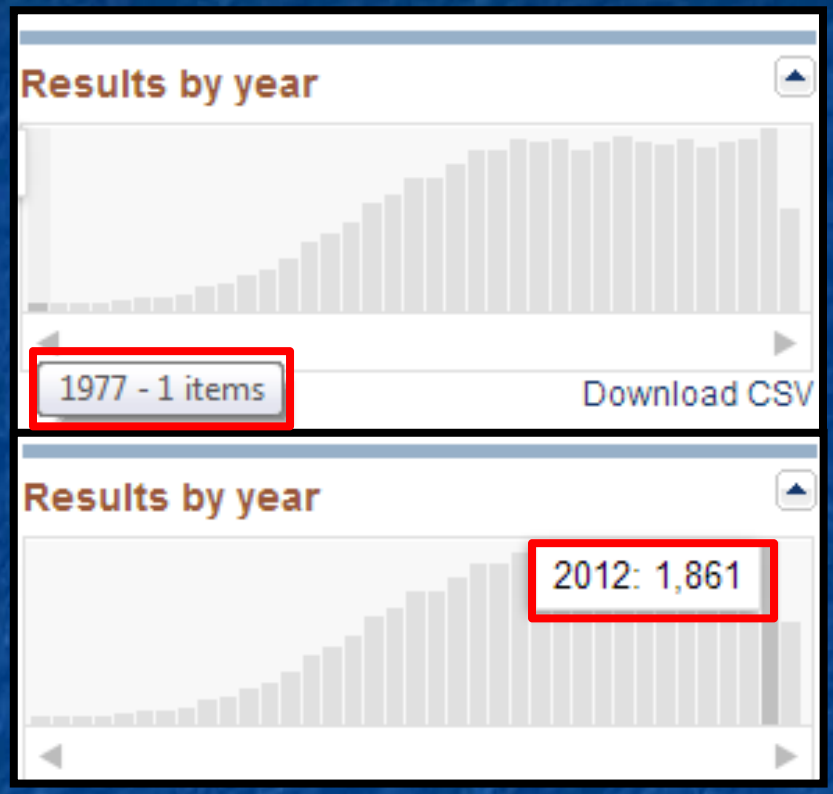
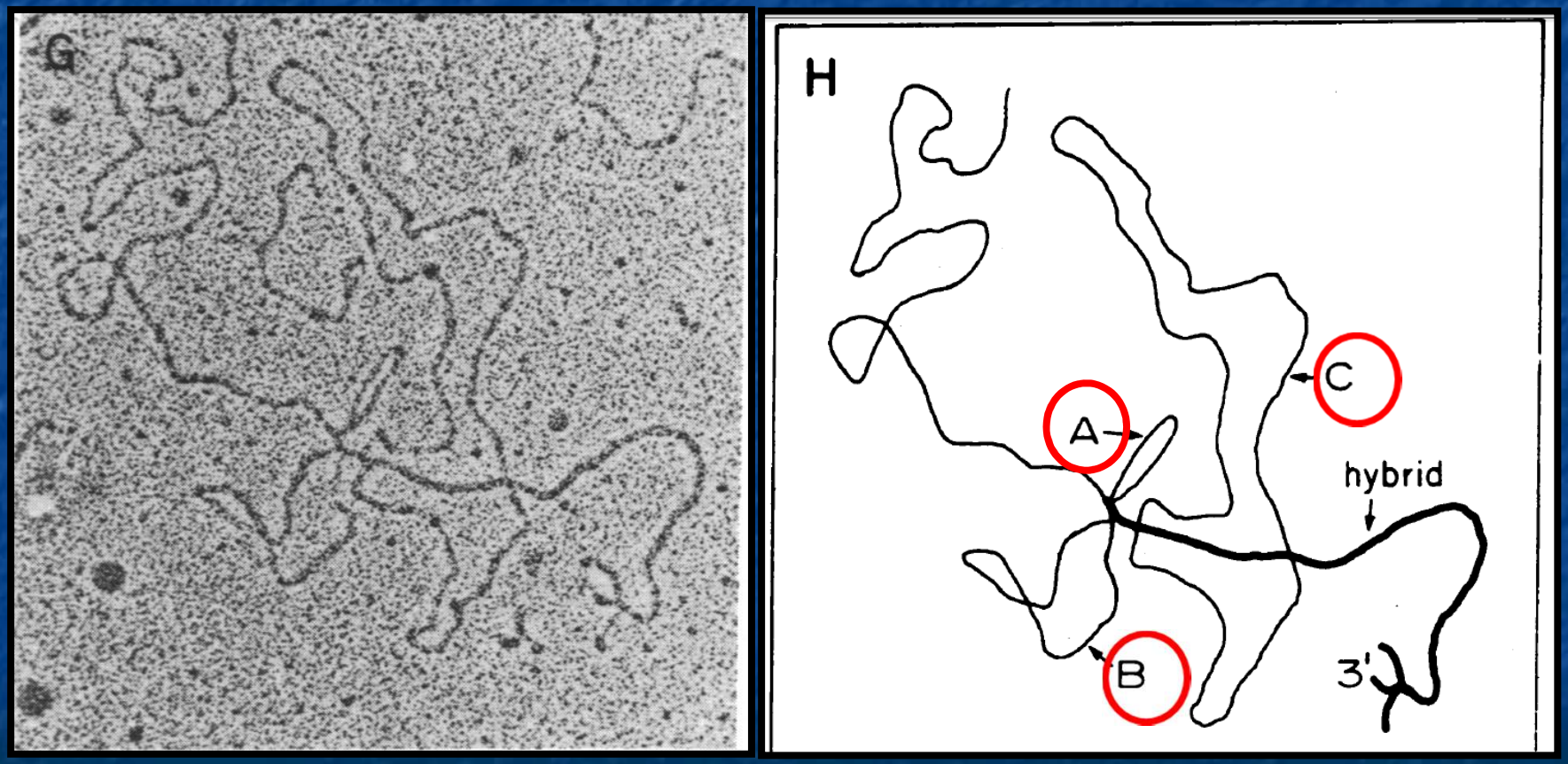
Overview

- The origin
- The U1snRNA – (m)RNA interaction: milestones and preconceptions
- Recognition and Selection
- Affinity and position
- Implications for Disease
- Correction Approaches

RNA splicing: two decades on top in research

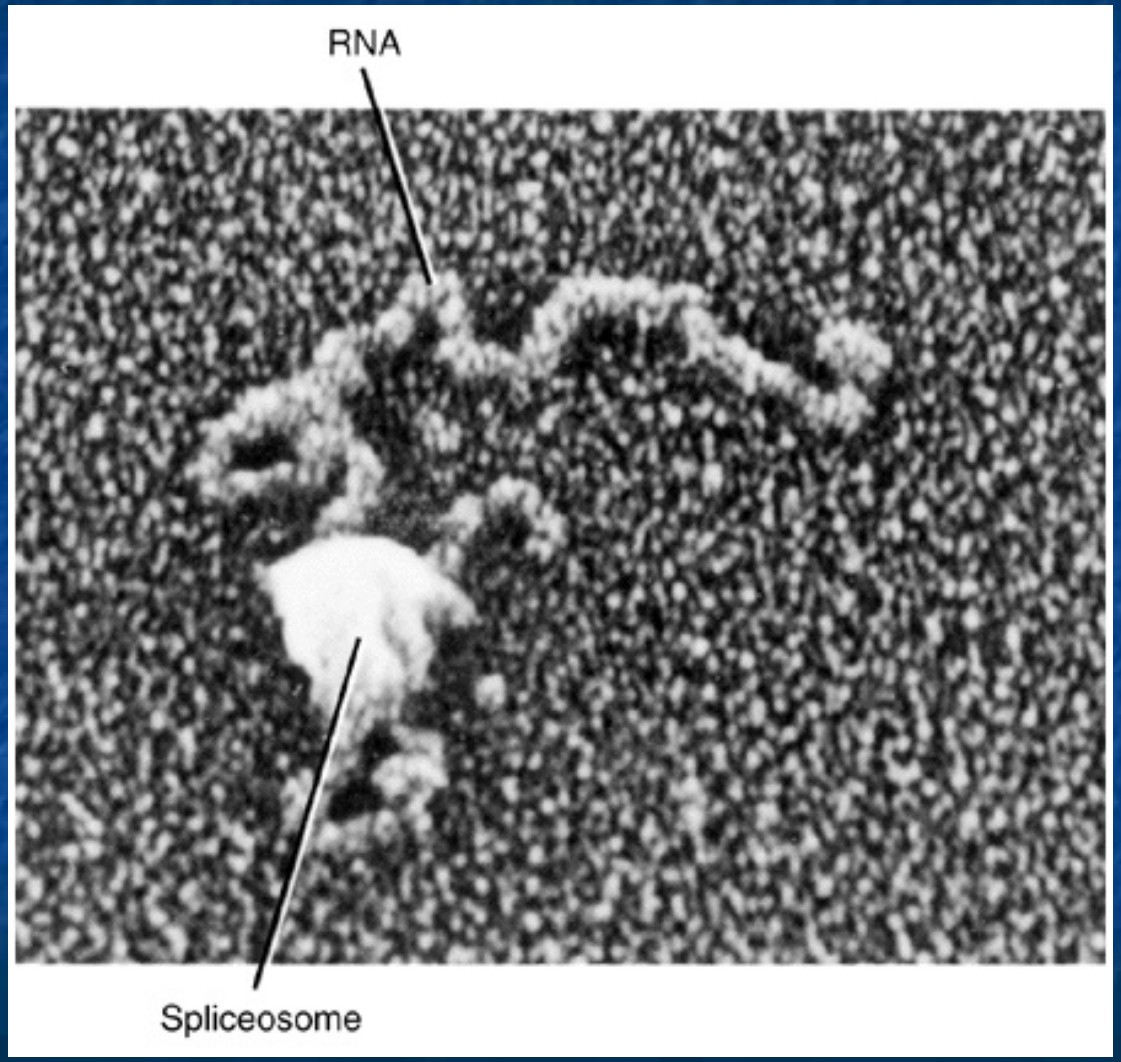


Introns and Exons: RNA Splicing
the origin



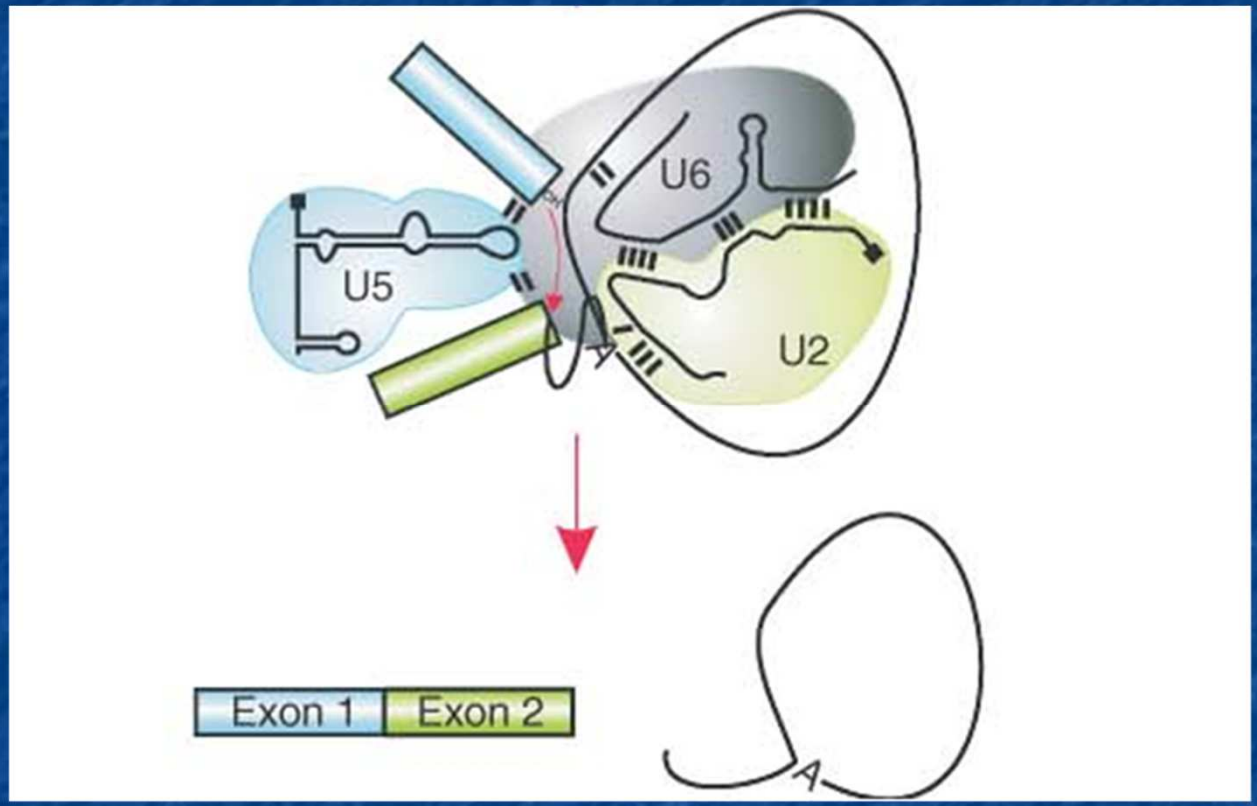
BERGET, MOORE AND SHARP 1977

Spliceosome

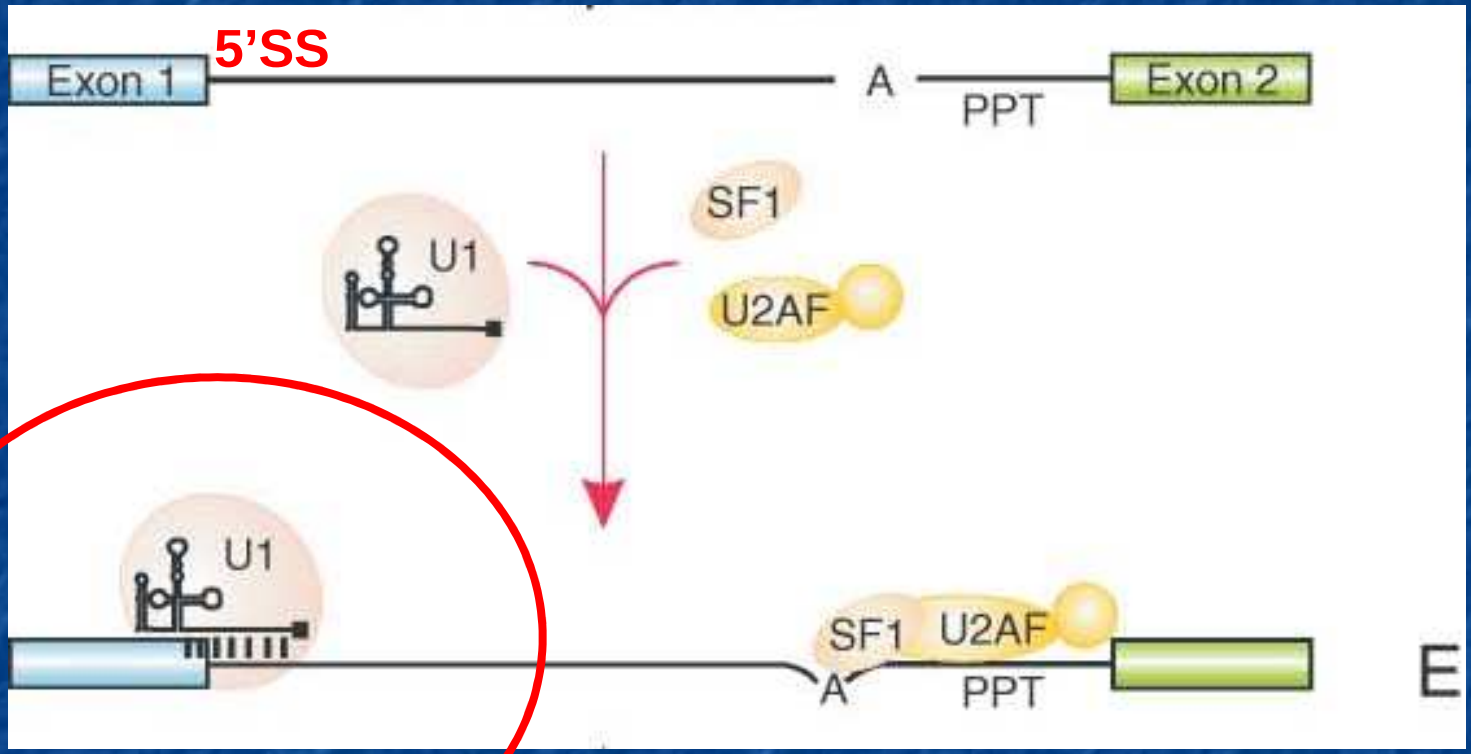


Brody and Abelson Science 1985

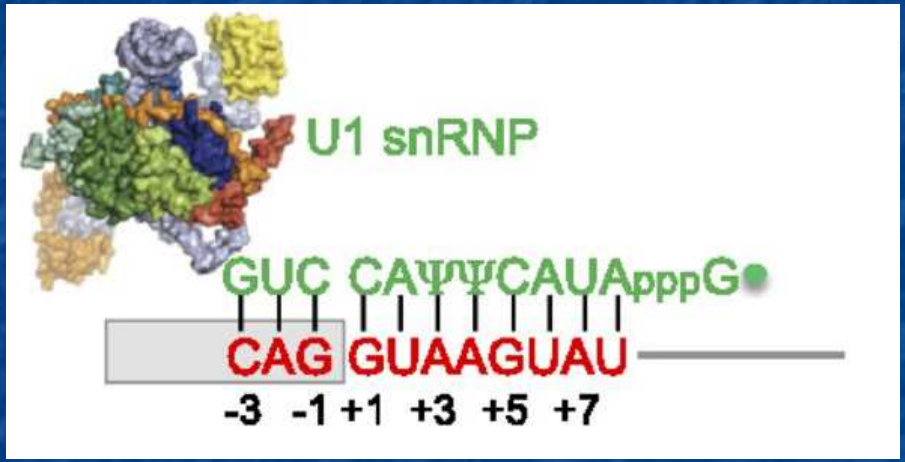
Spliceosome

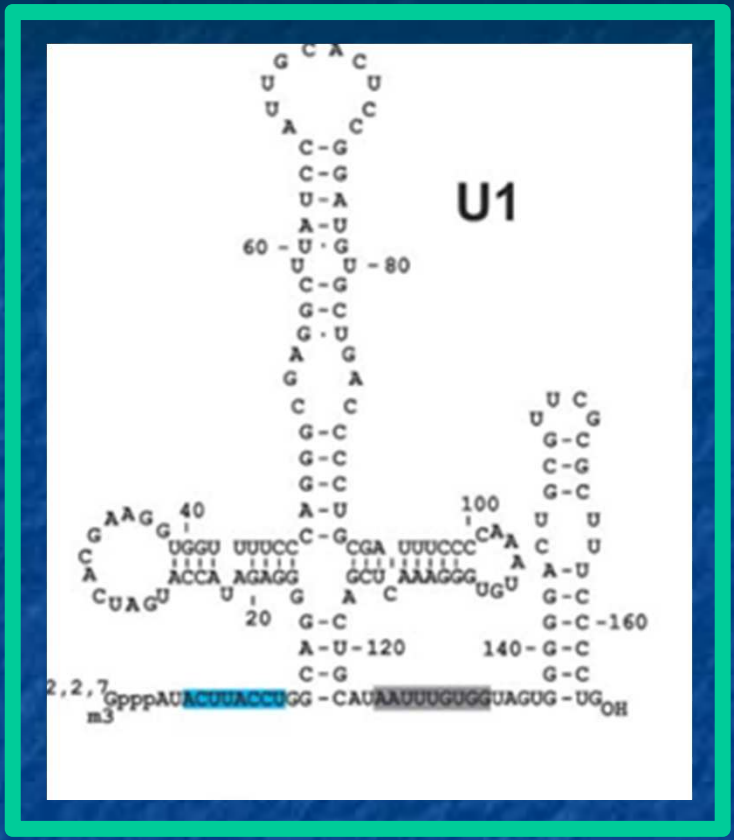


Splicing steps: focus on U1snRNA



Recognition of 5’ss by base-pairing to the 5’ end of U1 snRNA





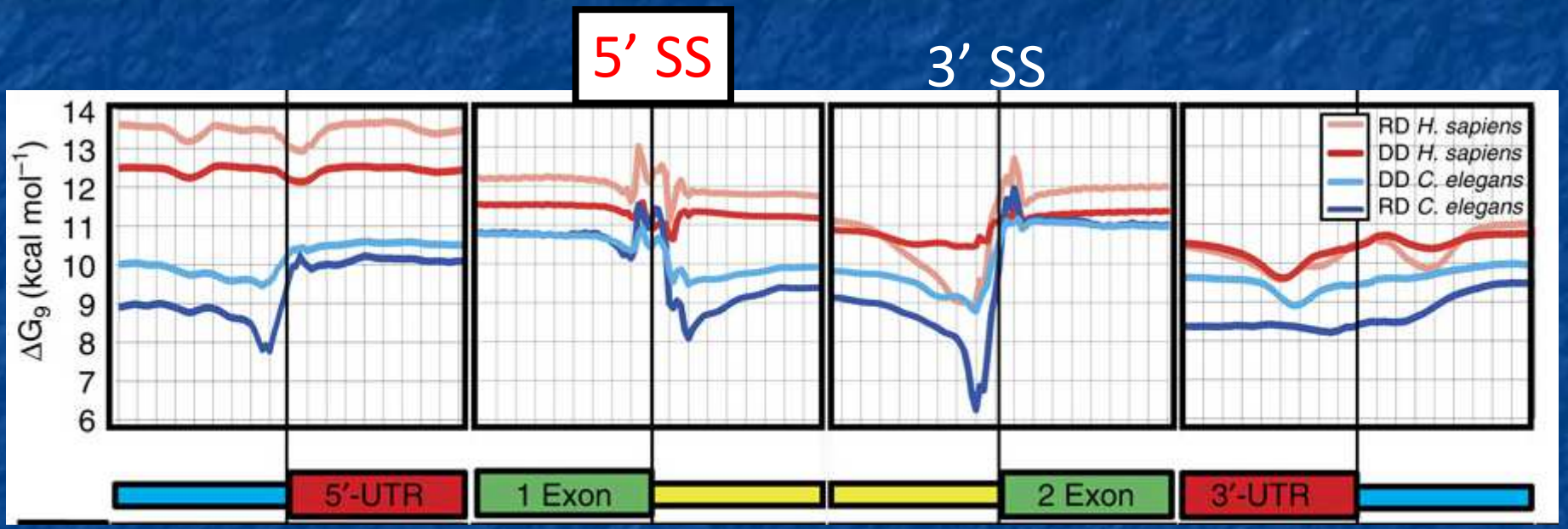
Milestones

- Numerous sequences emerged and revealed clear similarities among Splicing Sites
- The “consensus” sequence - the nucleotide most commonly found - was complementary to the sequence at the 5’ end of U1 snRNA

Milestones

a recognition mechanism based on affinity

The thermodynamic patterns of eukaryotic genes suggest mechanism for intron–exon recognition



Changes in physical properties might integrate the influences of proteins and sites

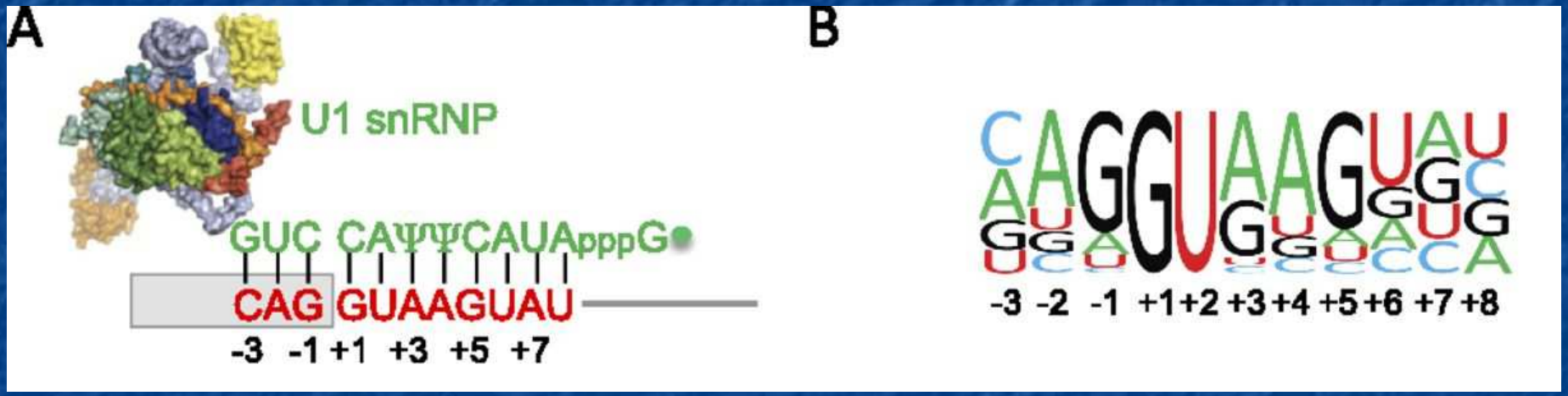
Nedelcheva-Veleva et Al Nat ure Communications 2013

Milestones and preconceptions

a constant register for base-pairing between all ss and U1 snRNA

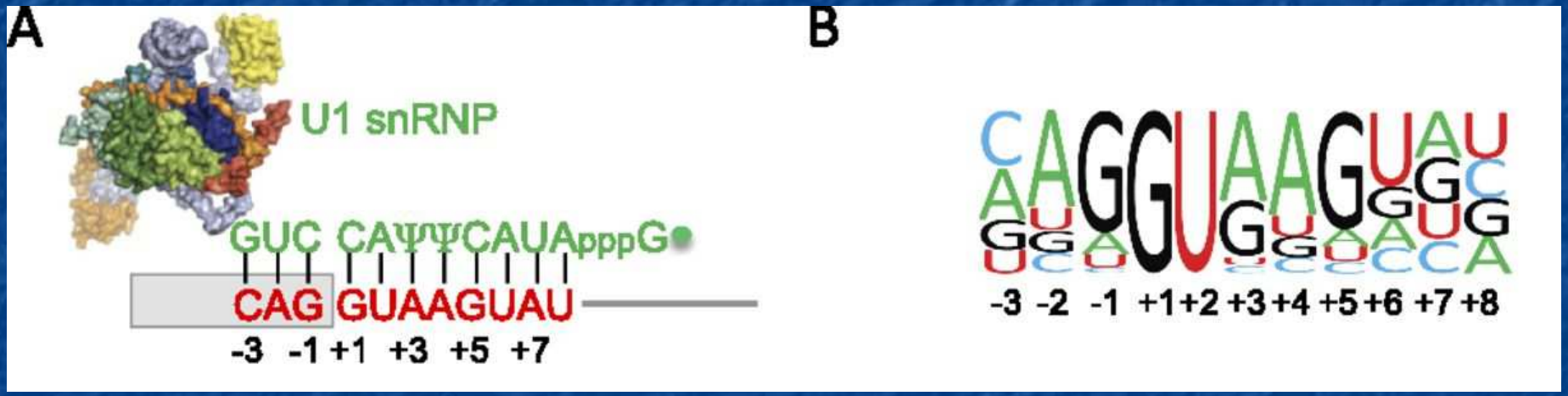
the “consensus” sequence was the “optimal” ss???

Consensus sequence of 5’ss base-paired to the 5’ end of U1 snRNA



Adapted from Roca X et al. Genes Dev. 2013;27:129-144

Consensus sequence of 5’ss base-paired to the 5’ end of U1 snRNA

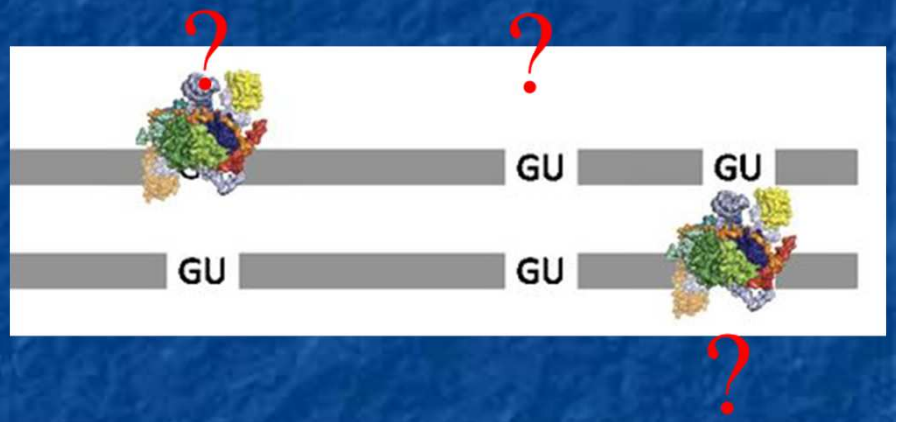


half of all transcribed GU are potential SS !!!!!!!!!!!!

Adapted from Roca X et al. Genes Dev. 2013;27:129-144

The model was inadequate 1

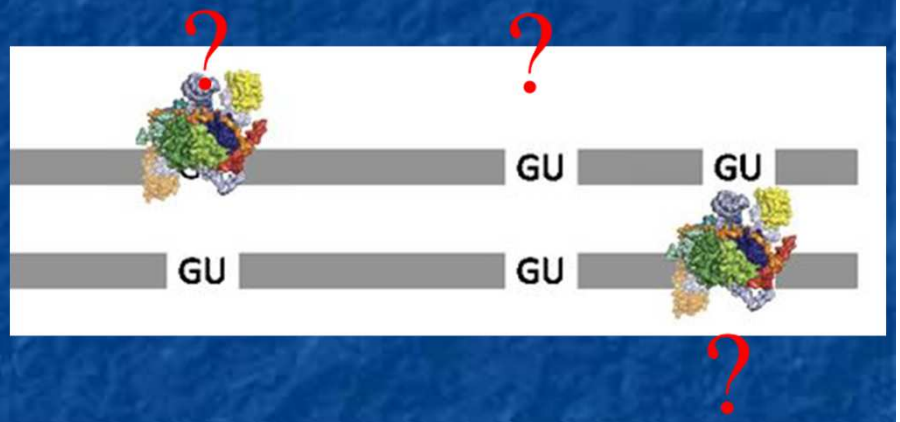
- genes comprise many more pseudo-5'ss than actual 5'ss
- some of them matched the consensus as well as or better than the used 5
- they (cryptic) were used when a natural site was inactivated (Treisman et al. 1983; Wieringa et al. 1983)
- use of alternative 5'ss was discovered- the ratio of use depended on the sequences of the sites that were in competition (Montell et al. 1982).



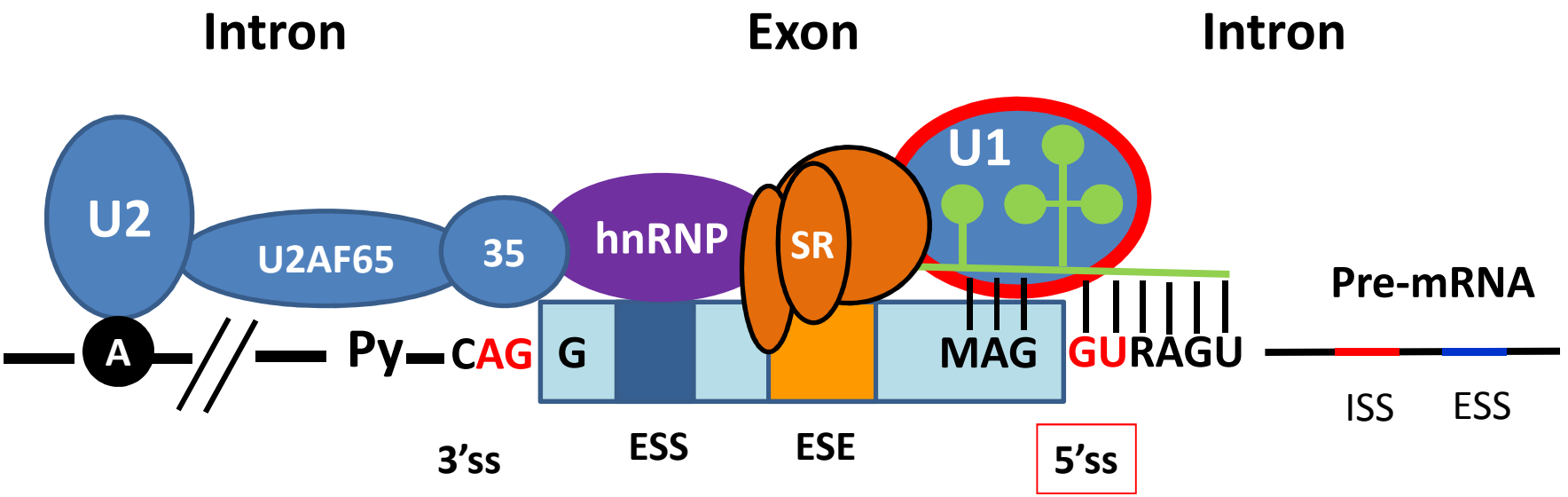
The model was inadequate 2

- sequence could not be the only determinant
- the use or avoidance of 5’ ss was not a simple intrinsic property of any sequence

- other snRNAs, regulatory proteins, splicing enhancers, and the relative positions of alternative ss contribute to selection



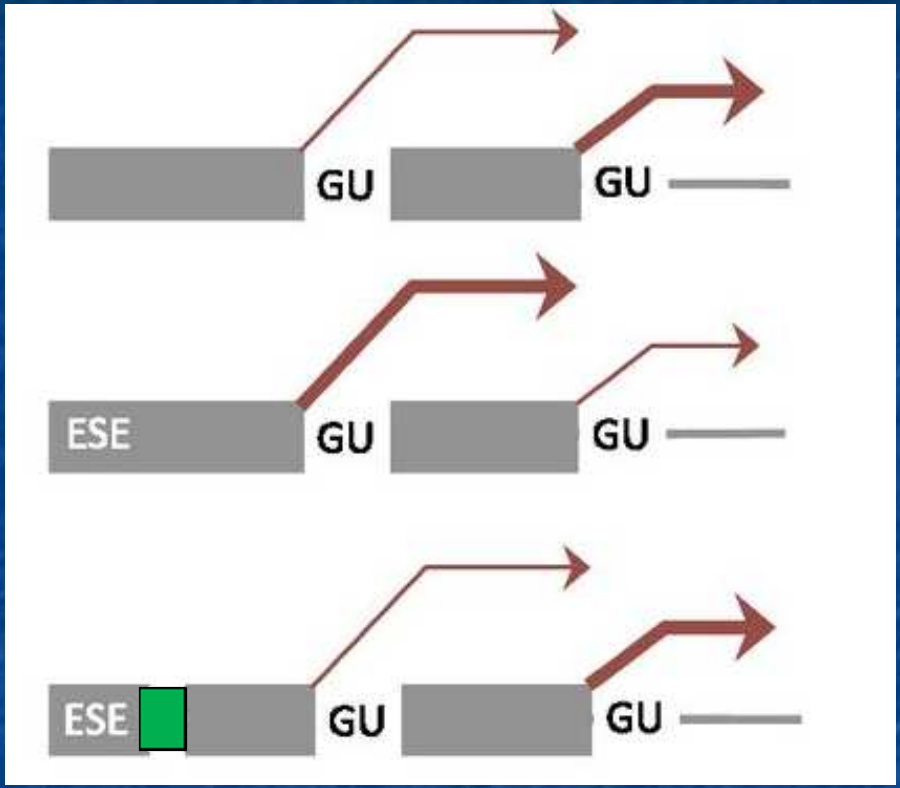
Proteins activate splicing on the basis of position and sequence of the sites



Exon definition

Affinity and position

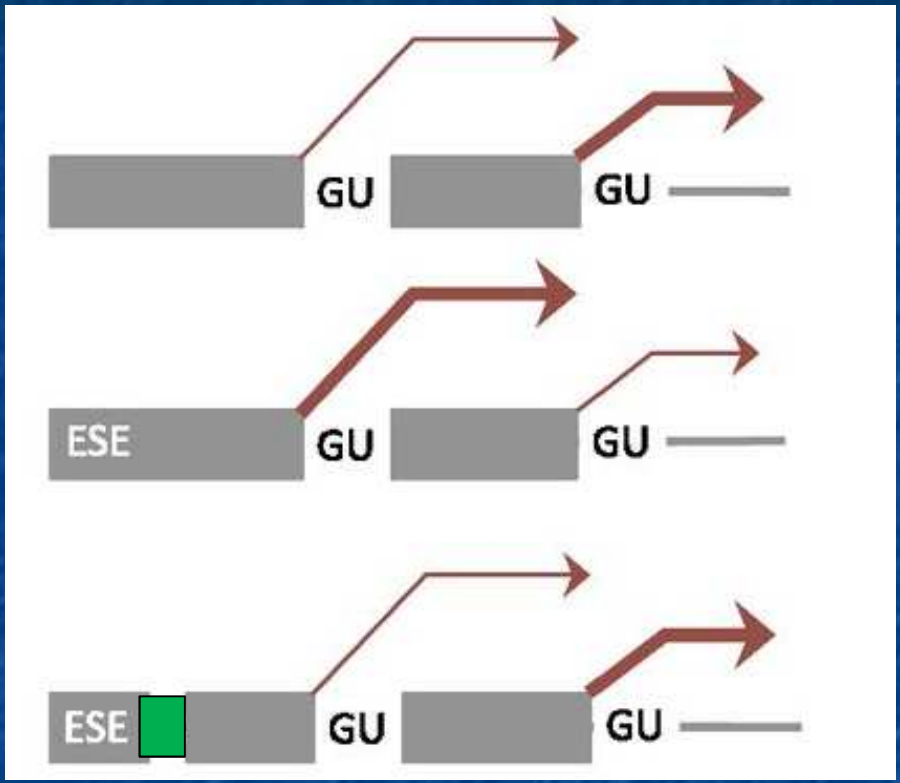
Effects of affinity for U1 snRNP and 5’ss position upon selection.



Insertion of a non-RNA linker between an ESE and the alternative 5’ss blocks its effects, arguing against a looping model (Lewis et al. 2012)

Adapted from Roca X et al. Genes Dev. 2013;27:129-144

Affinity, position and RNA continuity



the observed behavior is compatible with the exon as a rigid body (Hodson et al. 2012) high density of proteins associated to RNA in exons

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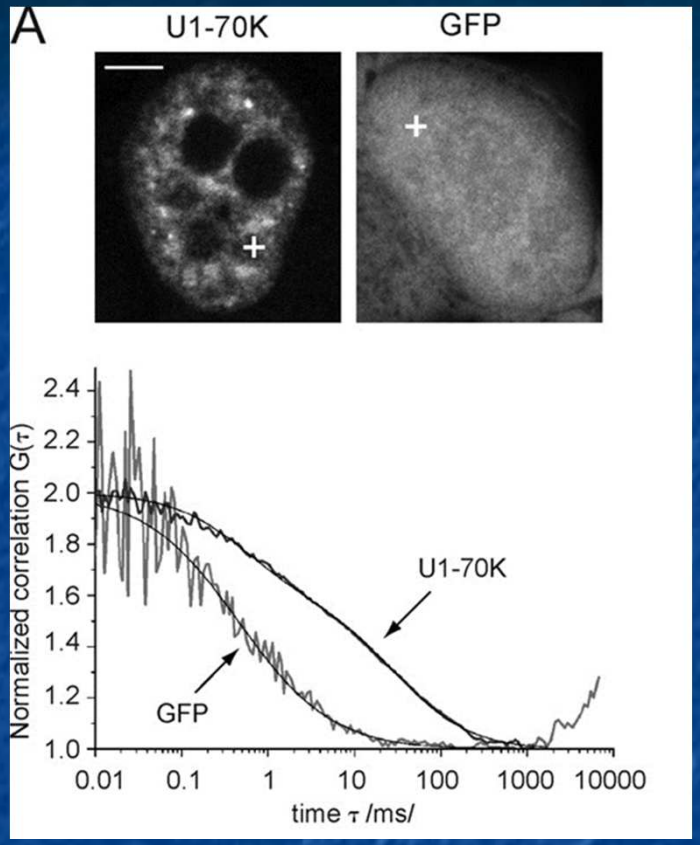
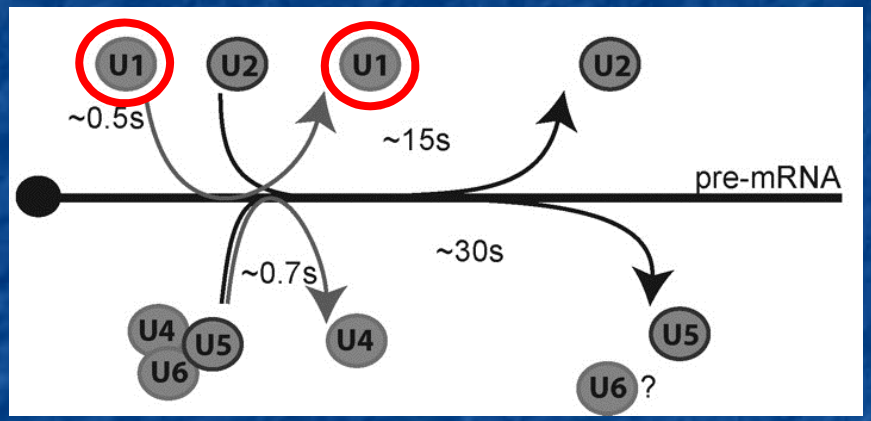
Adapted from Roca X et al. Genes Dev. 2013;27:129-144

Lifetimes of U1:pre-mRNA complexes



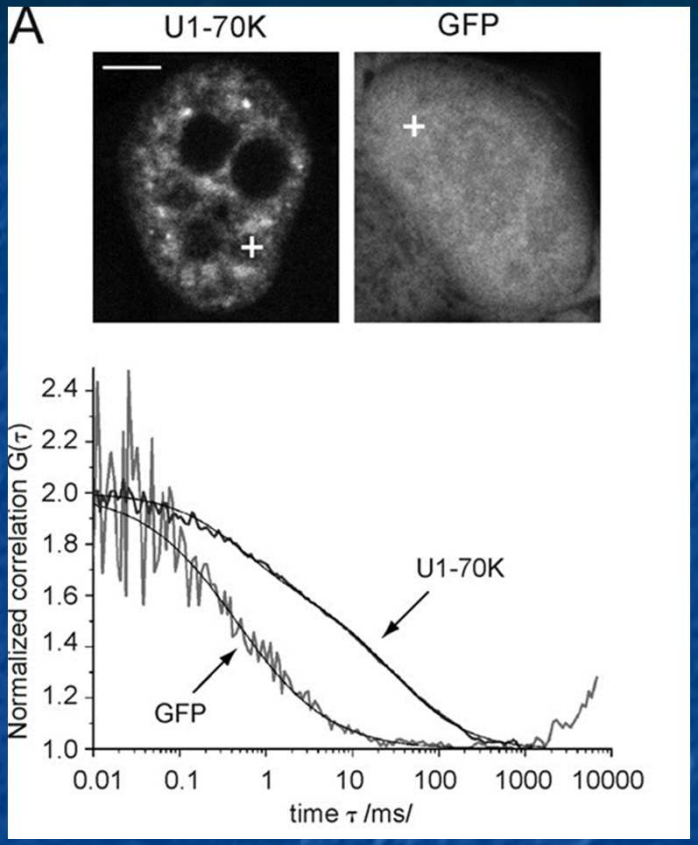


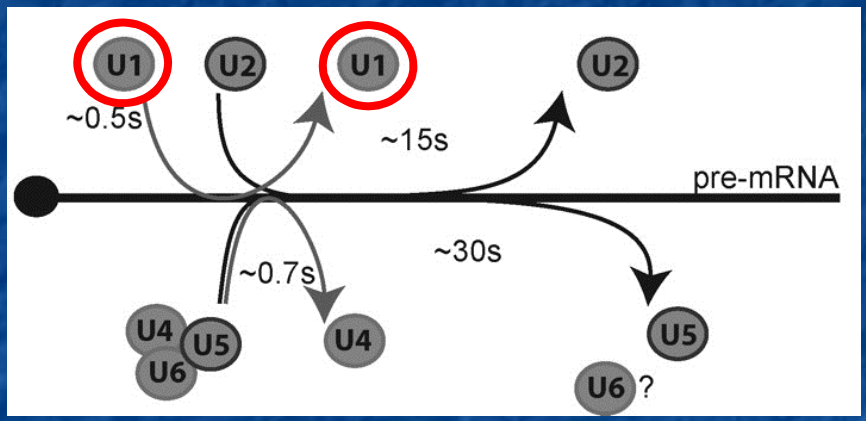
In vivo measurements suggest that snRNPs interact independently with pre-mRNA and that
the lifetime of bound U1 snRNP averages <1 sec

Huranová M et al. J Cell Biol 2010;191:75-86

JCB

Lifetimes of U1:pre-mRNA complexes





equilibrium between the candidate 5'ss and U1 snRNPs might be established well before the 5' ss selection

Huranová M et al. J Cell Biol 2010;191:75-86

JCB

A few answers ...

Most sites are recognized by U1 snRNP
part of them requires altered registers and bulges

Interplay of affinity and position plays a major role

Factors that modulate 5'ss usage act via a U1 snRNP by
+ stabilizing its interactions at a 5' ss
- competing for binding
- binding adjacent to the U1 snRNP and stabilizing it in an inactive conformation.

…and new questions

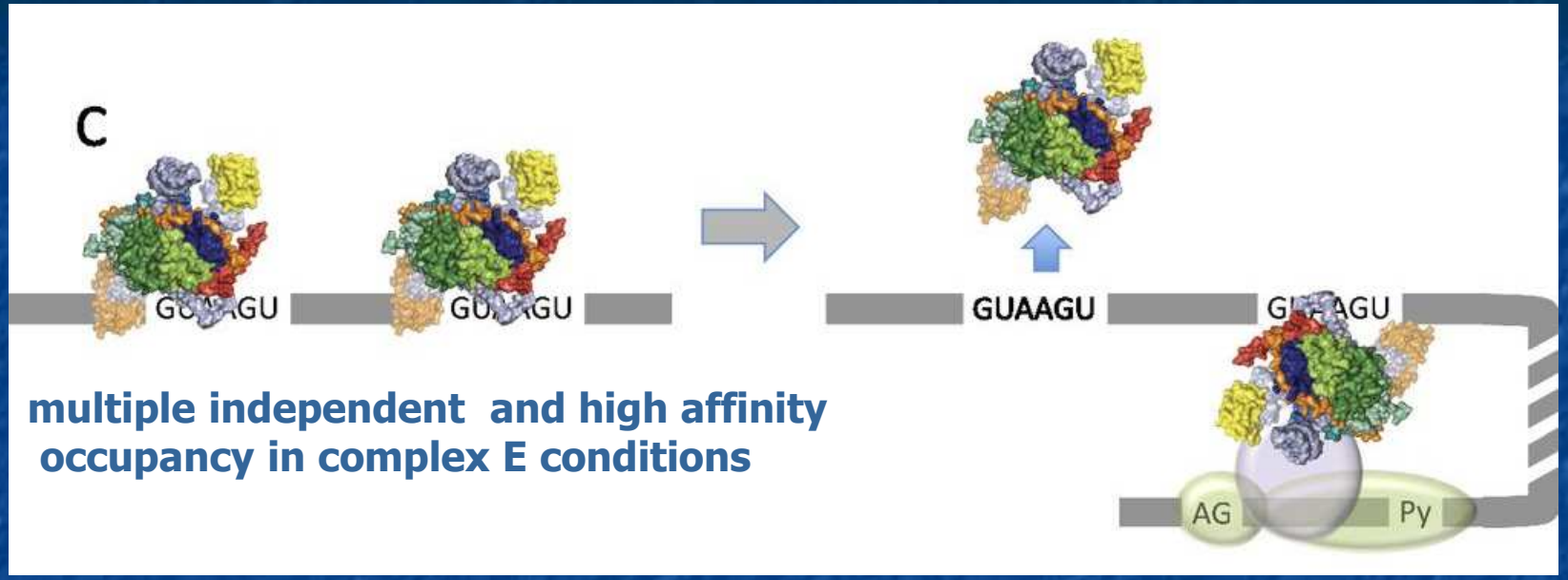
If U1 snRNPs mark the 5'ss to be used, how does the mechanism ensure that only one U1 snRNP marks each intron?

How does 5'ss recognition turn into selection?

Which are the mechanisms by which the recognition of numerous candidates might lead to selection of a single site?

How to establish the relation between basepairing strength and splicing outcomes?

Models for the effects of affinity for U1 snRNP and 5’ss position upon selection.



multiple independent and high affinity occupancy in complex E conditions

during formation of complex A, the intron-proximal U1 snRNP is selected, and the distal snRNP is displaced (Hodson et al. 2012). The surplus U1 snRNPs would dissociate

Adapted from Roca X et al. Genes Dev. 2013;27:129-144

Splicing and genetic diseases 1

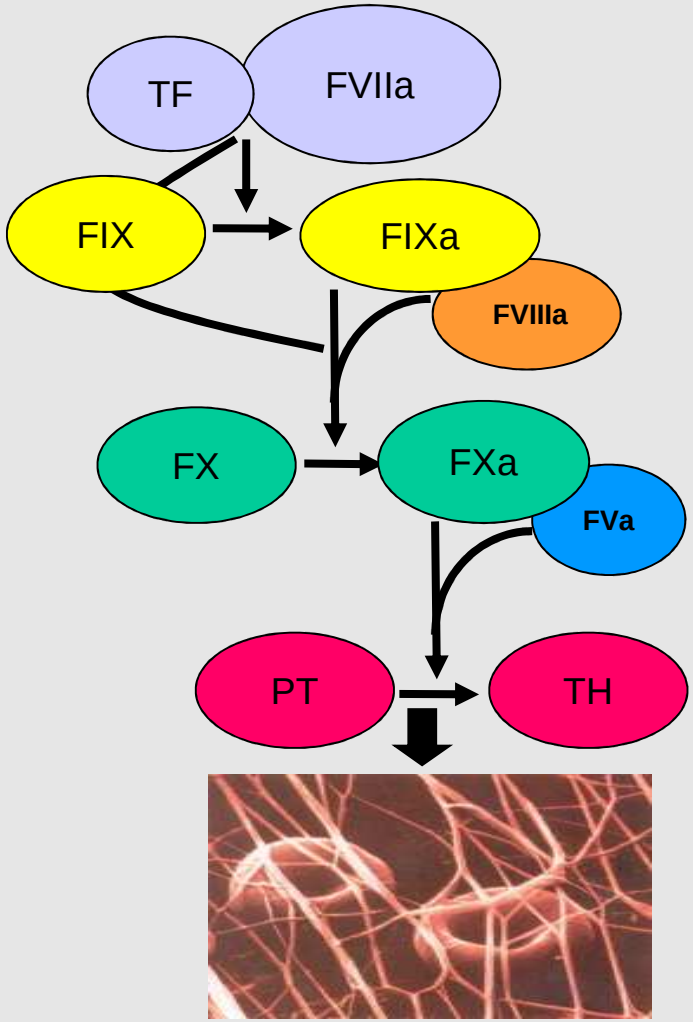
- Around 10-15 % of all disease-causing mutations affect splice sites (Krawczak et al. 2007) 50% F1 and ATM-ataxia telangiectasia genes
- Part of missense mutations and synonymous codons contain splicing mutations

Prediction of mutation severity by SS scoring methods are accurate but the molecular consequence are poorly defined

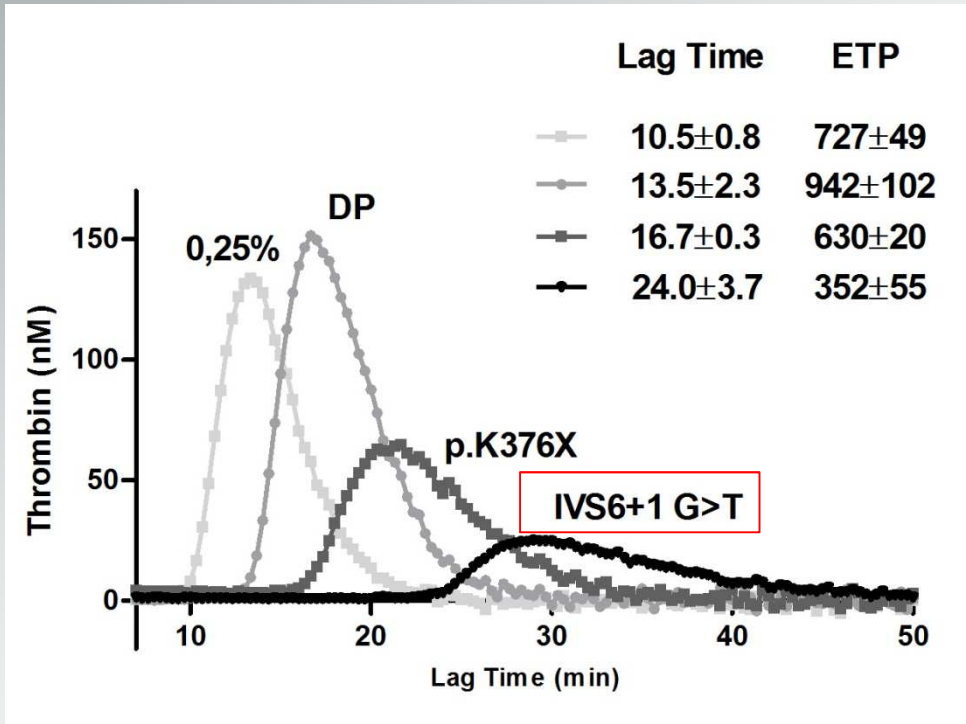
Molecular effects: reduction of correct splicing - skipping of the exon - activation of cryptic splice sites - intron retention.

- The most deleterious mutations at a 5'ss affect the nearly invariant GU

The Coagulation Cascade as a model to study splicing mutations



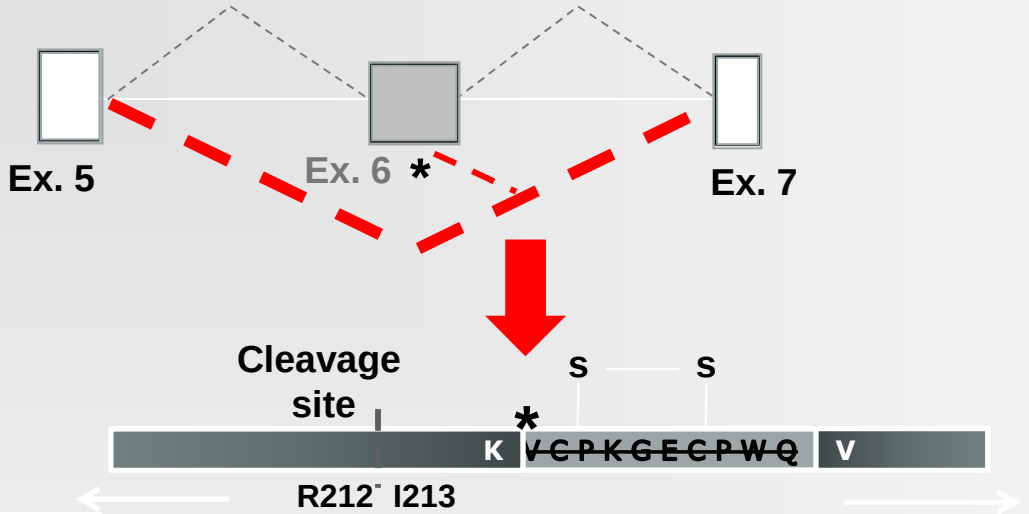
Coagulation FVII Deficiency: IVS6 +1 G>T a life threatening splicing mutation

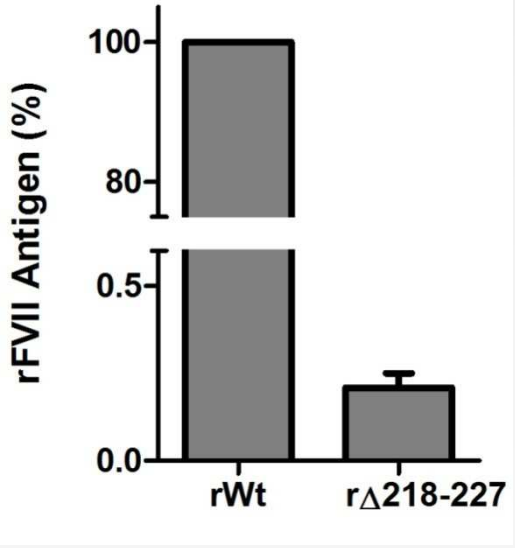


Cavallari et al 2012

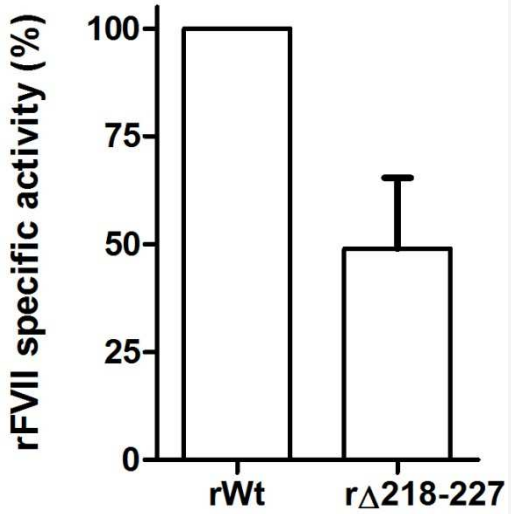
STER
Seven Treatment Evaluation Registry

IVS6 +1: alternative splicing and synthesis of trace amounts of a deleted but functional FVII

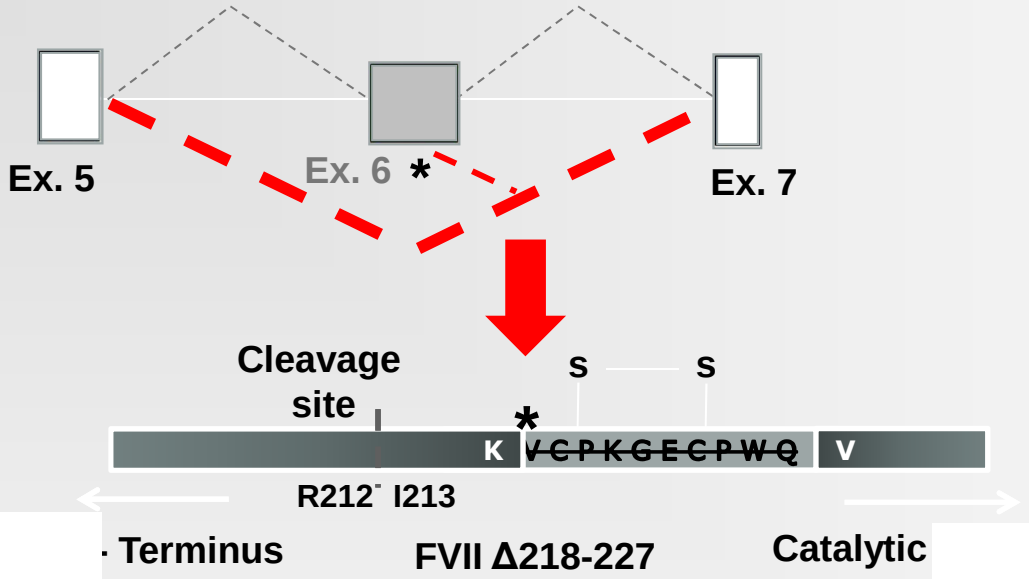


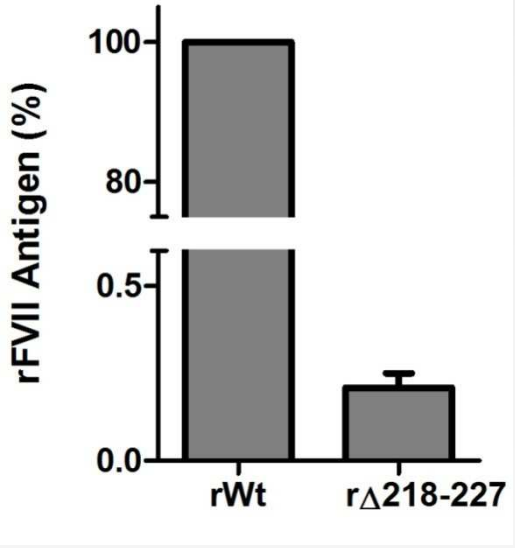


Cavallari et al 2012



IVS6 +1: alternative splicing and synthesis of trace amounts of a deleted but functional FVII





residual FVII levels in vivo? contribution to revert an otherwise perinatally lethal null-FVII deficiency?

Cavallari et al 2012