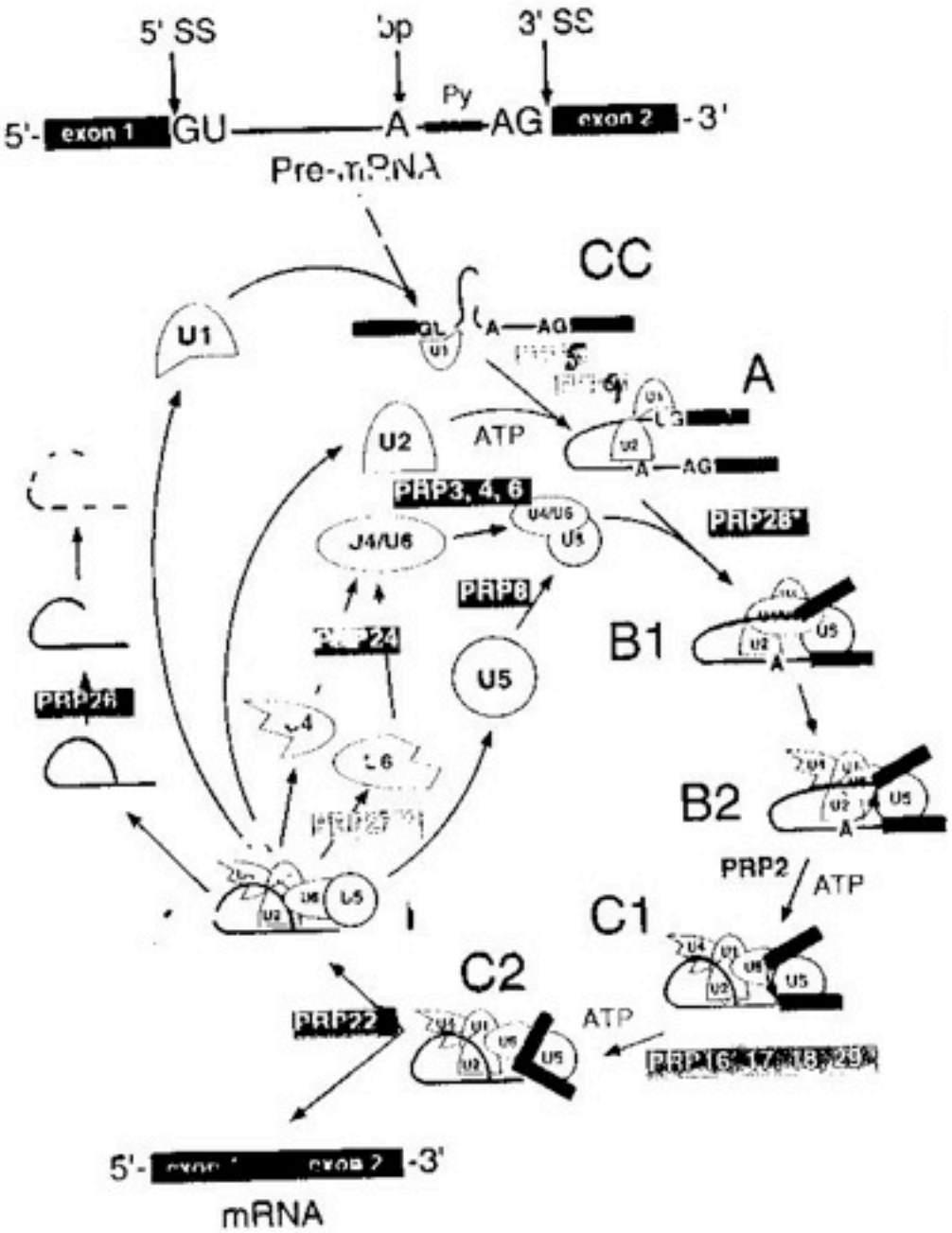


Figure 7. Transitions in the Spliceosome Cycle That Require a PRP Protein

The particular PRP mutant is listed beside the arrow indicating the transition in the cycle in vitro that requires the mutant protein.

PRP: Pre-RNA processing

Identificados por mutantes

Actividades de PRPs:

- -Unión a RNA
- -RNA Helicasas
- -Interacciones prot-prot.

prot-prot

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Flipping the Switch to an Active Spliceosome

Heather L. Murray and Kevin A. Jarrell*

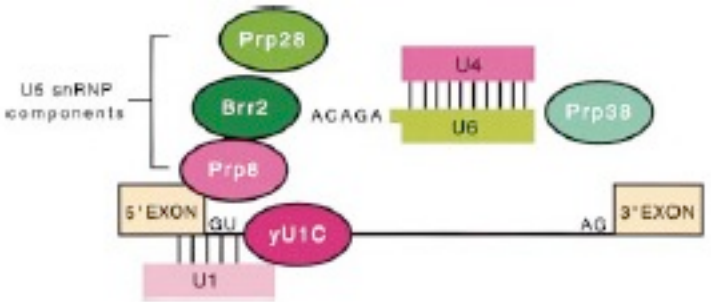

Figure 2. Factors Involved in Spliceosome Activation

Likely inhibitors of the process are shown in various shades of red, while likely stimulators are shown in green. Prp8 and Brr2 (Brr2 is also referred to as Rss1, Slt22, or Snu246) are known to be components of U5 snRNP (Lossky et al., 1987; Lauber et al., 1996) and are diagrammed accordingly. The diagram reflects the knowledge that human Prp28 (U5-100 kDa) is a component of U5 snRNP (Teigelkamp et al., 1997), and that human Brr2/Rss1/Slt22/Snu246 (U5-200 kDa) and human Prp8 (p220) have been shown to interact in vitro (Achsel et al., 1998).

Proteína clave es **Prp28**

- Participa en la Rxn de intercambio U1-U6
- Relacionada con DNA helicasas (unwindase, ATP dependiente)
- Roles:
  - -desenrrolla duplex U1-preRNA
  - -desestabiliza prot. que mantienen duplex U1-preRNA (yuc-1).
  - -desenrrolla U4-U6.
- Mutante en Prp-28 estabiliza unión U1-5'int

Prp-8: estabiliza duplex U4-U6

yuc-1: estabiliza duplex U1-5'int

Diversidad de proteínas mediante el uso de "casettes".

Splicing alternativo: debe ser regulado.

Necesidades de procesamiento:
- condiciones fisiológicas
- desarrollo embrionario.

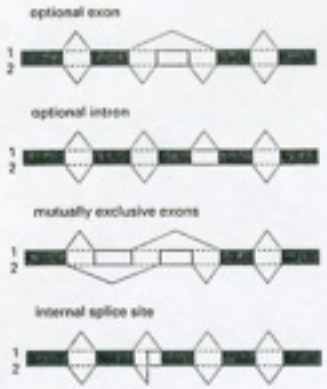

Figure 9–73 Four patterns of alternative RNA splicing. In each case a single type of RNA transcript is spliced in two alternative ways to produce two distinct mRNAs (1 and 2). The *dark blue boxes* mark exon sequences that are retained in both mRNAs. The *light blue boxes* mark possible exon sequences that are included in only one of the mRNAs; these boxes are joined by *red lines* to indicate where intron sequences (*yellow*) are removed. (Adapted with permission from A. Andreadis, M.E. Gallego, and B. Nadal-Ginard, *Annu. Rev. Cell Biol.* 3:207–242, 1987. © 1987 Annual Reviews, Inc.)

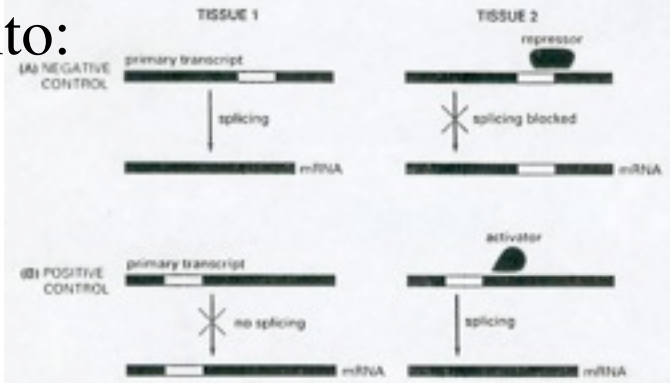

Figure 9–74 Negative and positive control of alternative RNA splicing. (A) Negative control, in which a repressor protein binds to the primary RNA transcript in tissue 2, thereby preventing the splicing machinery from removing an intron sequence. (B) Positive control, in which the splicing machinery is unable to remove a particular intron sequence without assistance from an activator protein.

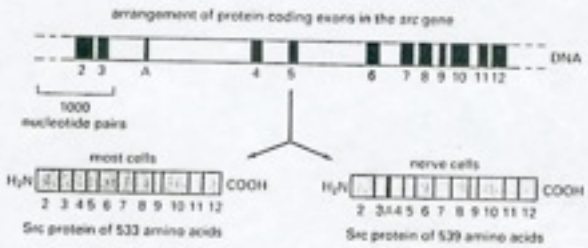

Figure 9–75 Regulated alternative RNA splicing produces cell-type-specific forms of a gene product. Here two slightly different tyrosine protein kinases are produced from the *src* gene because exon sequence A is included only in nerve cells. The neural form of the Src protein contains an extra site for phosphorylation and is also thought to have a higher specific activity. Only the protein-coding exons (*colored*) are shown in this diagram (exon 1, which forms the 5′ leader on the mRNA, is not shown). (After J.B. Levy et al., *Mol. Cell Biol.* 7:4142–4145, 1987.)

Cell, Vol. 118, 619–633, September 3, 2004, Copyright ©2004 by Cell Press

Alternative Splicing of *Drosophila* Dscam Generates Axon Guidance Receptors that Exhibit Isoform-Specific Homophilic Binding

Woj M. Wojtowicz, John J. Flanagan, S. Sean Millard, S. Lawrence Zipursky,* and James C. Clemens

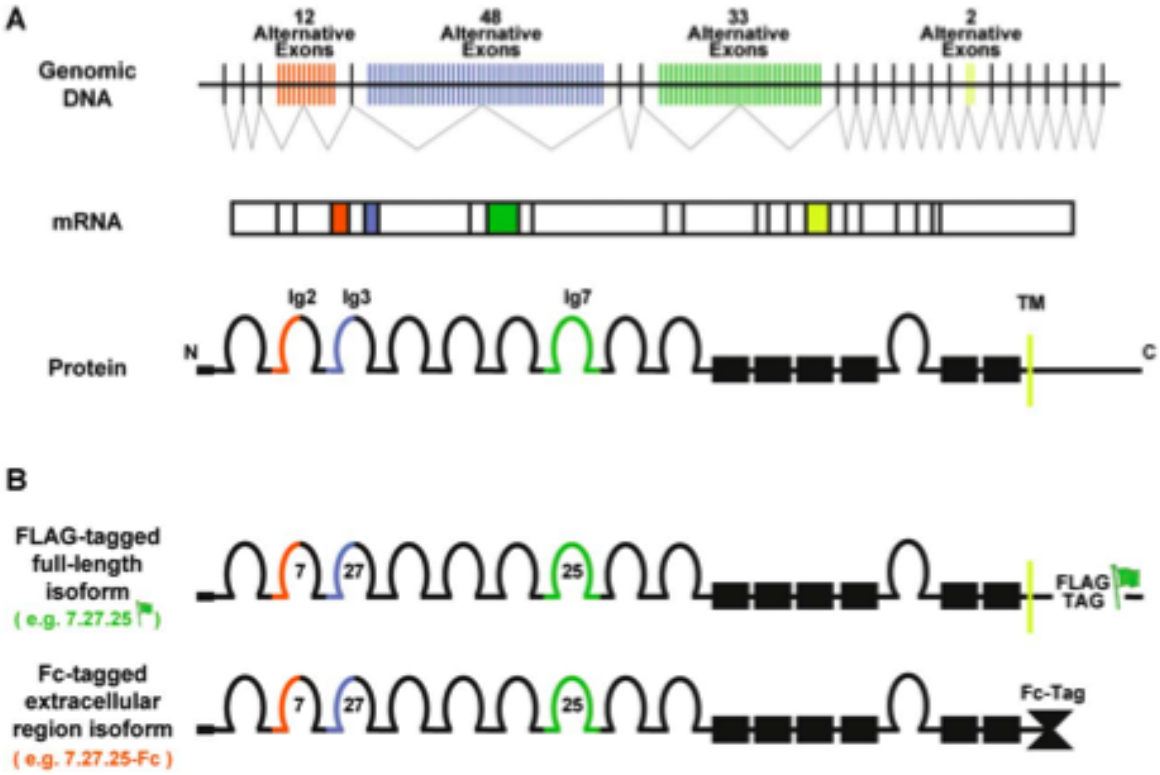

Figure 1. Alternative Splicing of Dscam Potentially Generates 38,016 Isoforms

(A) Schematic representation of *Dscam* gene, mRNA, and protein. The Dscam protein contains both constant and variable domains. The variable domains are encoded by alternative exons. Each block of alternative exons is indicated by a different color. A transcript contains only one alternative exon from each block. The *Dscam* gene encodes 12 alternative exons for the N-terminal half of Ig2 (red), 48 alternative exons for the N-terminal half of Ig3 (blue), and 33 alternative exons for Ig7 (green). There are two alternative transmembrane domains (yellow). (B) Schematic representation of Dscam proteins used in this study and explanation of isoform nomenclature. Immunoglobulin domain (Ig), horseshoe; fibronectin type III repeat (FNIII), black rectangle; transmembrane domain (TM), vertical rectangle. The NH_2 and COOH termini are indicated. Individual isoforms are denoted by the combination of alternative variable Ig domains. For instance, the isoform comprising Ig2 alternative 7, Ig3 alternative 27, and Ig7 alternative 25 is designated $Dscam^{7.27.25}$ or simply as 7.27.25. In this study, both full-length proteins and fragments of the extracellular domain were used. Full-length Dscam proteins expressed in COS cells and transgenic flies did not contain an epitope tag. Full-length proteins expressed in *Drosophila* S2 cells contained an internal FLAG epitope tag in the C-terminal cytoplasmic tail (denoted by a flag icon). For simplicity, the alternative transmembrane domain used in full-length isoforms is not indicated. Purified Dscam proteins containing different regions of the extracellular domain contained either a 6xHis tag (schematic not shown) or the Fc region of human IgG at the C terminus. Fc tags contain the hinge region, which dimerizes the Dscam proteins. 6xHis tags do not dimerize the Dscam proteins.

Splicing alternativo

Splicing enhancers: secuencias *cis* en el RNA

Unen las proteínas (factores *trans*) SR, ricas en Ser y Arg

- Dominio unión a RNA
- Dominio de activación (RS)

Paralelos con transcripción: experimentos de Maniatis

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The Function of Multisite Splicing Enhancers

Klemens J. Hertel and Tom Maniatis*

enhancers are thought to function by

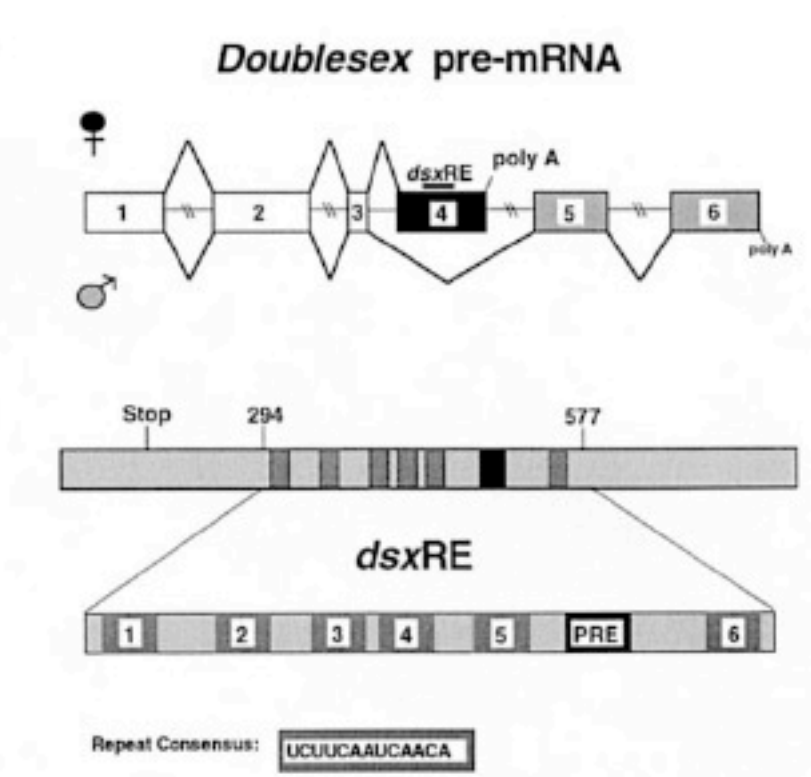
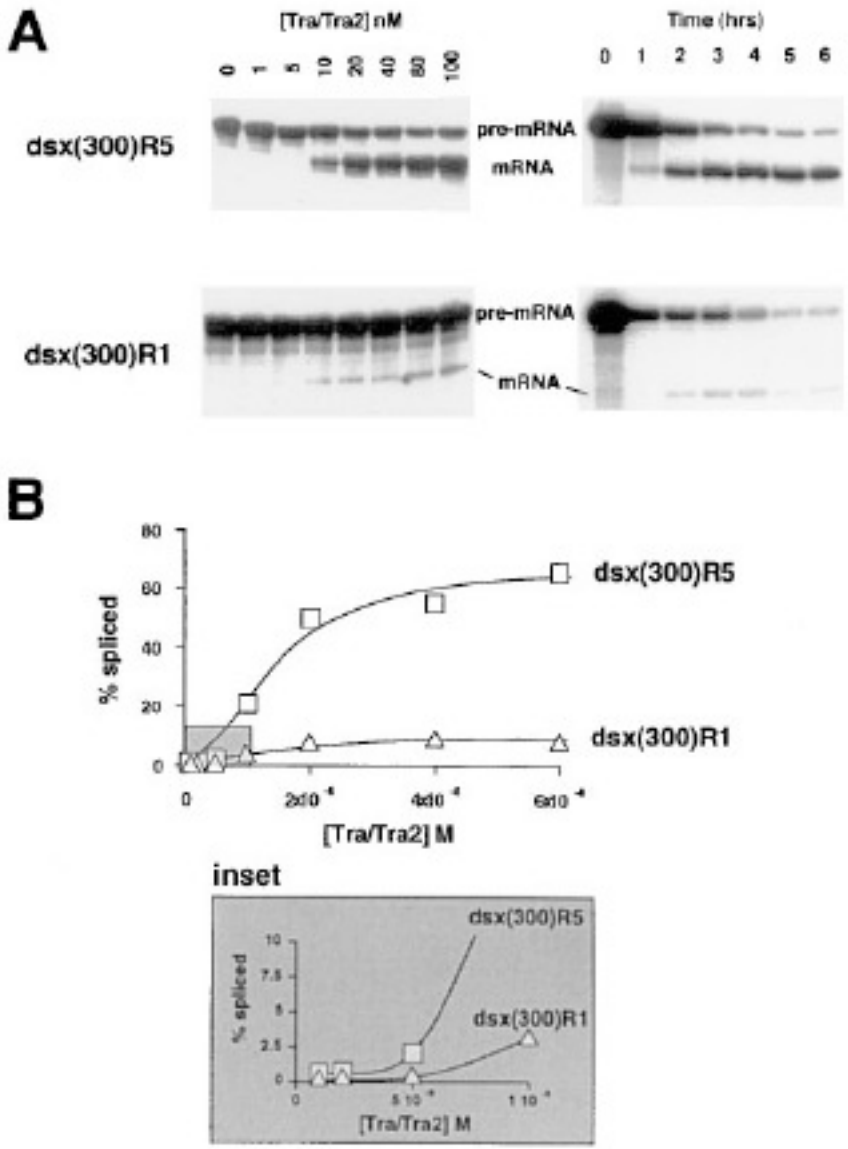


Figure 1. The Sex-Specific Splicing Pattern of the *Drosophila dsx* Pre-mRNA and the Organization of the *dsx* Splicing Enhancer (*dsx*RE)

(A) White boxes represent common exons 1, 2, and 3; the black box represents the female-specific fourth exon 4; and the gray boxes represent the two male-specific exons (5 and 6). Lines between the boxes represent introns; lines above and below the pre-mRNA illustrate the female- and male-specific splicing patterns. Sites of cleavage and polyadenylation are indicated by poly A.

(B) Organization of the female-specific splicing enhancer (*dsx*RE). The *dsx*RE is located approximately 300 nt downstream of the regulated 3′ splice site. Numbered boxes represent the six 13-nt repeat elements within the *dsx*RE. Consensus sequence of the repeat elements is shown below. The PRE is a purine-rich enhancer element that is distinct from the repeats.

(C) The structure of the *dsx* minigene substrates used in the in vitro splicing studies.



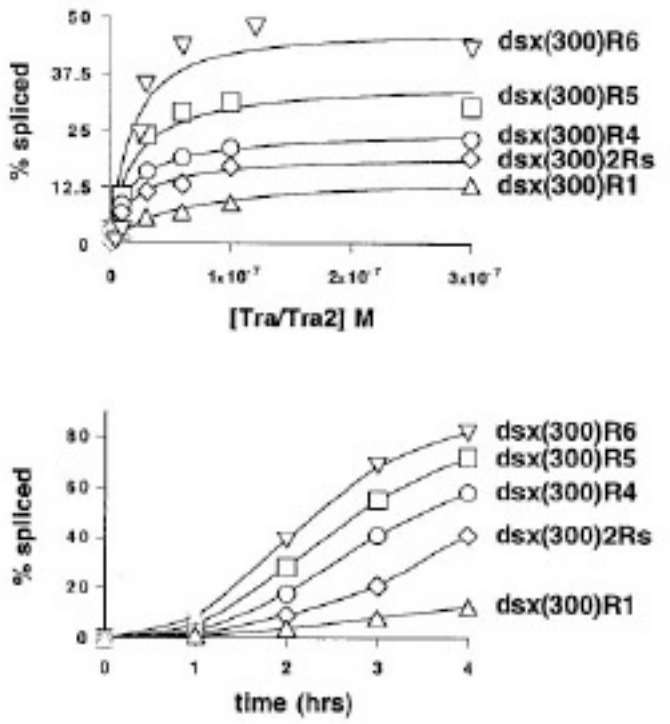


Figure 2. Splicing Efficiencies of Various Minigene Substrates as a Function of Tra/Tra2 Concentration or Time

(A) Splicing data for the substrates dsx(300)R5 and dsx(300)R1 after 2.5 hr incubation at various Tra/Tra2 concentrations and at 100 nM Tra/Tra2 over a time period of 6 hr.

(B) Quantitation of the data for the Tra/Tra2 titration in (A). Note the sigmoidal character for both substrates in the lower Tra/Tra2 concentration range (inset). The final extent of the fraction substrate spliced for dsx(300)R5 is reproducibly ~5-fold greater than for dsx(300)R1.

(C) Splicing efficiencies of all tested substrates as a function of Tra/Tra2 concentration.

(D) Kinetics of splicing for the substrates tested at Tra/Tra2 concentrations of 100 nM each.

Discussion

Recruitment of the Splicing Machinery

Transcriptional synergy has been observed with multisite transcriptional enhancers under conditions in which the binding sites are fully occupied by activator proteins (Carey et al., 1990; Lin et al., 1990; Choy and Green, 1993; Ohashi et al., 1994; Chi et al., 1995; Sauer et al., 1995). By contrast, our analysis of the effects of varying the number of *dsx* repeat sequences on the rate of splicing reveals a very different picture. As the number of repeats was increased an additive rather than synergistic increase in the efficiency and rate of splicing was observed. Even in the case where nonidentical enhancer elements were used to activate a weak 3′ splice site, additive rather than synergistic activation was observed. These results have two interesting mechanistic implications summarized in Figure 4B. First, there seems to be only one target for the enhancer complex. Based on previous studies this target might be the 35 kDa subunit of U2AF (Zuo and Maniatis, 1996). Second, the function of multiple repeats is to increase the probability of an interaction between an individual repeat and general splicing factors at the 3′ splice site rather than to facilitate the assembly of the *dsx*RE.

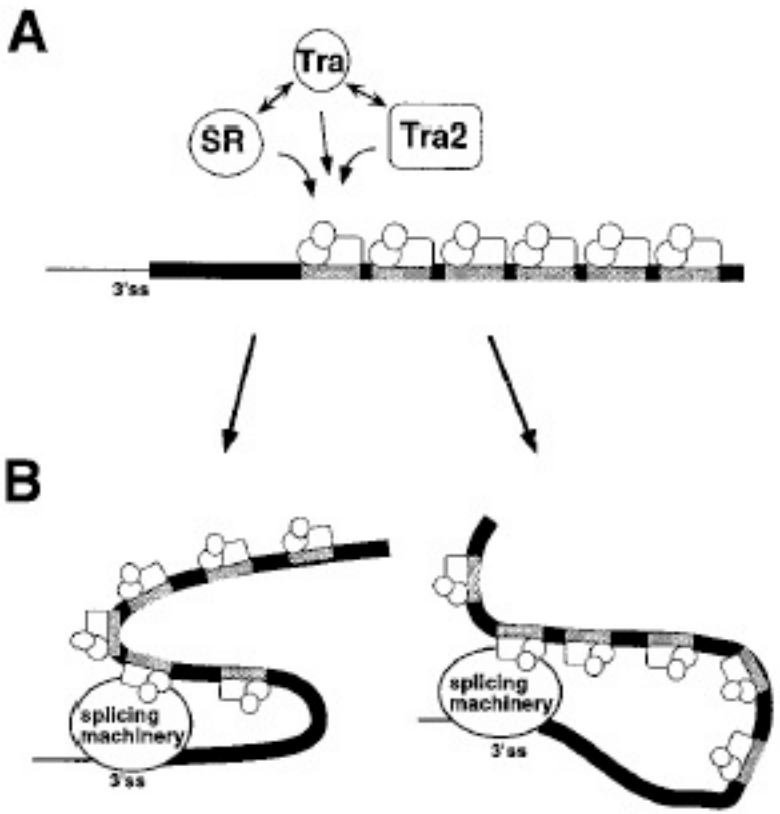


Figure 4. Tra/Tra2-Dependent Splice Site Activation of the *dsx* Pre-mRNA

(A) The sigmoidal character of Tra/Tra2 binding suggests highly cooperative interactions between Tra/Tra2 and factors contained within the nuclear extract. Since a Tra/Tra2/SR complex bound to an individual repeat does not significantly influence the formation of complexes on any other repeat elements, each repeat within the *dsx*RE can be described as an independent binding site.

(B) The additive character of the Tra/Tra2-dependent splicing kinetics suggests that only one complex at a time is capable of interacting with the splicing machinery. With only one target site available, no cooperativity is observed at the level of enhancer-dependent recruitment of the splicing machinery to the regulated, weak 3′ splice site.

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Arginine/Serine-Rich Domains of SR Proteins Can Function as Activators of Pre-mRNA Splicing

Brenton R. Graveley and Tom Maniatis*

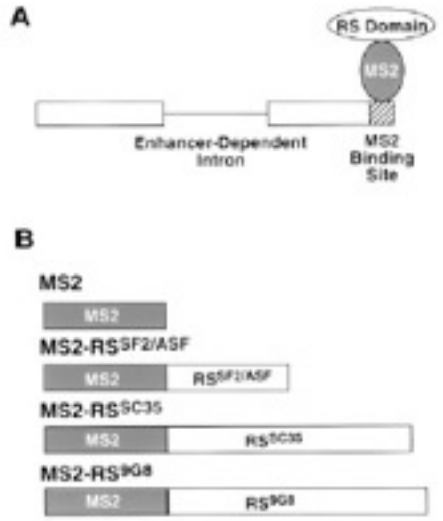
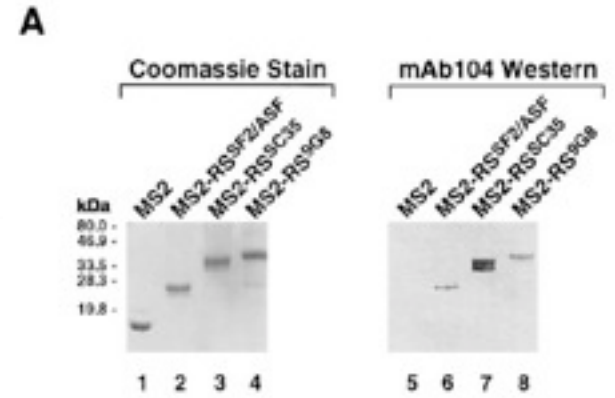


Figure 1. Experimental Strategy and Design

(A) Schematic diagram of experimental approach. A pre-mRNA splicing substrate is generated containing an enhancer-dependent intron in which the splicing enhancer is replaced by a binding site for the MS2 bacteriophage coat protein. In parallel, a hybrid protein is generated containing MS2 and an RS domain.

(B) Domain structure of the proteins generated in this study. MS2-$RS^{SF2/ASF}$, MS2-RS^{SC35}, and MS2-RS^{9G8} contain the RS domains of SF2/ASF (amino acids 198–248 [Cáceres and Krainer, 1992; Zuo and Manley, 1992]), SC35 (amino acids 117–221 [Fu and Maniatis, 1992]), and 9G8 (amino acids 123–238 [Cavaloc et al., 1994]), respectively, fused C-terminal of MS2.



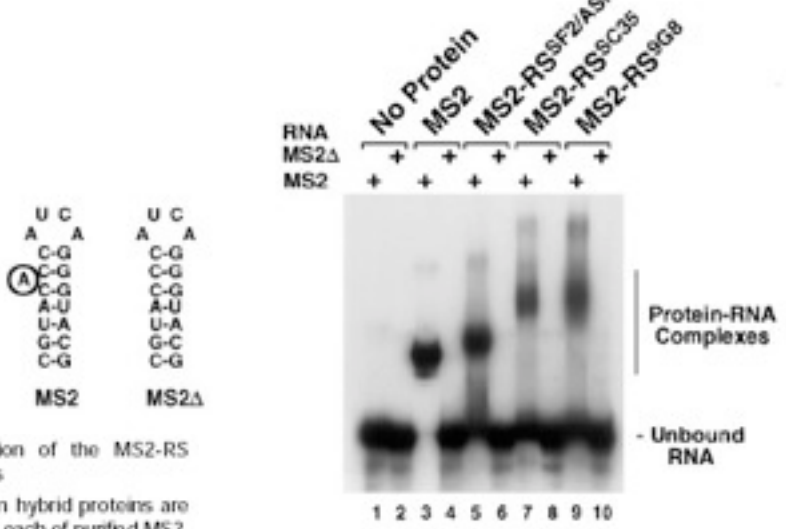


Figure 2. Characterization of the MS2-RS Domain Hybrid Proteins

(A) The MS2-RS domain hybrid proteins are phosphorylated. One μg each of purified MS2, MS2-$RS^{SF2/ASF}$, MS2-RS^{SC35}, and MS2-RS^{9G8} were separated on a 15% SDS–polyacrylamide gel and either stained with Coomassie brilliant blue (left) or probed with MAb 104 (right).

(B) The MS2-RS domain hybrid proteins bind specifically to MS2 RNA. Two 40 nt RNAs were generated that contained a wild-type (MS2) or mutant (MS2Δ) MS2 binding site. MS2Δ lacks a bulged adenosine required for MS2 binding (Carey et al., 1983). The sequences and structures of the relevant portions of these RNAs are shown on the left. Approximately 100 ng each of MS2, MS2-$RS^{SF2/ASF}$, MS2-RS^{SC35}, and MS2-RS^{9G8} was incubated with the MS2 or MS2Δ RNAs, and the reactions were resolved on a native polyacrylamide gel.

Genes que tienen splicing enhancer-dependientes

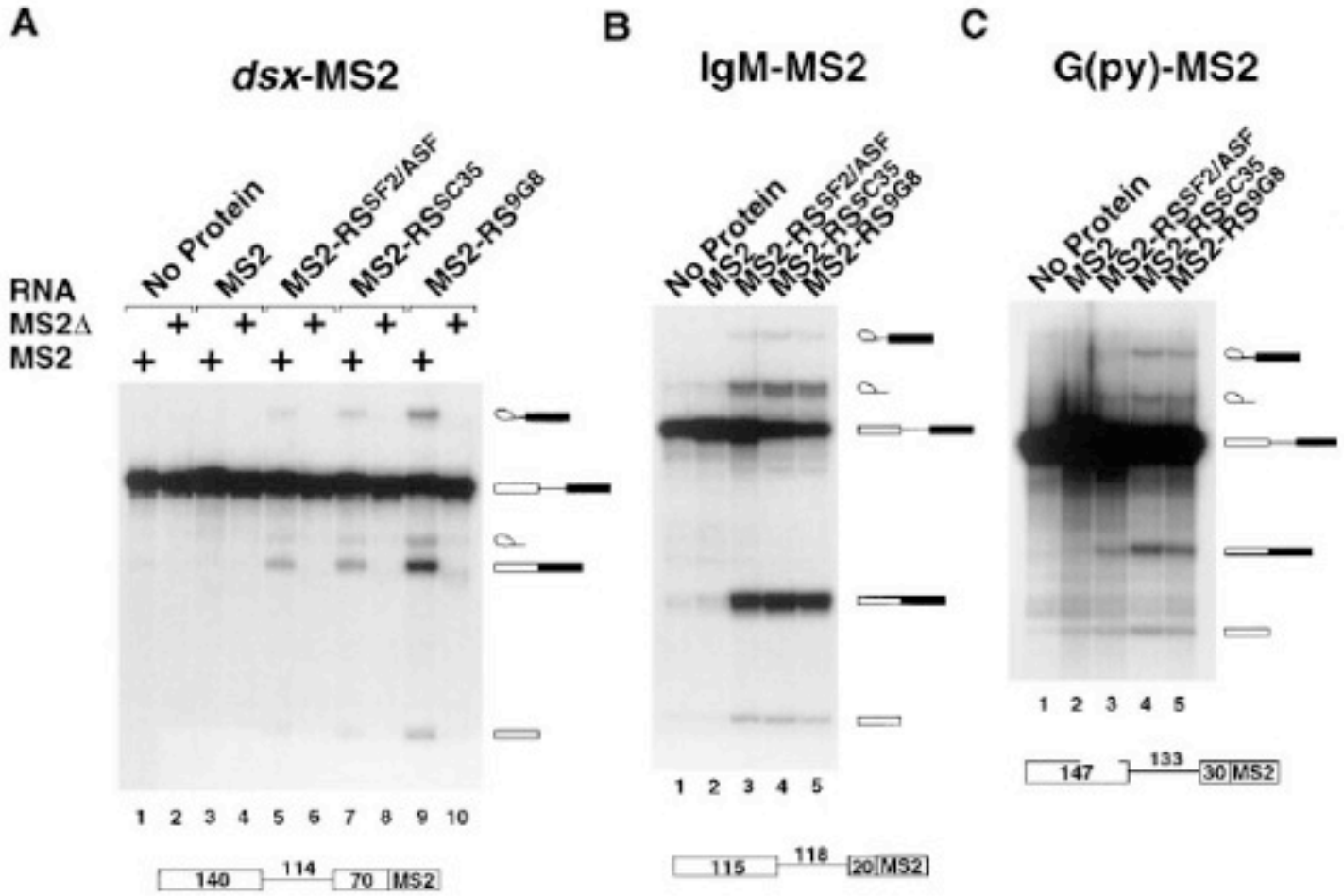


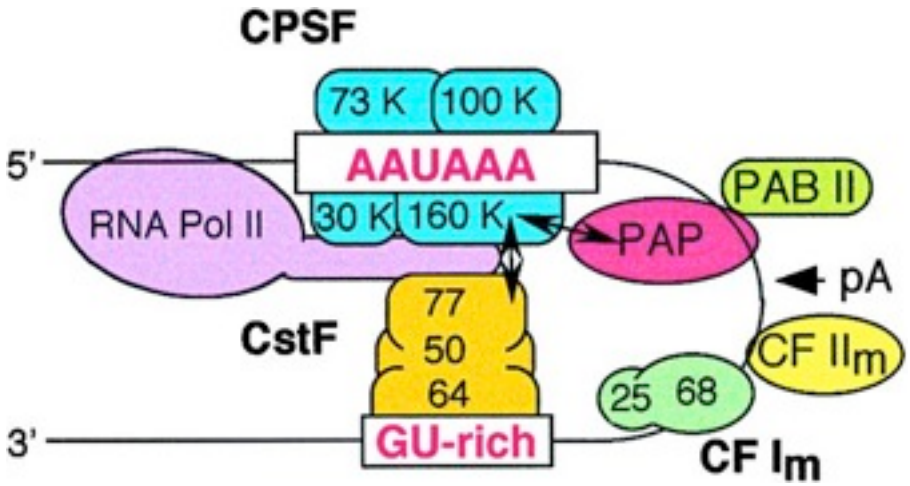
Figure 3. MS2-RS Domain Hybrid Proteins Can Functionally Substitute for a Splicing Enhancer When Bound to RNA

Splicing reactions were carried out in HeLa cell nuclear extract using RNA substrates containing an MS2 binding site downstream of the weak 3′ splice sites of the *D. melanogaster doublesex* (*dsx*-MS2) (A), mouse immunoglobulin μ (IgM-MS2) (B), and human β-globin (G(py)-MS2) (C) pre-mRNAs. In vitro splicing reactions contained 15% HeLa nuclear extract either alone or supplemented with approximately 100 ng of MS2, MS2-$RS^{SF2/ASF}$, MS2-RS^{SC35}, or MS2-RS^{9G8}. The reactions were carried out for 2 hr at 30°C, and the reaction products resolved on 12% denaturing polyacrylamide gels. The *dsx*-MS2Δ pre-mRNA contains the mutant MS2 binding site in which the single bulged adenosine required for MS2 binding has been deleted. Identities of the spliced products are indicated.

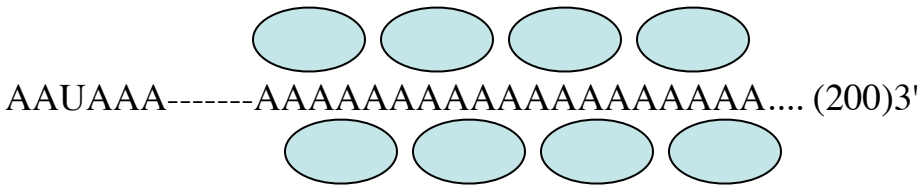
Poliadenilación: Estabilidad + transporte de mRNA (Entender la estructura de un gen eucariótico)

RNA Pol II: multiples sitios de término río abajo de la secuencia AAUAAA (11-30 bases 5' del corte y agregado de poliA.

3' generado por:
-Endonucleasa CF I, CF II
y CstF (cleavage specificity factor)
-Poli (A) polimerasa (PAP)
-CPSF: une AAUAAA



PAB: poly(A) binding protein



*Prot NS-1 del virus influenza secuestra CPSF, PAB II