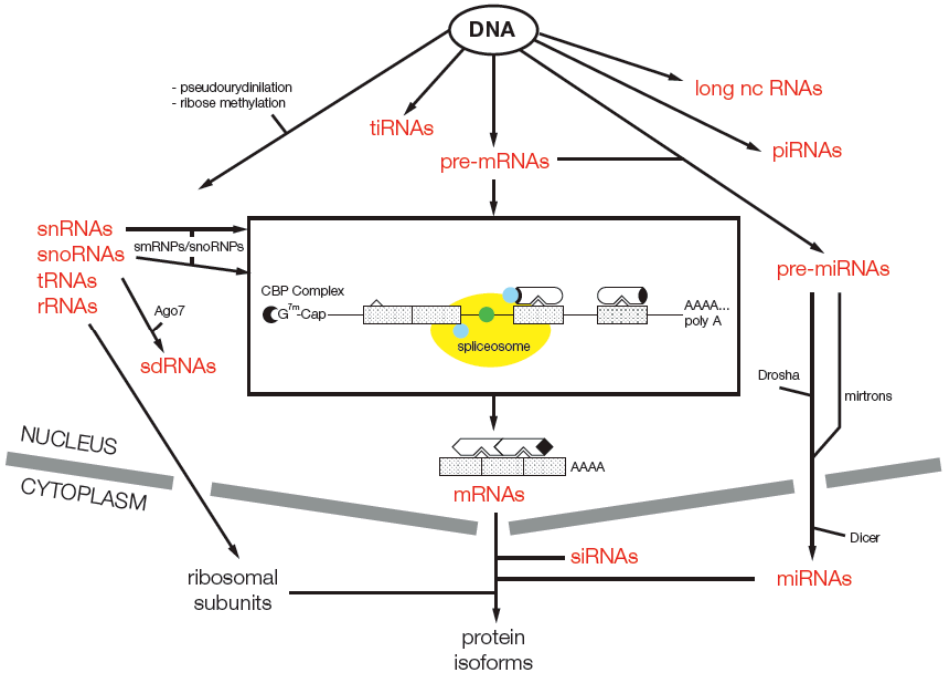
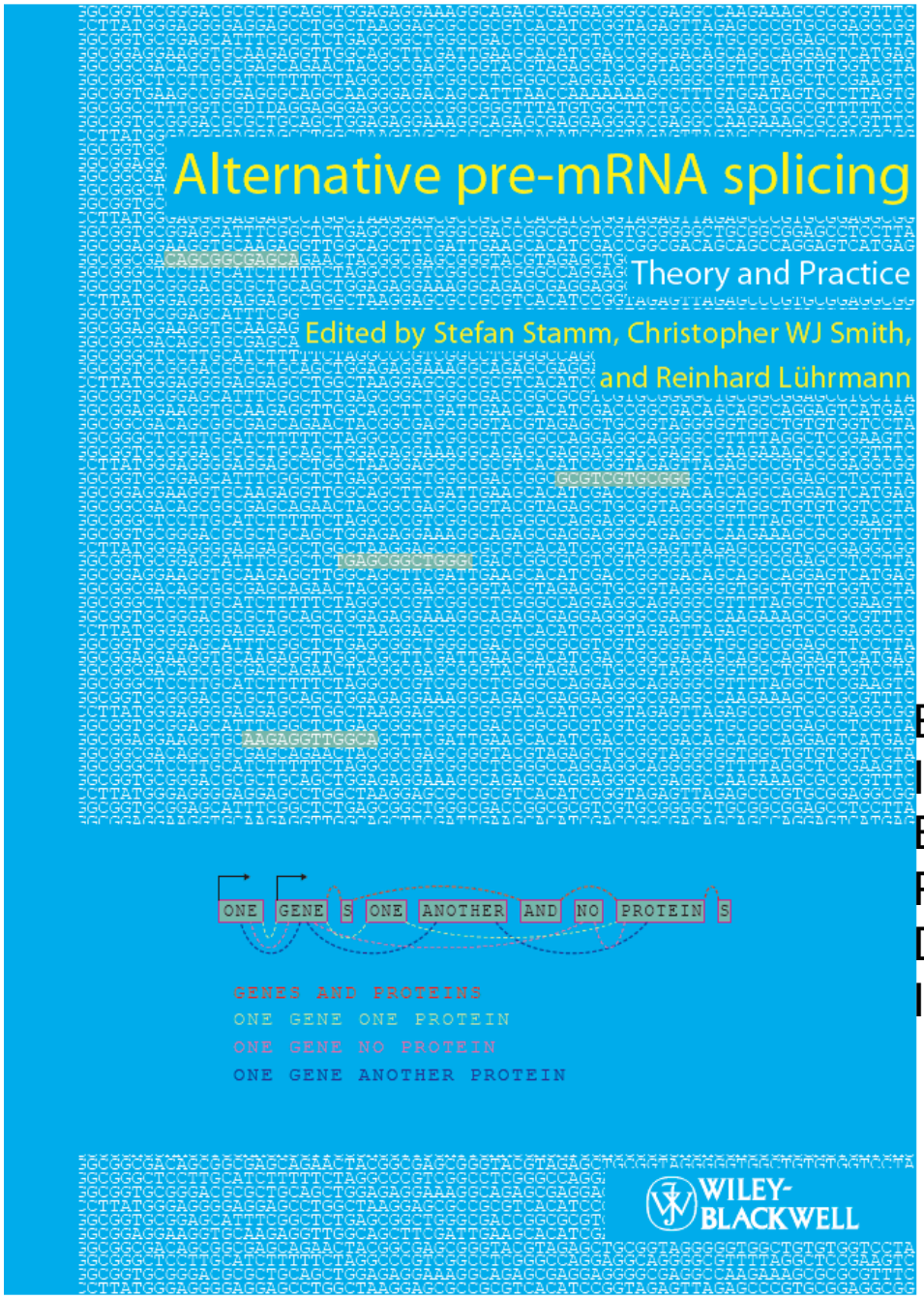
Life of a mRNA

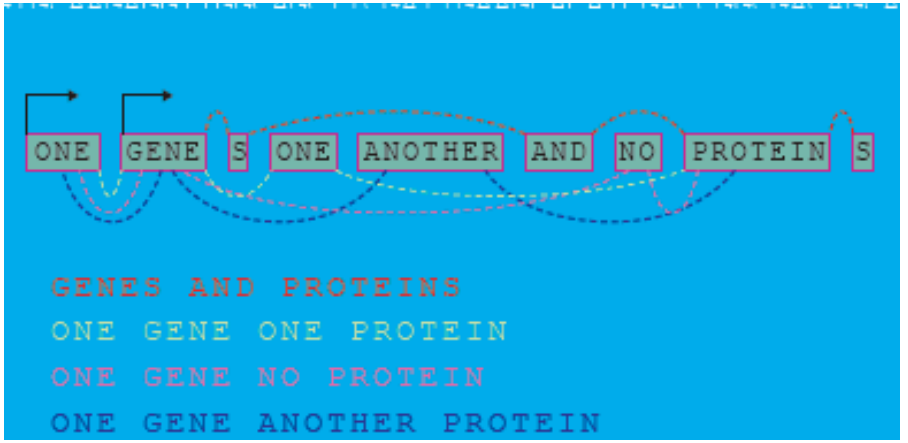
Pre-mRNA processing is coupled to transcription, RNA export, translation and degradation. Pre-mRNA processing allows to increase the concentration content of DNA

Transcription
Capping
Pre-mRNA (alternative) splicing
polyAdenylation
RNA degradation





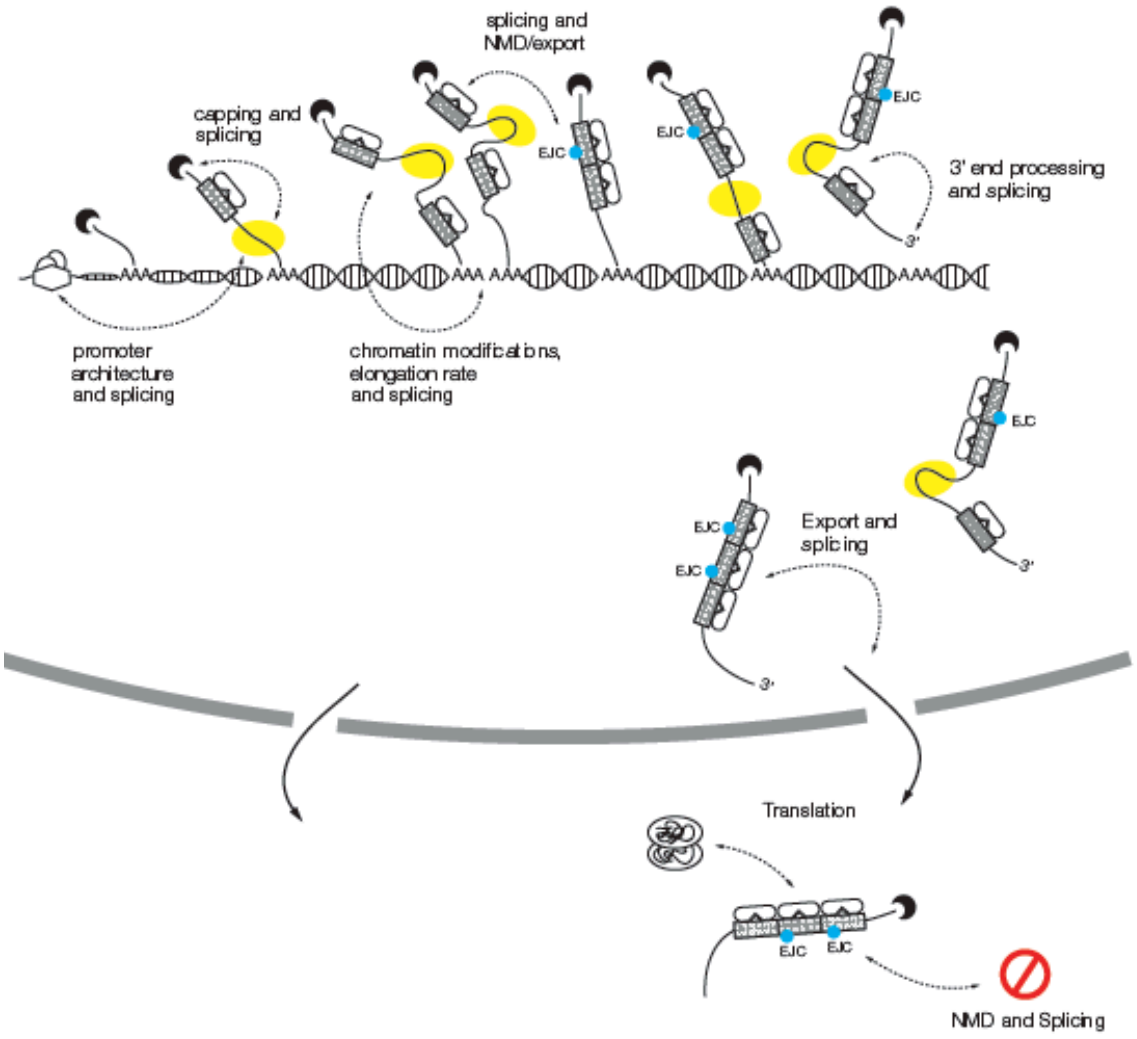
Function of alternative splicing



- Binding properties
- Intracellular localisation
- Enyzmatic and signaling activity
- Protein stability
- Domains with posttranslational modifications
- Ion channels

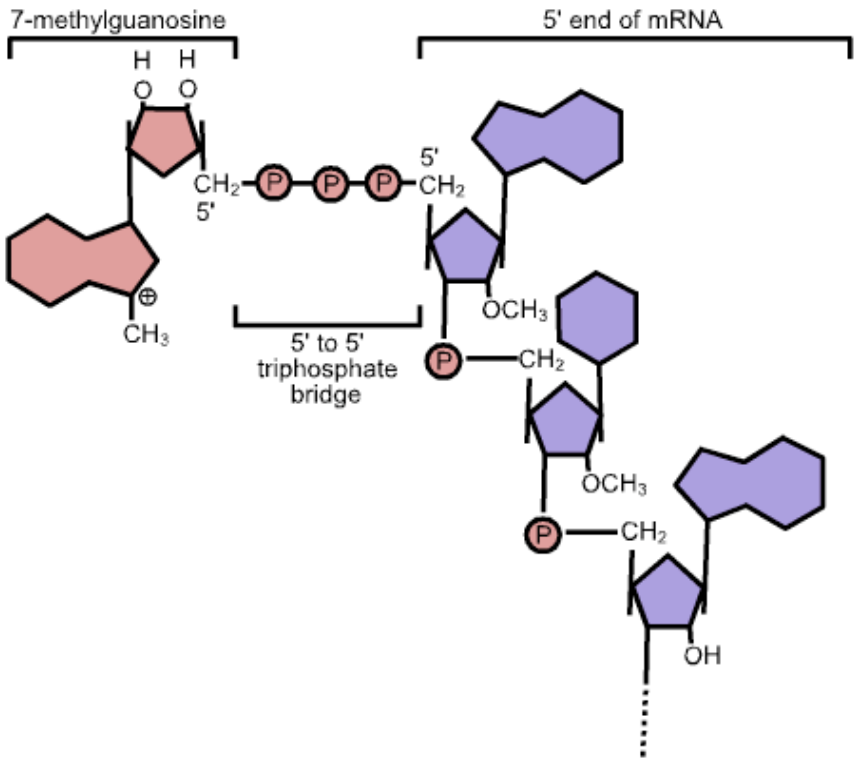
Function of alternative splicing. Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, Toiber D, Thanaraj TA, Soreq H. Gene. 2005 Jan 3;344:1-20. Epub 2004 Dec 10. Review.

RNA processing steps are coupled to transcription



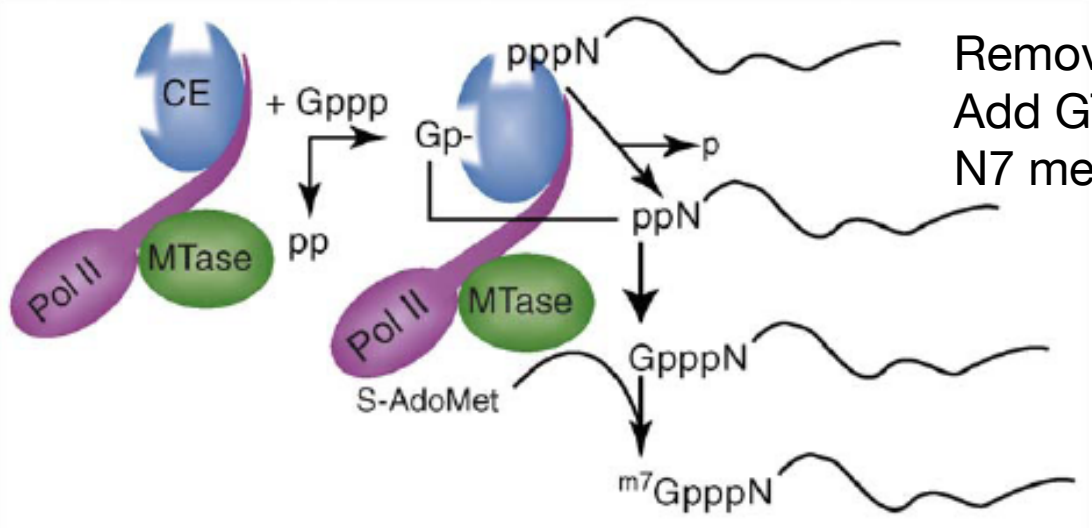
When testing splicing (RNA metabolism) in vivo, there are indirect effects

Capping



Cap 0, cap1, cap 2 Methyl groups from 5’ to 3’

Mechanism of cap synthesis

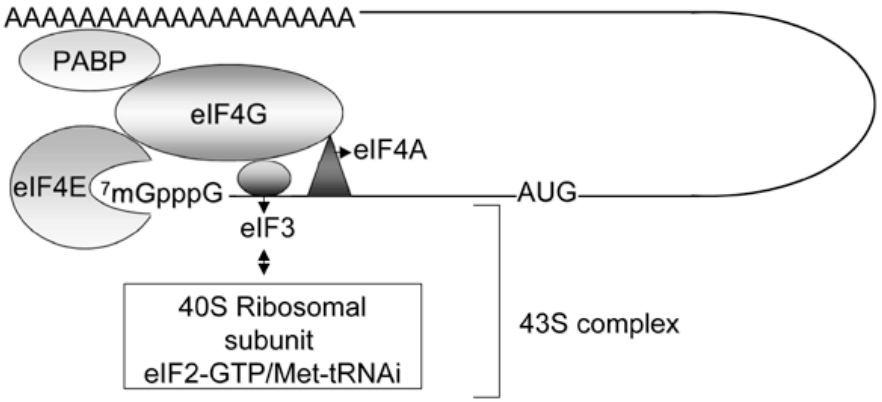


Remove one of three terminal phosphates
Add GTP
N7 methylate the G residue

CE: Capping enzyme
Mtase: methylase
S-Ado Met: S-adenosyl methionine

Where does capping occur ?

Function of the cap



CBP: cap binding protein

RNA stability

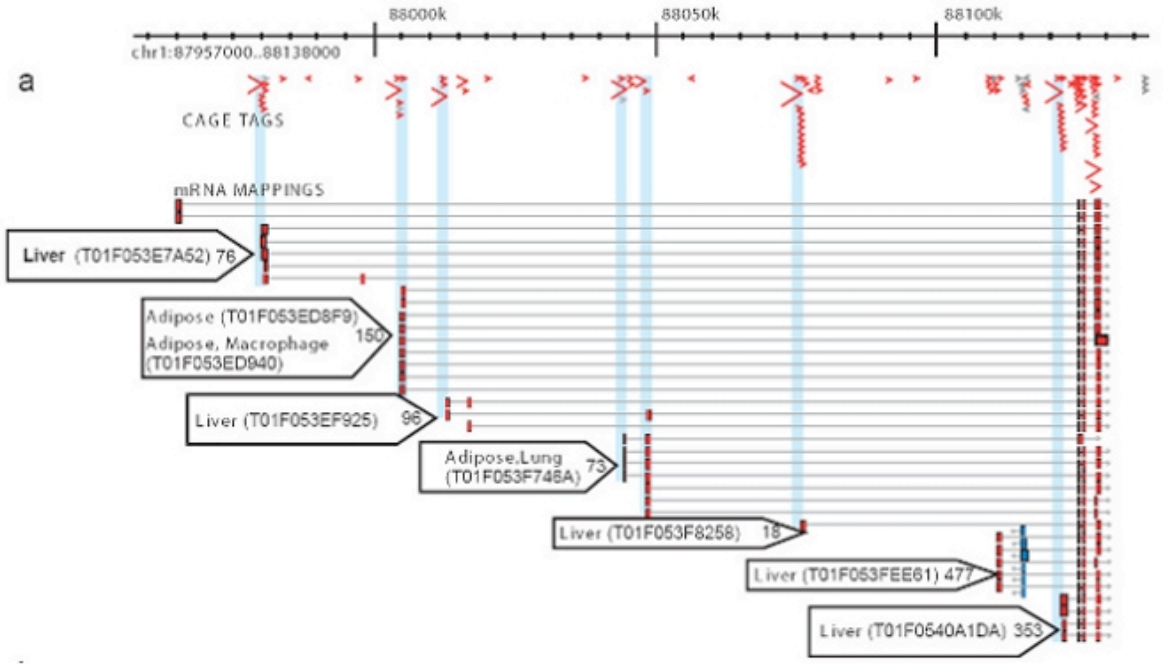
RNA splicing

RNA polyadenylation

RNA nucleocytoplasmatic transport

RNA translation

CAGE tags to map translational start sites

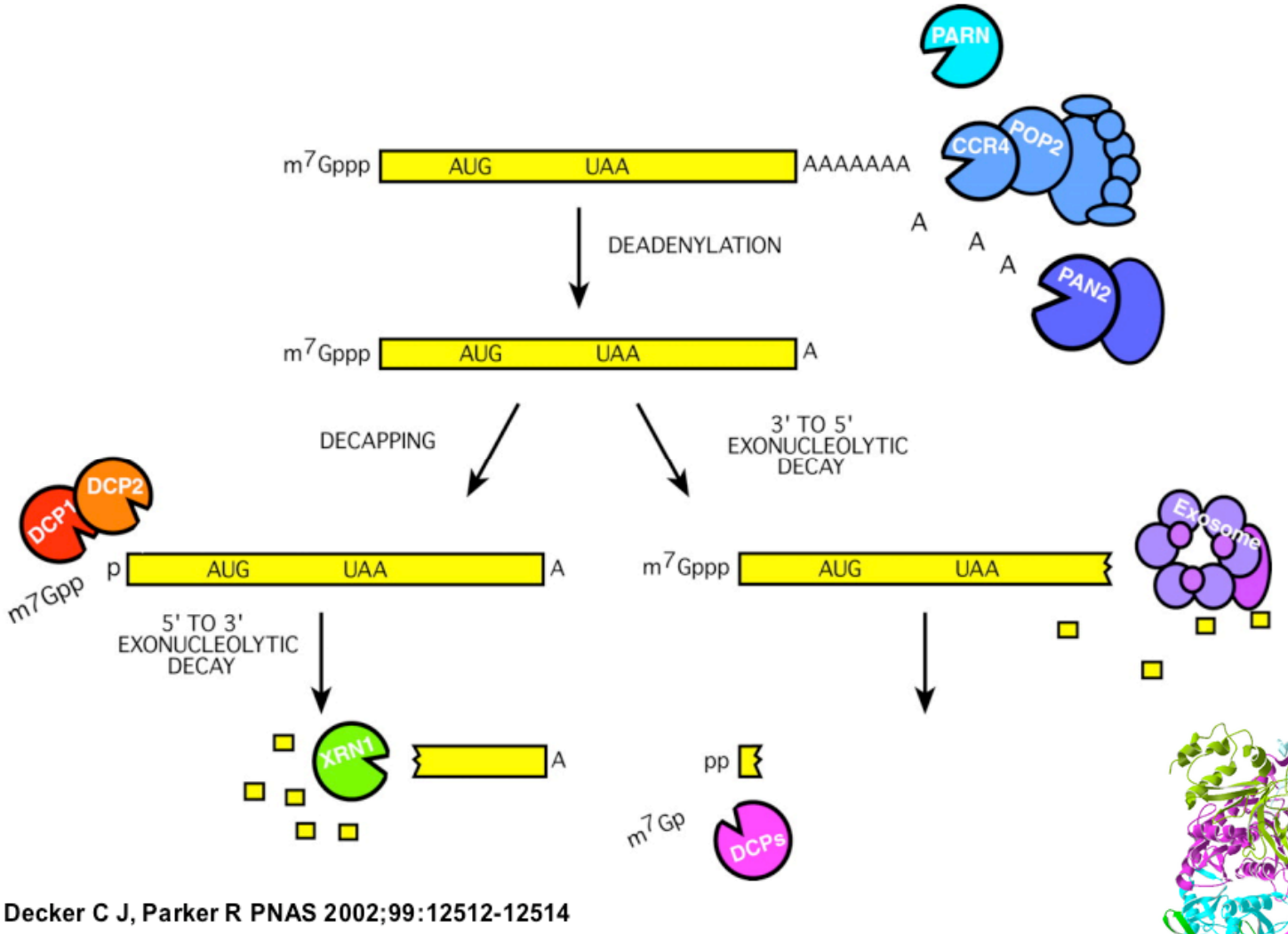


Depending on the source tissue analyzed, CAGE tags can be mapped to different positions in a ~100 kB upstream region of the mouse UDP-glucuronyl transferase gene, identifying different transcriptional start sites. The frequency of a given CAGE tag is a direct measure for the abundance of the respective transcript variant in the different tissues. (from Carninci et al., 2006)

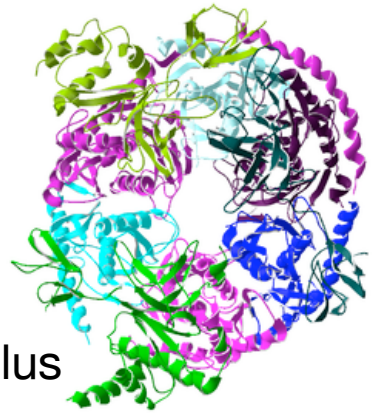
CAGE, cap analysis of gene expression

How would YOU clone capped, full length RNA?
Where do you find cage tags?

Uncapping

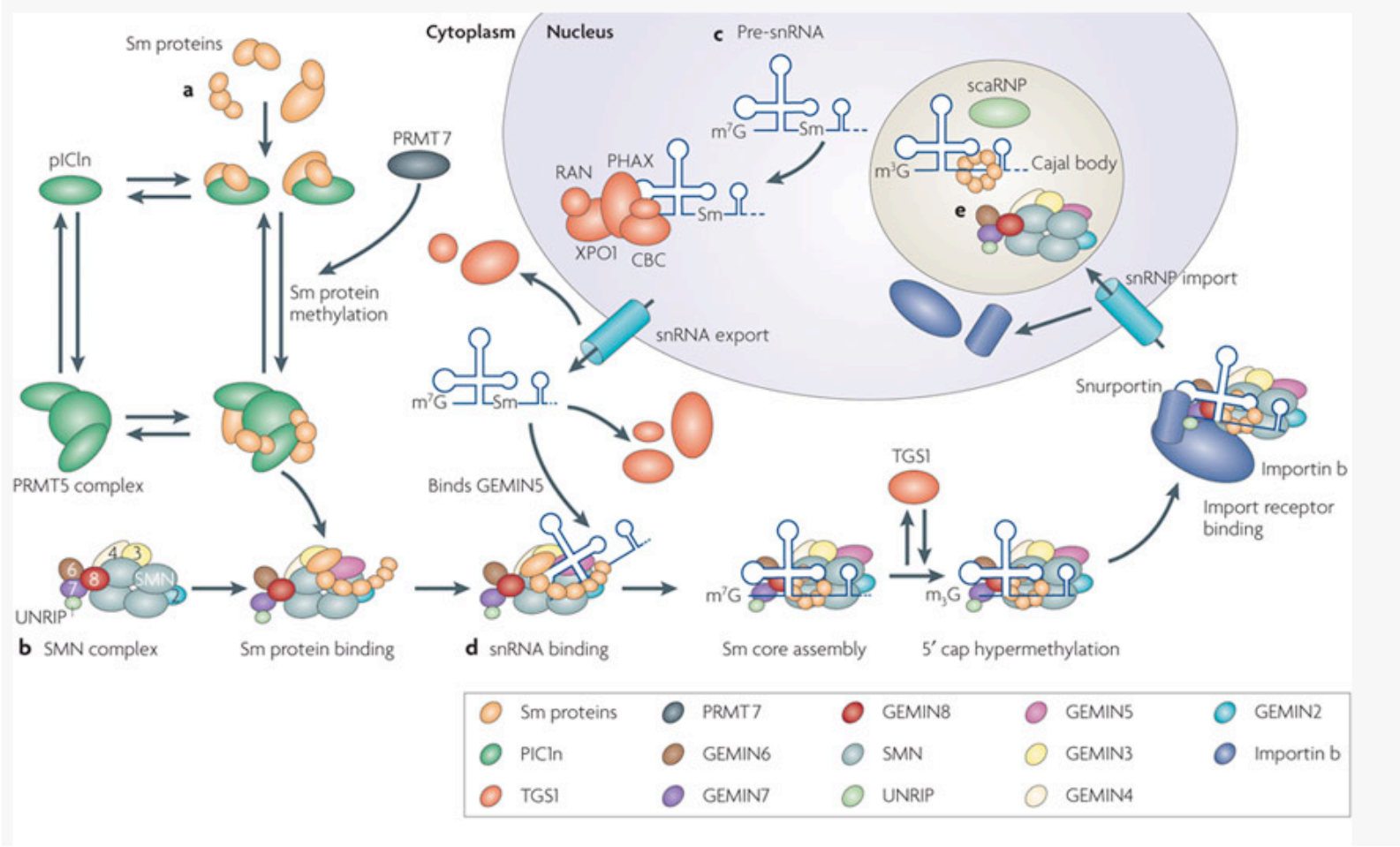


Decker C J, Parker R PNAS 2002;99:12512-12514



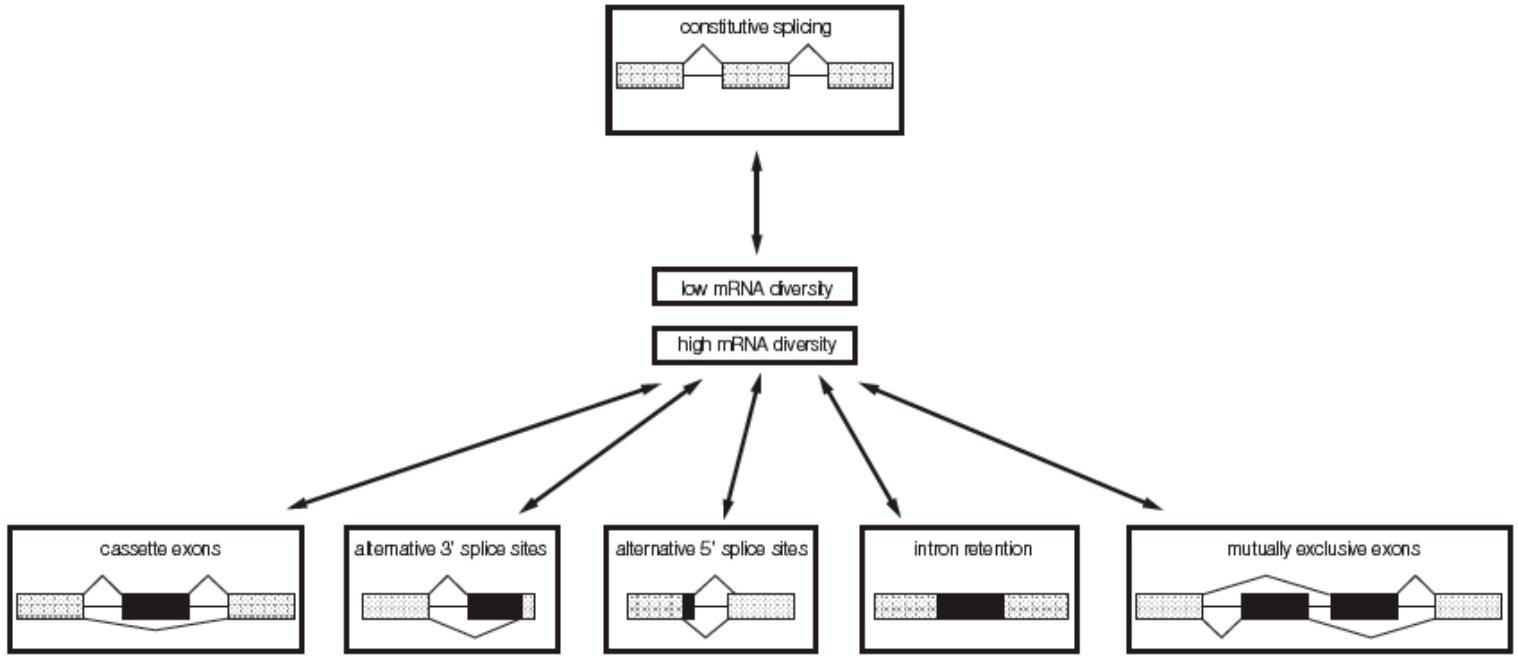
Exosome: RNA degradation complex, cytoplasma, nucleus, nucleolus

Trimethyl G cap: Capping in the cytosol



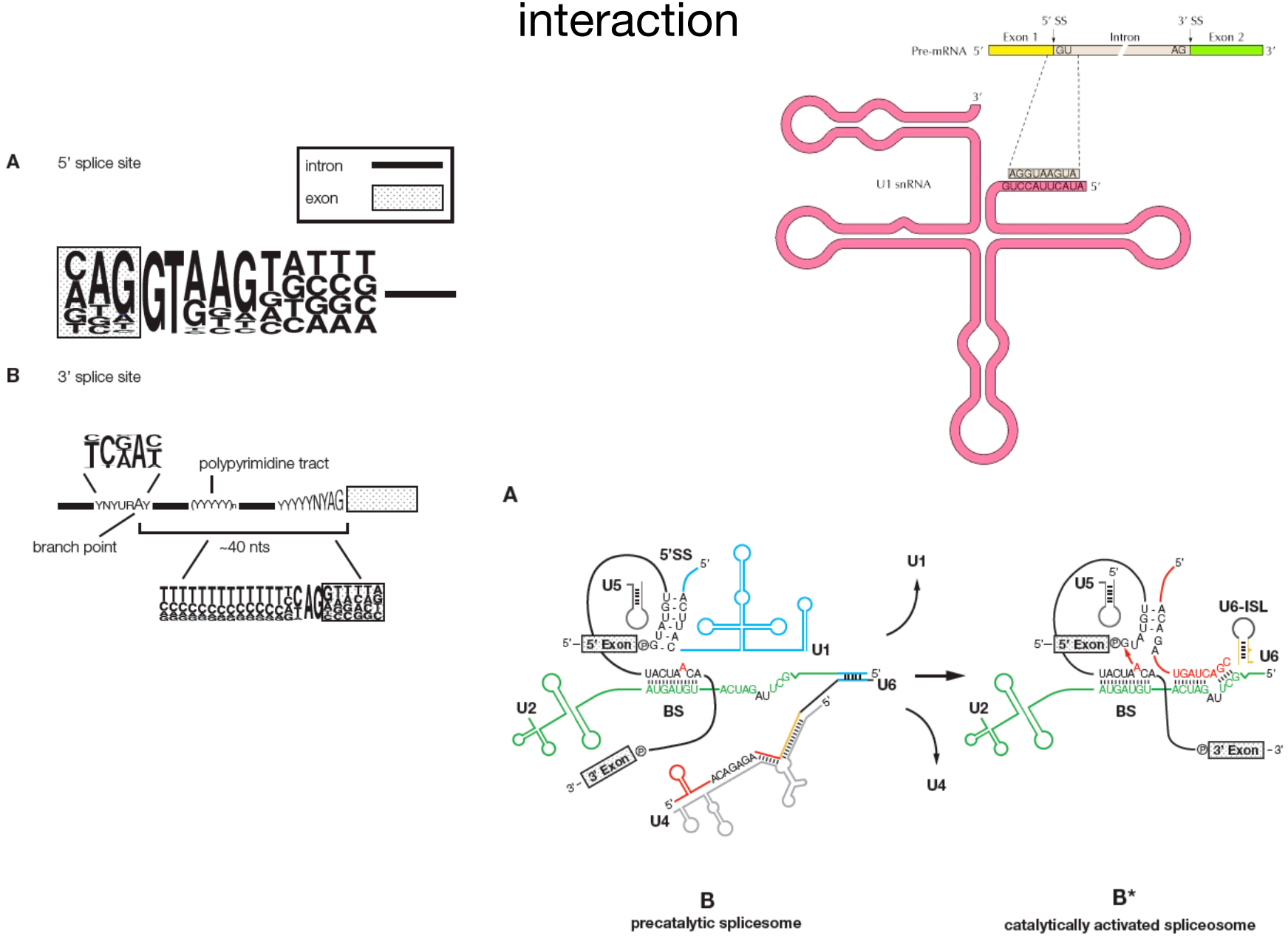
What is the function of the TMG cap?

(alternative) Splicing

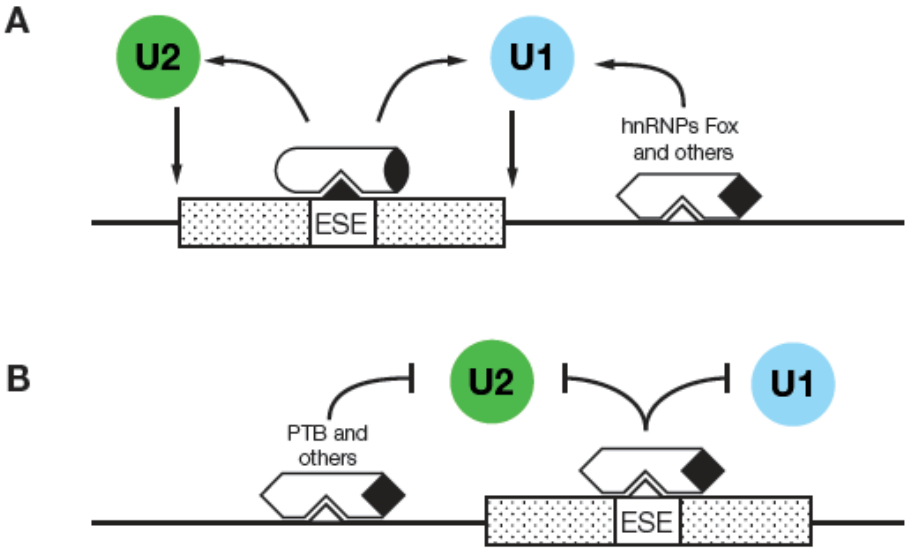


Almost all protein coding polII transcripts undergo splicing, >90% are alternatively splicing

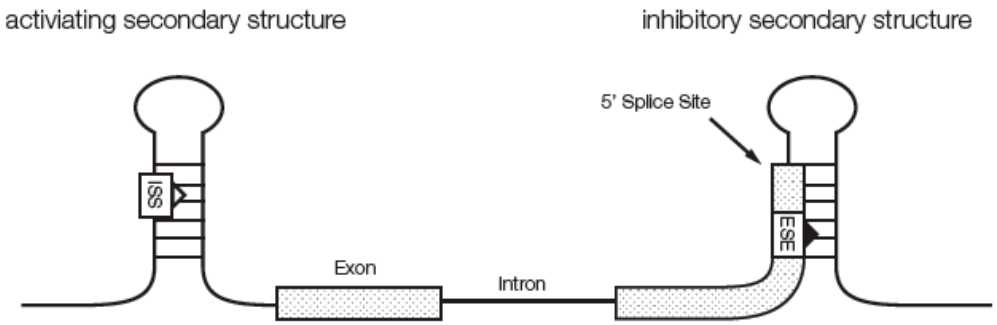
Splice sites are degenerate and are recognized by RNA:RNA interaction



Recognition of splice sites is aided by proteins binding to RNA

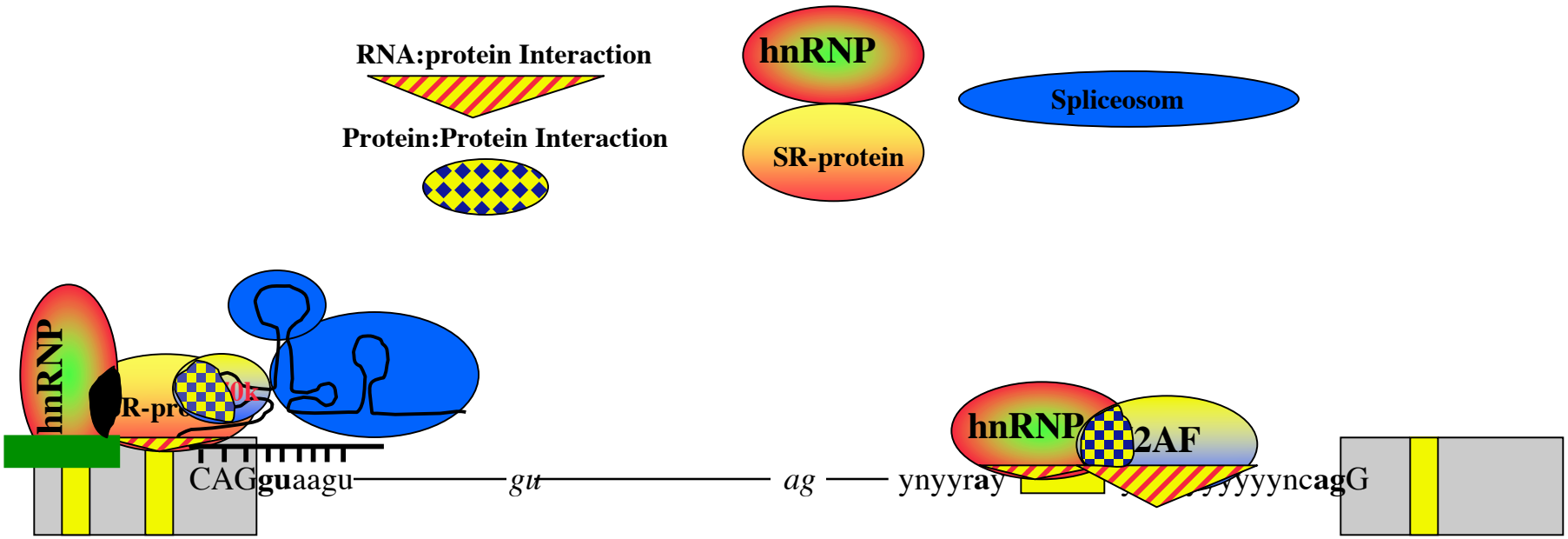


Exon definition model



Sequestration of regulatory sites in cis

Splicing complexes are transient



Combinatorial control, integration of weak protein:protein, RNA:RNA, protein:RNA interactions

- **Phosphorylation regulates splice site selection**
- **Small RNAs regulate the selection of splice sites**

Exon Recognition is determined by multiple elements

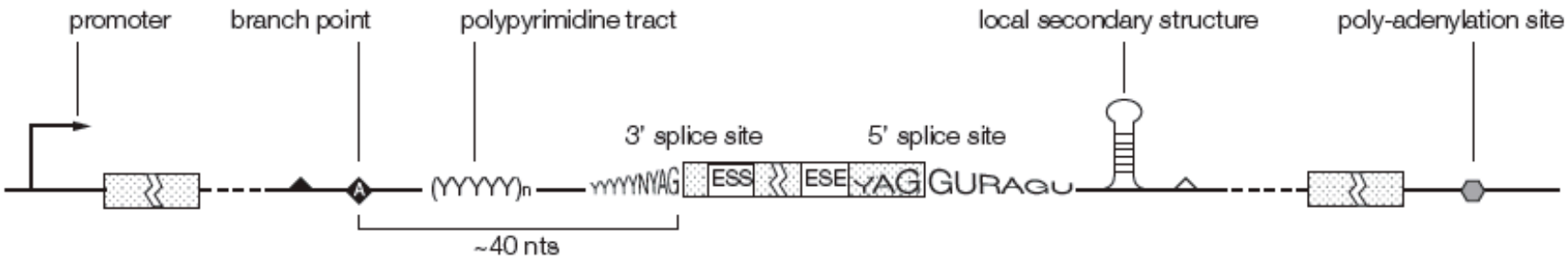
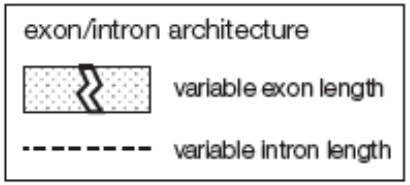


Table 4. Characterization of Dr. Venter's and Dr. Watson's exomes. Numbers for Dr. Watson's exome are taken from [20].

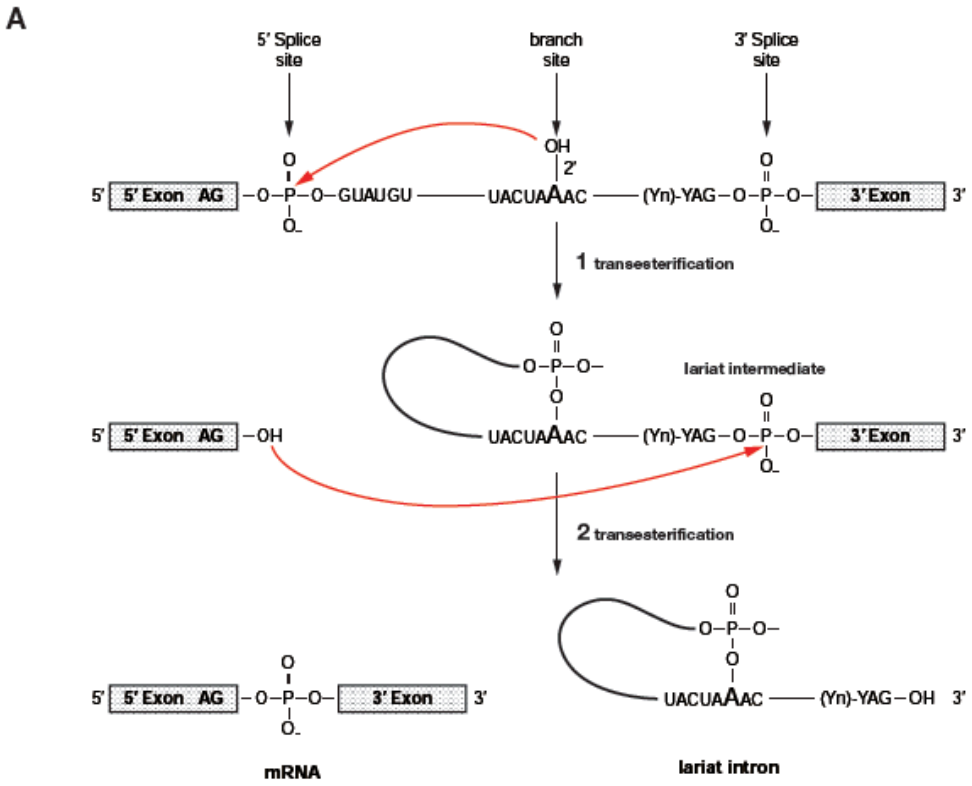
	Dr. Venter's Exome	Dr. Watson's Exome
Total Number of Nonsynonymous SNPs	10,389	10,569
Number of Novel Nonsynonymous SNPs	772 (7% of total nsSNPs)	1,573 (15% of total nsSNPs)
% nsSNPs predicted to affect protein function*	14% (7,781 predicted on)	20% (3,898 predicted on)
Number of Coding Indels	739	345**

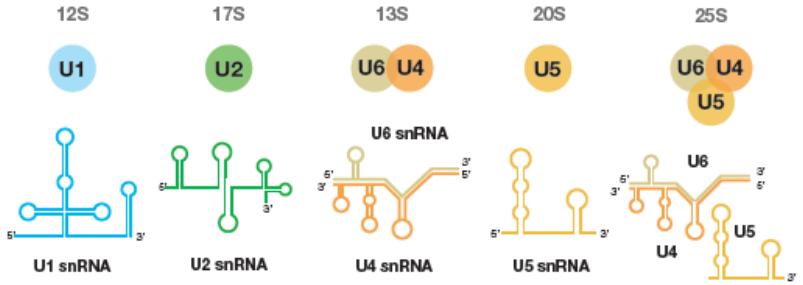
*Different prediction algorithms were used [30,33], and this may account for the difference between the two exomes.
**Indels of size 2 bp and greater were considered; 1 bp indels were discarded. If we removed 1 bp indels from Dr. Venter's exome in order to compare with Dr. Watson's exome, Dr. Venter would have 423 coding indels.
doi:10.1371/journal.pgen.1000160.t004

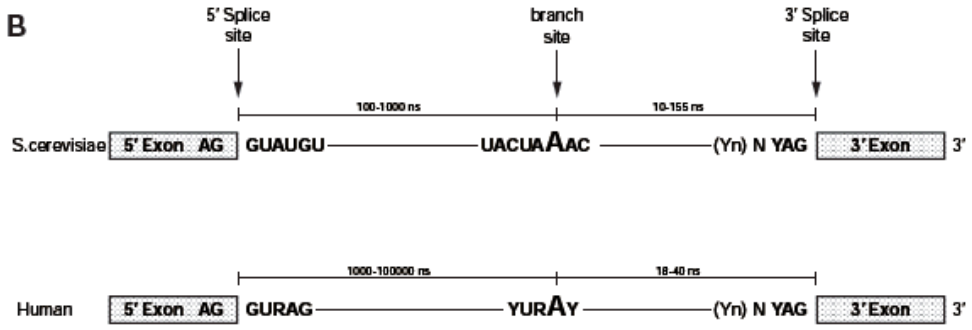


Numerous mutations have effect on pre-mRNA processing

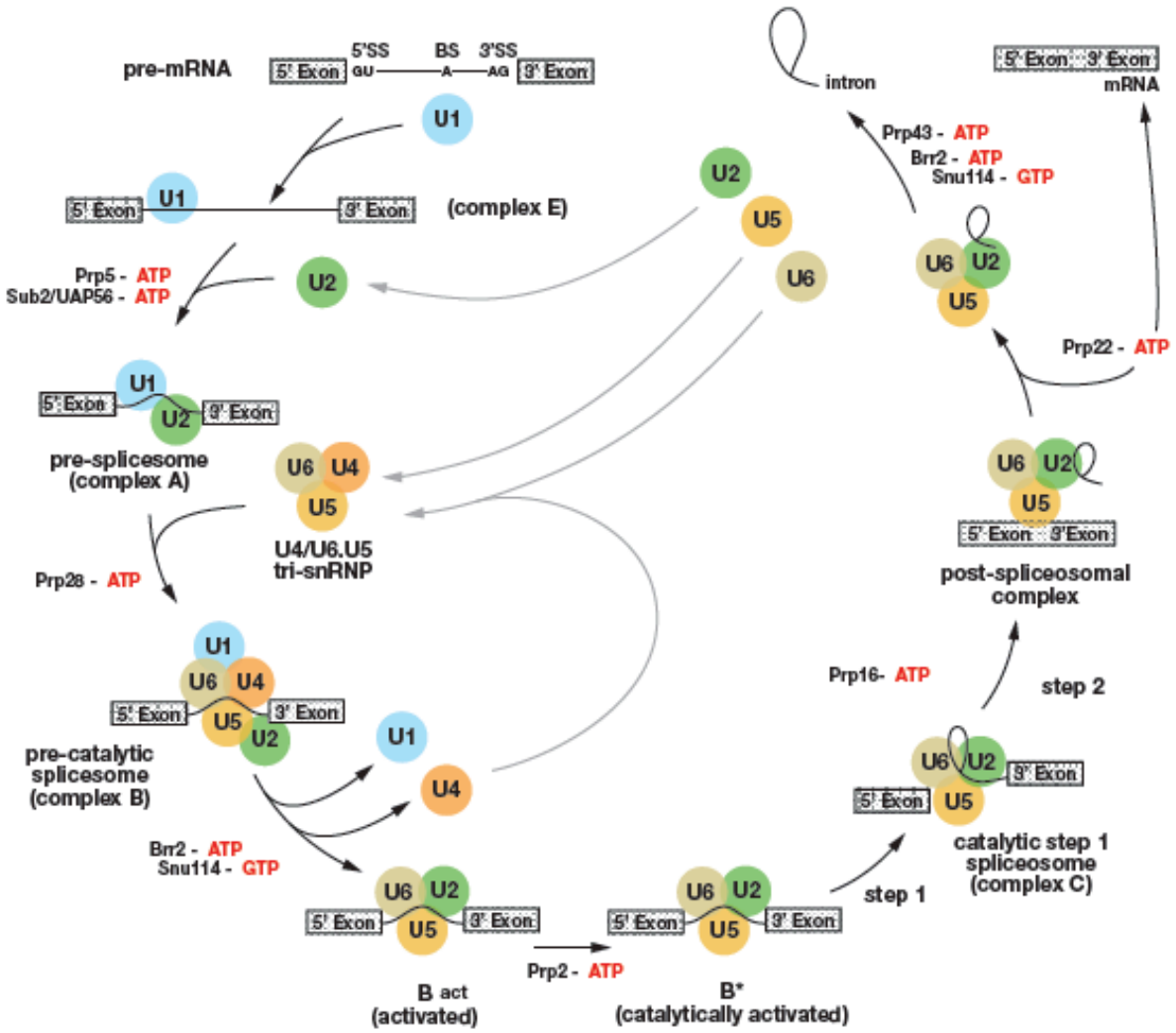
Splicing is carried out by the spliceosome

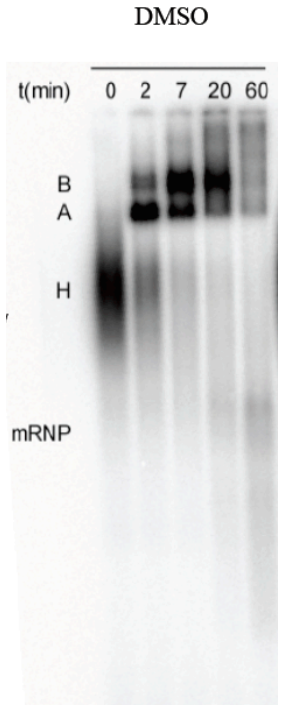






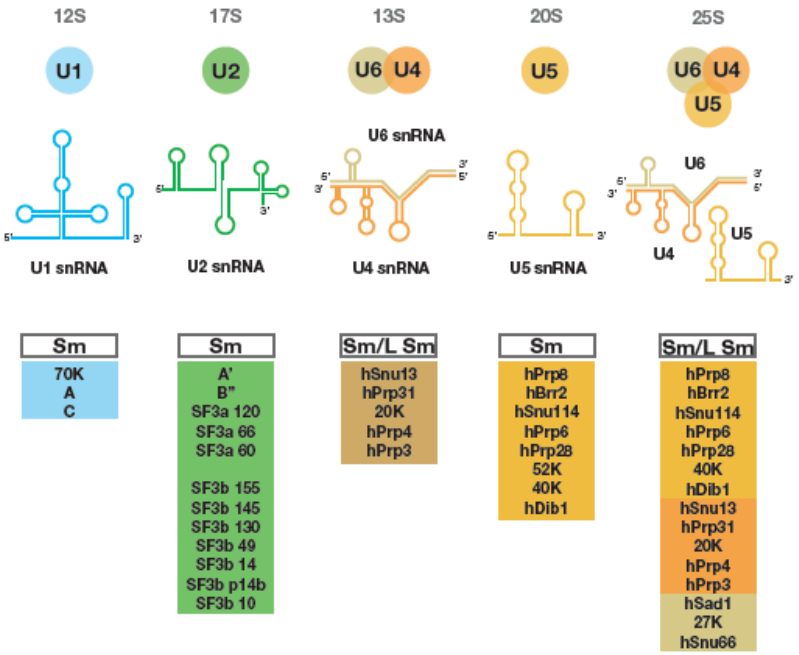
Spliceosomal cycle

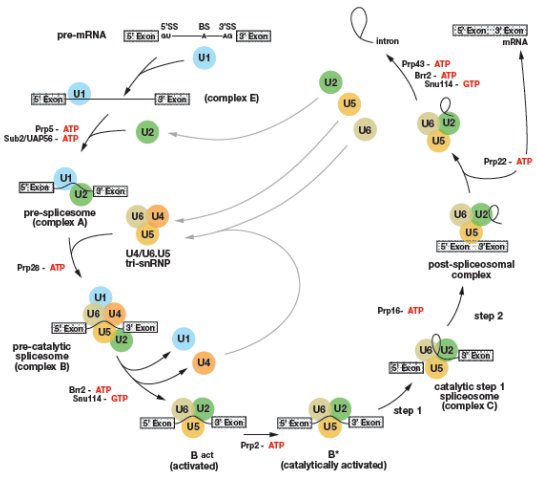




Looking at splicing complexes in a gel

RNAs are the backbone of the spliceosome





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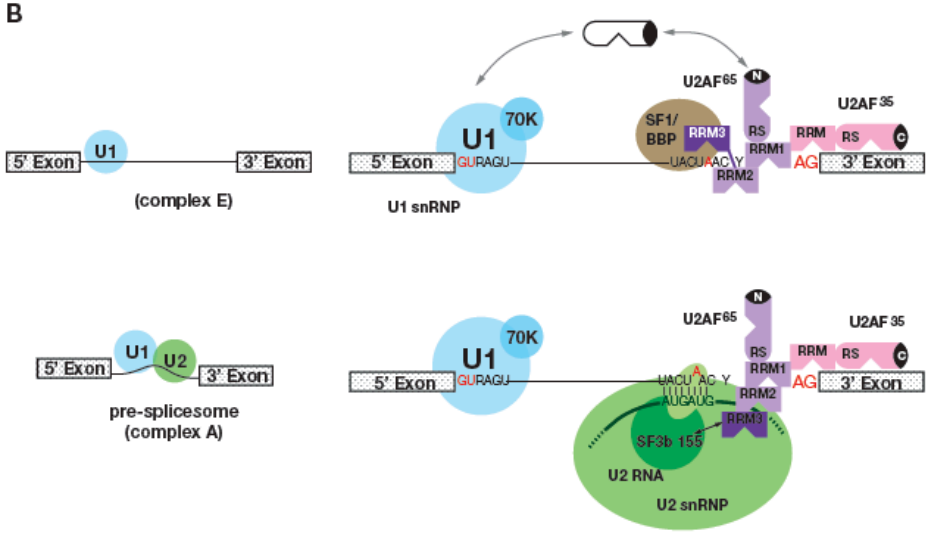
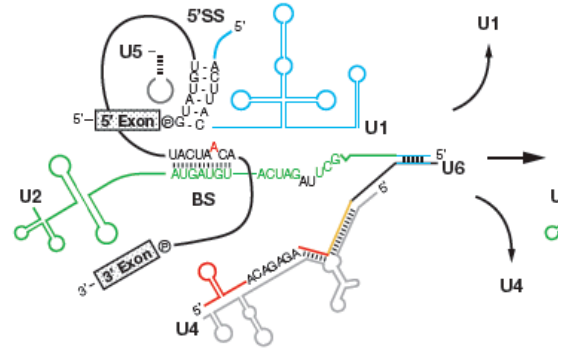
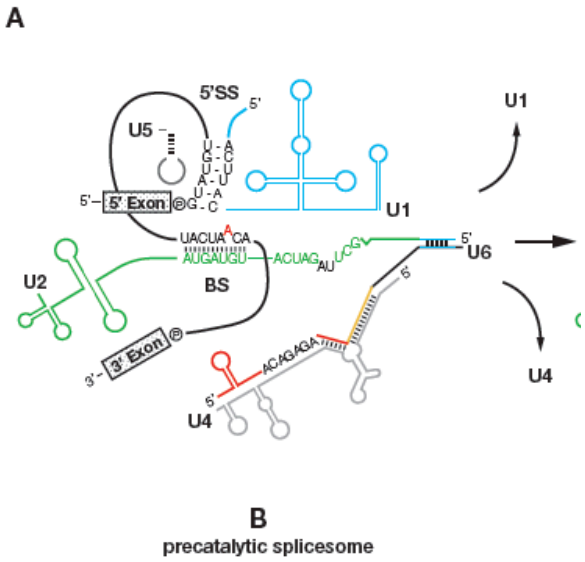
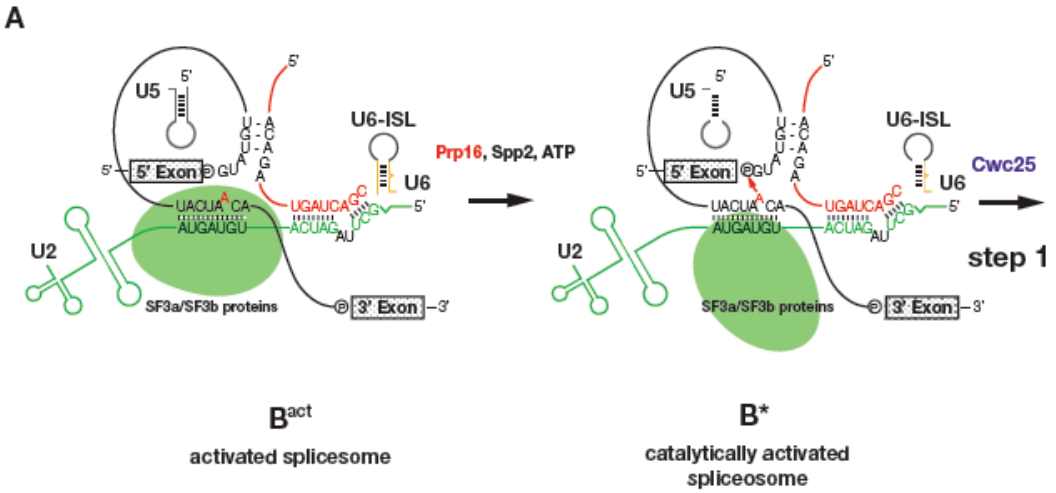


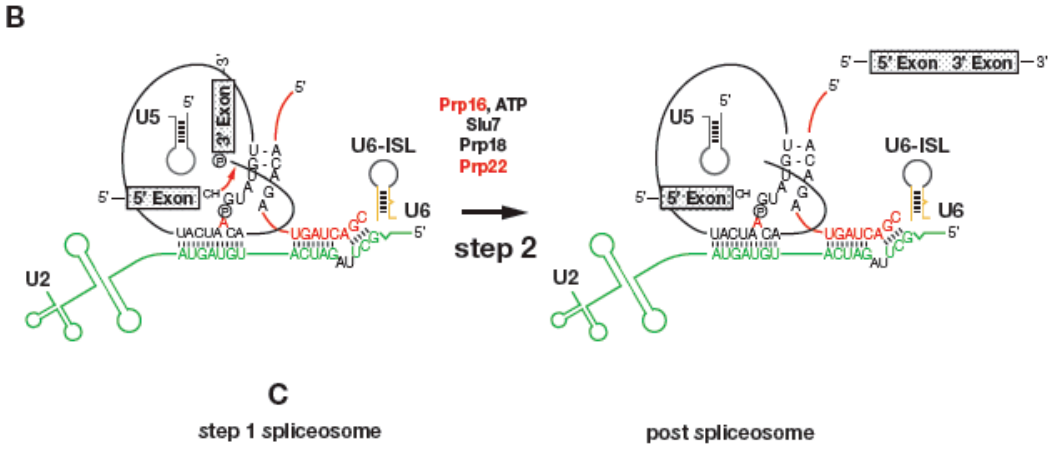
A



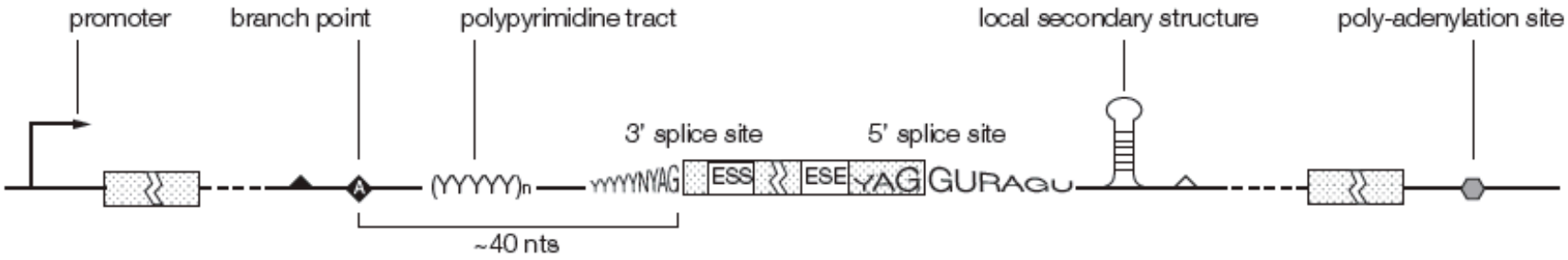
RNA rearrangements during the splicing reaction

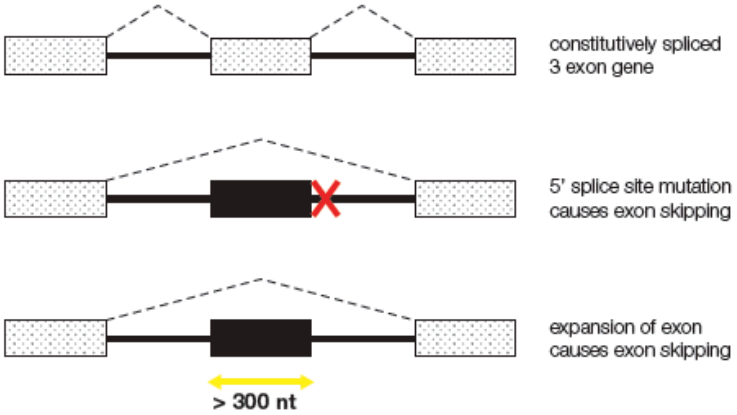


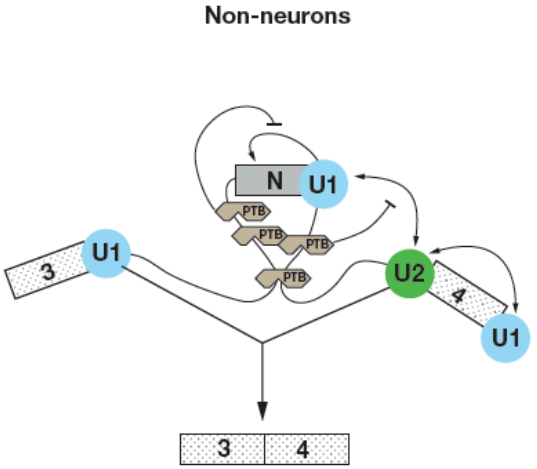


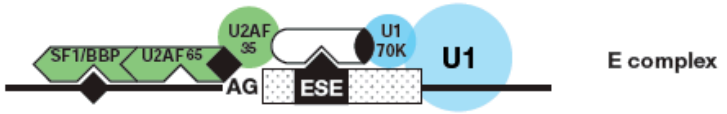


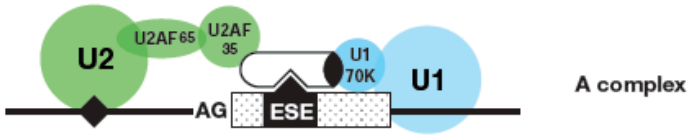
Alternative splicing: Competition between elements

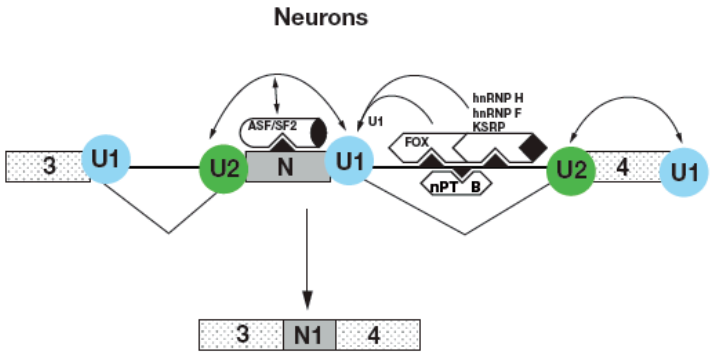






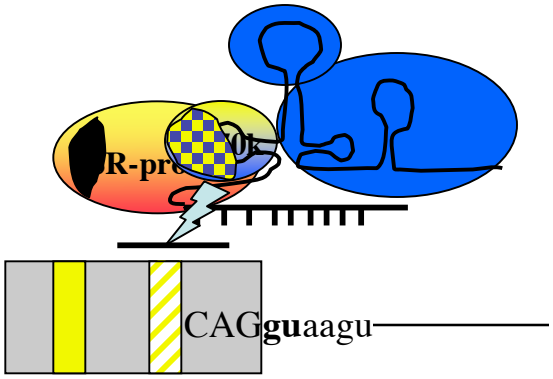






Diseases caused by mutations affecting splicing

FTDP-17	dementia	Tau exon 10
thrombasthenia of glanzmann and naegeli	Blood coagulation	platelet glycoprotein IIIa
Menke Disease	Copper metabolism	MNK
Leukodystrophy	lymphocytes	Arylsulfatase A
Immunodeficiency	lymphocyte	Adenosine deaminase
Immunodeficiency	lymphocyte	TNFRSF5
Cerebrotendinous xanthomatosis	lipid-storage disease, also called cerebral cholesterinosis	CYP27A1
Marfan syndrome	Connective tissue	Fibrillin-1
Acute intermittent porphyria	Porphobilinogen deaminase	Heme biosynthesis
Tyrosinemie	metabolite	Fumarylacetoacetat hydrolase
Leigh's encephamyelopathy	metabolite	Pyruvate dehydrogenase E1 alpha
Homocystinuria	Metabolite	Methionine synthase
SBCAD:	Metabolite	short branced chain acyl-CoA dehydrogenase
Bardet Biedl syndrome	metabolite	MGC1203
Hutchinson-Gilford progeria (HGPS)	Nuclear structure	Lamin A
Neurofibromatosis (NF)	cancer	NF-1
Duchenne muscular dystrophy	muscle	Dystrophin, exon 23

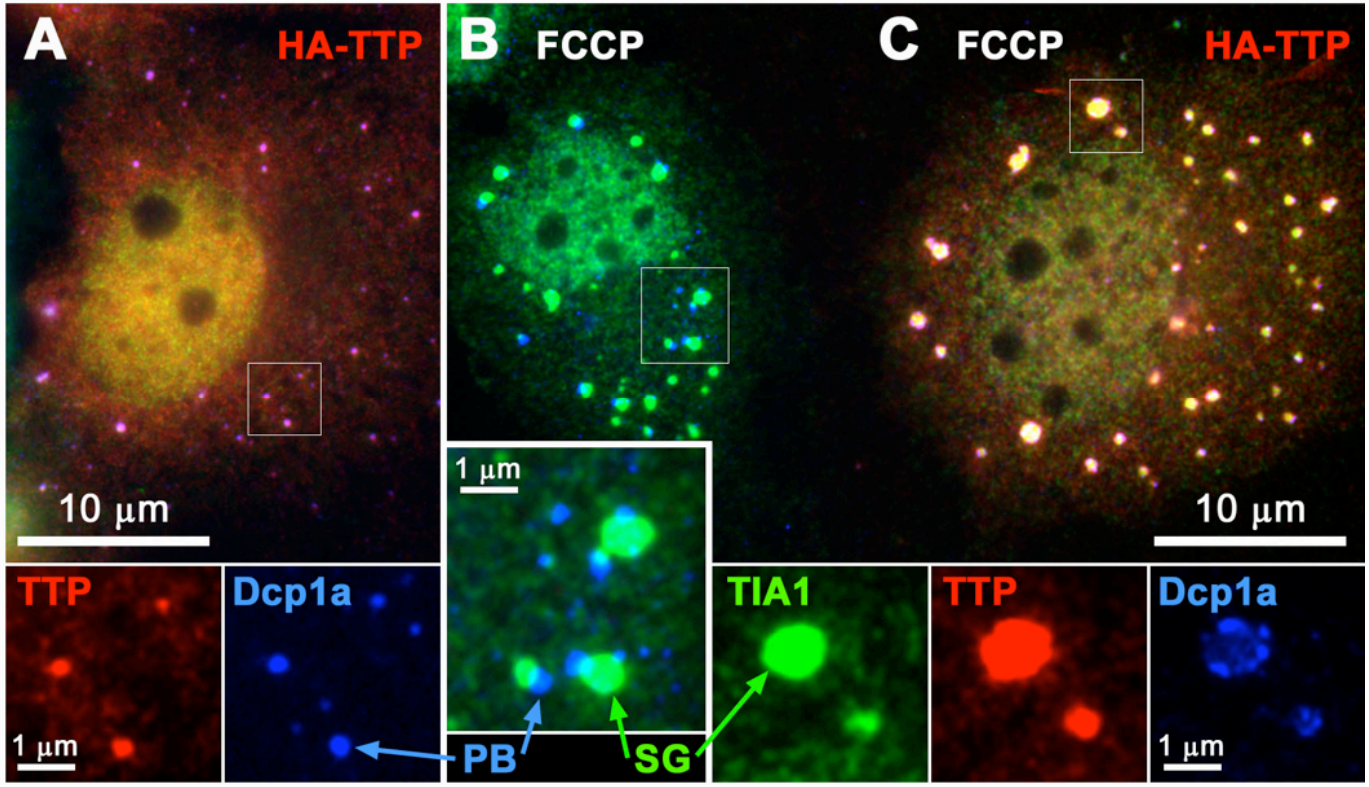


Mostly rare diseases, informative for the splicing process

Alternative splicing and disease, Prog. Mol. Subcell. Biol., 2006, Springer
Buratti et al., Nucl. Acids Res. 34, 2006: 3494-3510
Tazi et al. (2009): Biochim Biophys Acta 1792:14

P bodies: the end of the RNA?

Processing bodies (p bodies)
Dcp bodies, GW body



Summary and outlook

RNA is the first read-out of the genetic information
RNA is more than a messenger, RNA ‘interprets’ the genetic information
RNA is processed, which changes the readout of the genetic information
RNA can have enzymatic activity
RNA is structural more diverse than DNA
Proteins have evolved that stabilize the structure of RNA

Understand how SNPs affect RNA processing and genetic readout
Contribution to complex diseases?
Understanding the rules that govern RNA processing