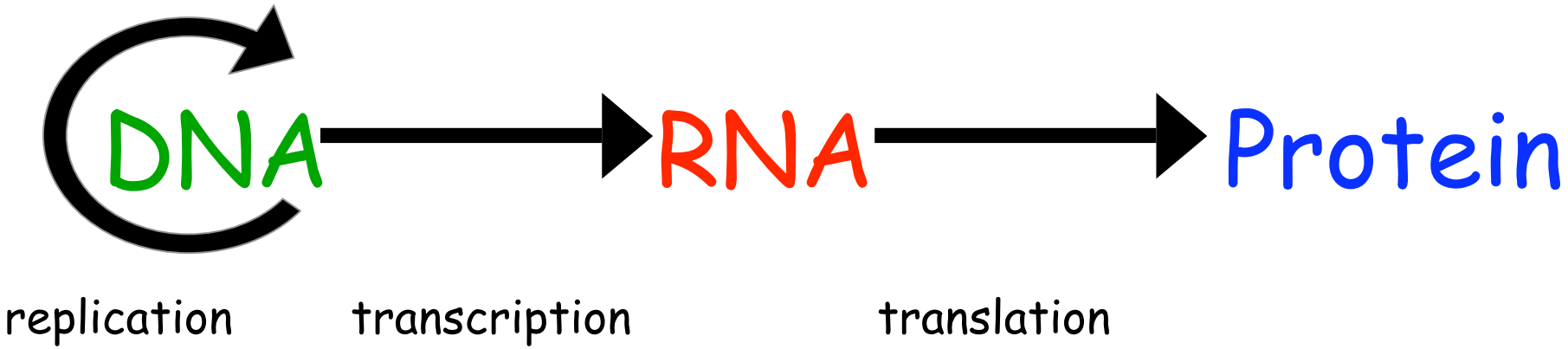
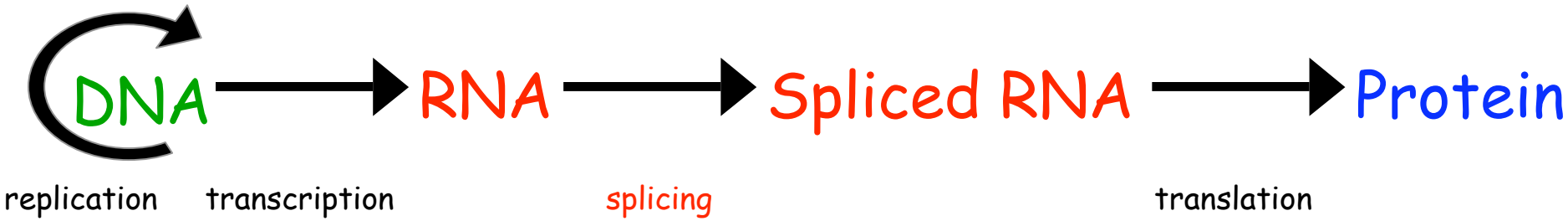
Novel RNAs along the Pathway of Gene Expression

(or, The Expanding Universe of Small RNAs)

Central Dogma



Central Dogma



Base Pair Rules

DNA

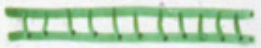
A = T

G ≡ C

RNA

A = U

G ≡ C

DNA usually 2 strands 

RNA usually 1 strand BUT

- Lupus and the Discovery of snRNPs (pronounced snurps)

- Current Challenges in Splicing

- MicroRNAs: the latest novel RNAs in Gene Regulation

Immune System

Antibodies

normally against:

- bacteria
- viruses
- (cancer cells)

abnormal against:

- own cellular components

= Autoantibodies

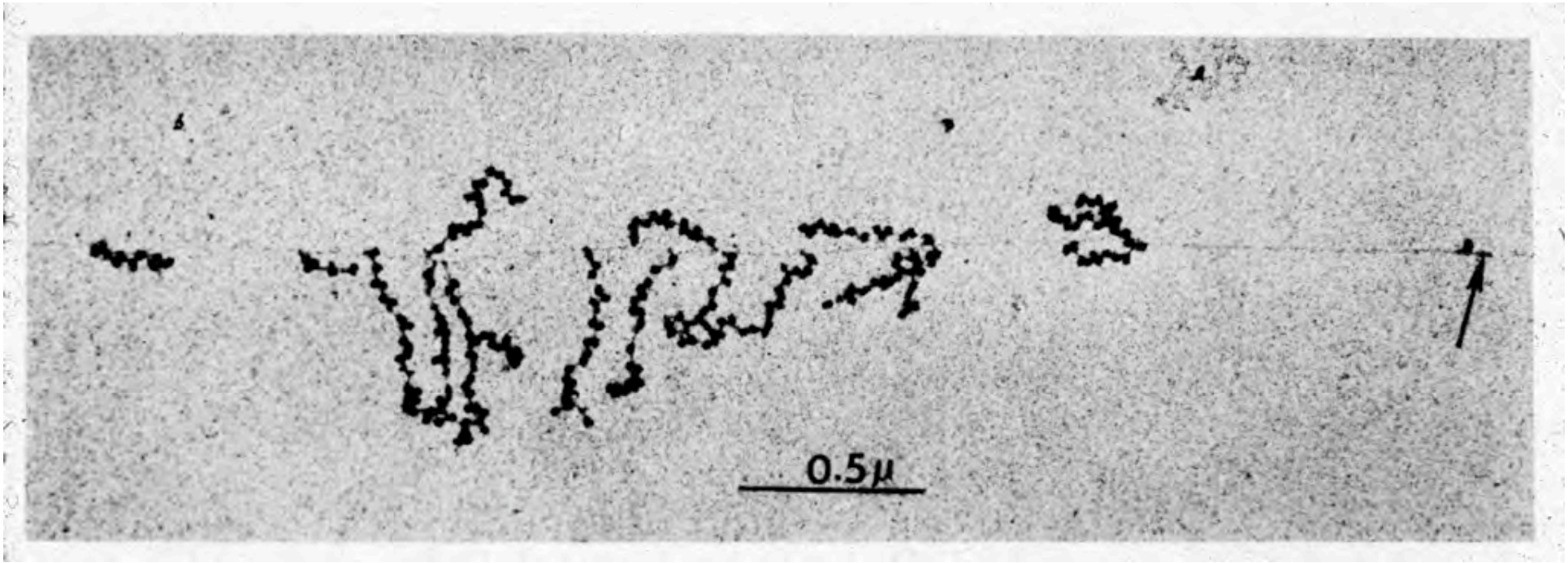
Autoantibodies

antibody

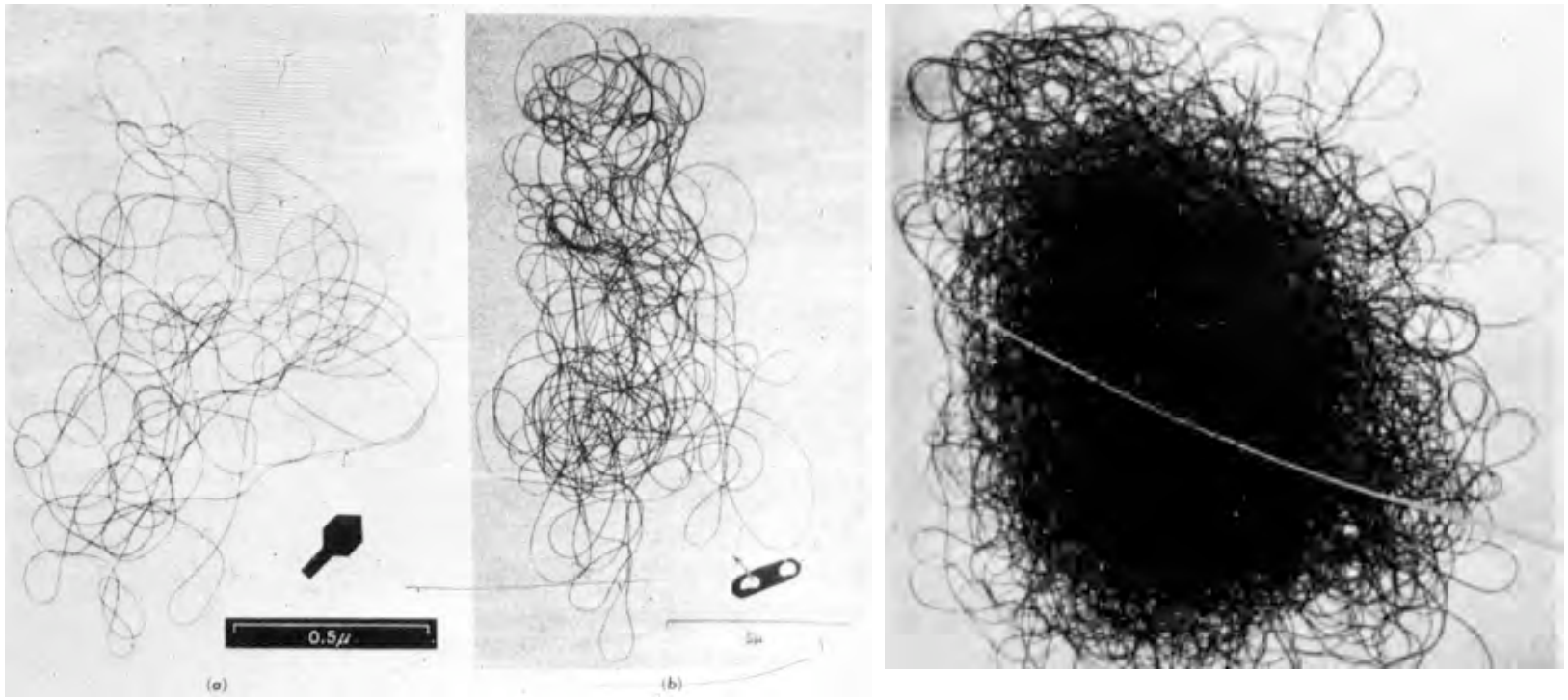
cellular component

immune complex

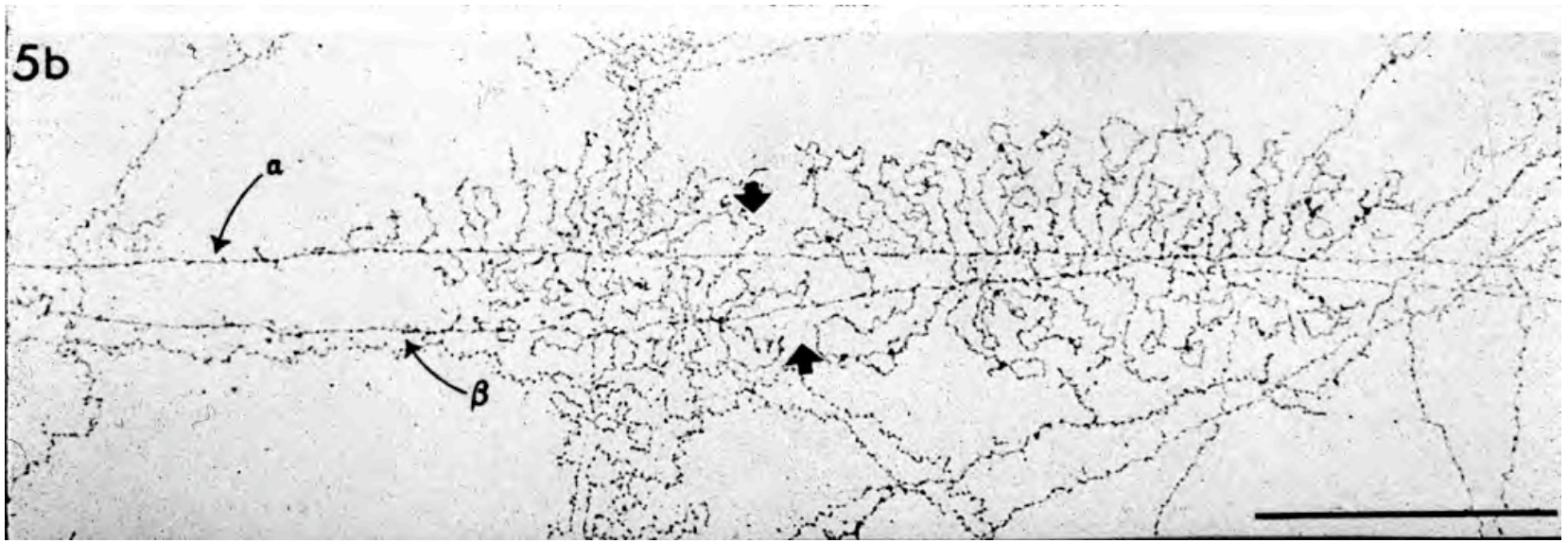
DNA ⟶ RNA ⟶ Protein



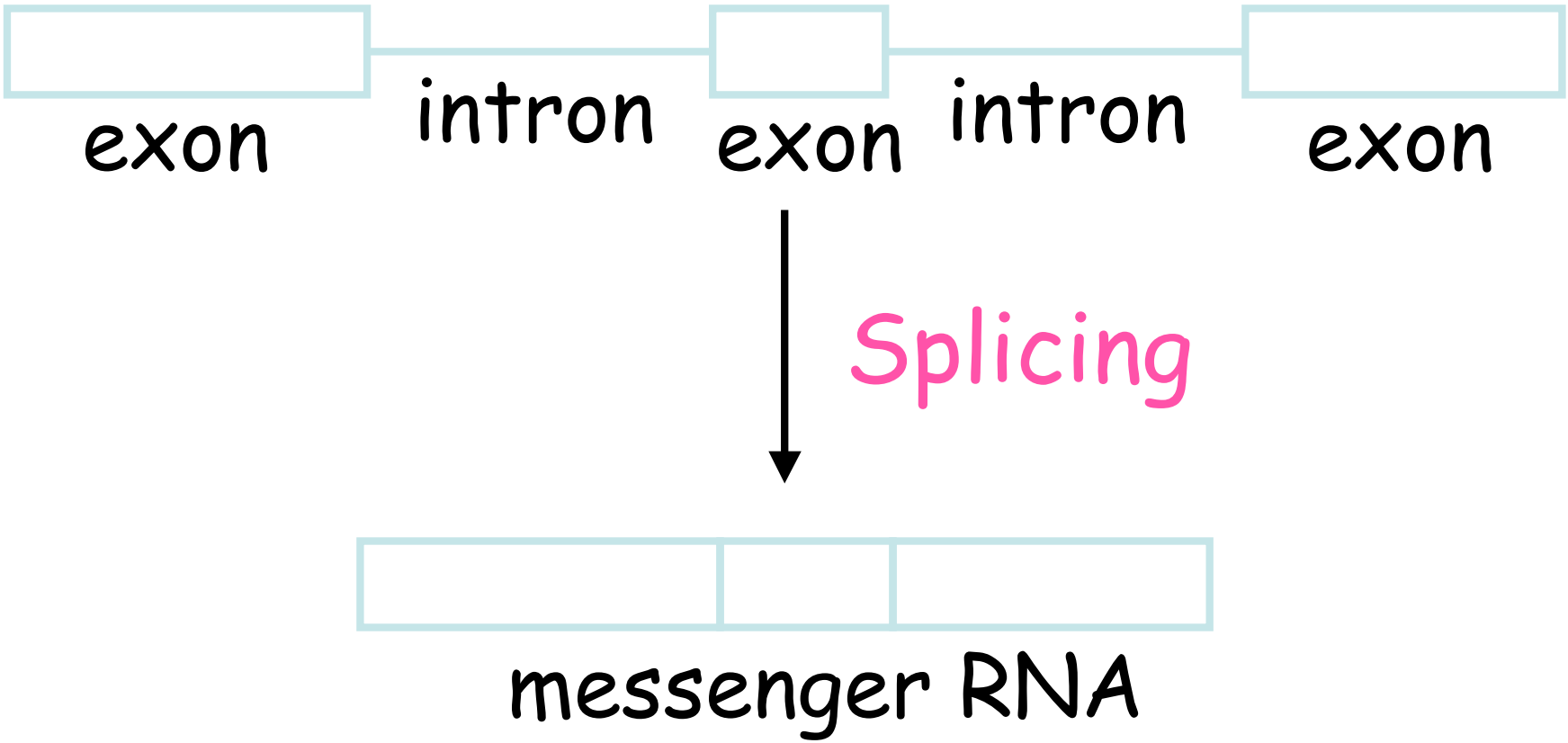
Amount of DNA in Phage, Bacterium, and Mammalian Cell



Nascent RNAs Are Coated with Proteins



Genes in Pieces



Introns

Even though the 3-letter code remains

wpsjkdmspsxmzpyrtgkslqrabkbdifqgpmwxoltfjvsydorwmbxlzfreualksdjhzxmt intact,

many genes are interrupted by long segments of "gibberish"

pjsgelvnyisgqzhbmsjq2qqndpqpoewirualsdfjzxcmvn\rlj DNA containing no 3-letter

words specifying amino acids

Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors

IT is commonly accepted that antibodies do not penetrate living cells. In only one study anti-purine and anti-nucleoside antibodies were found to penetrate fertilised sea urchin eggs and modify their development[1]. Such penetration has been considered unusual and the addition of anti-DNA antibodies does not affect mammalian tissue cells in culture[2]. Direct immunofluorescence of skin biopsies of patients with mixed connective tissue disease (MCTD) using fluorescent anti-IgG has occasionally shown speckled intranuclear fluorescence[3–5] but it is doubted that IgG entered the cells while still viable. Patients with MCTD have high titres of antibody to nuclear ribonucleoprotein (RNP)[6,7] which also gives a nuclear speckled pattern on cell substrates in direct immunofluorescence[8]. Should the antibodies to cellular components and nucleic acids which occur in autoimmune diseases be able to penetrate living cells, a novel mechanism of immunologically mediated damage and/or dysfunction could operate. We show here that anti-RNP IgG can penetrate viable human mononuclear cells (MNC), by their surface Fc receptor, and react with their nuclear RNP.

We thank the Fondo de Fomento Educativo, Mexico, for financial support.

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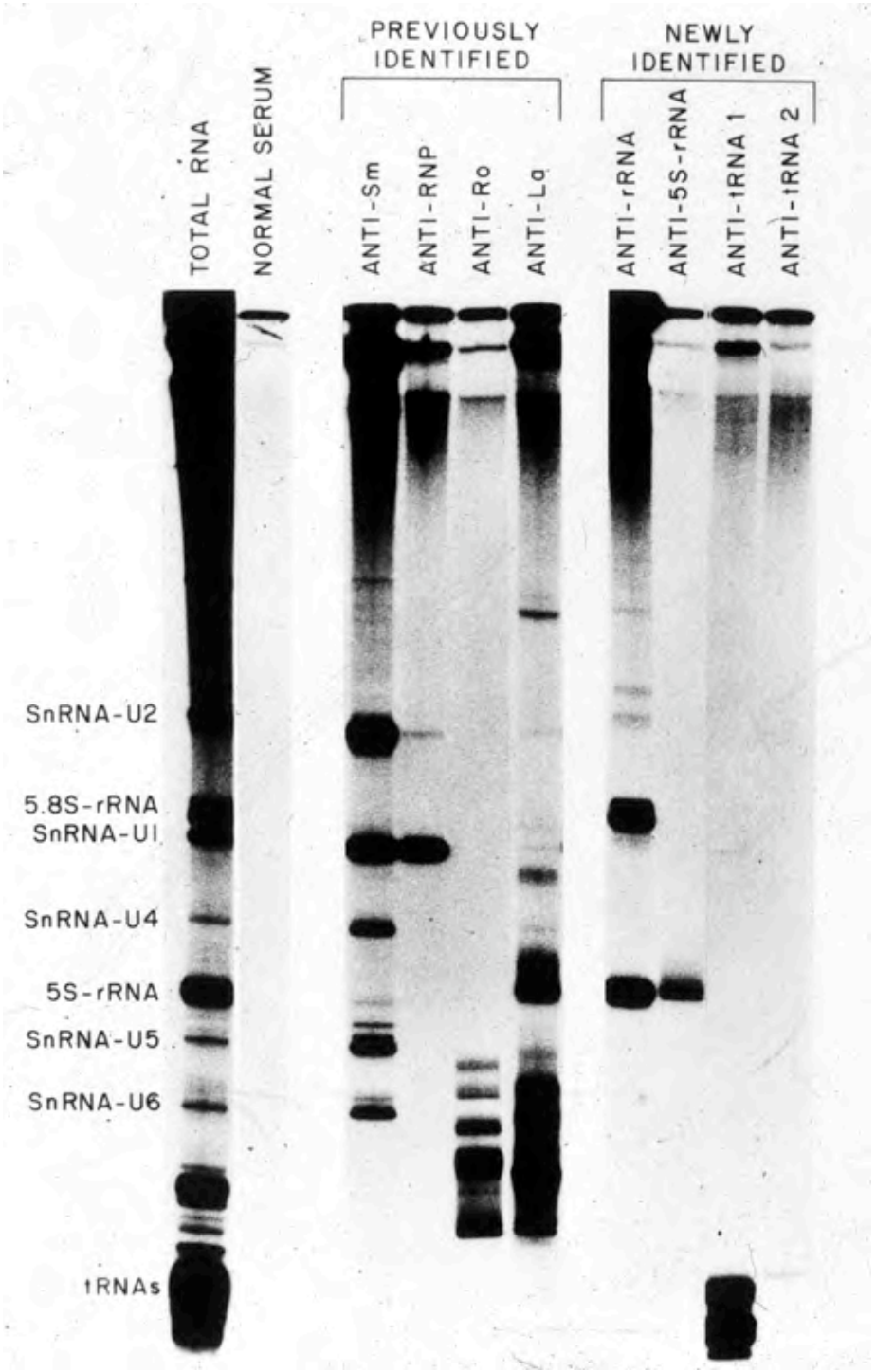
1. Rosenkranz, H. S., Erlanger, B. F., Tanenbaum, S. W. & Beiser, S. M. *Science* **145**, 282–284 (1964).

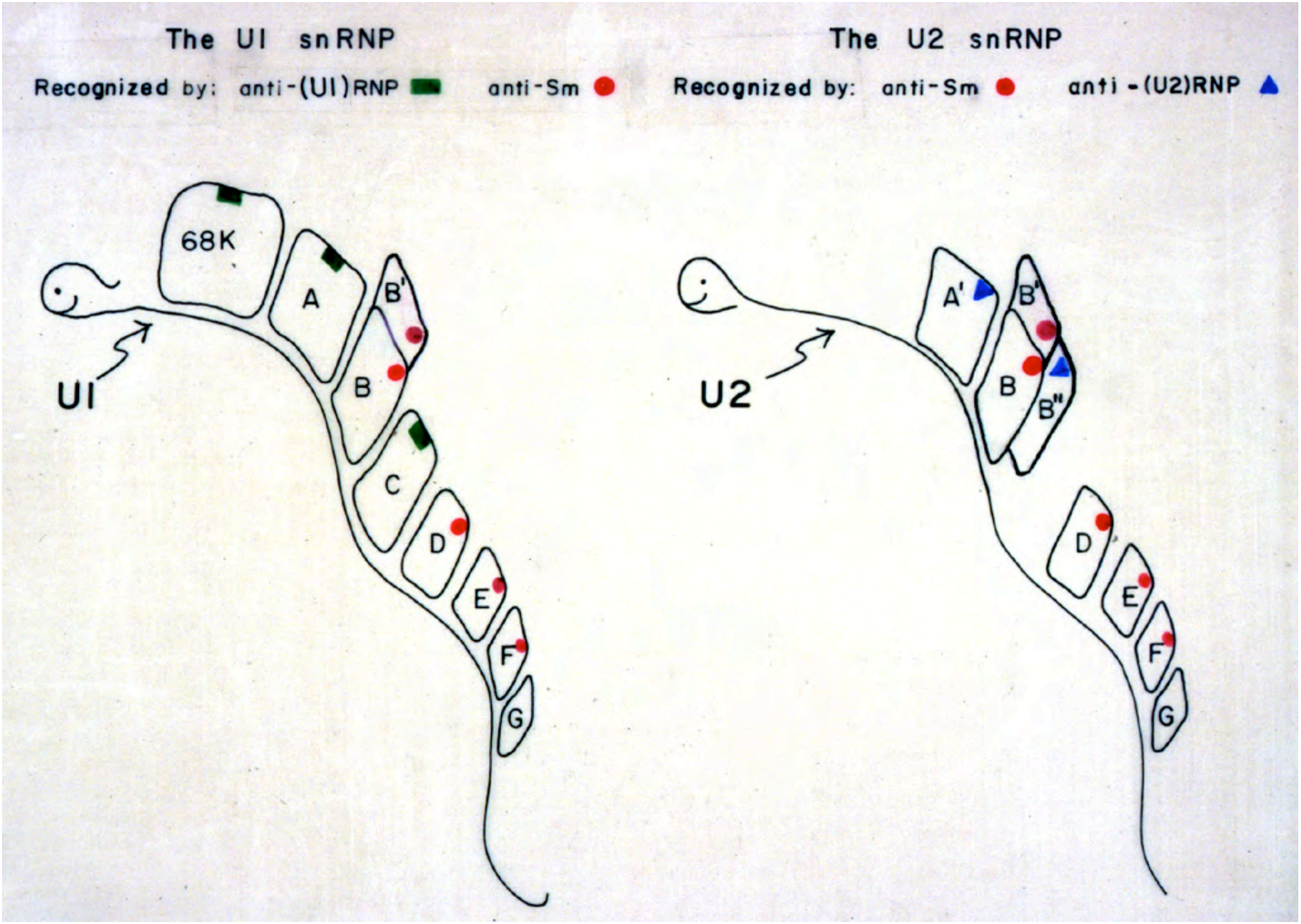
Autoantibodies

antibody

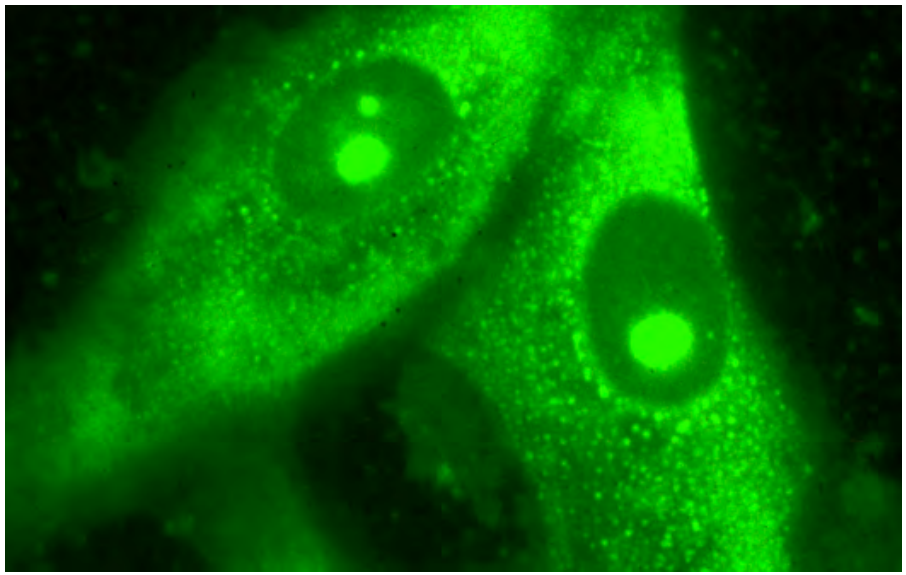
cellular component

immune complex

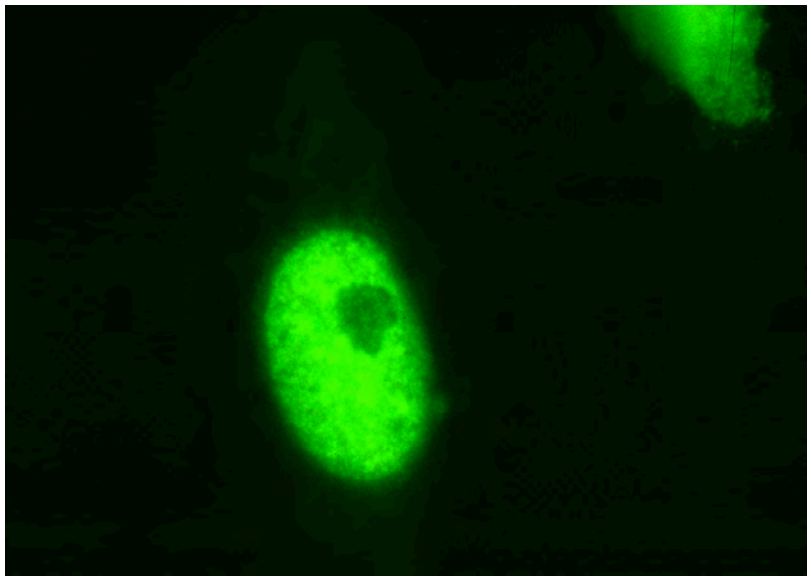

PREVIOUSLY IDENTIFIED
NEWLY IDENTIFIED
TOTAL RNA
NORMAL SERUM
ANTI-Sm
ANTI-RNP
ANTI-Ro
ANTI-La
ANTI-rRNA
ANTI-5S-rRNA
ANTI-tRNA 1
ANTI-tRNA 2
SnRNA-U2
5.8S-rRNA
SnRNA-U1
SnRNA-U4
5S-rRNA
SnRNA-U5
SnRNA-U6
tRNAs


The UI snRNP
Recognized by: anti-(UI)RNP
anti-Sm
68K
A
B'
B
C
D
E
F
G
UI
The U2 snRNP
Recognized by: anti-Sm
anti-(U2)RNP
A'
B'
B
B"
D
E
F
G
U2

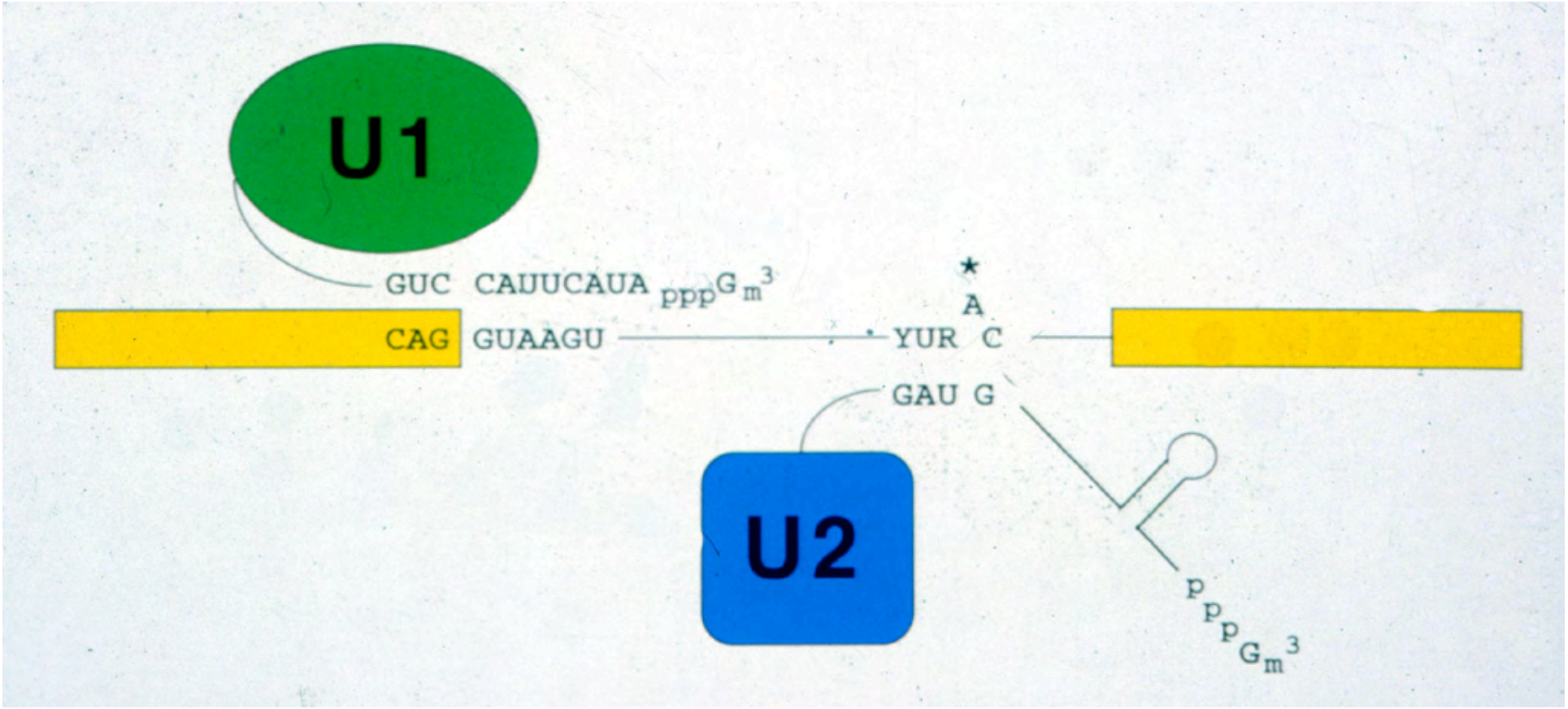
anti-ribosome



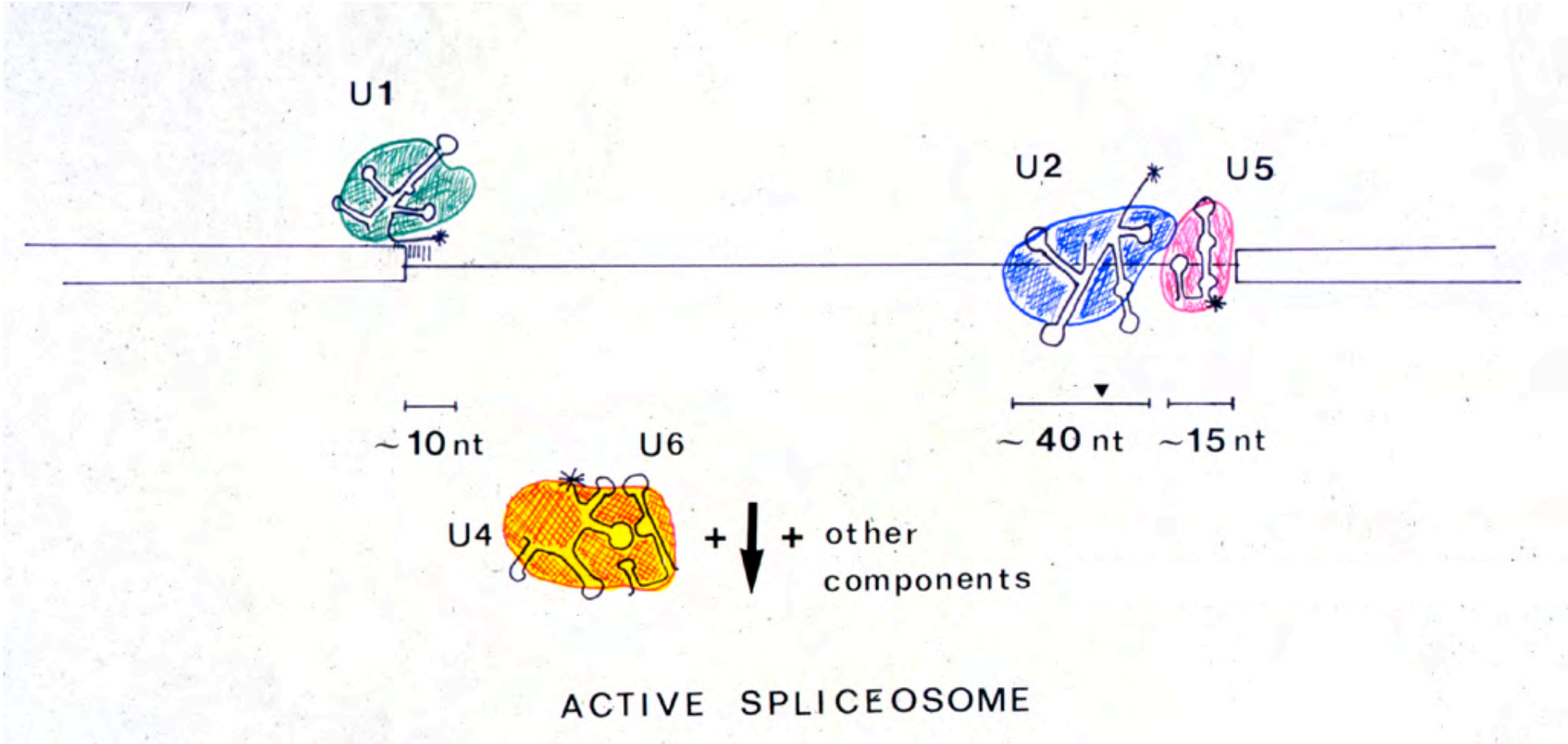
anti-RNP



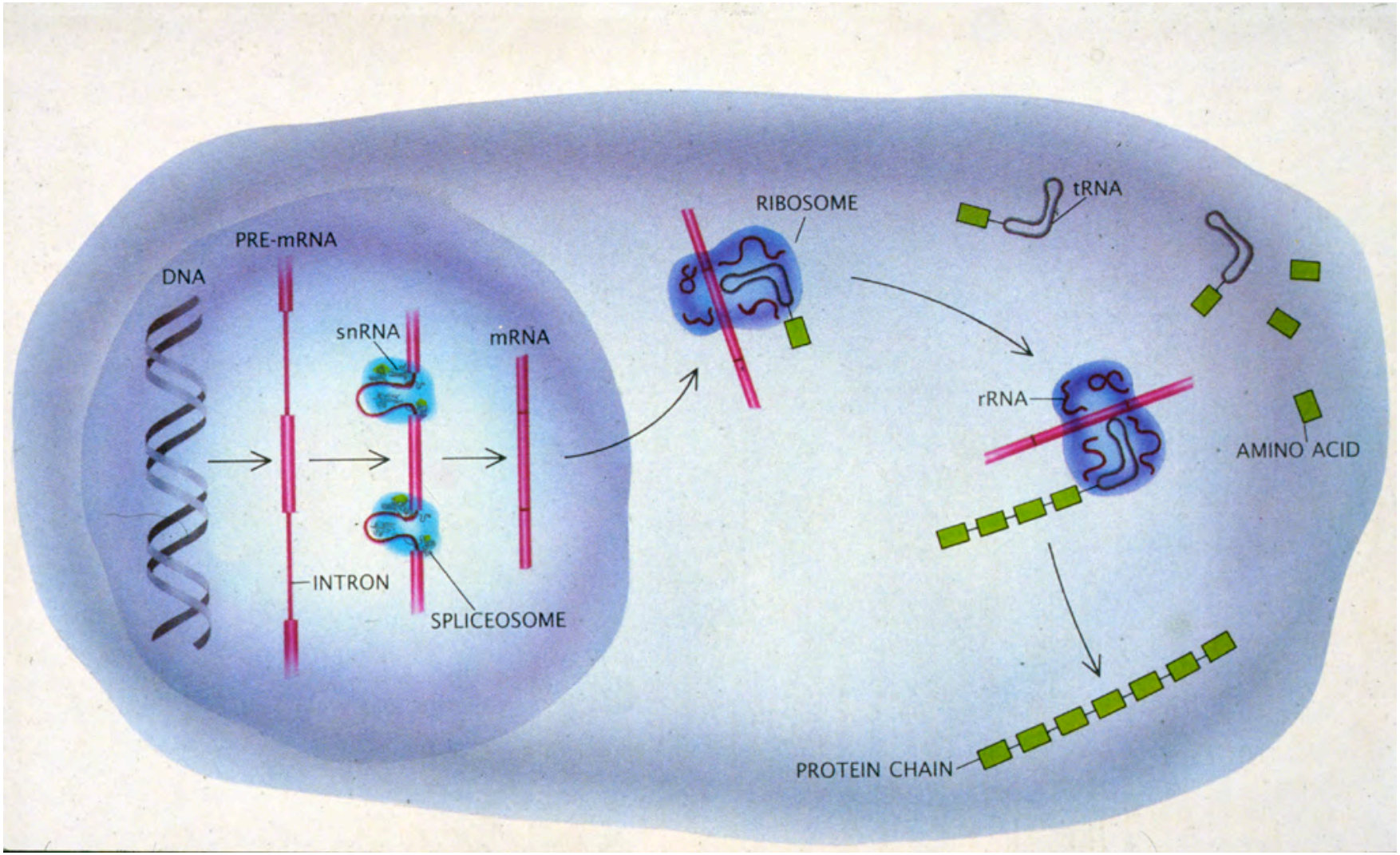
Base Pairing Interactions in Splicing



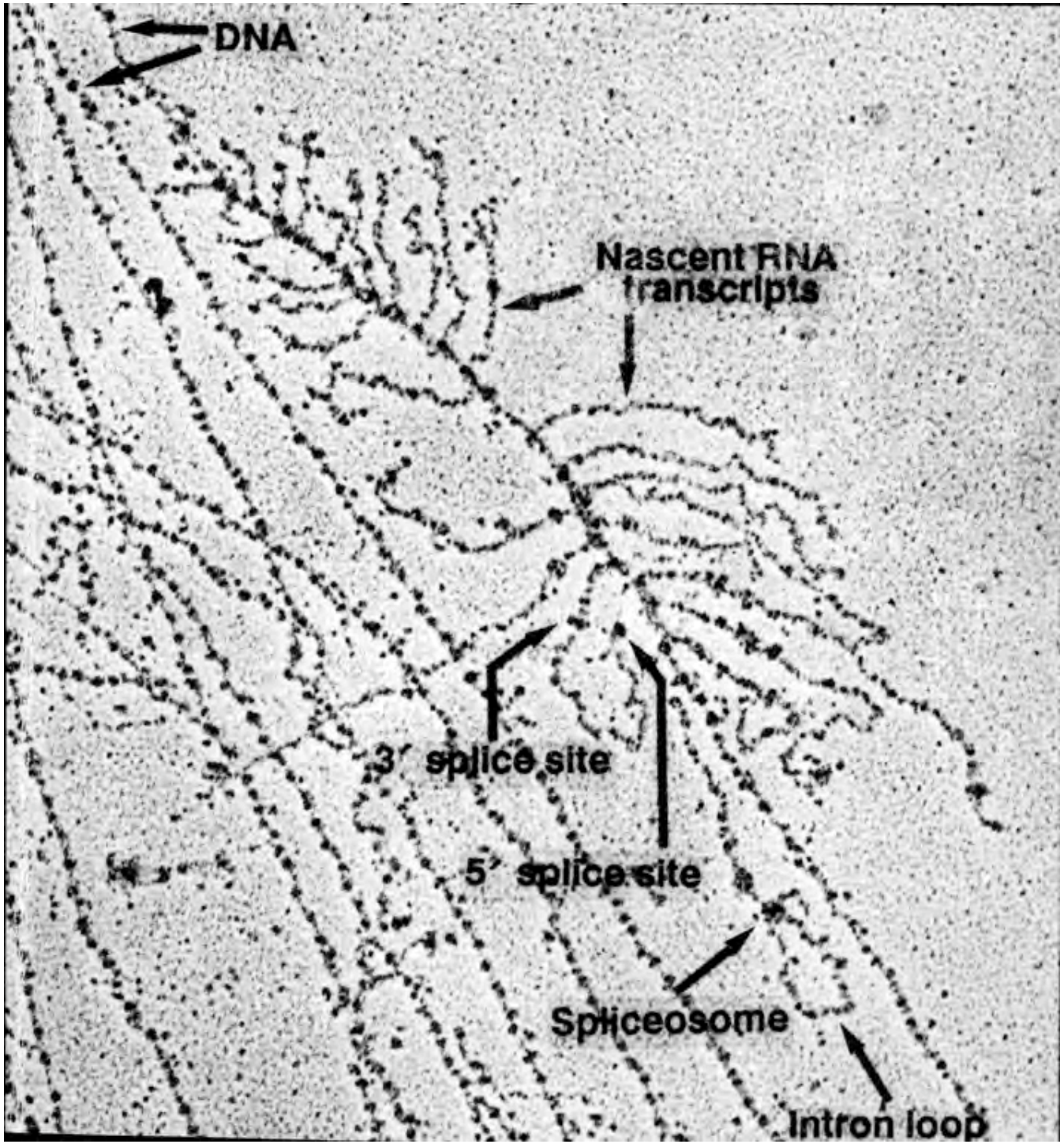
SnRNP Binding Determines Minimum Intron Size

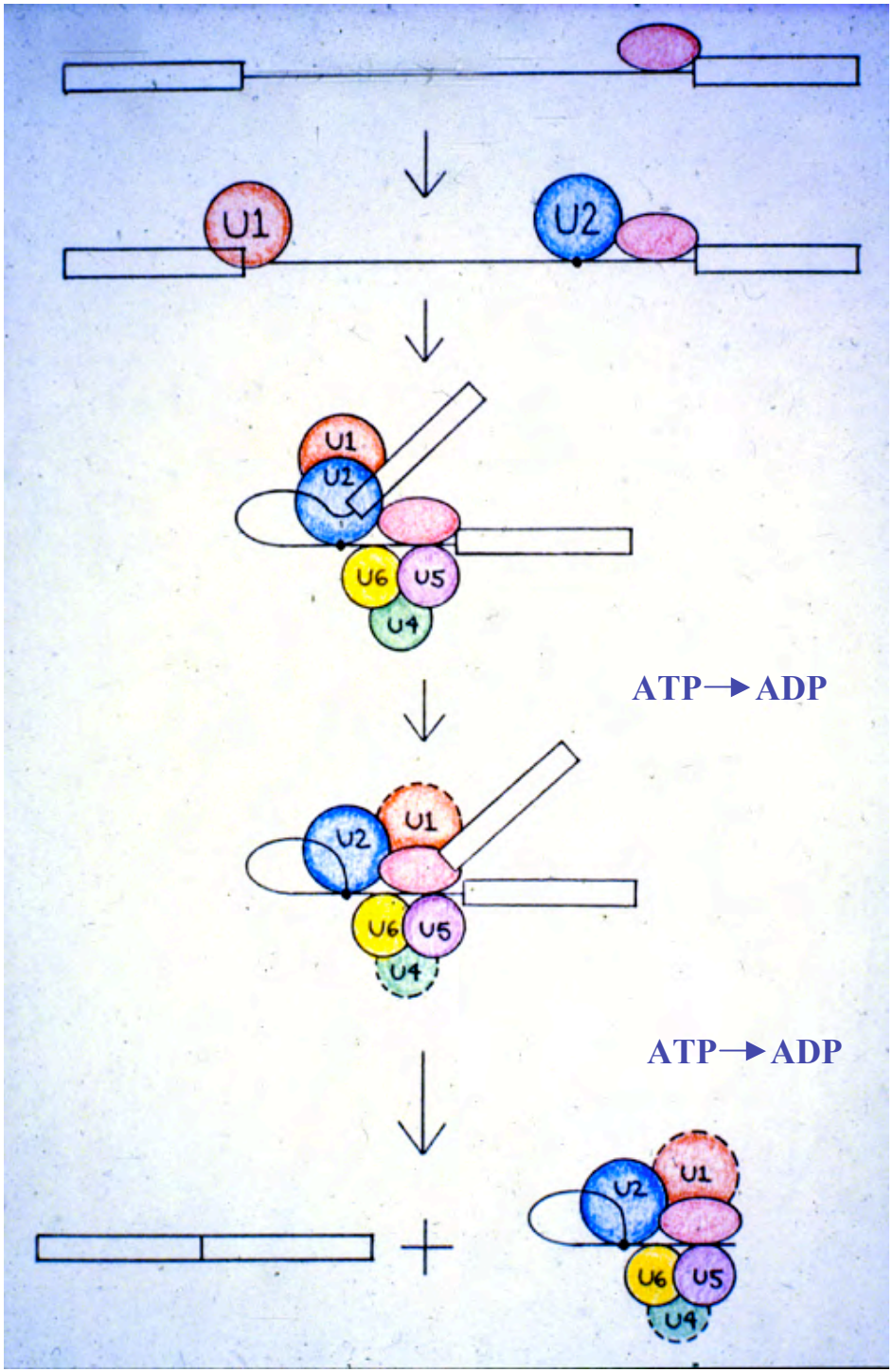


Splicing in higher cells:



an extra step in gene expression


DNA
Nascent RNA transcripts
3' splice site
5' splice site
Spliceosome
Intron loop

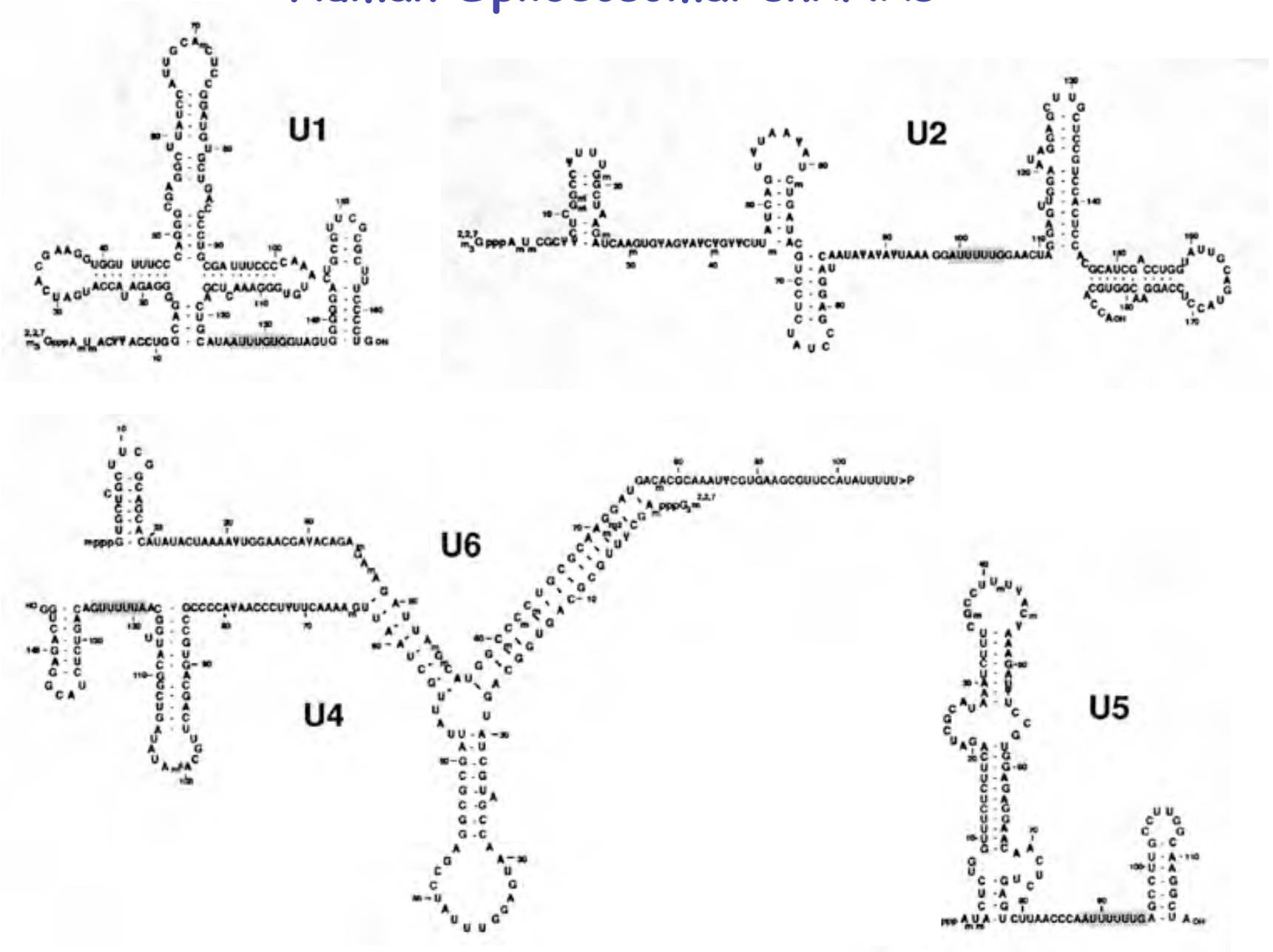

U1
U2
U1
U2
U6
U5
U4
ATP→ADP
U2
U1
U6
U5
U4
ATP→ADP
U2
U1
U6
U5
U4

- Lupus and the Discovery of snRNPs
  (pronounced snurps)

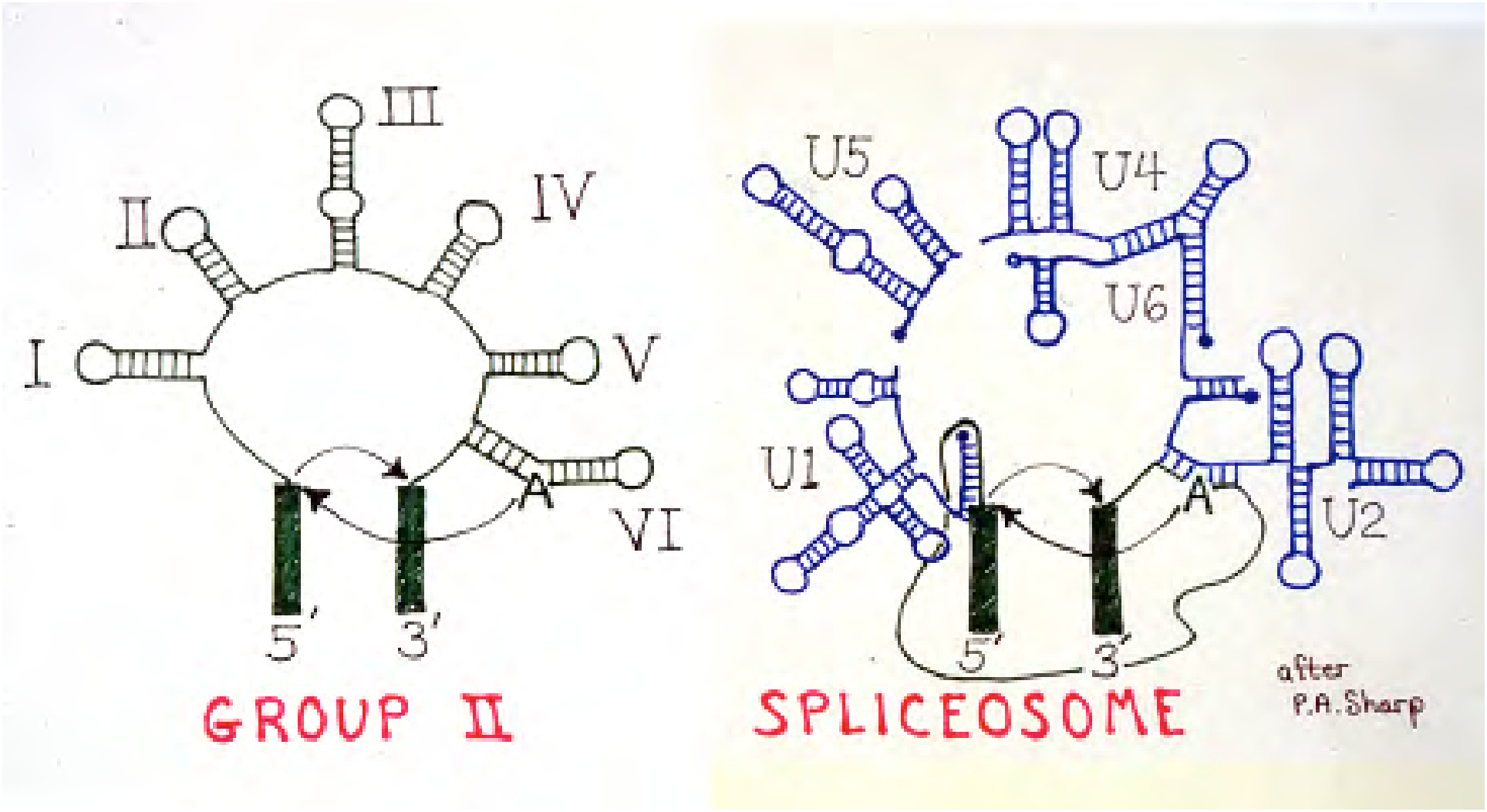
- Current Challenges in Splicing
  - Is the spliceosome a ribozyme?
  - How is alternative splicing regulated?
  - Coordination of splicing with other events in gene expression.

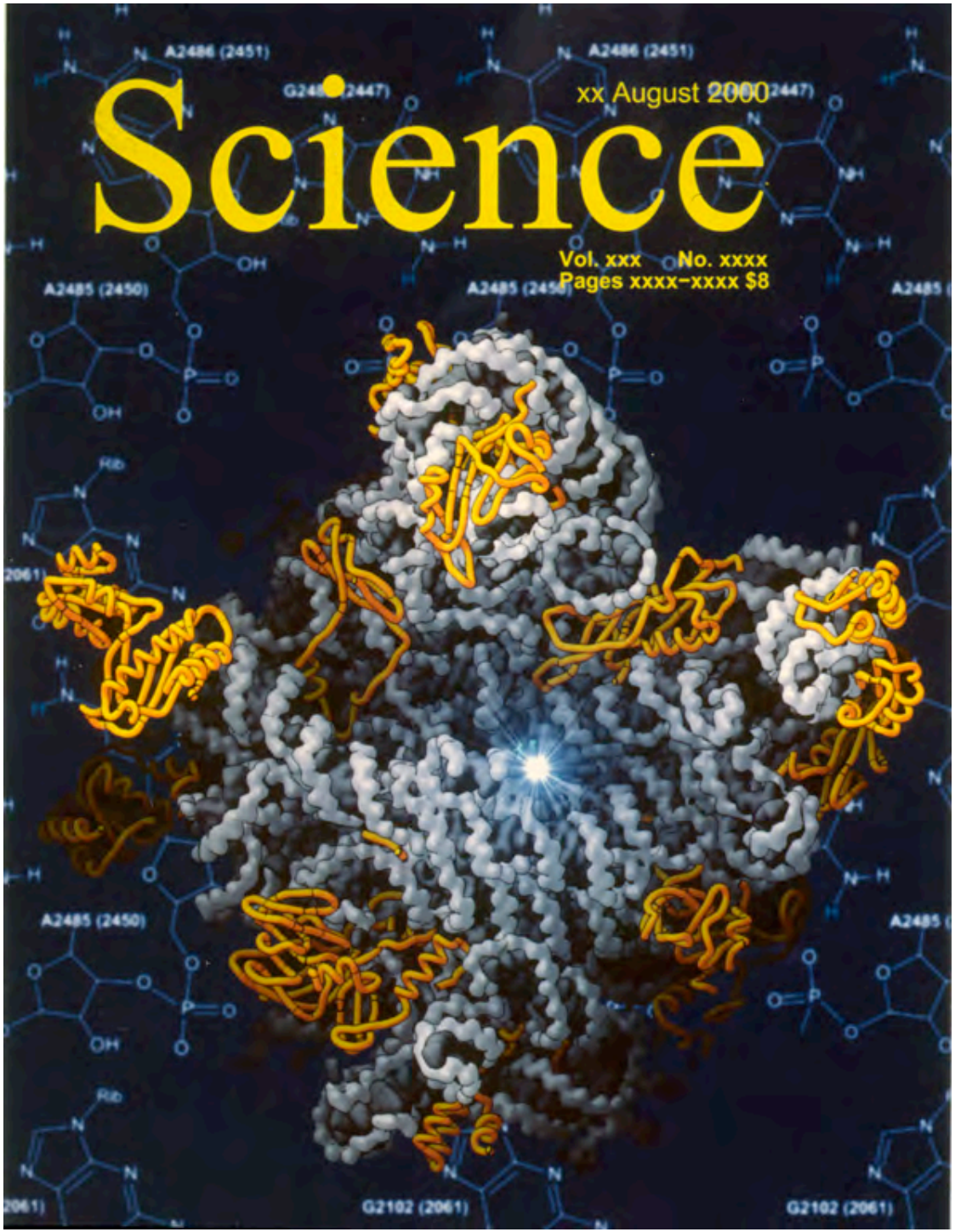
- MicroRNAs: the latest novel RNAs in

Human Spliceosomal snRNAs



The Spliceosome: a Group II Intron in Pieces?





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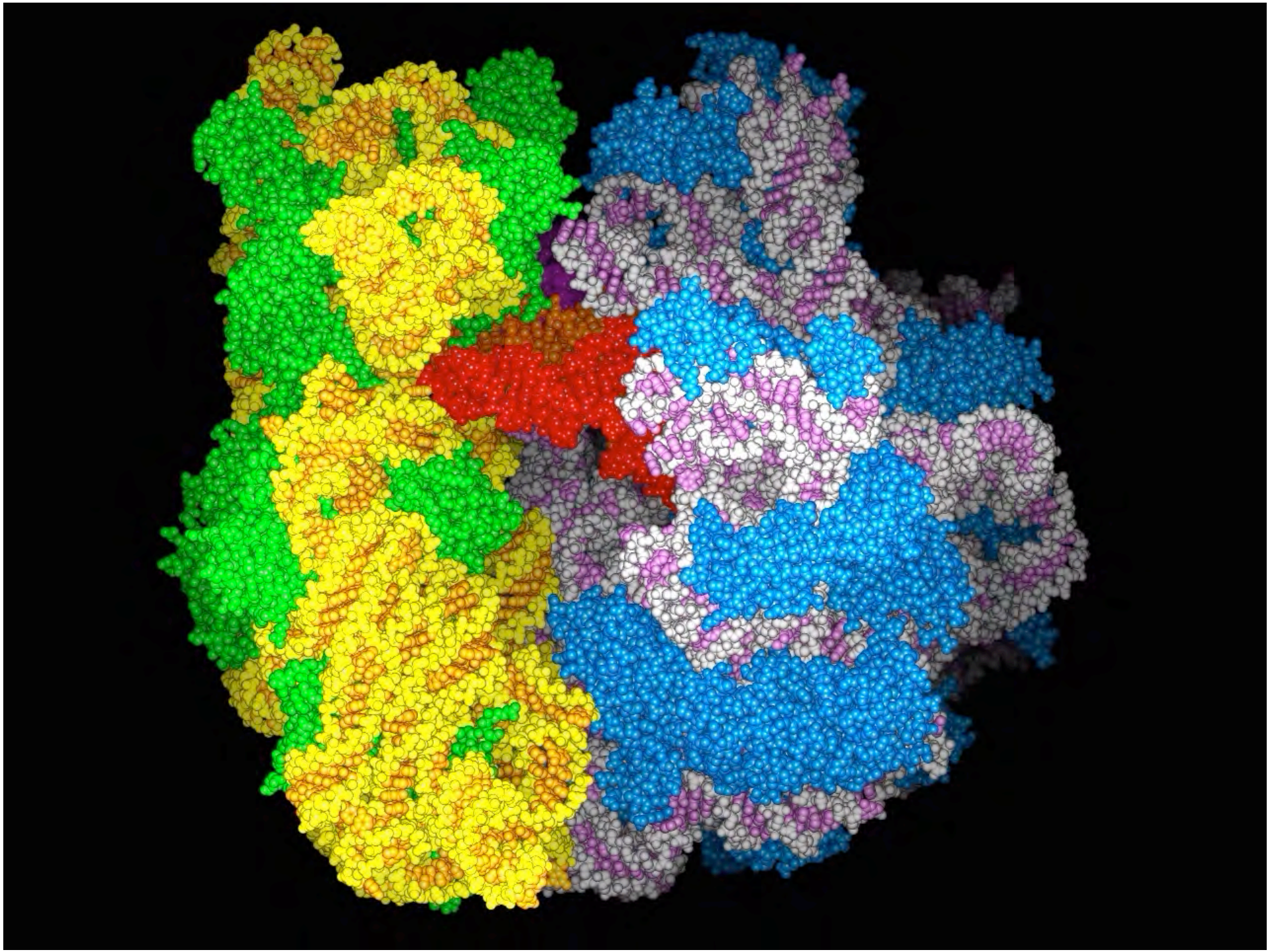
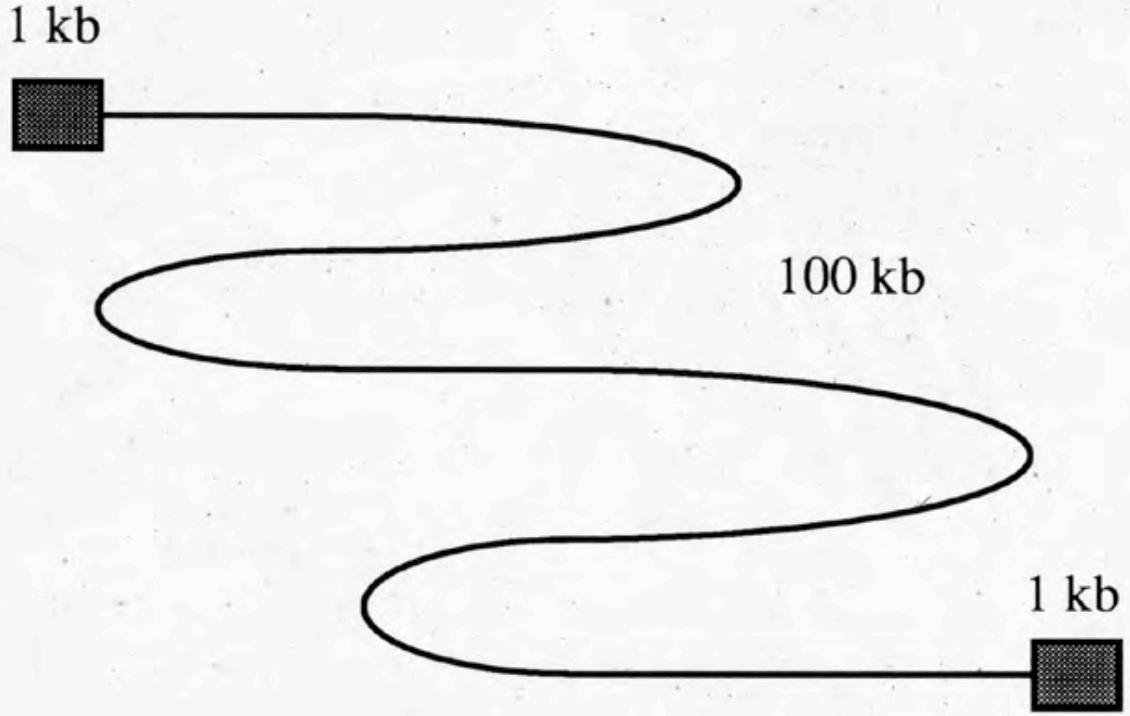
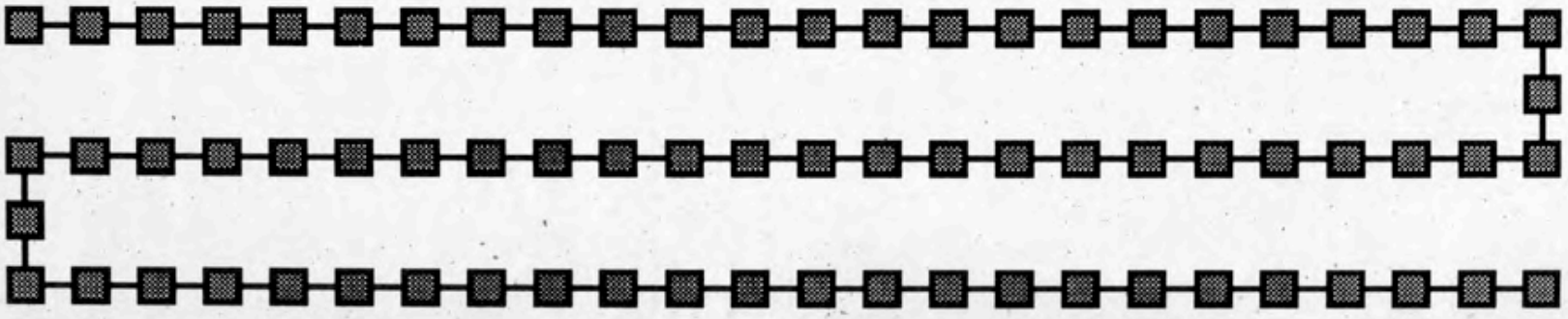


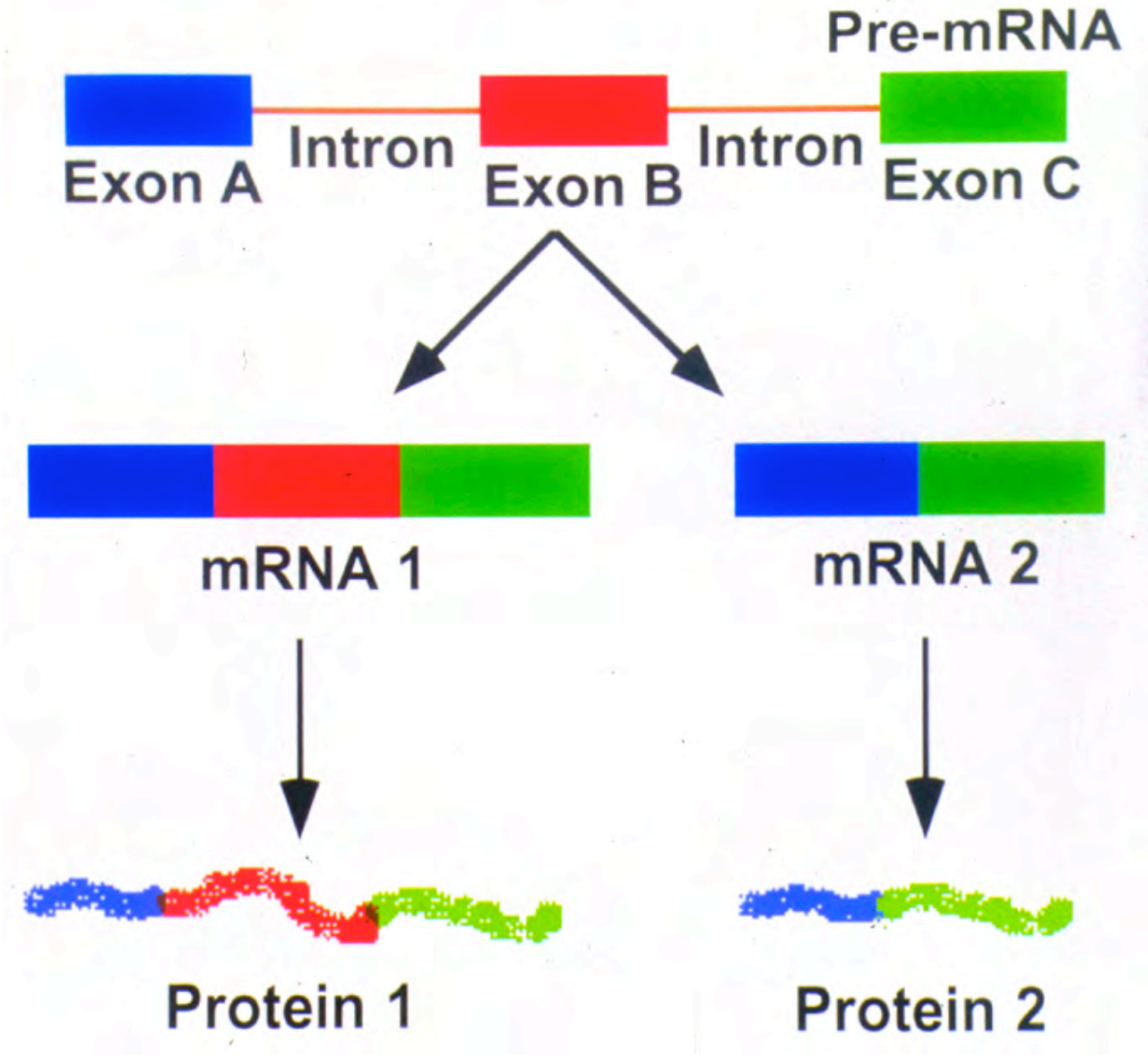
In some genes, the introns can be very large:



In others, there are a large number of introns:



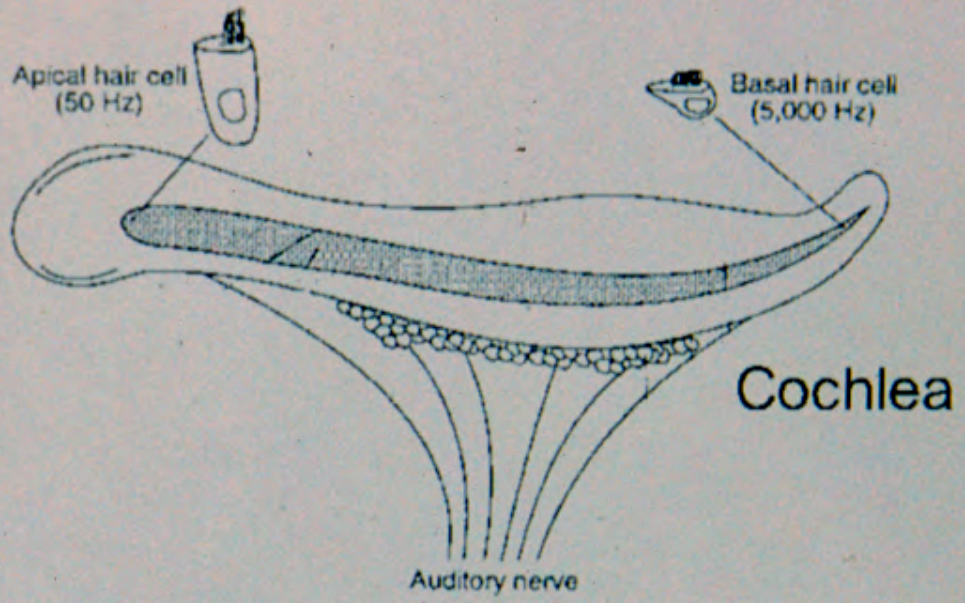
ALTERNATIVE SPLICING CAN PRODUCE DIVERSE PROTEINS FROM A SINGLE GENE.



Introns Are Larger than Exons

J. Valcarcel

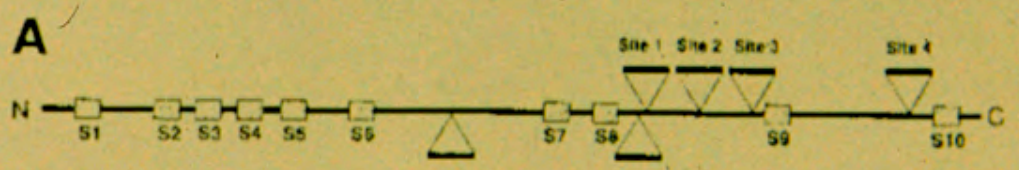
Alternative Splicing of Slo K^+ Channel Transcripts Contributes to Frequency Tuning of Auditory Hair Cells.



Cochlea

Figure 1. Tonotopic and Morphological Gradients of the Chicken's Cochlea

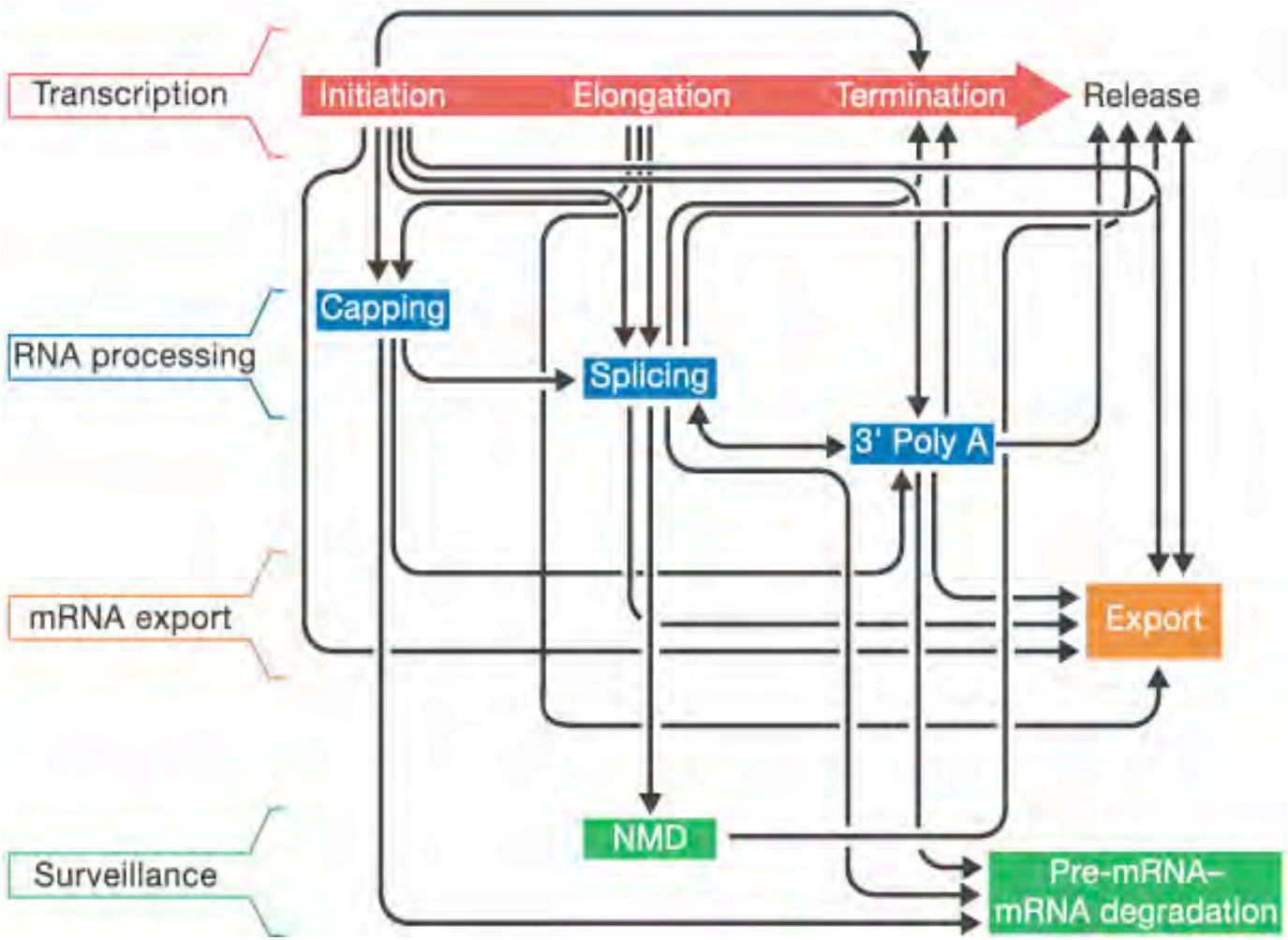
From Rosenblatt et al. Neuron 19 p1061 (1997).



Slo Protein Sequence

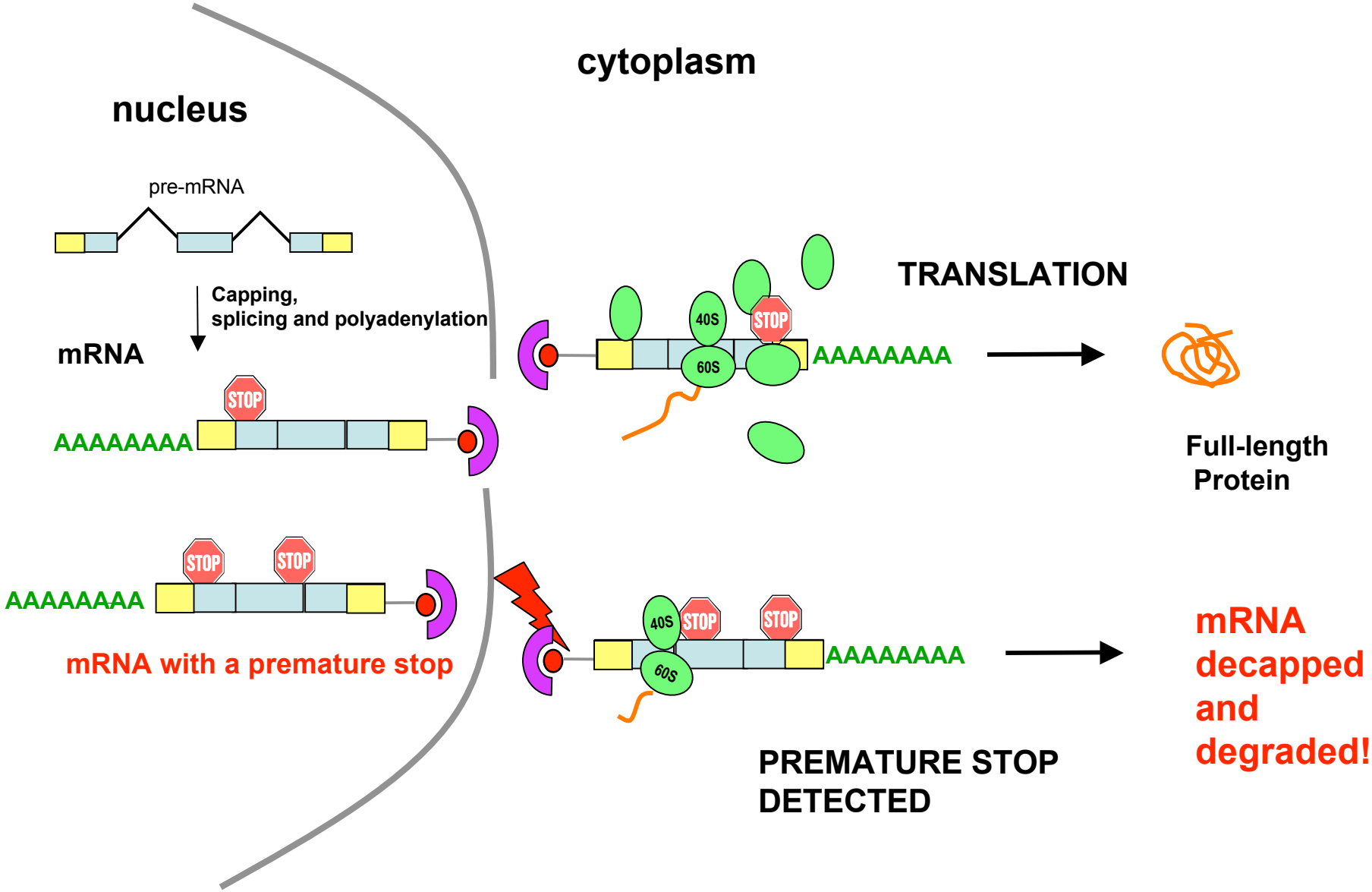
From Tseng-Crank et al. Neuron 13 p1315 (1994).

COUPLING OF STEPS IN GENE EXPRESSION



Maniatis & Reed (2002) *Nature* **416**, 499-50

Nonsense Mediated mRNA Decay (NMD)



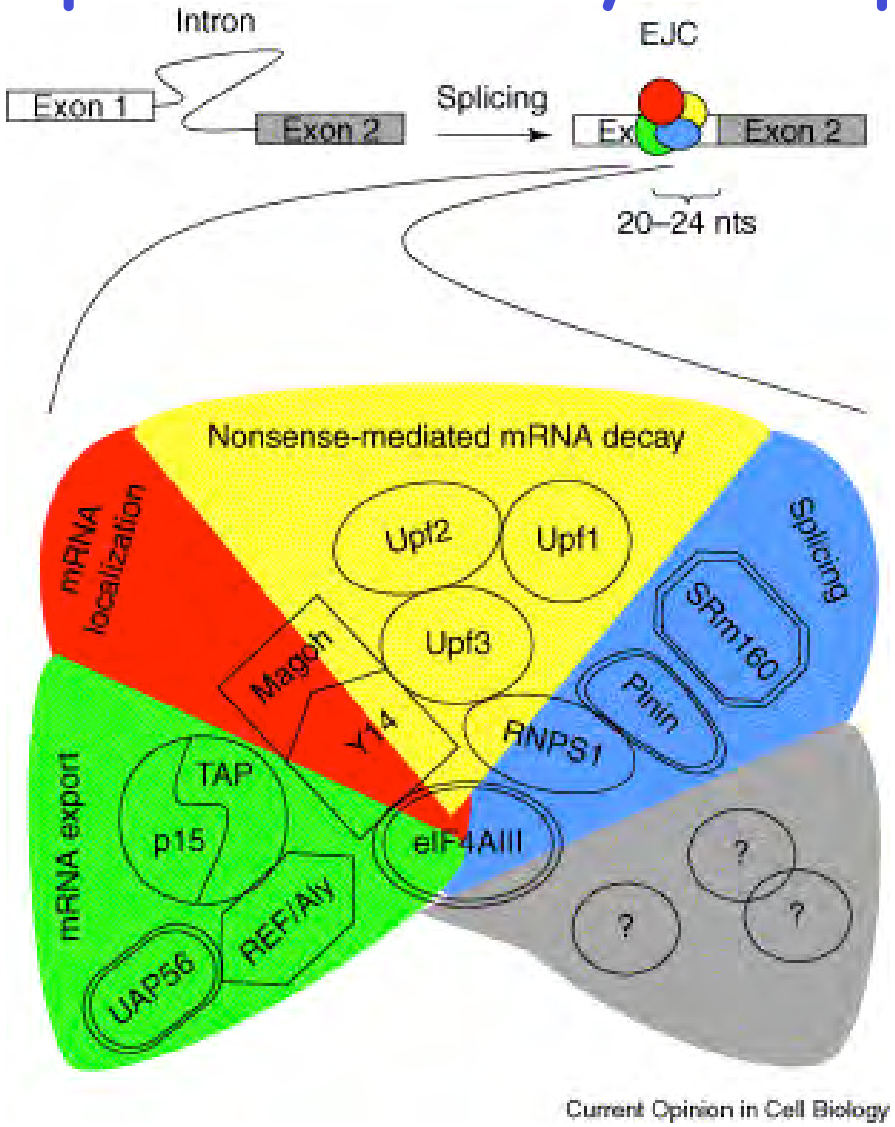
Nonsense-Mediated mRNA Decay (NMD):

mRNA Surveillance

- Detects presence of a premature termination codon (PTC) – arising from mutation or incomplete processing.
- Prevents synthesis of truncated, potentially deleterious proteins.
- Conserved in eukaryotes – from yeast to man.
- Important for making disease genes recessive and for lymphocytes.
- Requires active translation.
- At least three factors – Upf1, 2, and 3 – involved

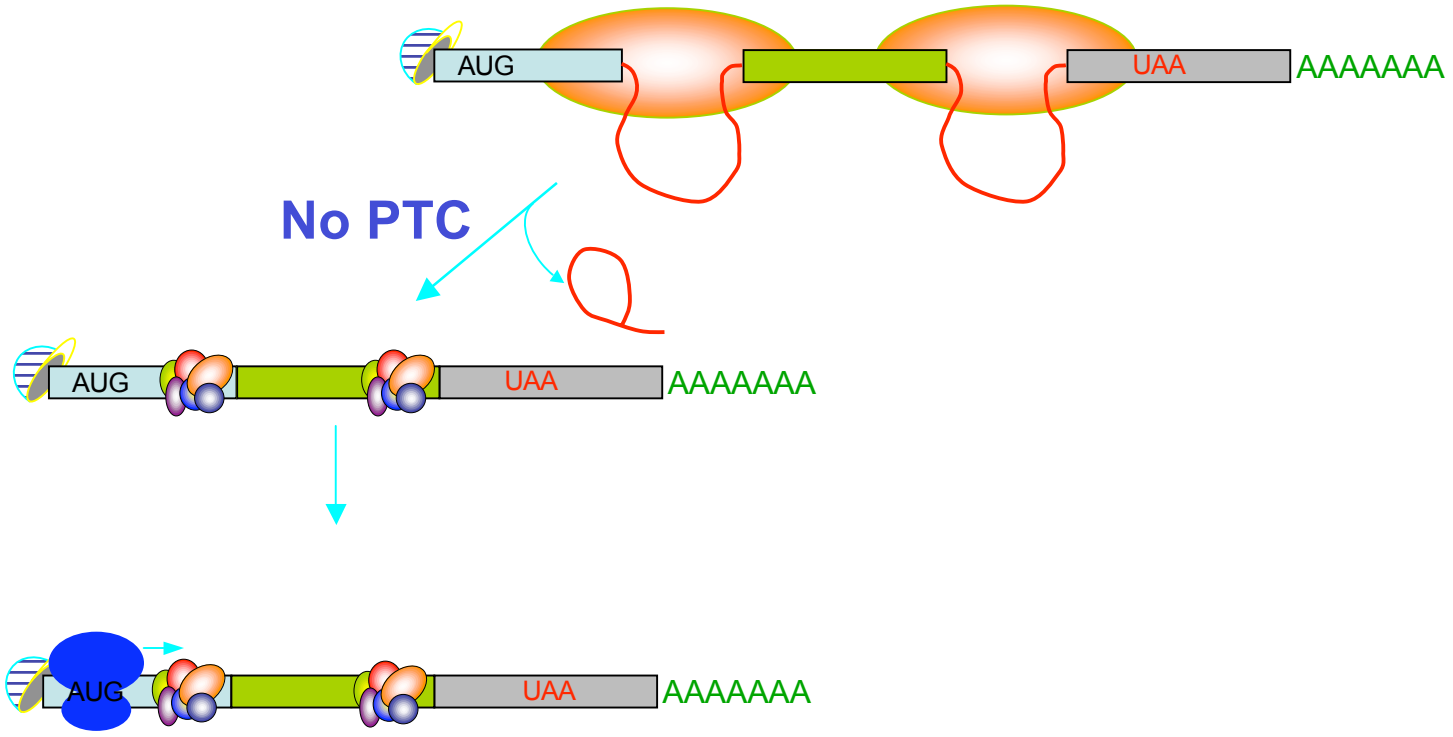
An Exon Junction Complex (EJC) Is Deposited on the Spliced mRNA by the Spliceosome

EJC first described by LeHir, Izaurralde, Maquat, and Moore (2000) ***EMBO J.*** ***19****, 6860.*



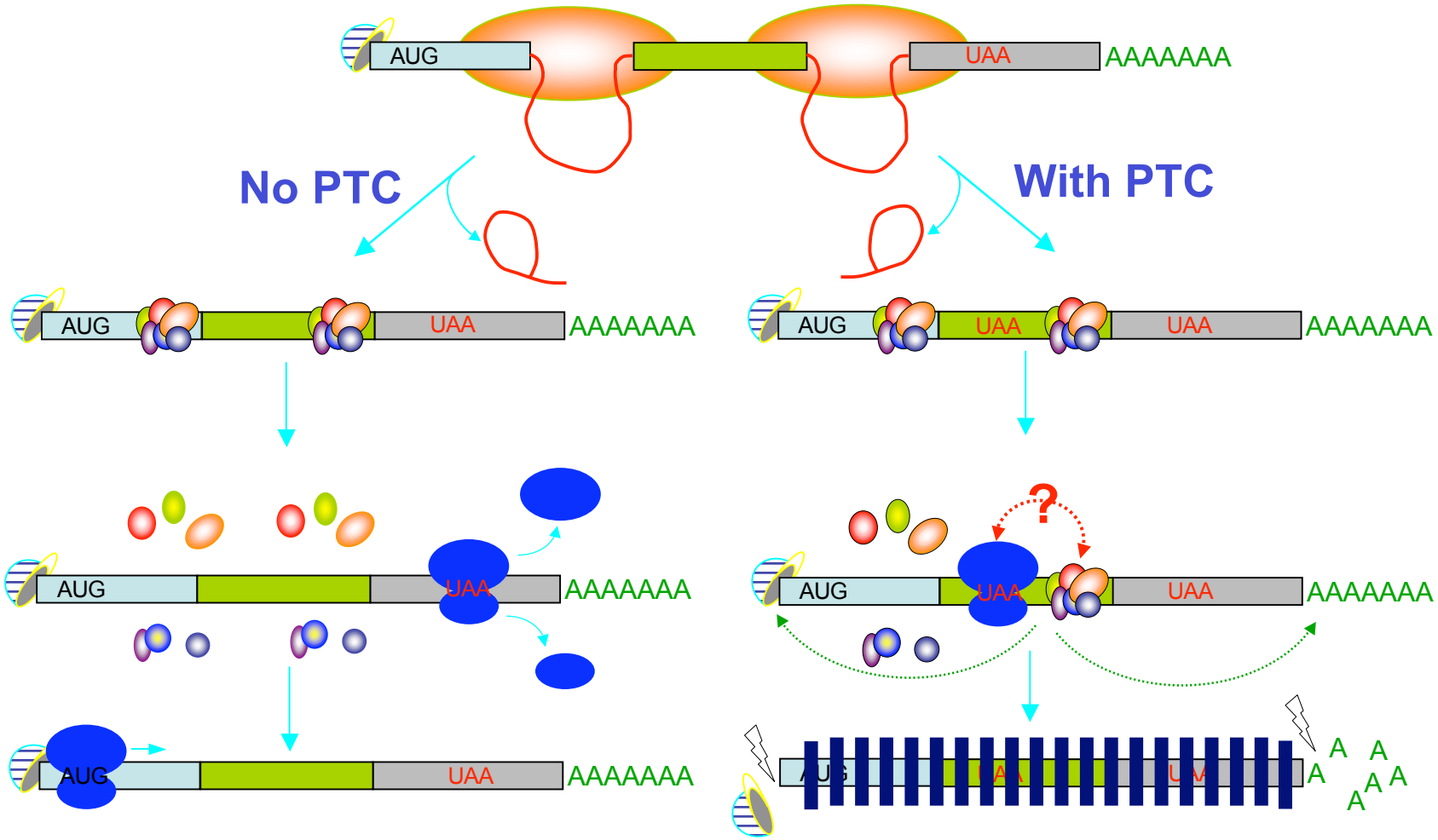
Tang, Nott and Moore, **Current Opinion in Cell Biology** (2004) **16**, 279.

Nonsense-Mediated mRNA Decay (NMD)



Zhang and Krainer

Nonsense-Mediated mRNA Decay (NMD)



Multiple rounds of translation

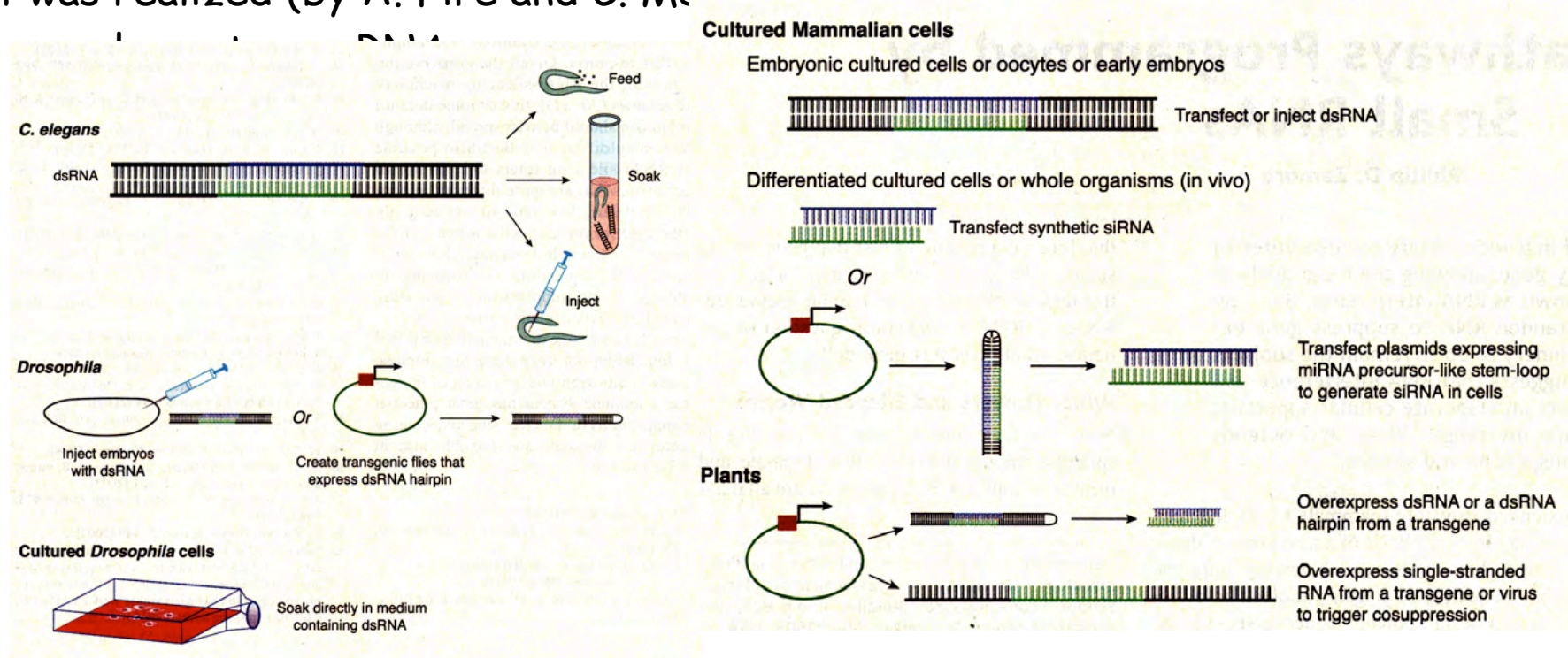
mRNA degradation
(from both 5' and 3' ends)

- Lupus and the Discovery of snRNPs (pronounced snurps)
- Current Challenges in Splicing
- MicroRNAs: the latest novel RNAs in Gene Regulation

RNA Interference (RNAi)

•RNAi (RNA interference) was discovered when attempts at overexpression from transgenes in worms and plants led to no expression whatsoever!

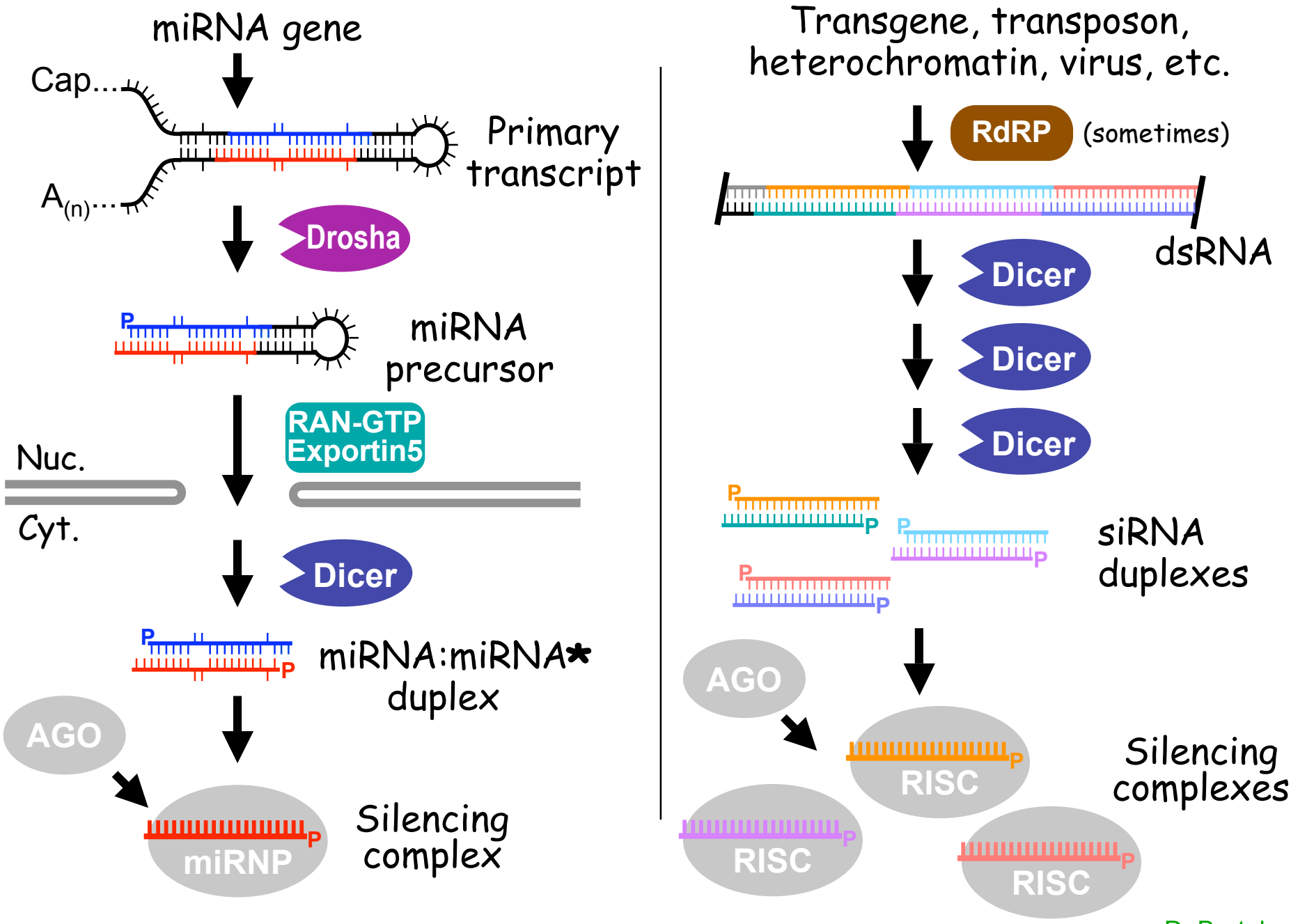
•It was realized (by A. Fire and C. Mello) that dsRNA triggers the degradation of

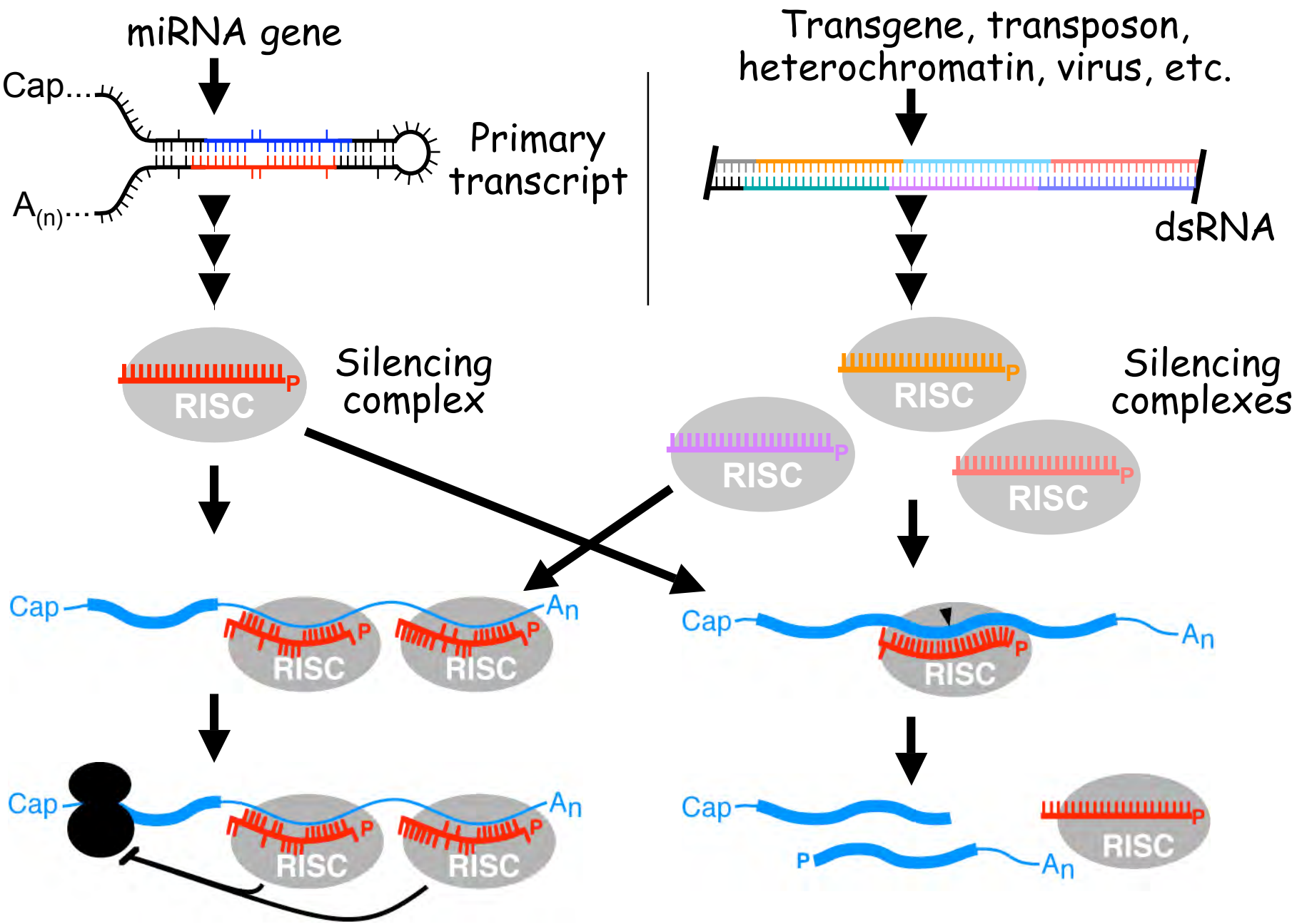


Thus, RNAi is a wonderful tool for functional gene knockouts.

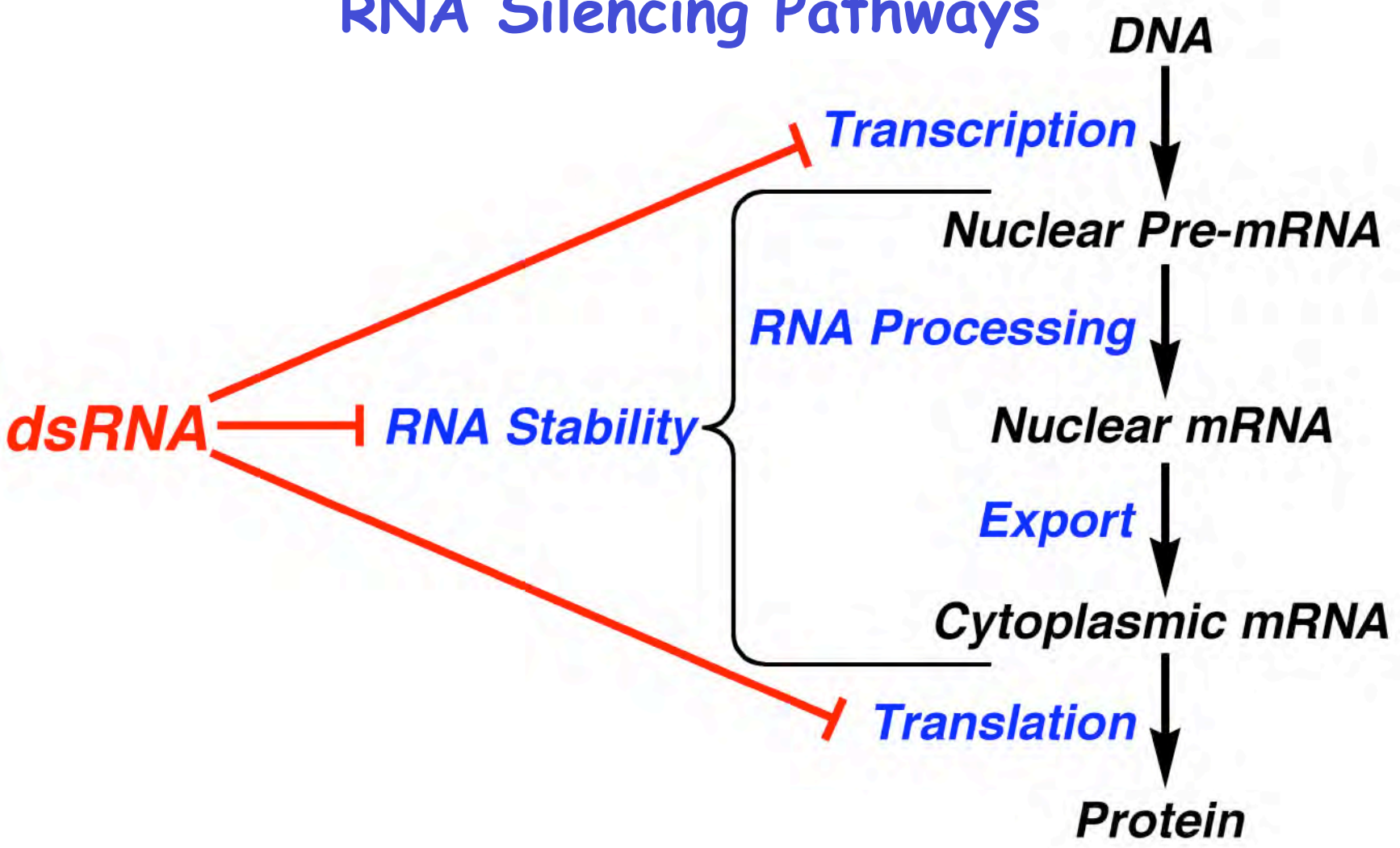
BUT, why is this machinery present in cells???

To process 100s of microRNAs that function to regulate translation, transcription and even chromosome remodeling!


miRNA gene
Cap...
A(n)...
Primary transcript
Drosha
miRNA precursor
RAN-GTP
Exportin5
Nuc.
Cyt.
Dicer
miRNA:miRNA* duplex
AGO
miRNP
Silencing complex
Transgene, transposon, heterochromatin, virus, etc.
RdRP
(sometimes)
dsRNA
Dicer
Dicer
Dicer
siRNA duplexes
AGO
RISC
RISC
RISC
Silencing complexes
D. Bartel


miRNA gene
Cap...
A(n)...
Primary transcript
Transgene, transposon, heterochromatin, virus, etc.
dsRNA
RISC
Silencing complex
Silencing complexes
Cap
An

All the Critical Steps in Eukaryotic Gene Expression Are Subject to Contol by RNA Silencing Pathways



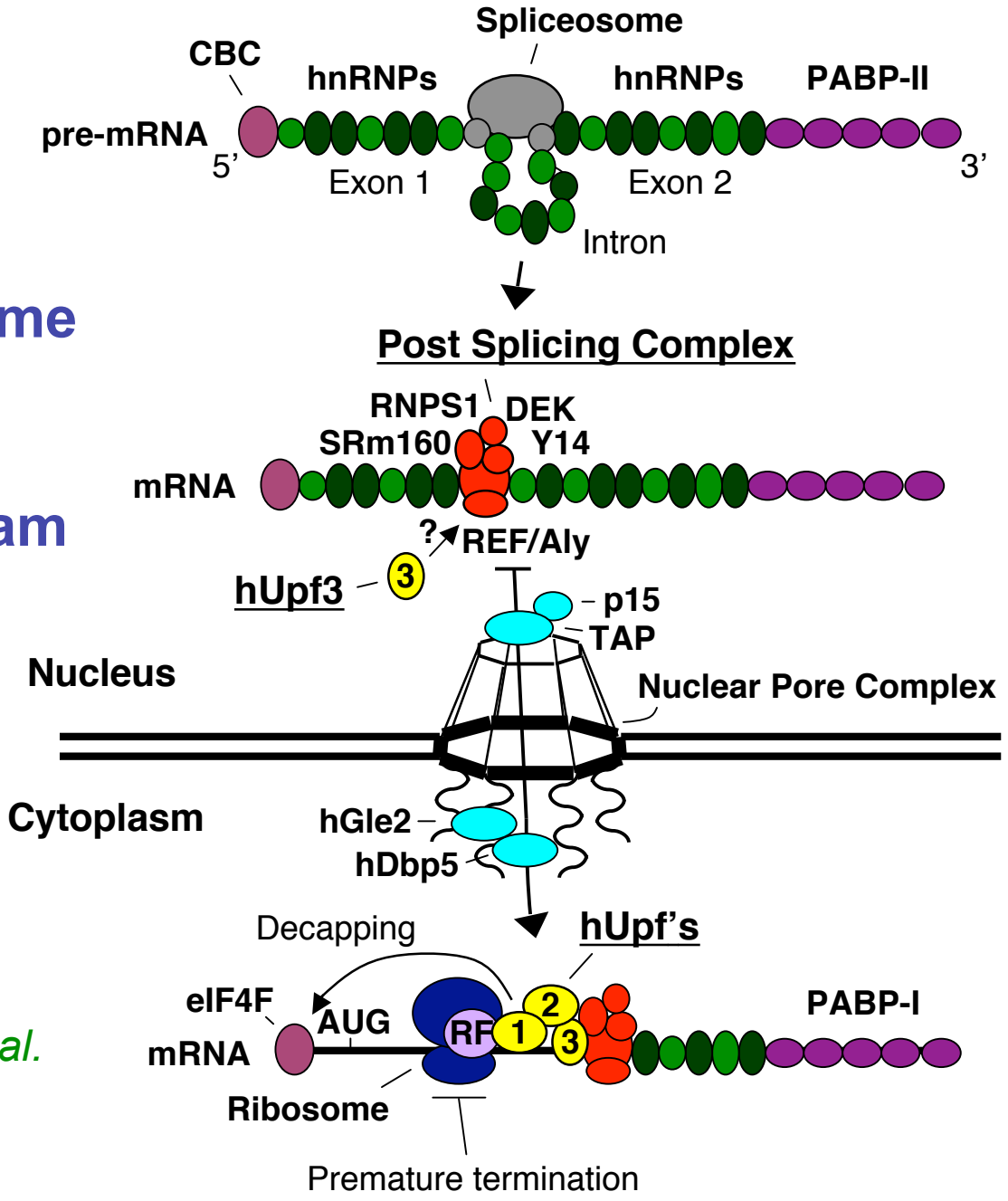
RNA Silencing Pathways Are Ancient, Diverse and Essential

dsRNA can trigger:

- ***mRNA degradation (animals, plants, protists, fungi)***
- ***Transcriptional repression (animals, plants, fungi)***
- ***Centromeric heterochromatin formation (animals, fungi)***
- ***Translational repression (animals, plants)***
- ***Macronuclear DNA elimination (protists)***

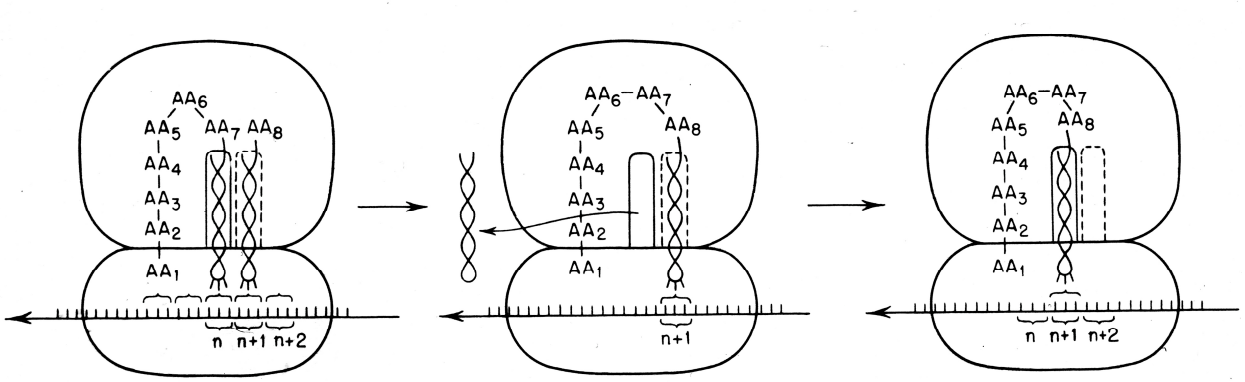
Splice Girls

Nonsense-mediated mRNA decay (NMD) occurs when a ribosome stalls at a nonsense codon located upstream of an exon junction complex (EJC).



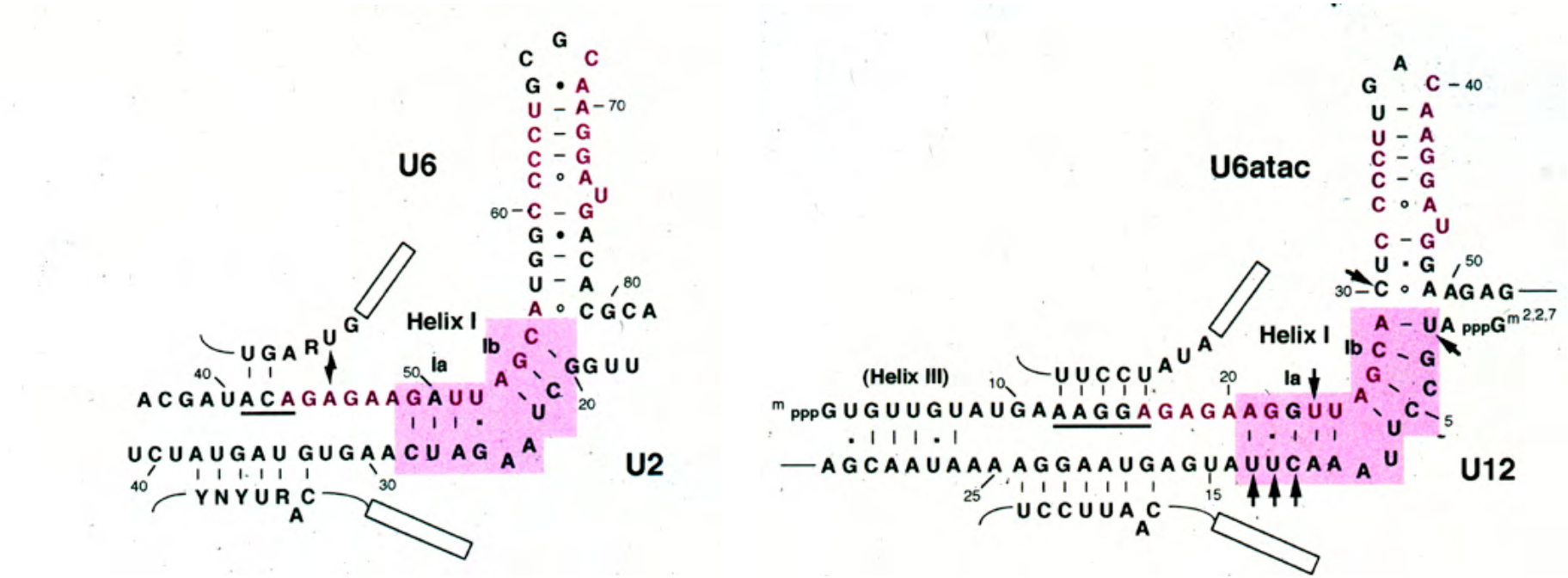
EJC first described by LeHir et al. (2000) ***EMBO J.19****, 6860.*

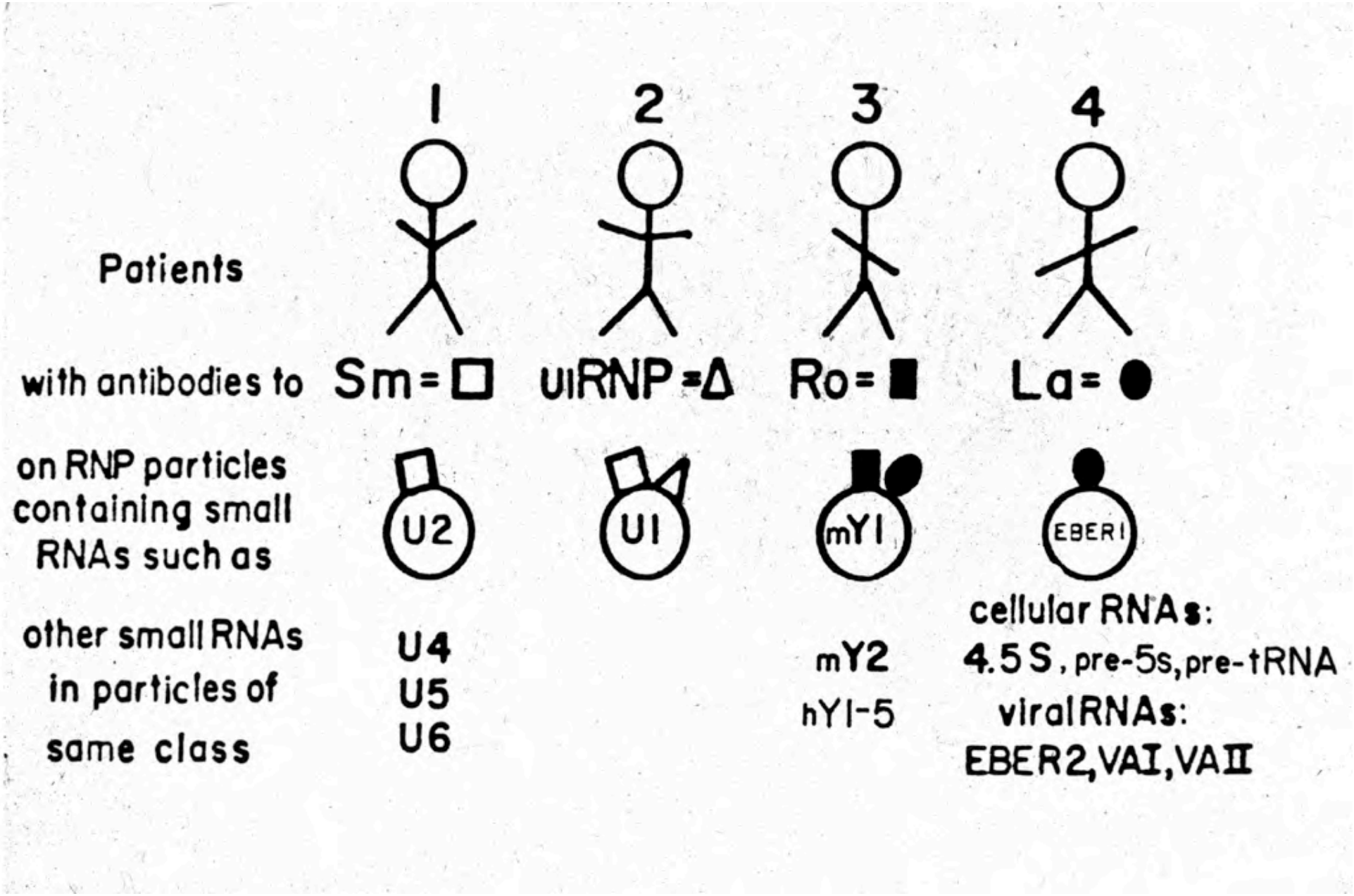
The Synthesis of Proteins Upon Ribosomes



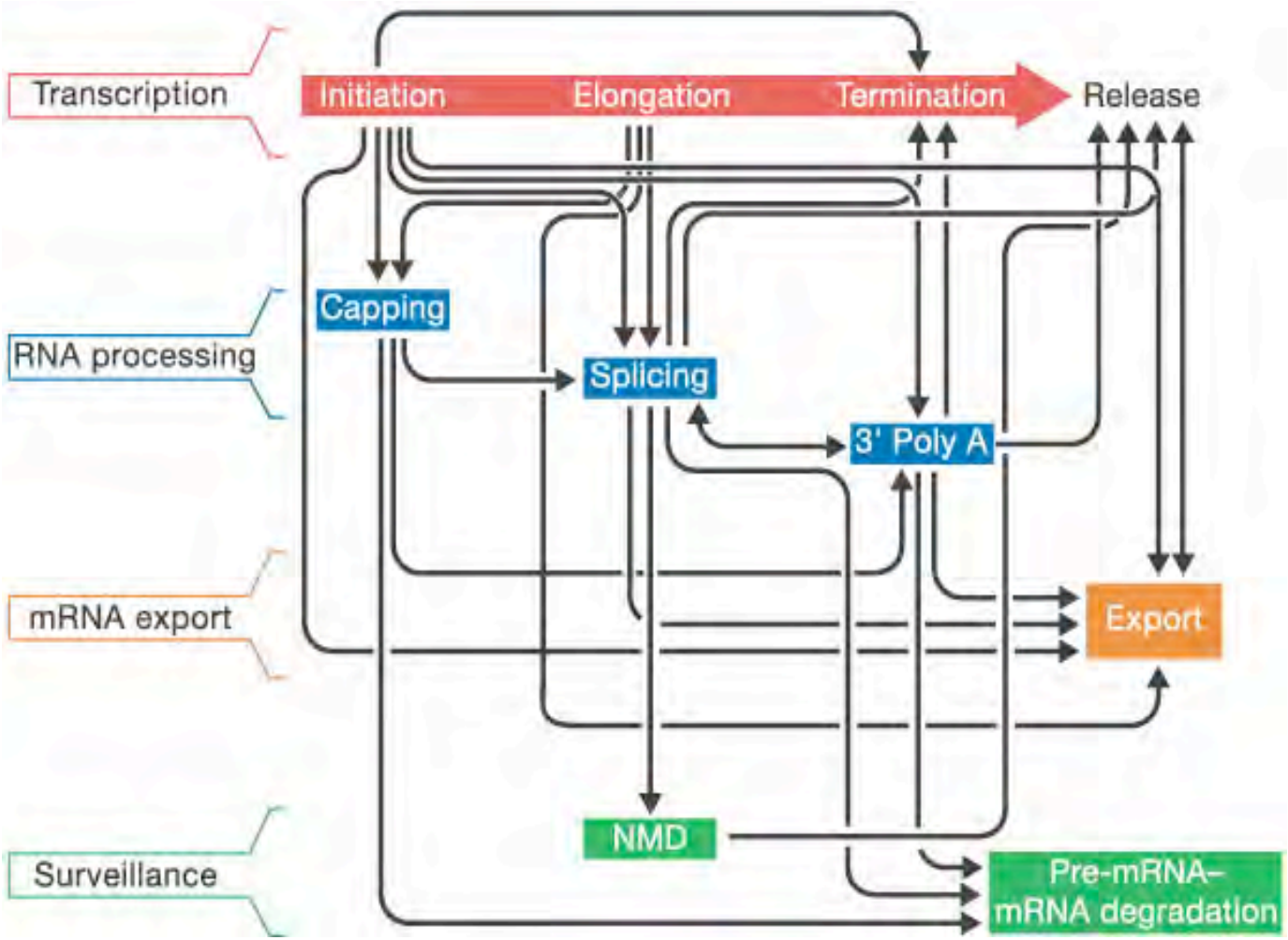
J.D. Watson (1964) *Bull. Soc. Chim. Biol.* **46**, 1399-1425

Catalytic Cores of the Major and Minor Spliceosome




Patients
1
2
3
4
with antibodies to
Sm=□
uIRNP=Δ
Ro=■
La=●
on RNP particles containing small RNAs such as
U2
U1
mY1
EBER1
other small RNAs in particles of same class
U4
U5
U6
mY2
hY1-5
cellular RNAs:
4.5 S, pre-5s, pre-tRNA
viral RNAs:
EBER2, VAI, VAII

COUPLING OF STEPS IN GENE EXPRESSION



Maniatis & Reed (2002) *Nature* **416**, 499-50