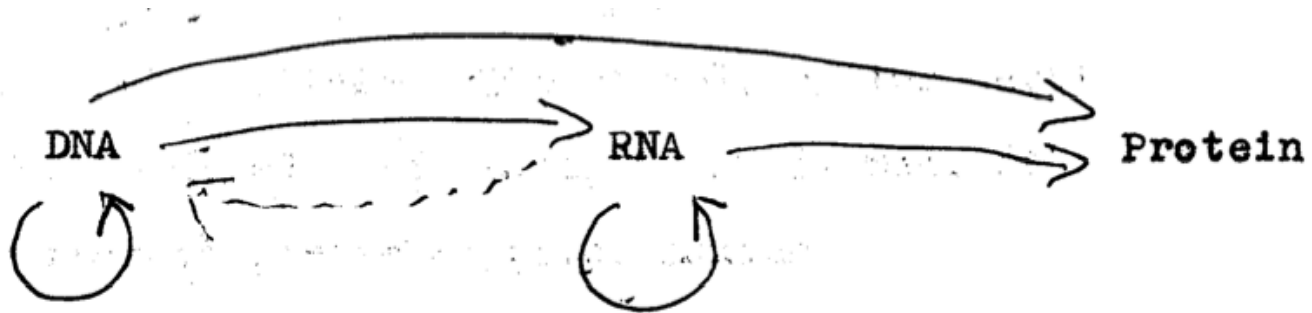


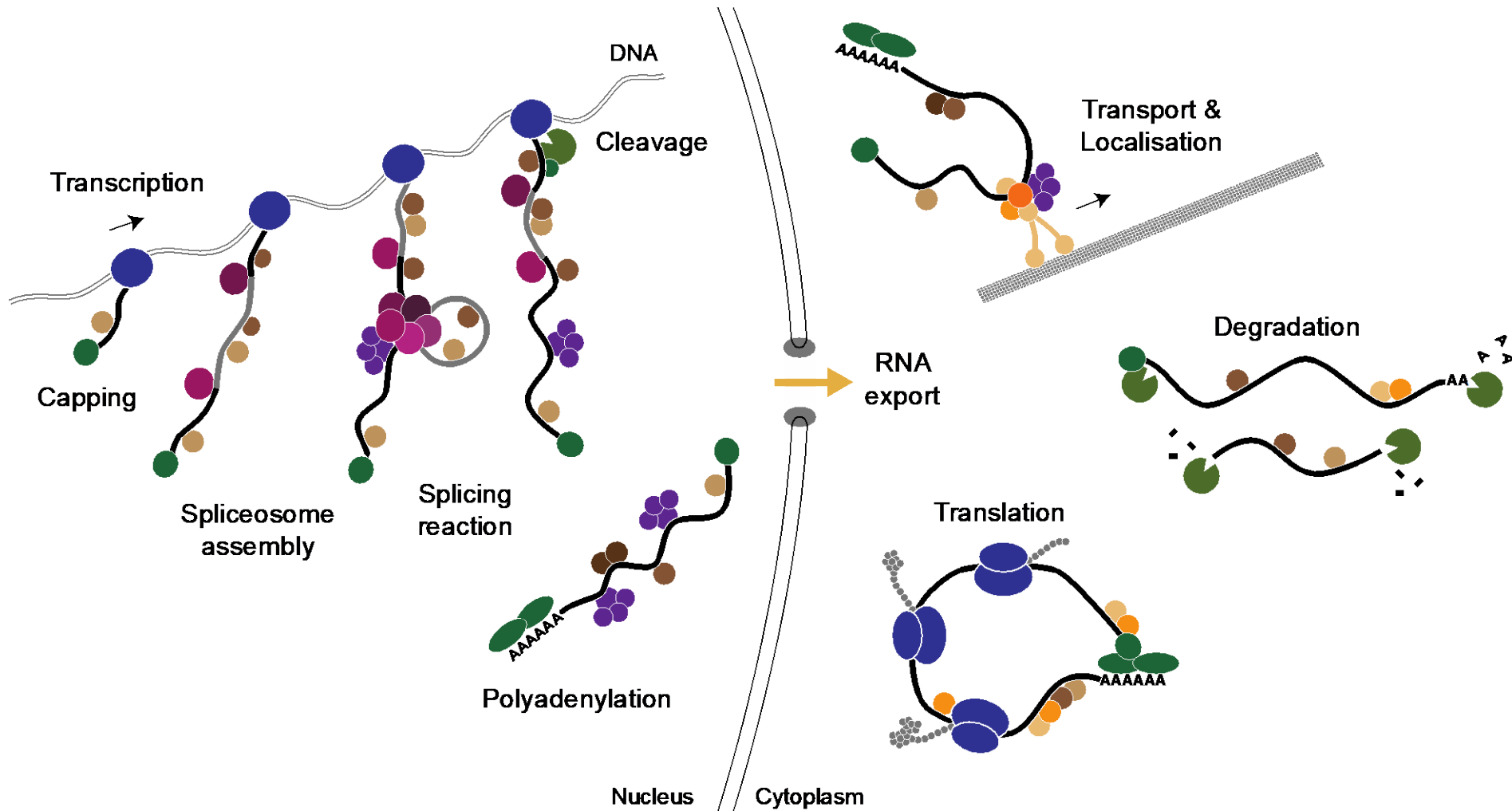
Das zentrale Dogma der Molekularbiologie

“DNA makes RNA makes protein”

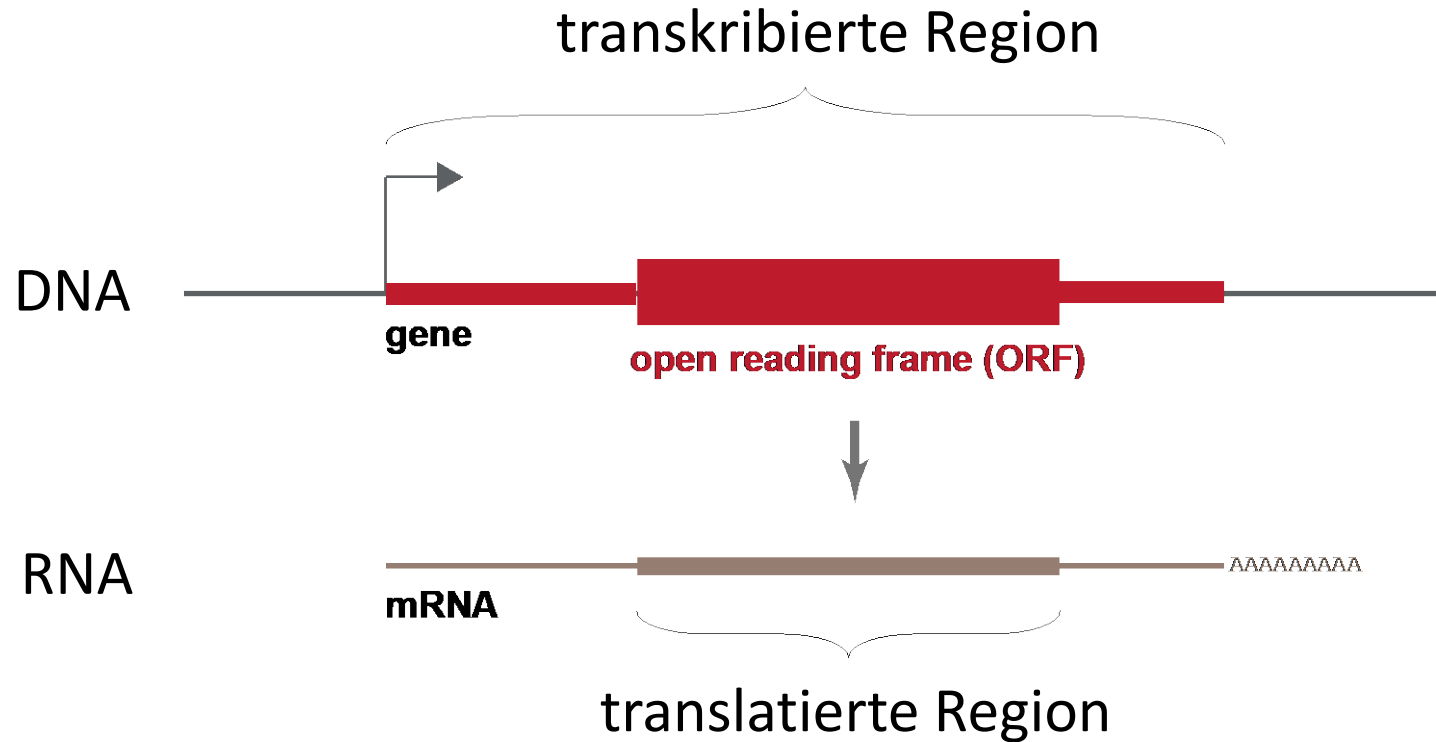


Francis Crick, 1956

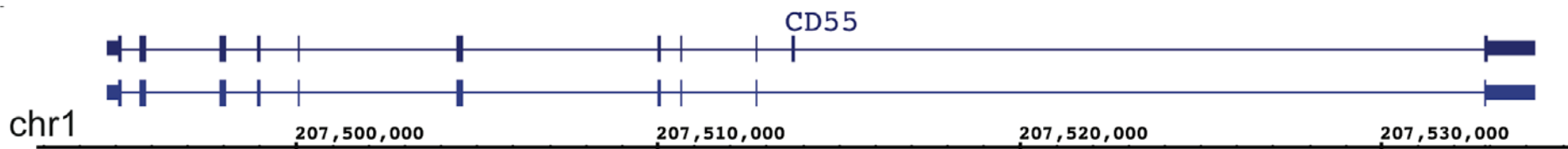
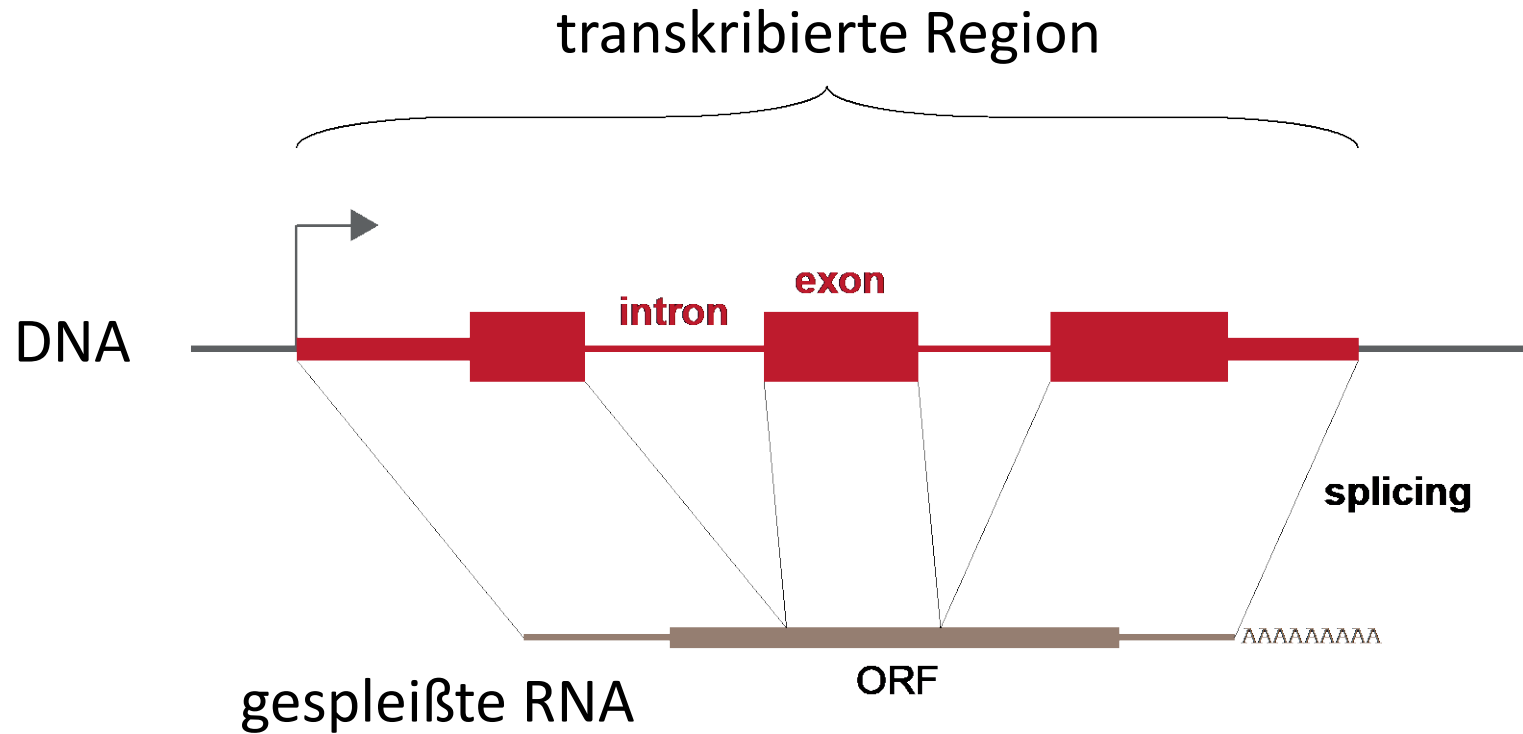
Der komplexe Lebenszyklus einer RNA



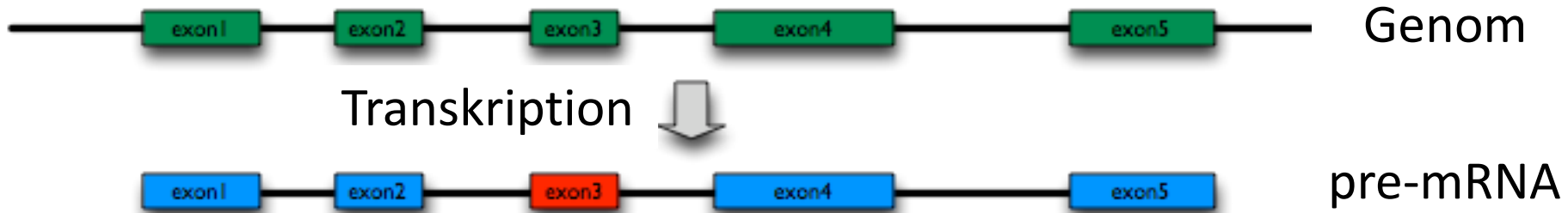
Der Aufbau einer eukaryotischen mRNA



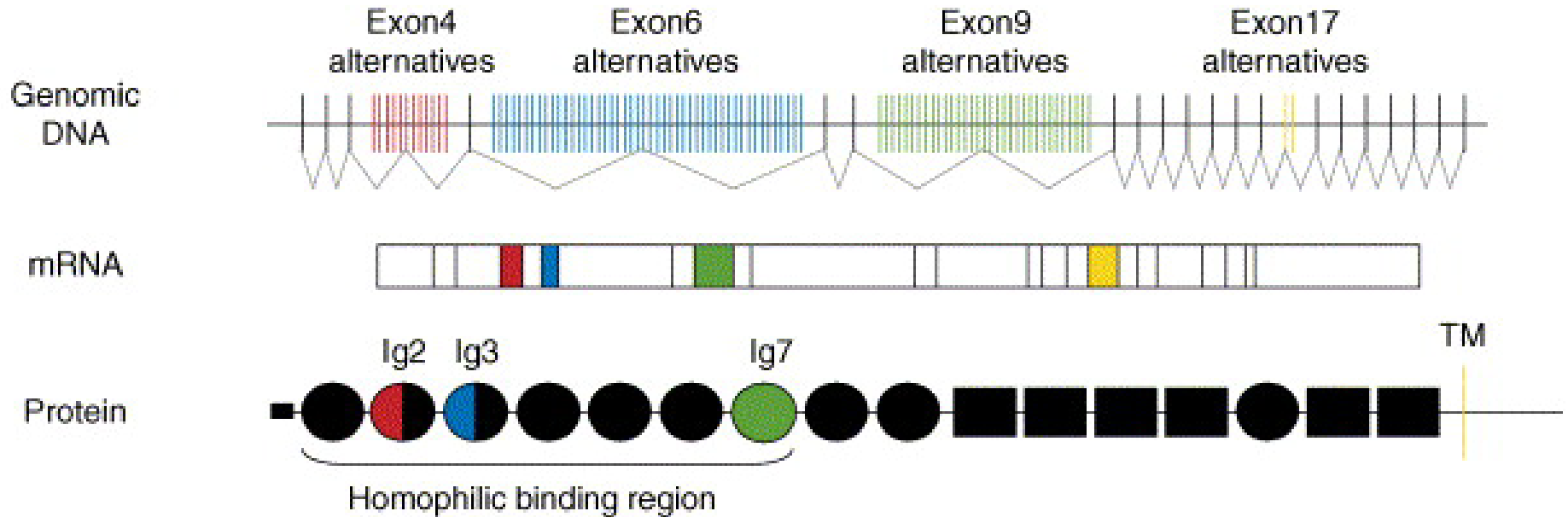
Der Aufbau einer eukaryotischen mRNA



Alternatives Spleißen (AS) erzeugt mRNA- und Protein-Isoformen

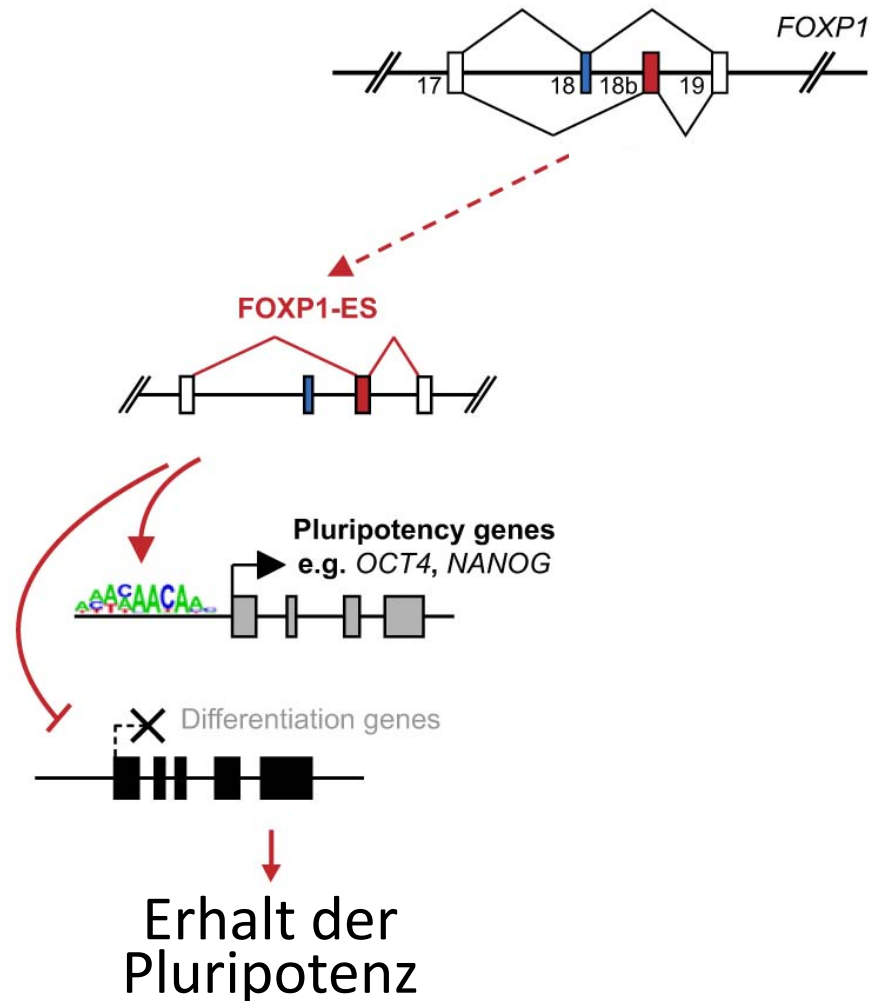


Beispiel I: Proteindiversifizierung von Dscam in *Drosophila*

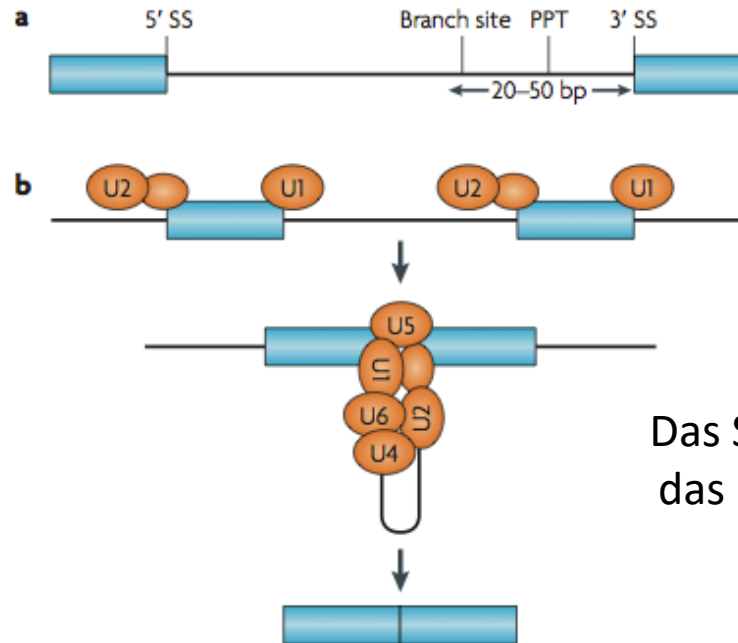


- neuronaler Zelloberflächen-Rezeptor
- mehr als 38.000 verschiedene Isoformen
- Interaktion identischer Rezeptoren vermittelt Abstoßung

Beispiel II: AS als Schalter in embryonalen Stammzellen

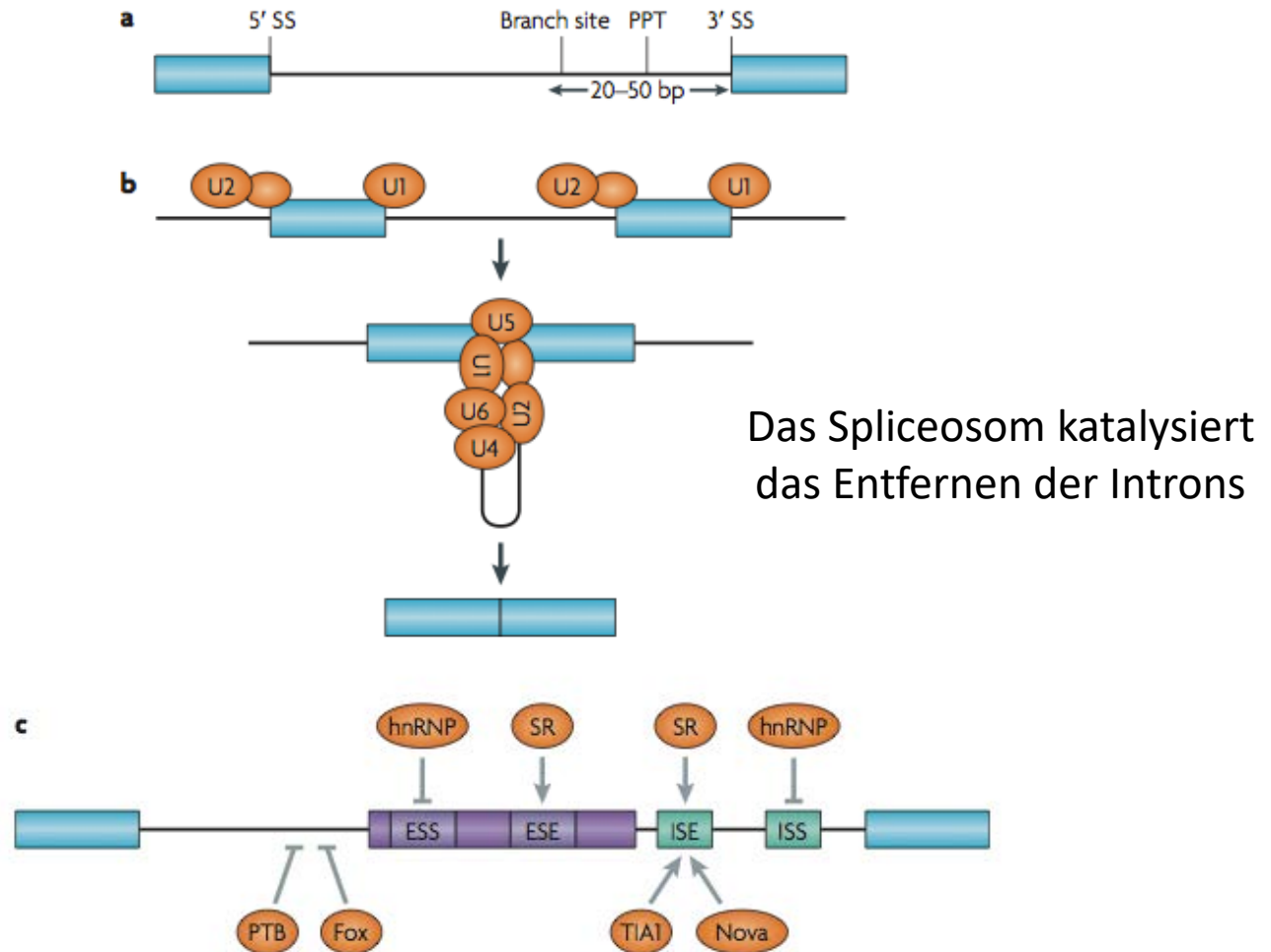


RNA-bindende Proteine (RBPs) bestimmen die Spleißreaktion



Das Spliceosom katalysiert das Entfernen der Introns

RNA-bindende Proteine (RBPs) bestimmen die Spleißreaktion



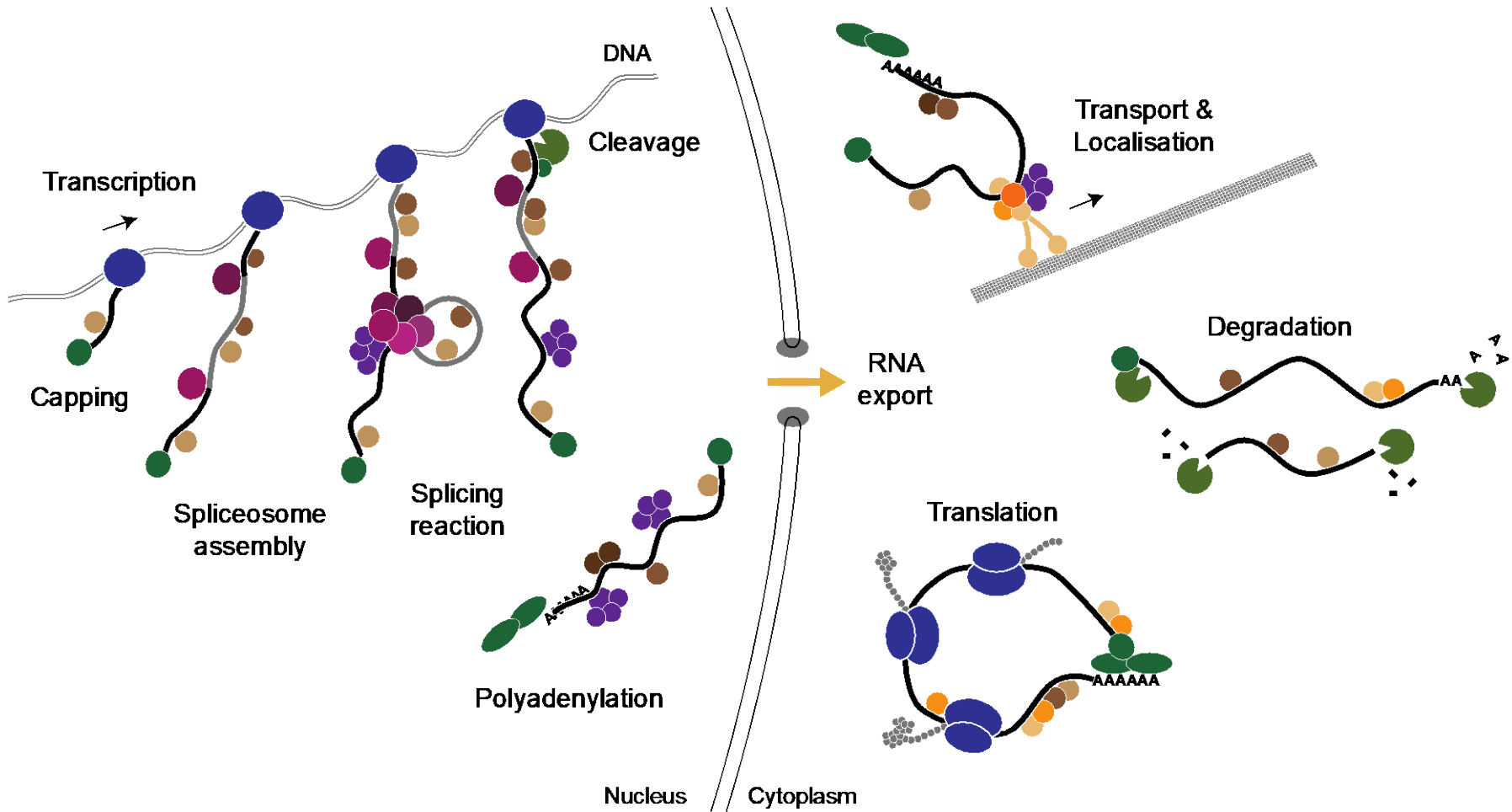
Genomic Views of Splicing Regulation



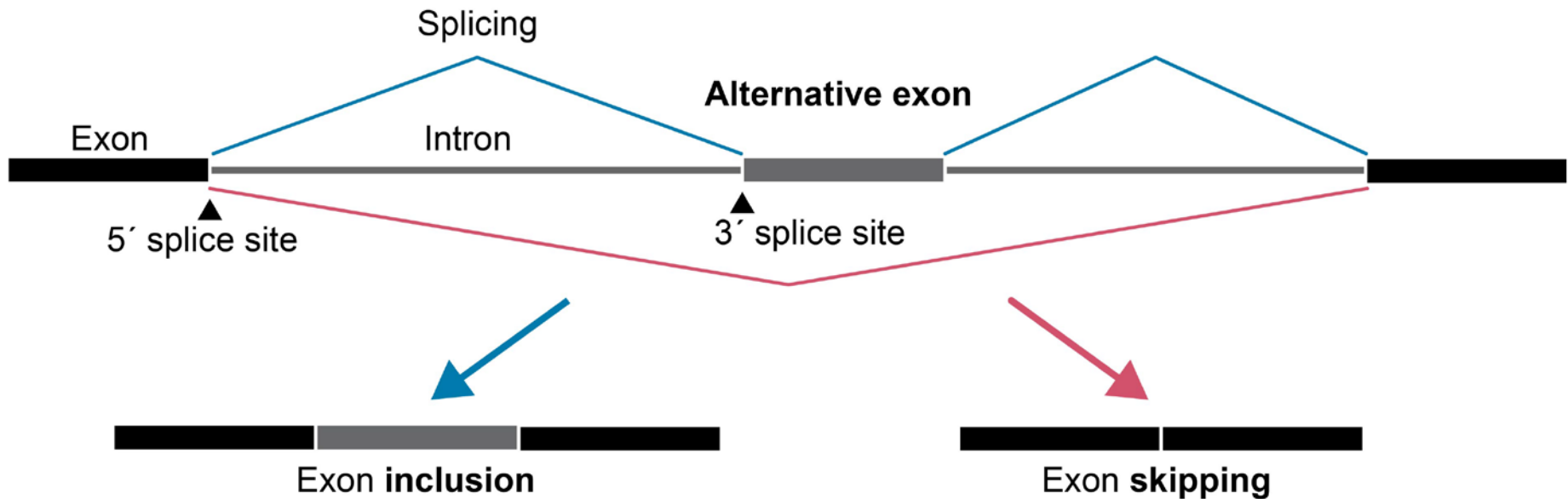
Image:
Heng-Chang Chen

Julian König
Institute of Molecular Biology (IMB), Mainz

Gene expression is extensively controlled at the posttranscriptional level

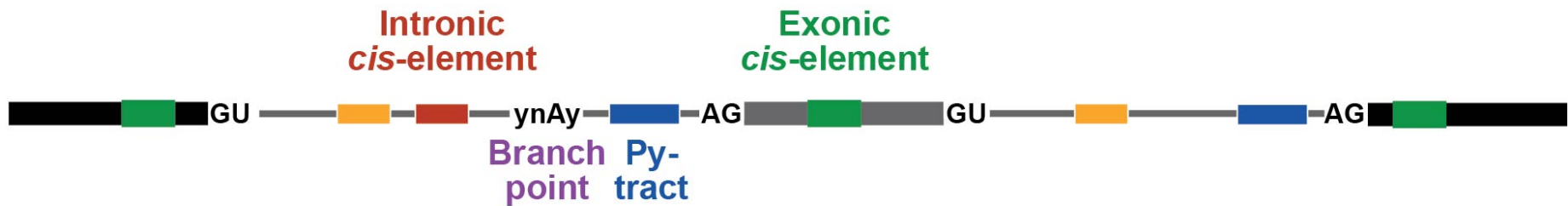


Alternative splicing is crucial to generate proteome diversity

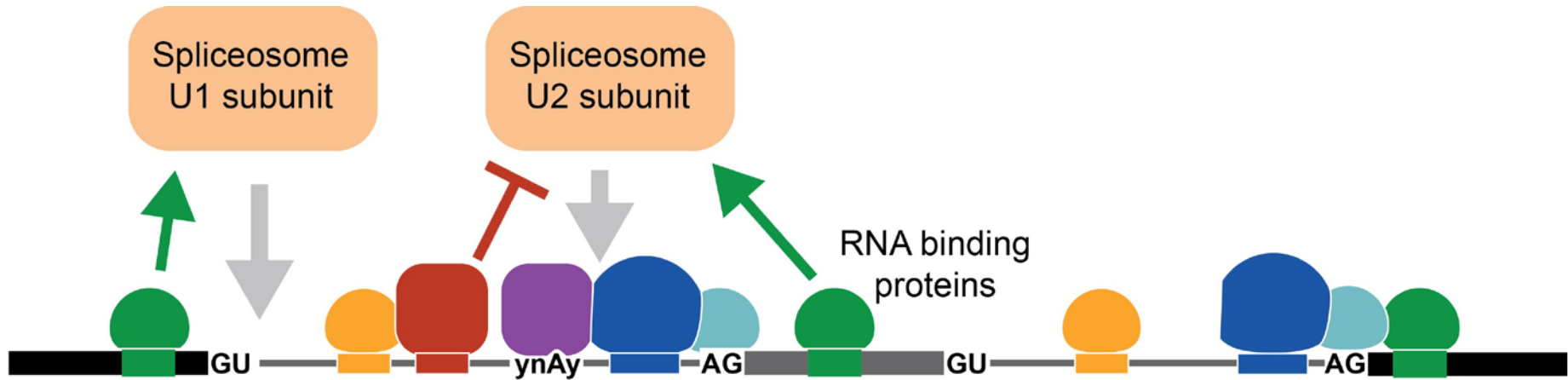


Important for tissue identity & targeted in genetic disorders and cancer

cis-regulatory elements encode splicing regulation



RNA-binding proteins promote or repress spliceosome recruitment

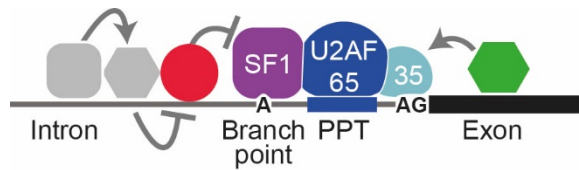


How the *cis*-elements are interpreted by RNA-binding proteins is called **the splicing code**

Decipher the regulatory code of splicing.

Predict, understand and treat erroneous splicing
in genetic disease and cancer.

3' splice site definition



Ribosome-associated quality control

MKRN1 (human)

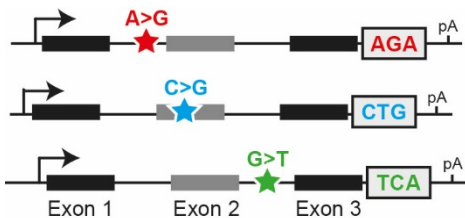


- Zink finger (C₃H)
- PAM2-like
- RING domain

Splicing

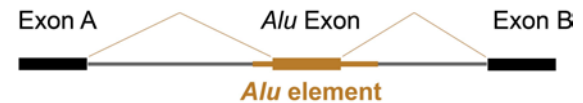


cis-regulatory elements and the splicing code



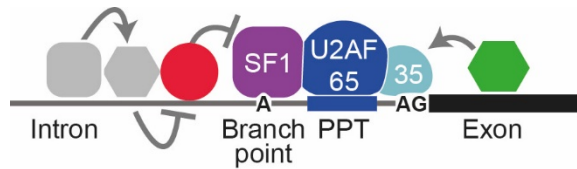
Translation

Alu element exonization in evolution and disease



Project 1: Ribonucleoprotein complex assembly

3' splice site definition



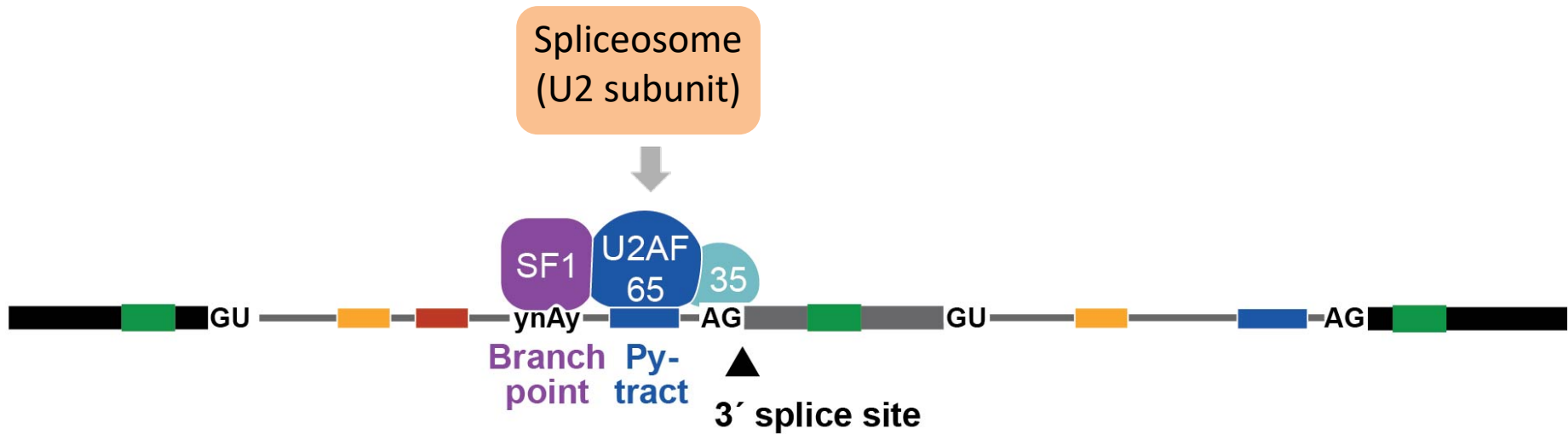
Splicing



Translation

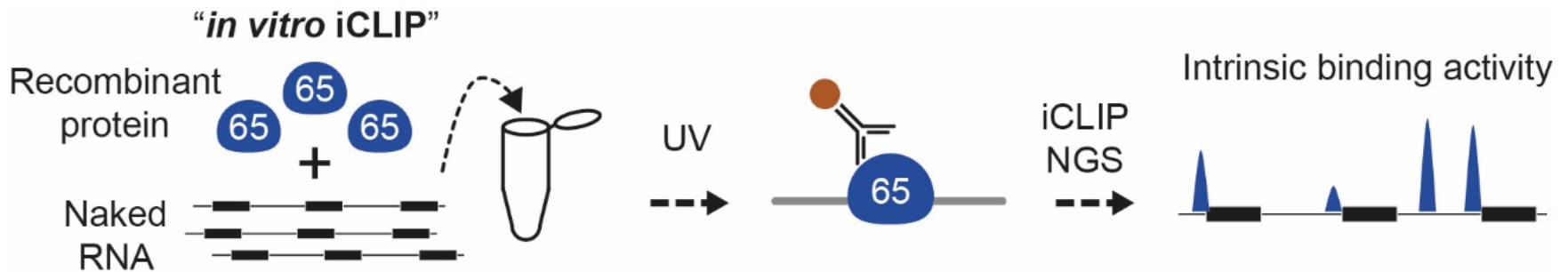
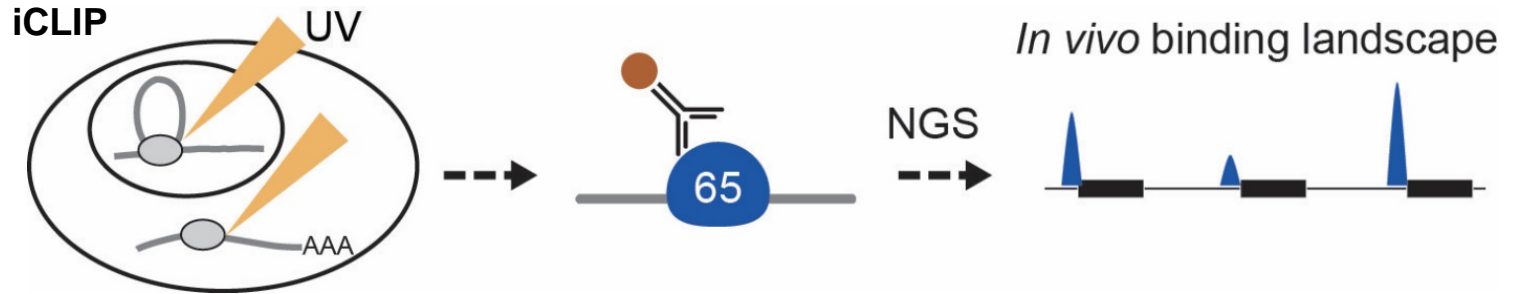
How do ribonucleoprotein complexes assemble at the 3' splice site?

U2AF65 is a central player in 3' splice site definition

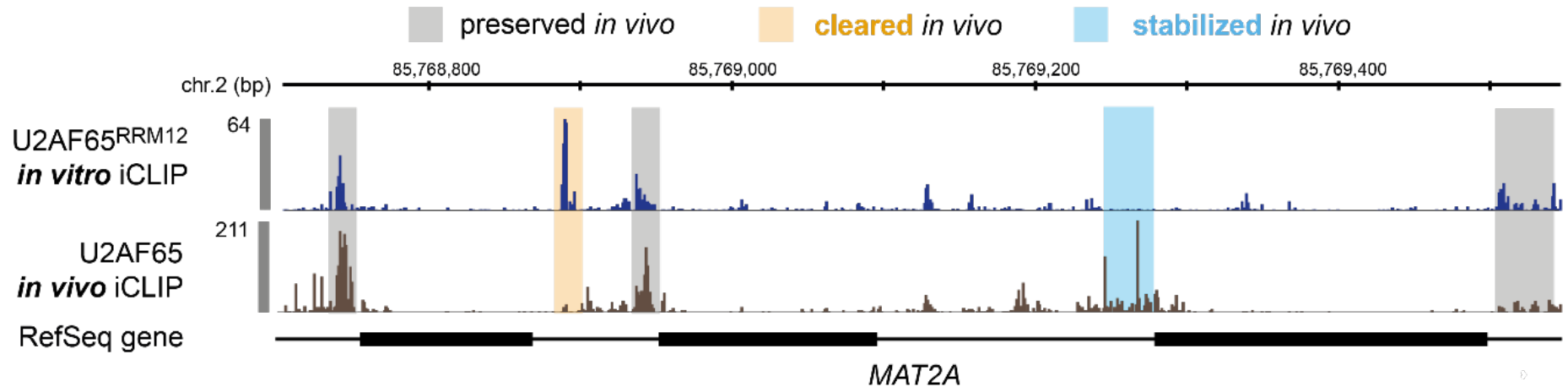


How does U2AF65 specifically recognize splice sites?

in vitro iCLIP reveals the intrinsic binding activity of U2AF65

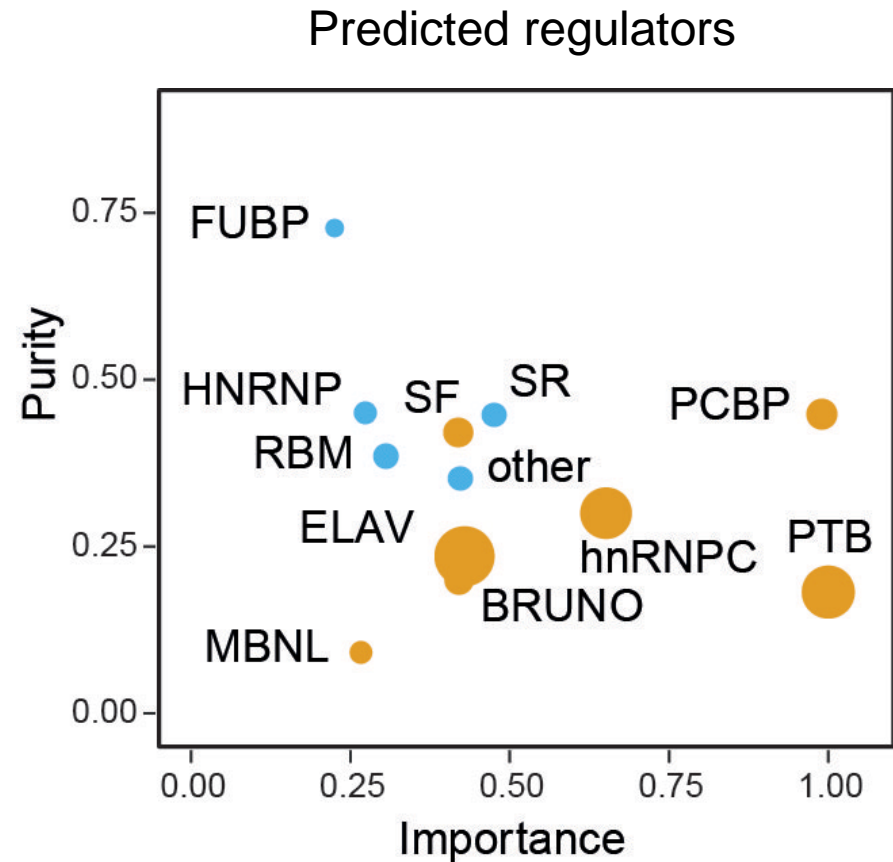
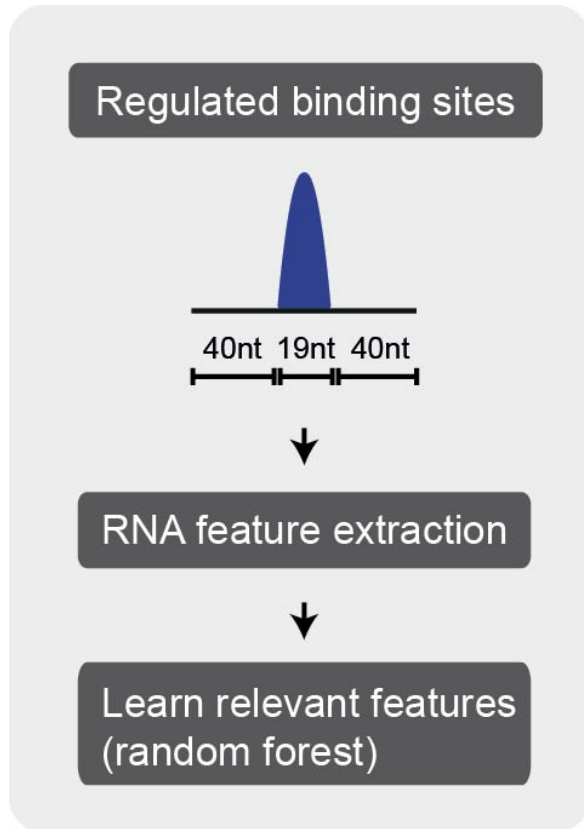


In vitro vs. *in vivo* comparison pinpoints regulatory hotspots



What causes the differences?

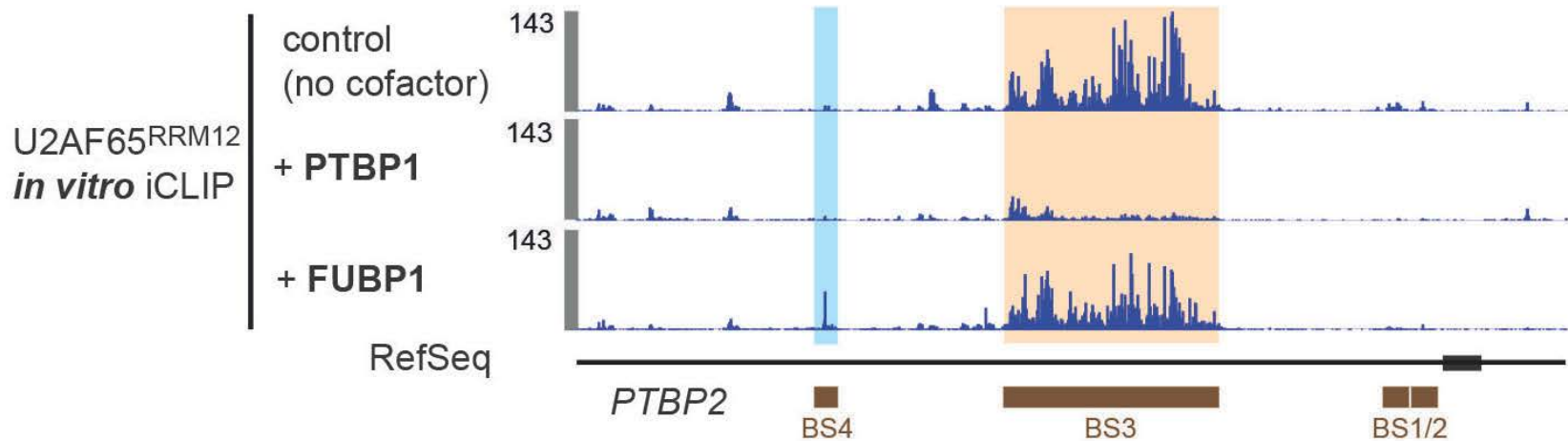
Machine learning predicts regulators of U2AF65 binding



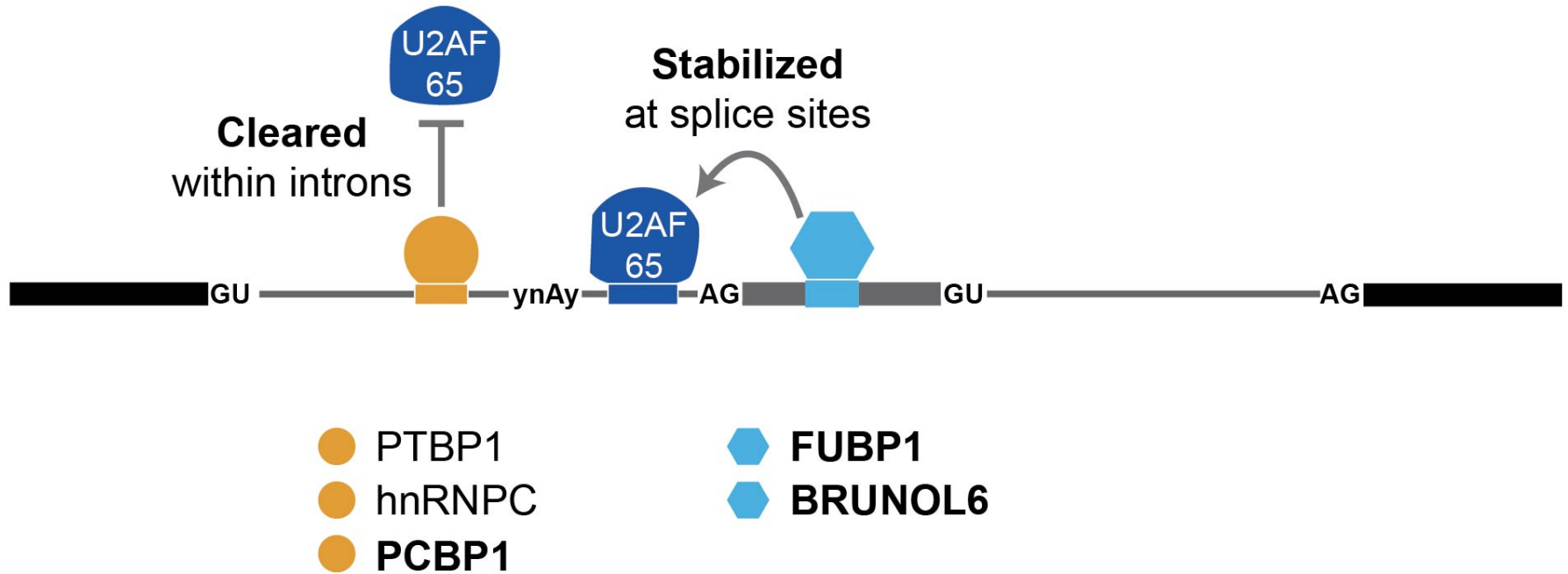
Binding at **cleared** & **stabilized** sites

PTBP1 and FUBP1 shape U2AF65 binding

■ FUBP1-regulated sites ■ PTBP1-regulated sites



in vitro iCLIP uncovers regulation of U2AF65 by co-factors



Decipher the regulatory network of selected splicing events

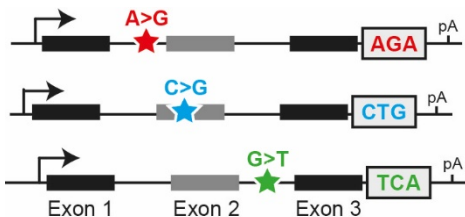
Predict disease-causing mutations

Splicing

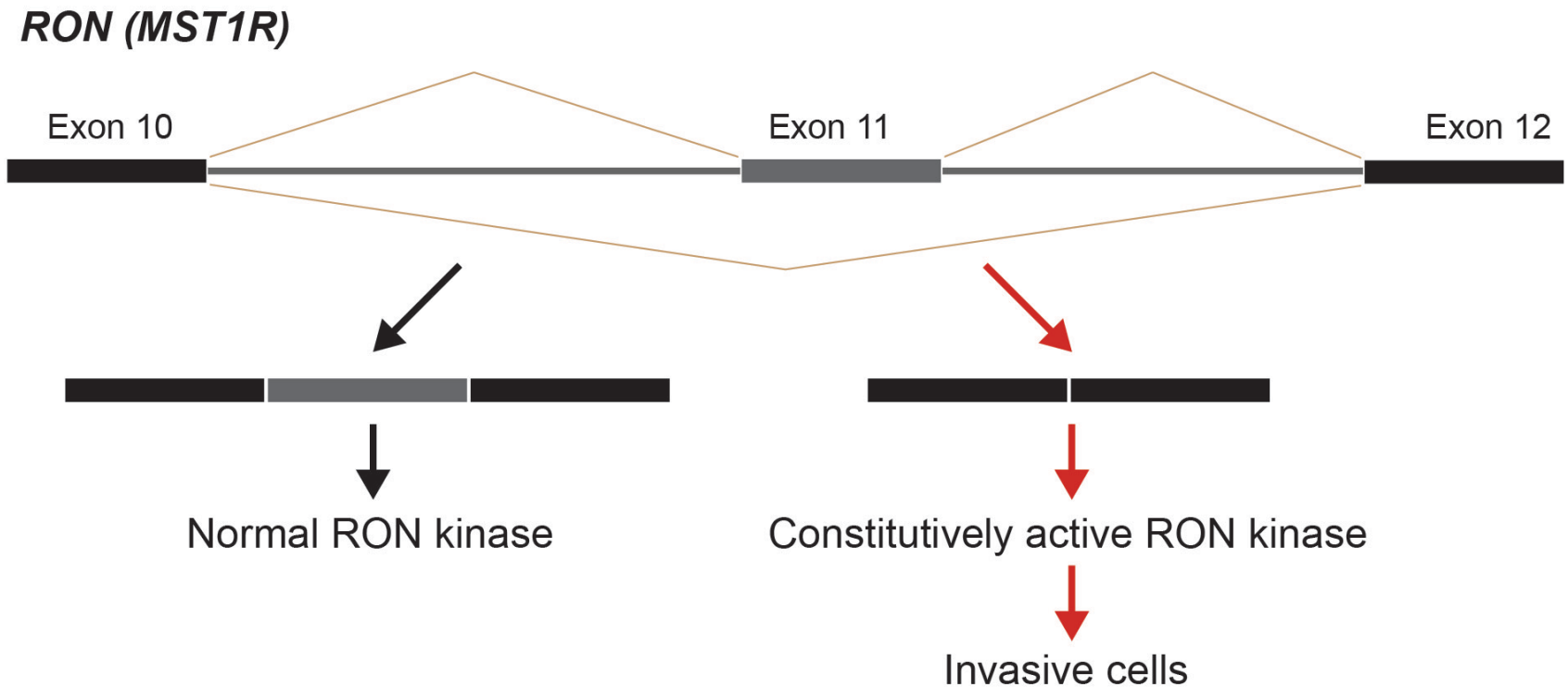


Translation

cis-regulatory elements
and the splicing code

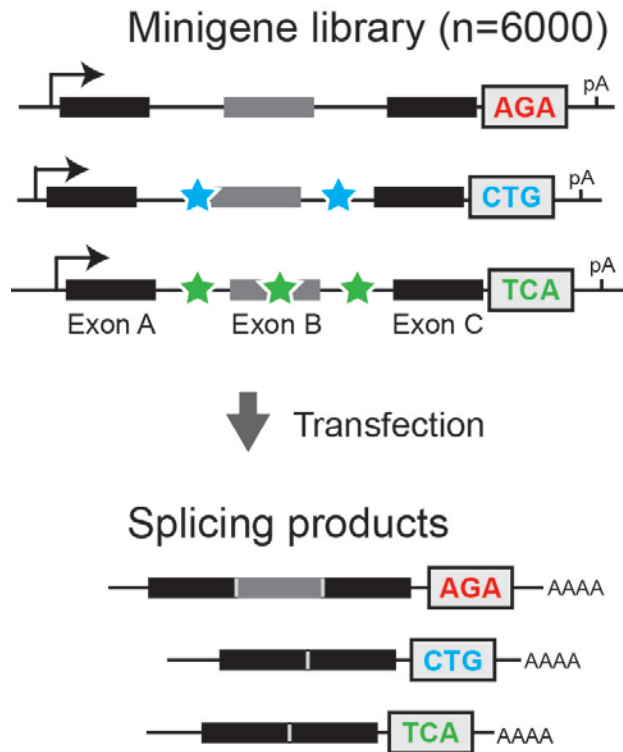


Aberrant splicing of *RON* promotes invasive cancer cells

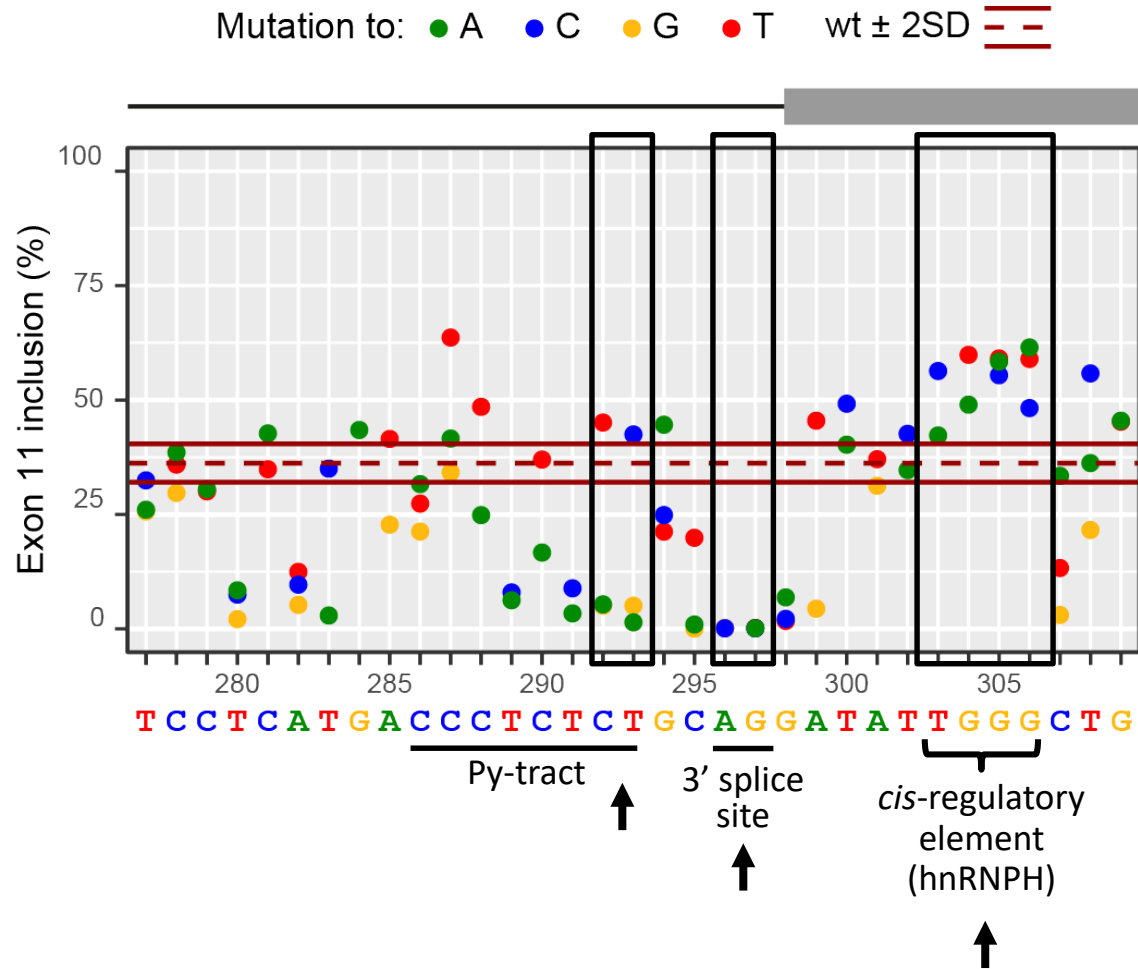


We aim for a complete map of *cis*-regulatory elements that control *RON* splicing

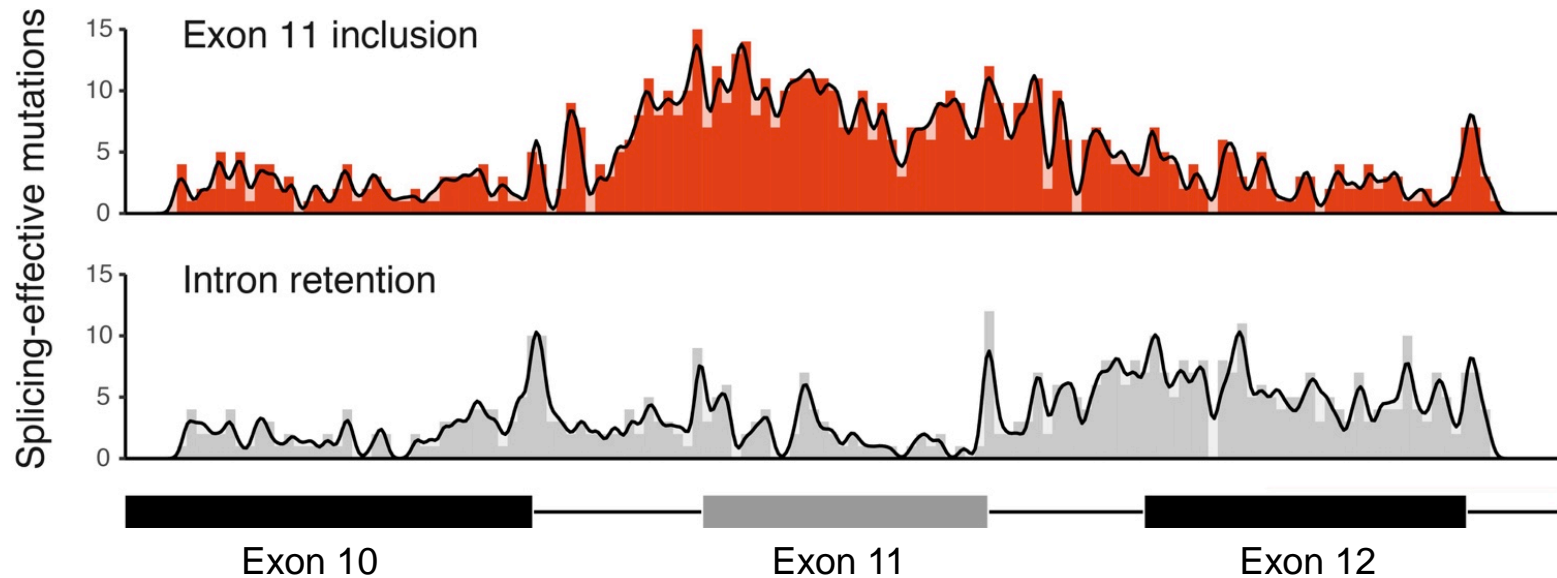
High-throughput screen for *cis*-regulatory elements



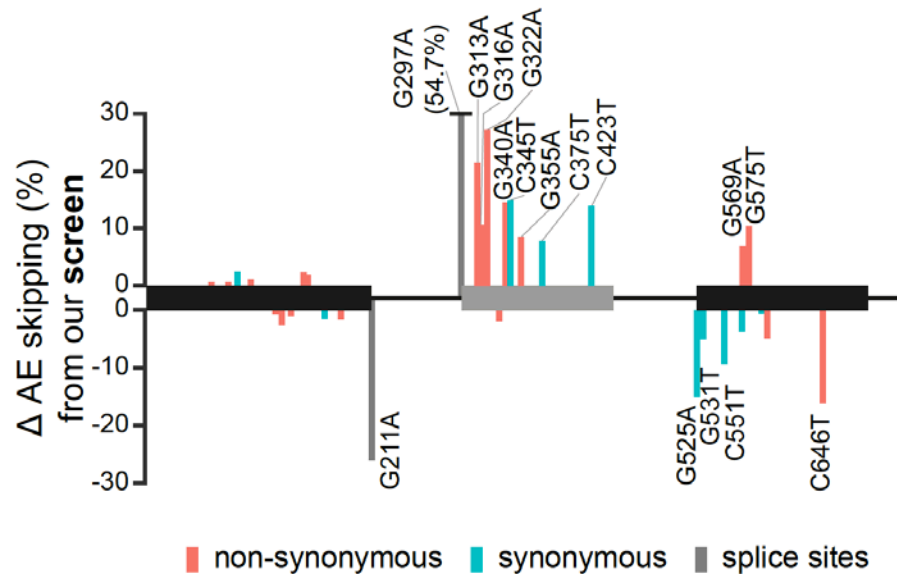
Quantification of mutation effects with nucleotide resolution



The dense regulatory landscape of *RON* exon 11

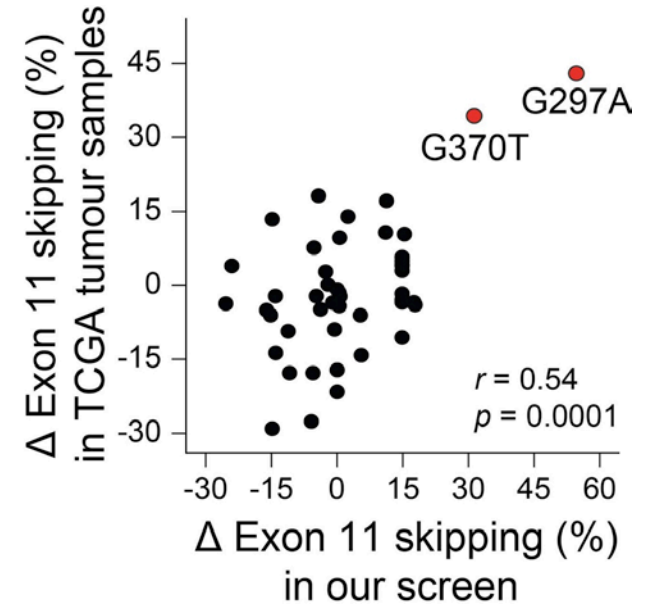


The identified mutations modulate splicing in cancer patients



COSMIC

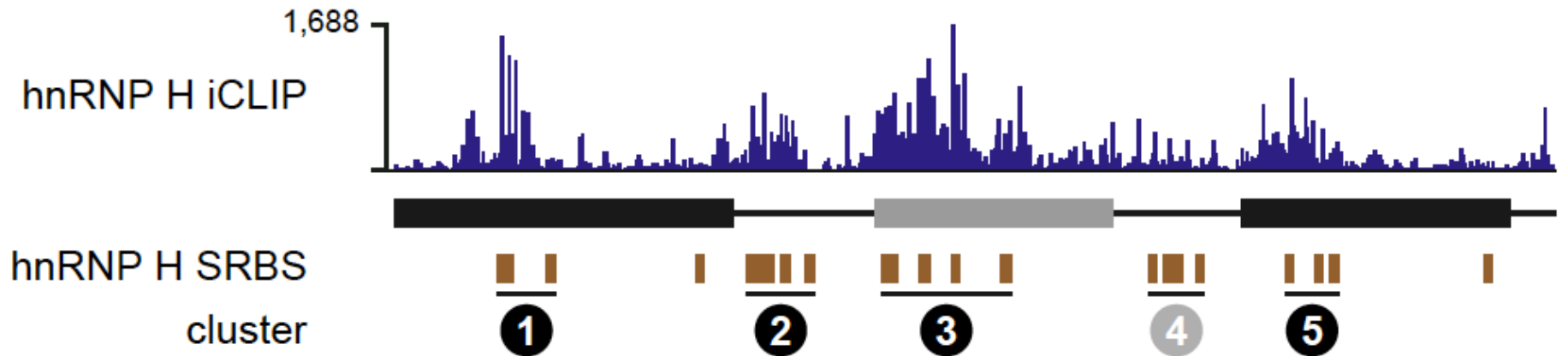
(Catalogue of Somatic Mutations in Cancer)



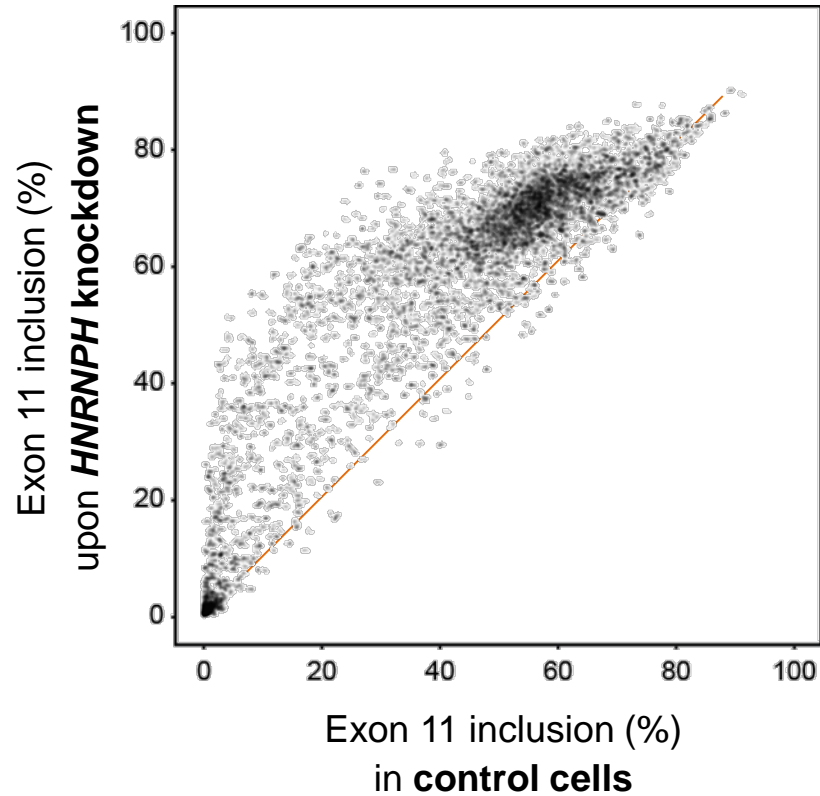
TCGA

(The Cancer Genome Atlas)

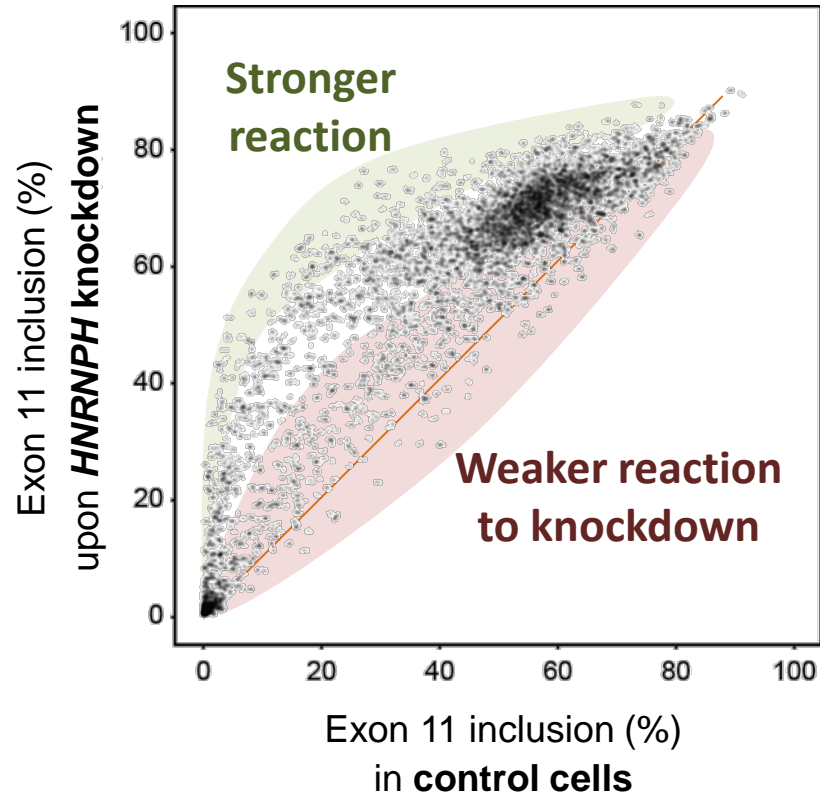
hnRNP H has several binding sites around *RON* exon 11



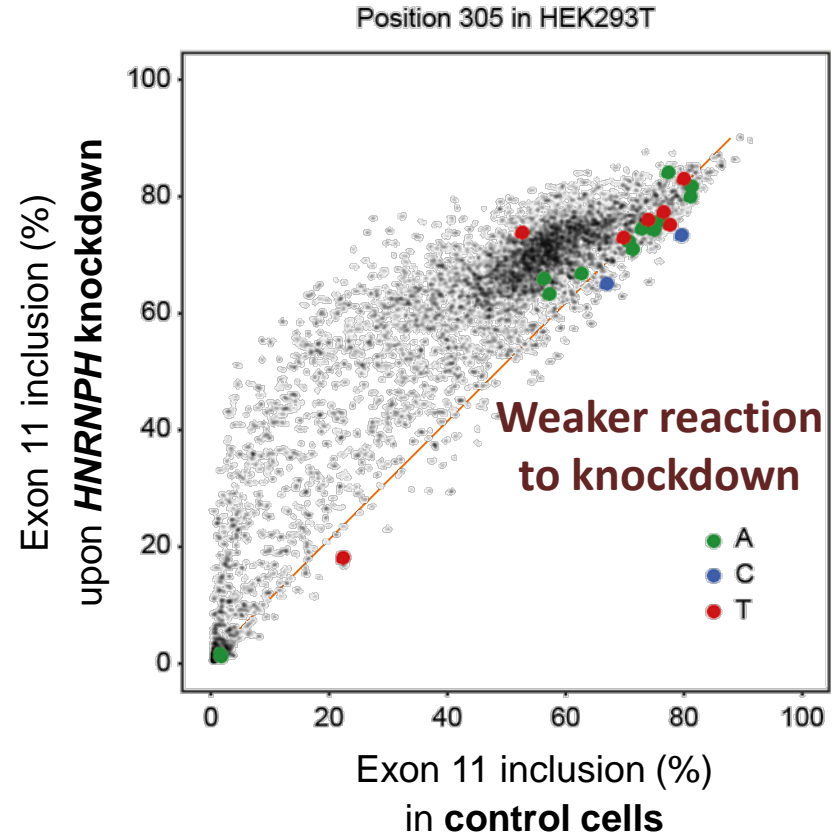
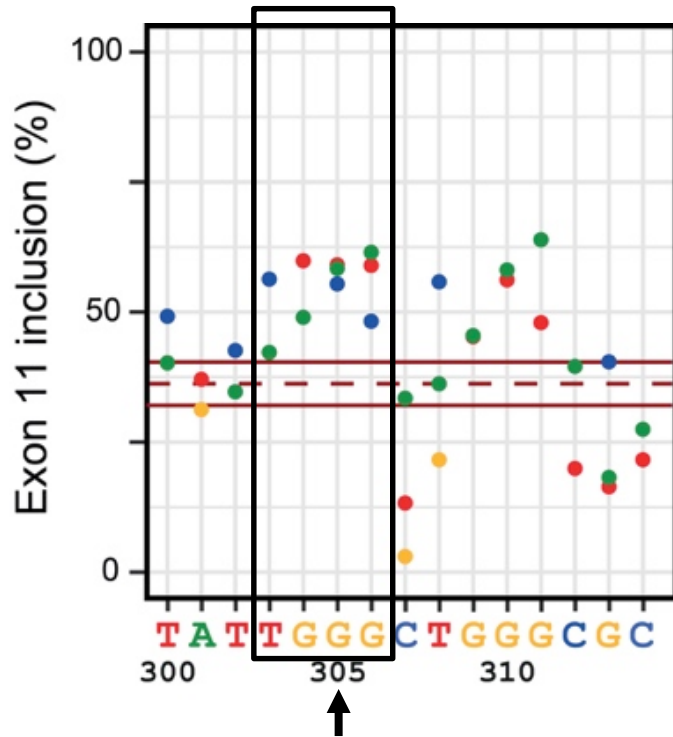
Epistatic interactions link RBPs to *cis*-regulatory elements



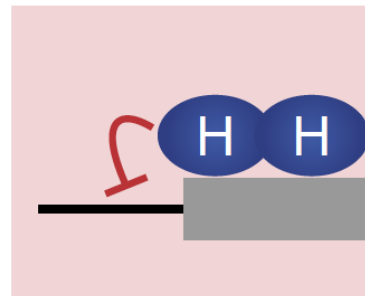
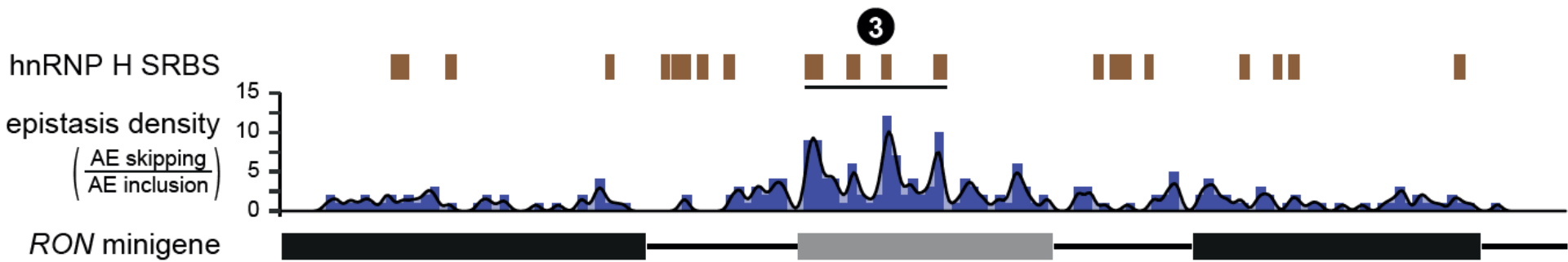
Epistatic interactions link RBPs to *cis*-regulatory elements



Mutants in hnRNP H binding sites are less affected by knockdown

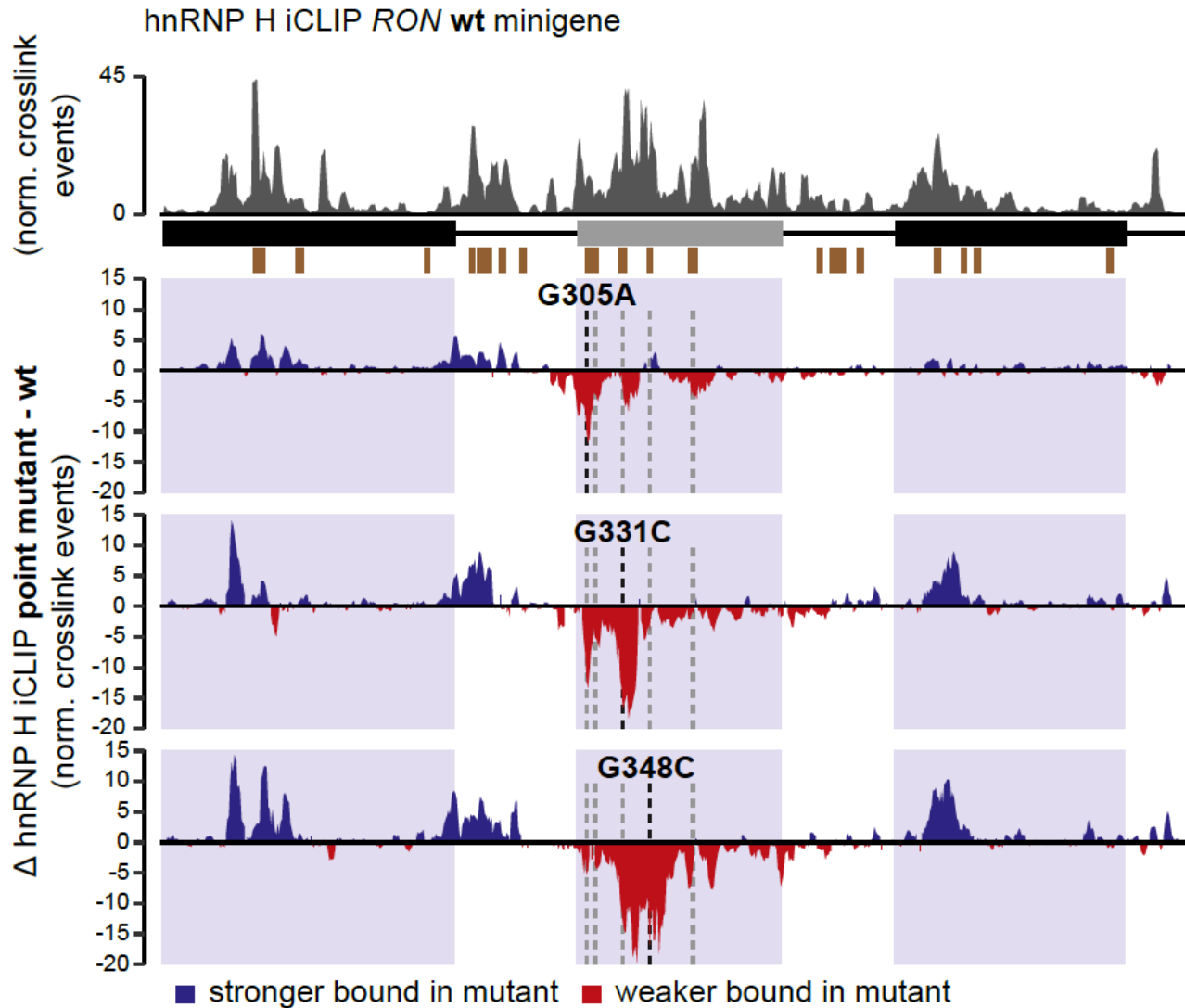


Epistatic effects point to most relevant hnRNP H binding sites

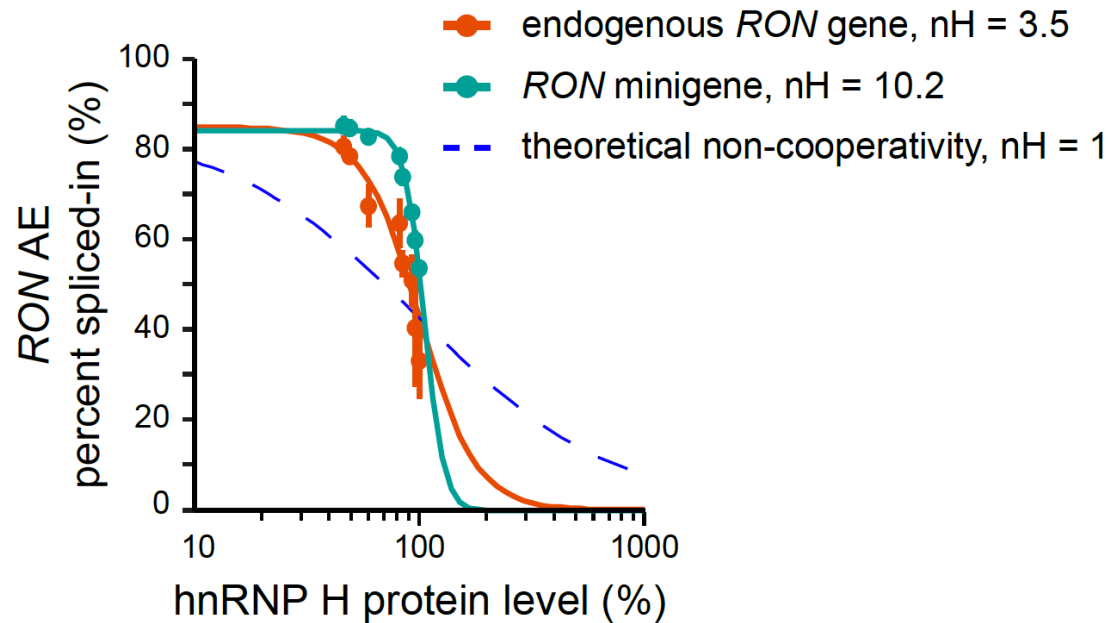


Cooperative binding?

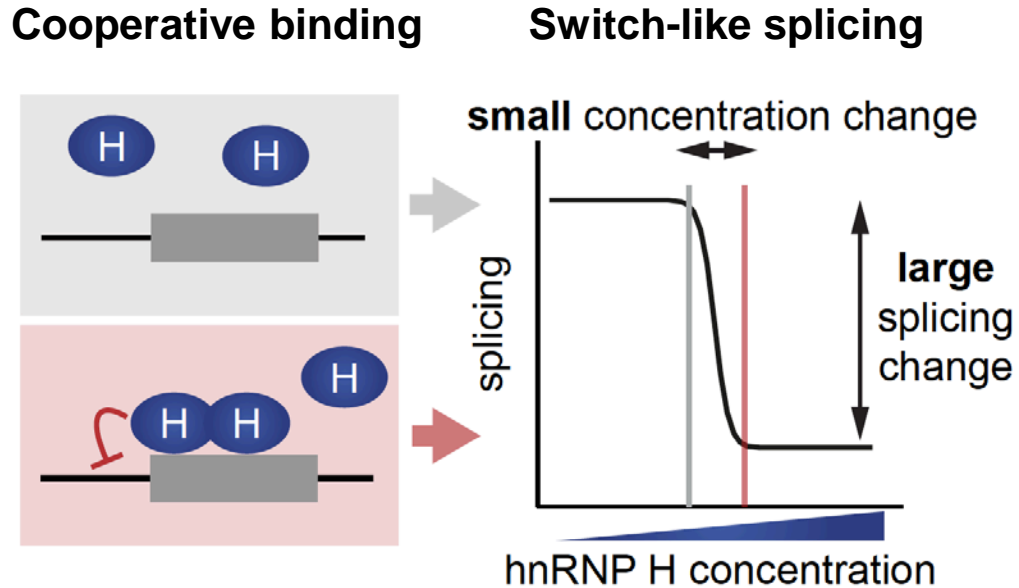
hnRNP H shows cooperative binding in the alternative exon



hnRNP H levels drive a switch-like splicing response



Cooperative binding results in a switch-like splicing response



Thank you!



König group:

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Mariela Cortes Lopez

Laura Schulz

Andreas Buchbender

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IMB administration

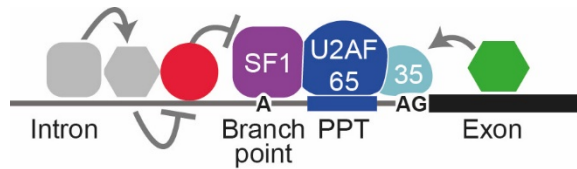
IMB core facilities

Maria Mendez-Lago (Genomics)

Anke Busch (Bioinformatics)

Thank you!

3' splice site definition



Splicing



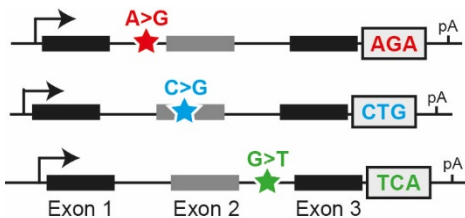
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