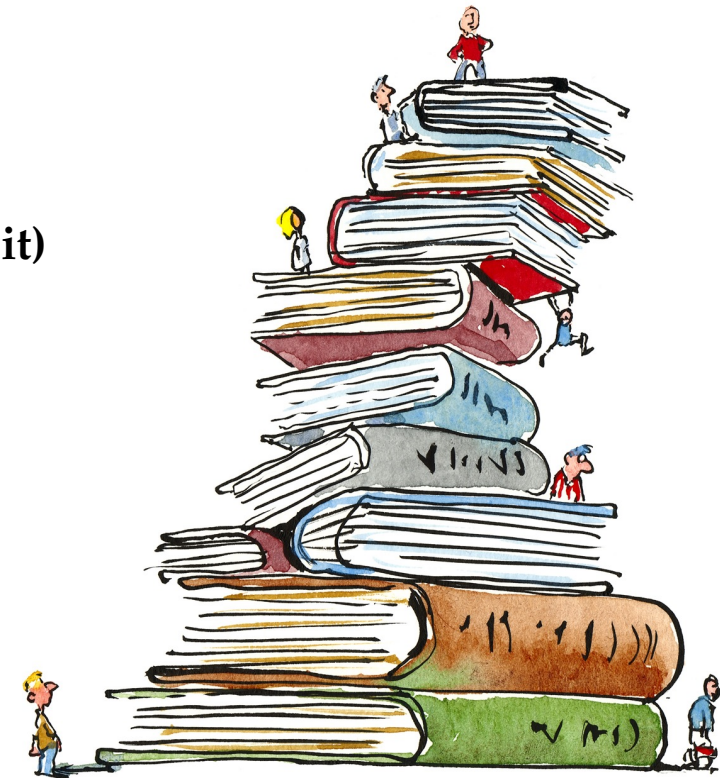


Gene Expression Regulation in Eukaryotes (GERE)

A.A. 2026-2027

- **Module 1*:** Prof. Alessandro Fatica (alessandro.fatica@uniroma1.it)
- **Module 2:** Prof. Mariangela Morlando

*midterm exam for Module 1 in late January or early February



REGE Syllabus - Modules 1 and 2

Course Topics

- Mechanisms of transcriptional regulation
- Transcription termination
- mRNA processing
- Alternative splicing and alternative polyadenylation as regulatory mechanisms of gene expression
- RNA processing and degradation machinery (exosome, TRAMP)
- Non-coding RNAs in gene expression regulation (siRNA, microRNA, piRNA, lncRNA, circRNA)
- Genomic imprinting and X-chromosome dosage compensation
- RNA quality control (control of pervasive transcription, NMD, NSD, NGD)
- RNA export
- mRNA degradation (decapping, deadenylation, P-bodies)
- Translational control of mRNA
- Epitranscriptomics: RNA modifications and gene expression regulation (U-tail, m5C, m6A, Ψ / Y)
- RNA editing

Methodologies

- Nucleic acid and protein quantification, study of protein/nucleic acid interactions, protein and RNA immunoprecipitation
- Next-generation sequencing
- Epigenetic analysis (ChIP-seq, CUT&RUN, CUT&Tag, ATAC-seq)
- Yeast as a model system for eukaryotic functions, cell culture and its applications, the mouse model system
- Genome editing
- Methodologies for studying translation
- Methodologies for studying chemical modifications of RNA

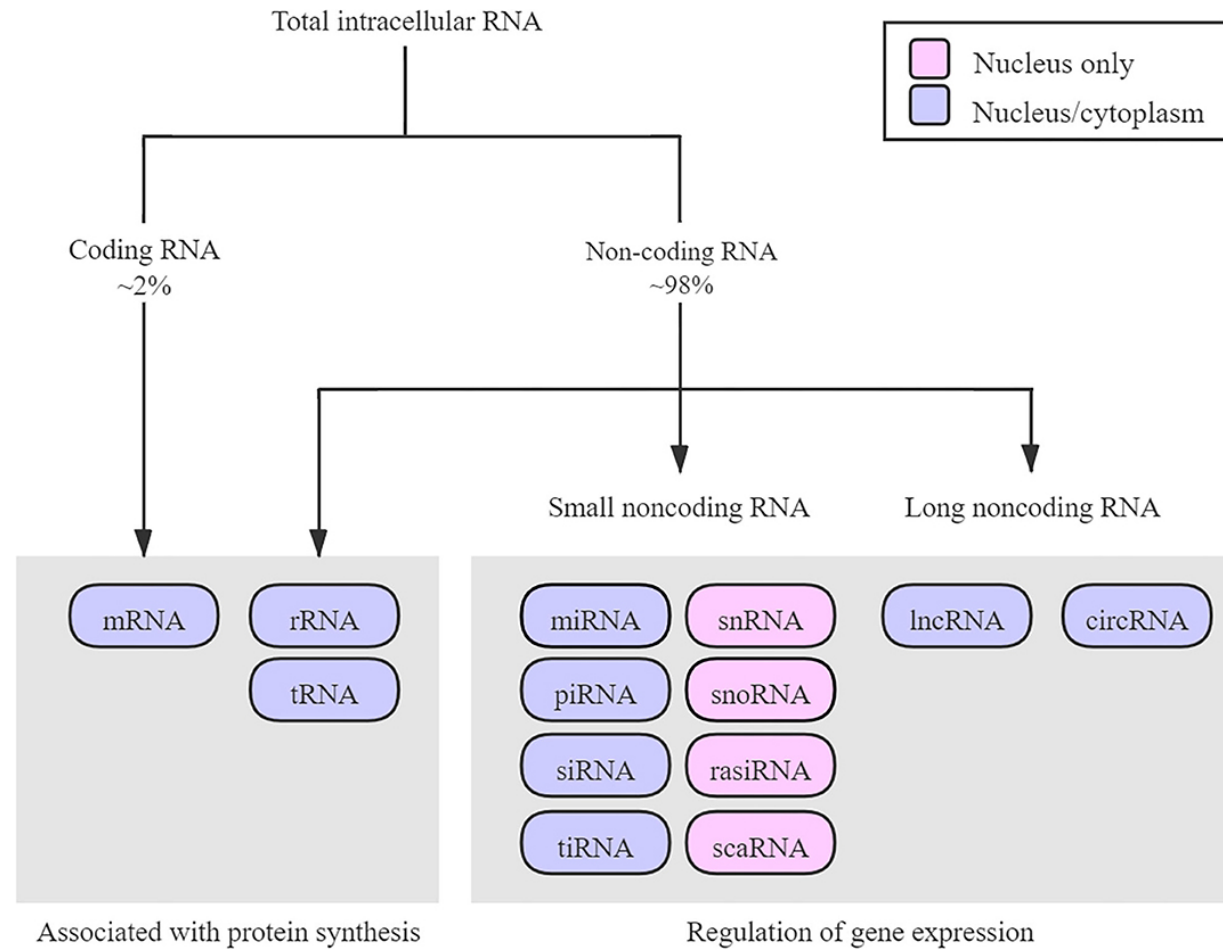
Teaching materials on the website: <http://elearning2.uniroma1.it/>

Methodologies for the Study of Gene Expression

What is gene expression?

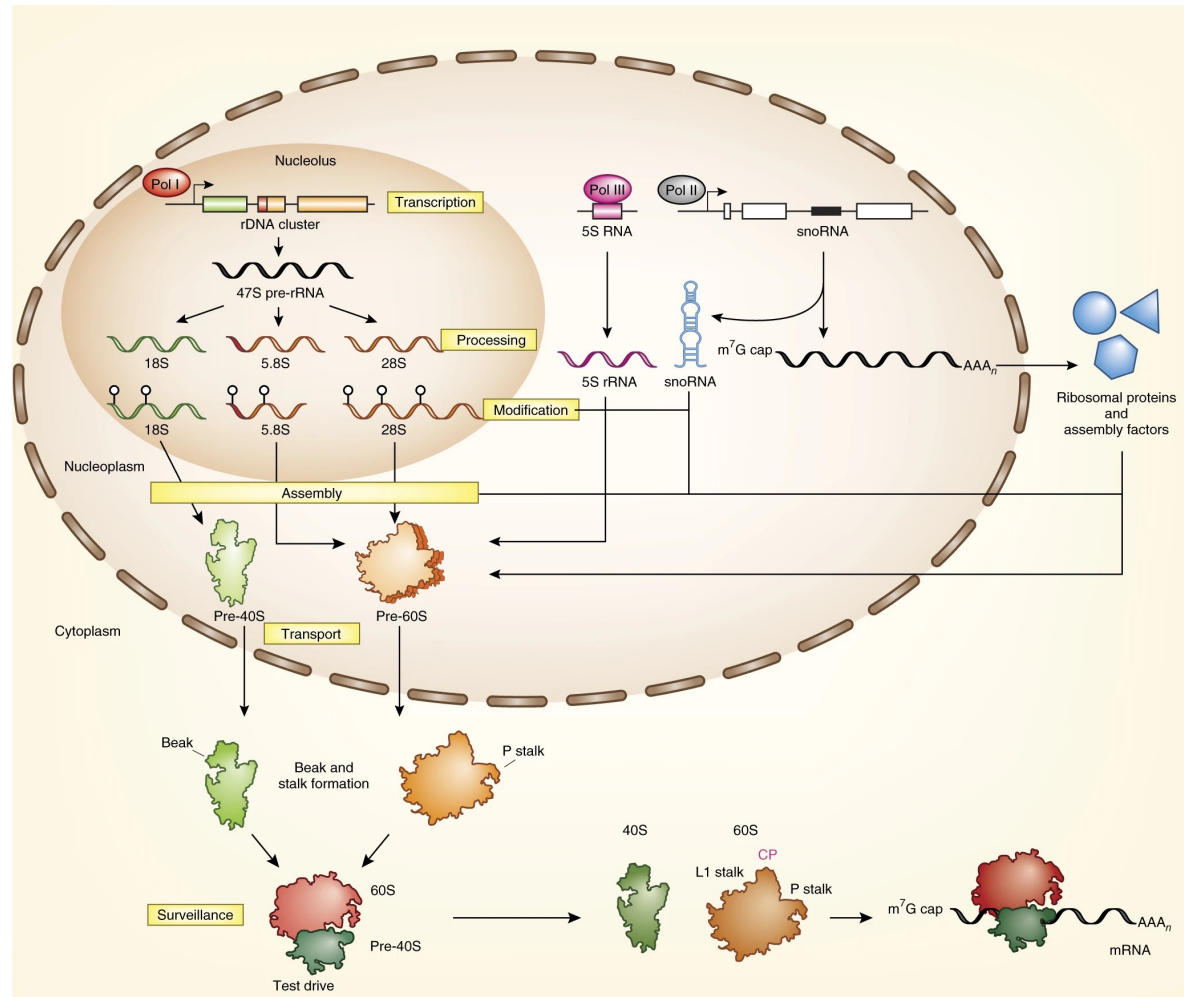
Gene expression is the fundamental process through which the information encoded in a gene is used to synthesize a functional gene product, usually a **protein** or a **functional RNA molecule**.

Classes of cellular RNA



Ribosomal RNA (rRNA)

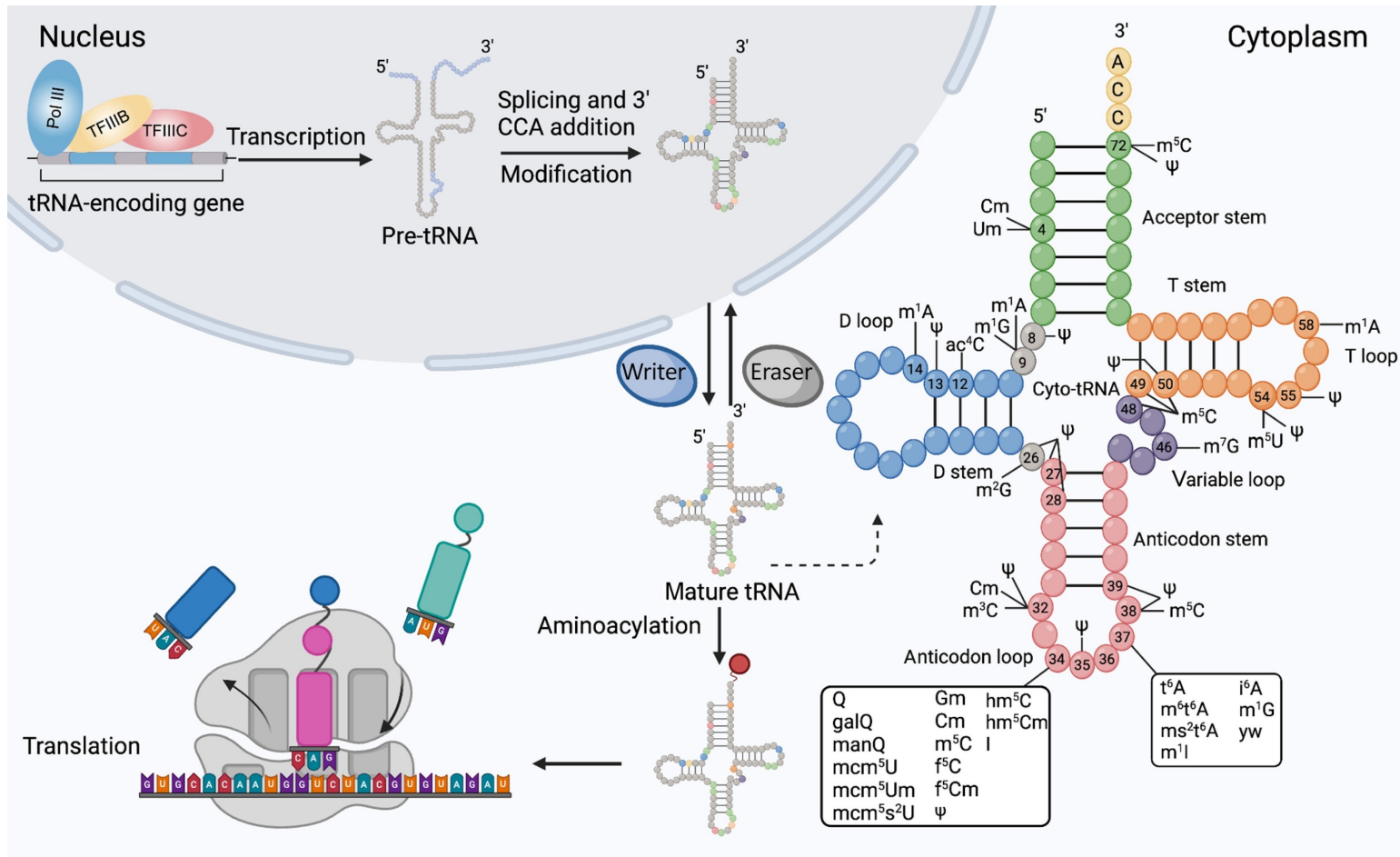
80%-90%
Total RNA



To be expressed, rRNA must be processed and modified

Transfer RNA (tRNA)

10%-15%
Total RNA

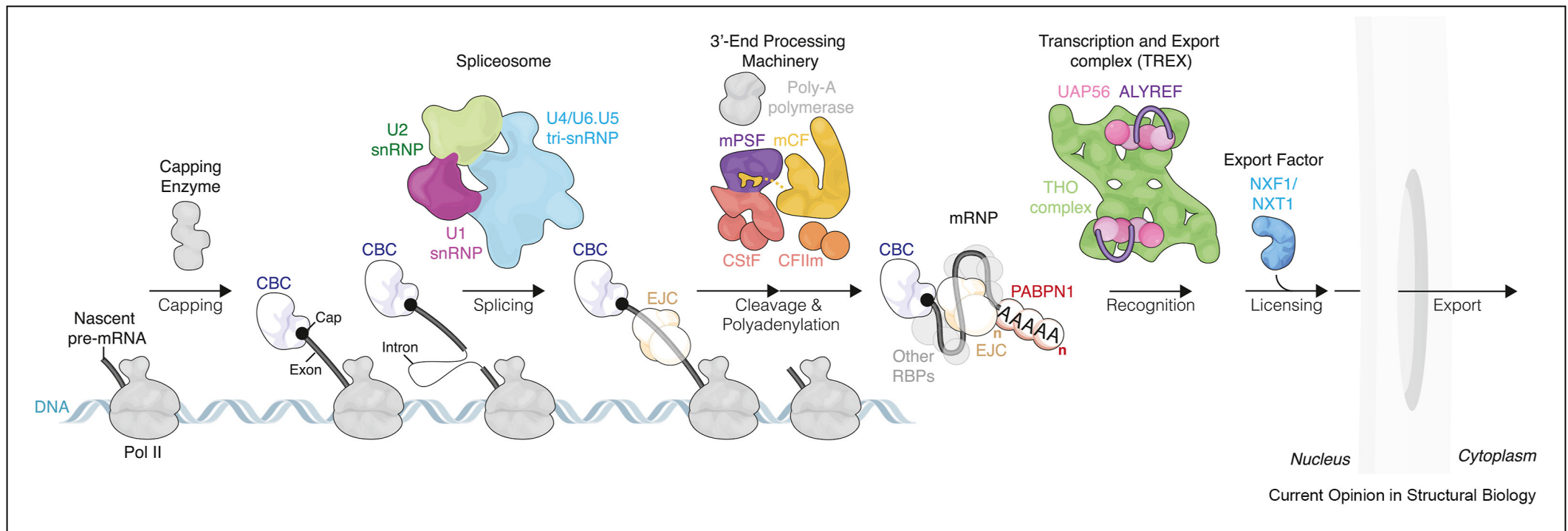


Trends in Cell Biology

To be expressed, tRNA must be processed and modified

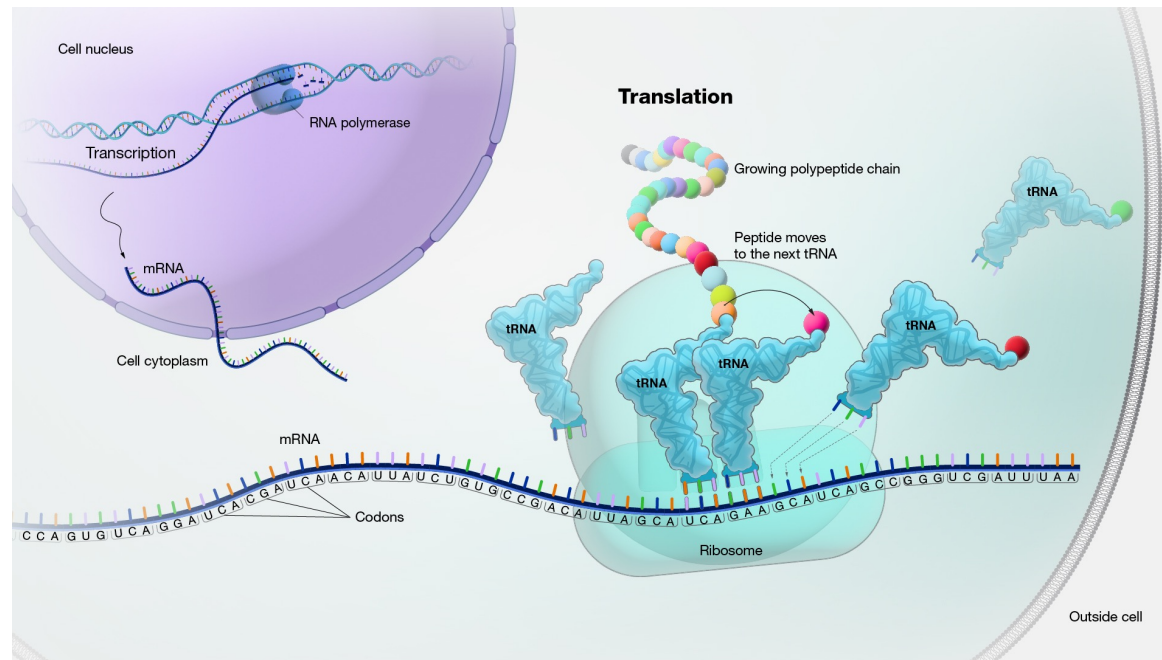
mRNA

1%-5%
Total RNA



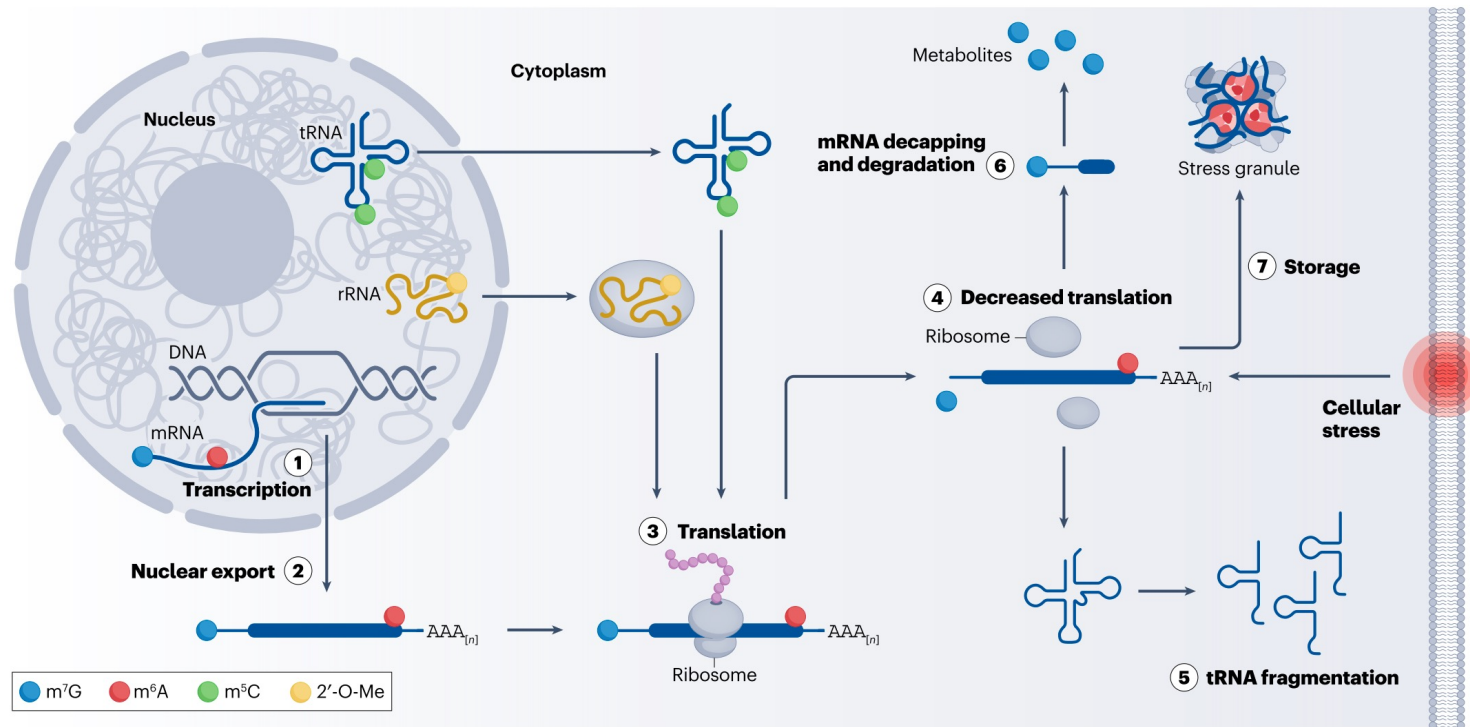
To be expressed, mRNA must be processed

Translation



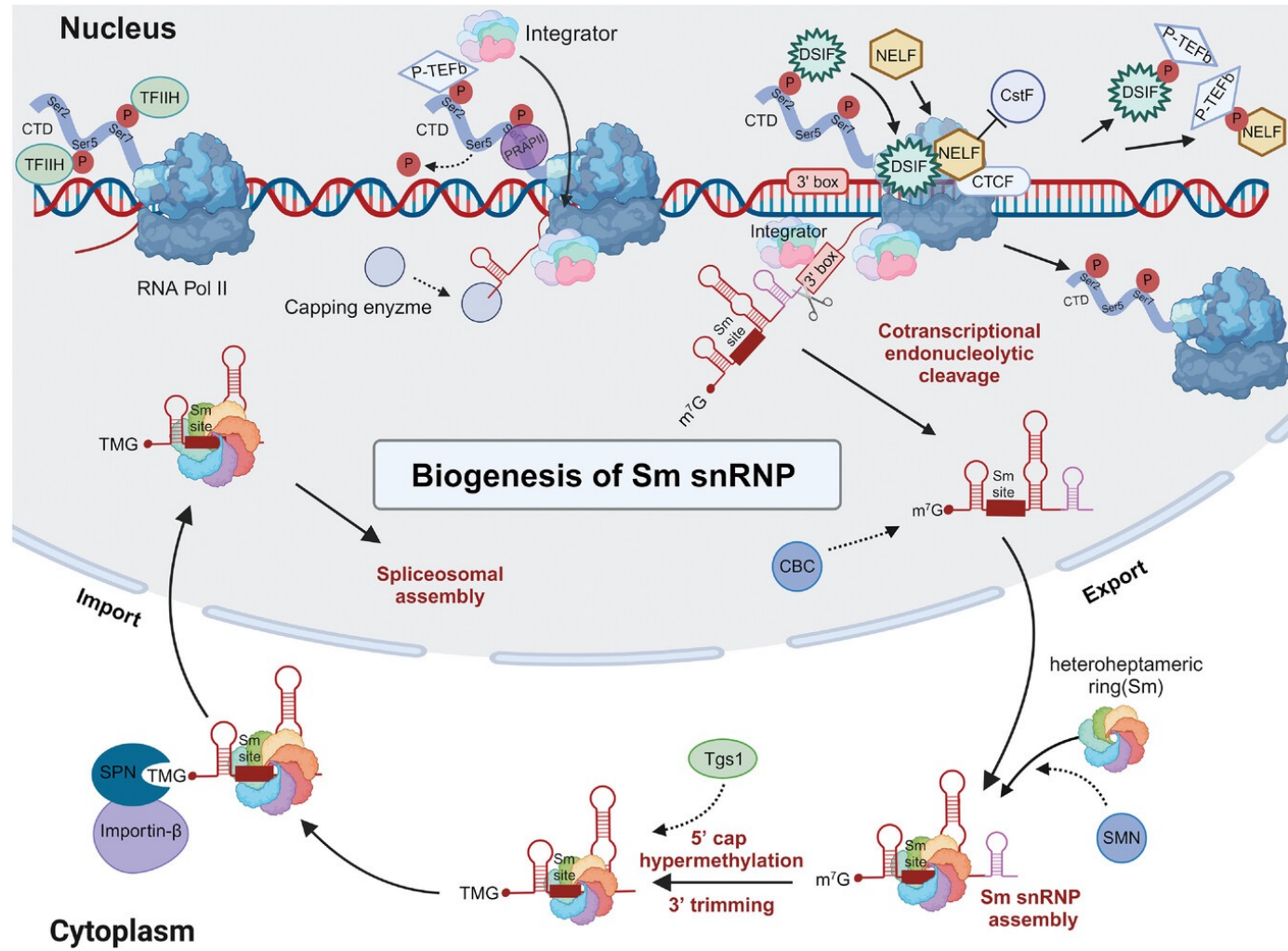
To be expressed, mRNA must be translated

Chemical modifications of RNA can impact gene expression at various levels

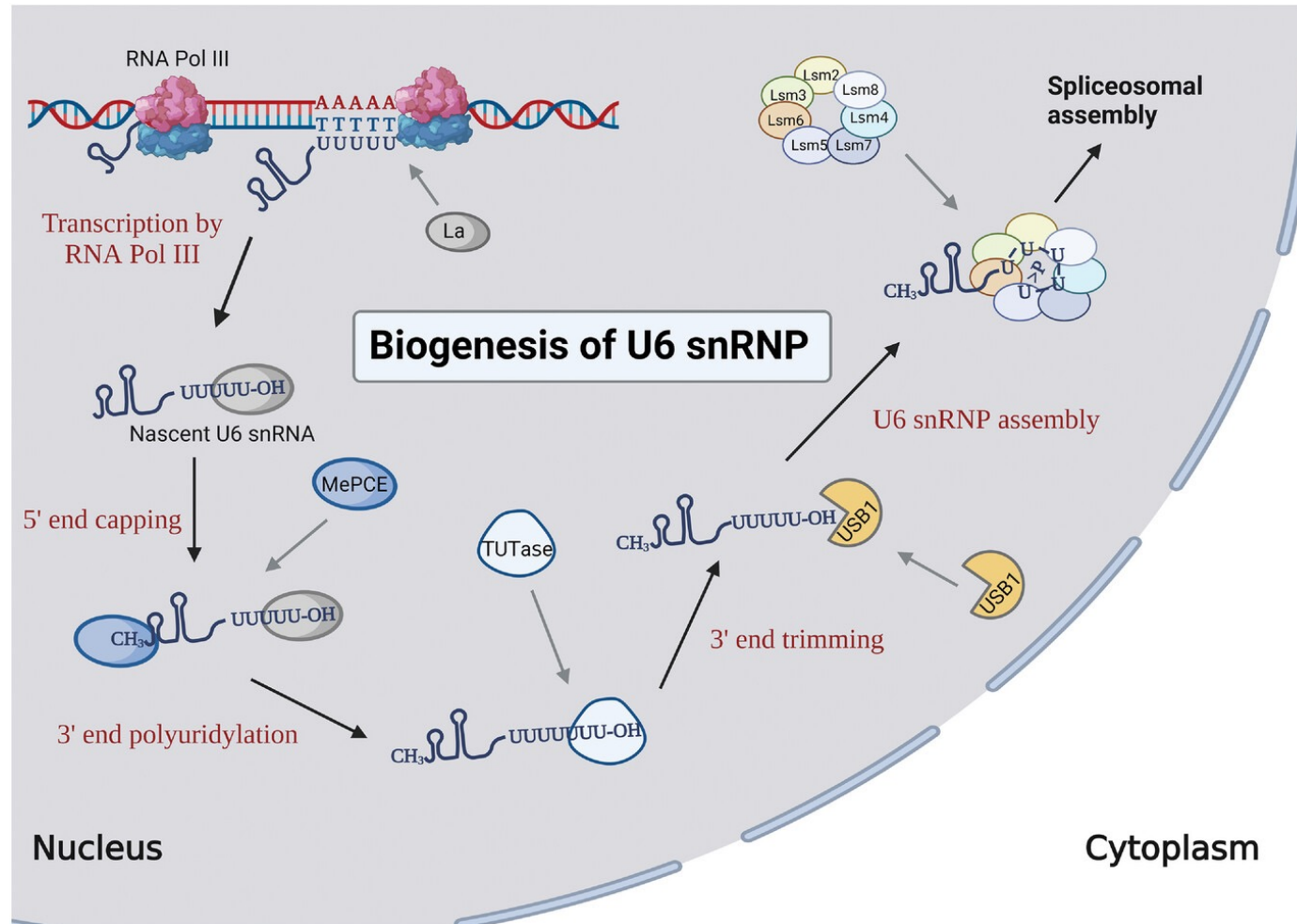


Just like in rRNAs and tRNAs, chemical modifications of mRNA also affect its expression.

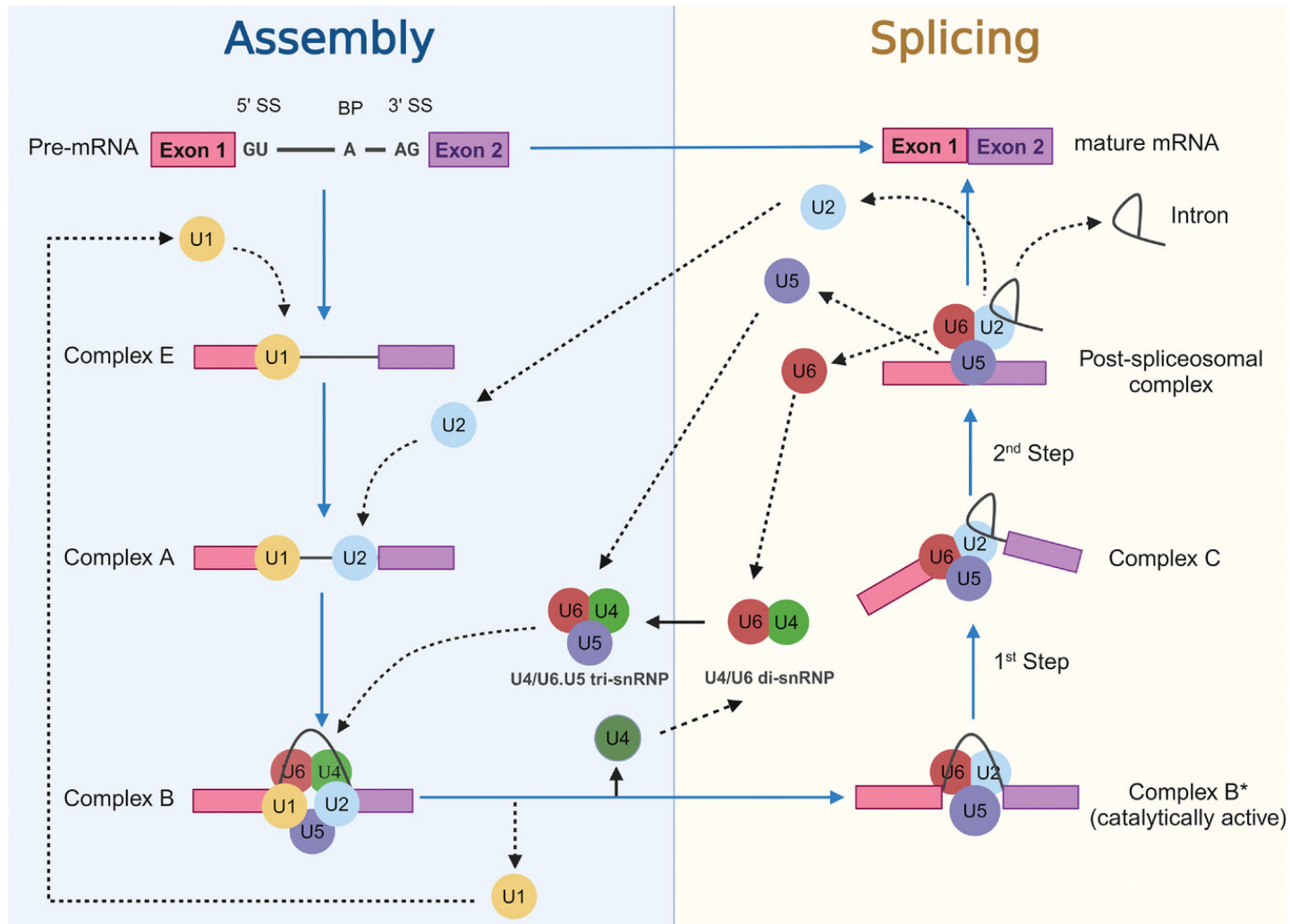
Sm-snRNA (e.g. U1, U2, U4, U5)



Lsm-snRNA (U6)

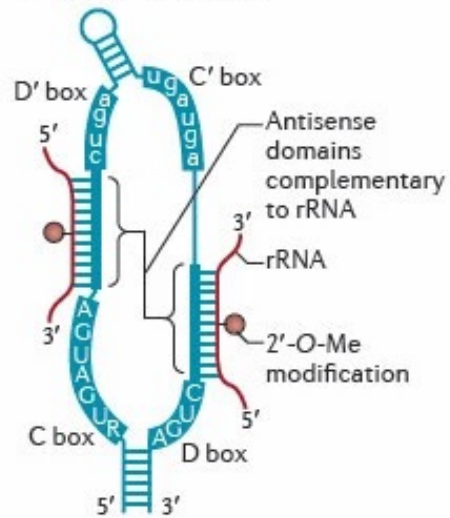


Pre-mRNA splicing

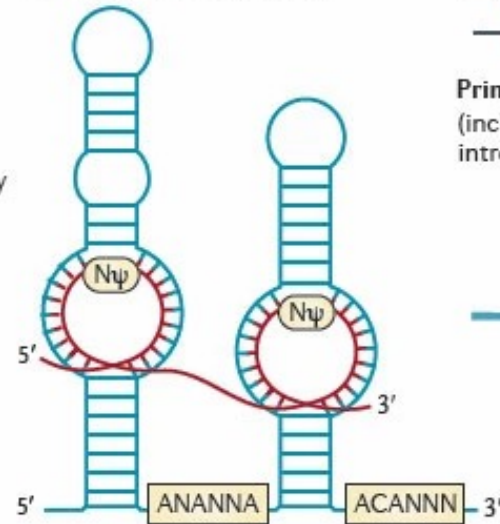


snoRNA

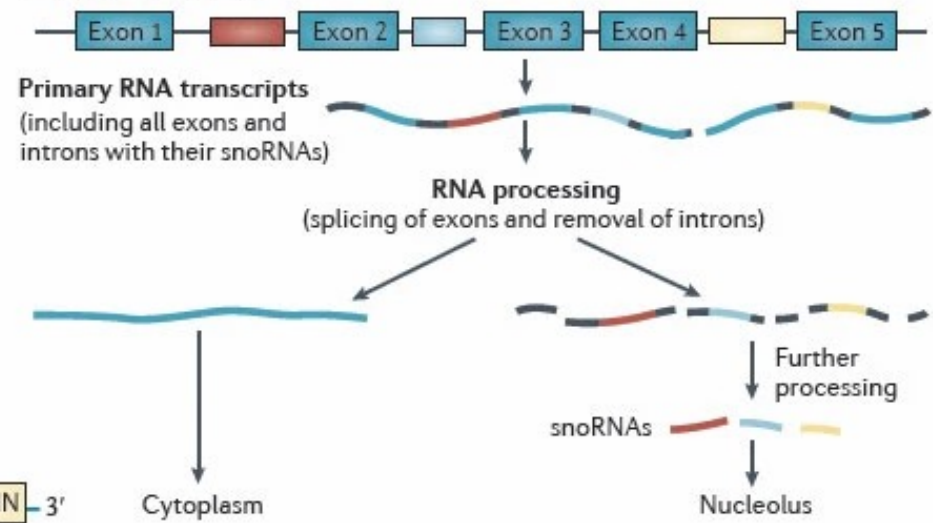
a C/D box snoRNAs



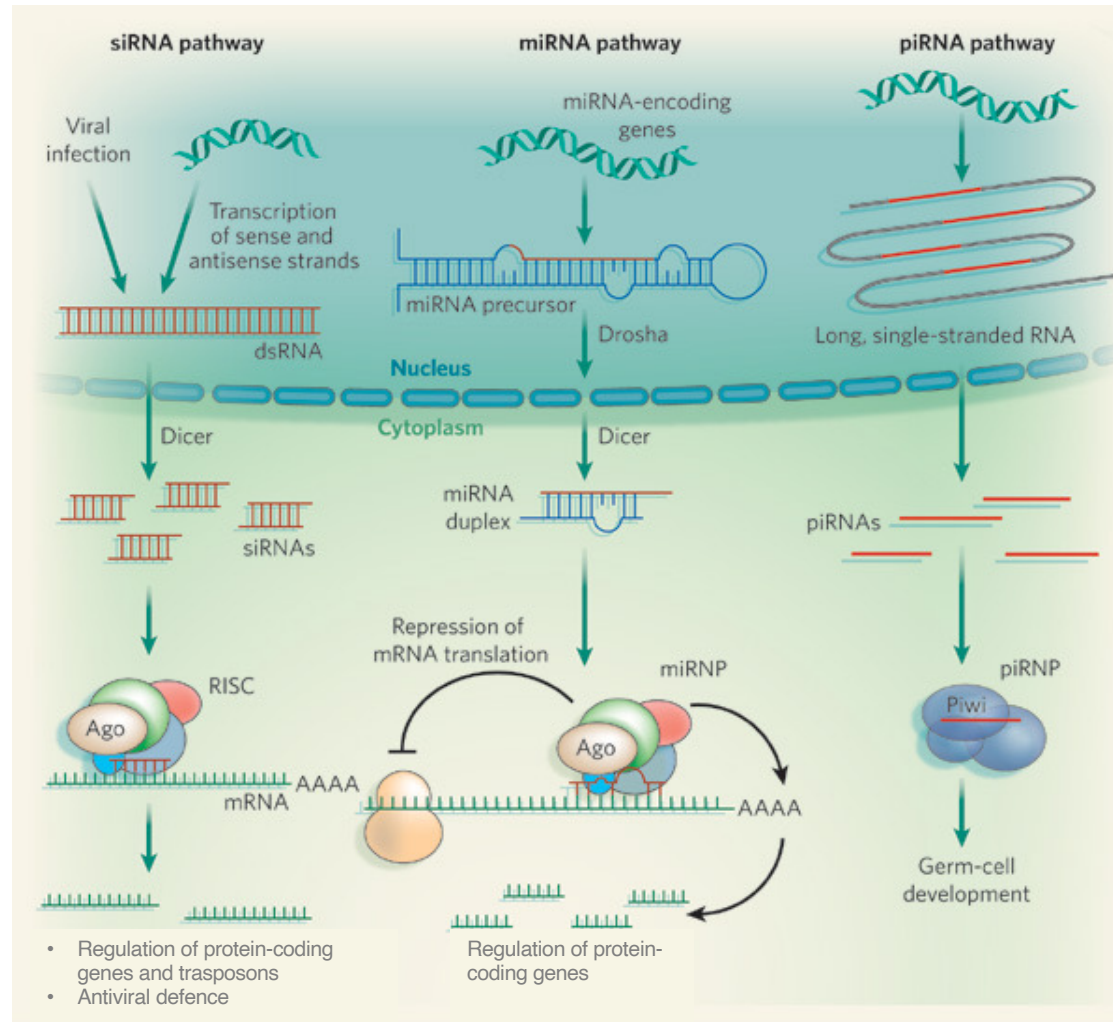
b H/ACA box snoRNAs



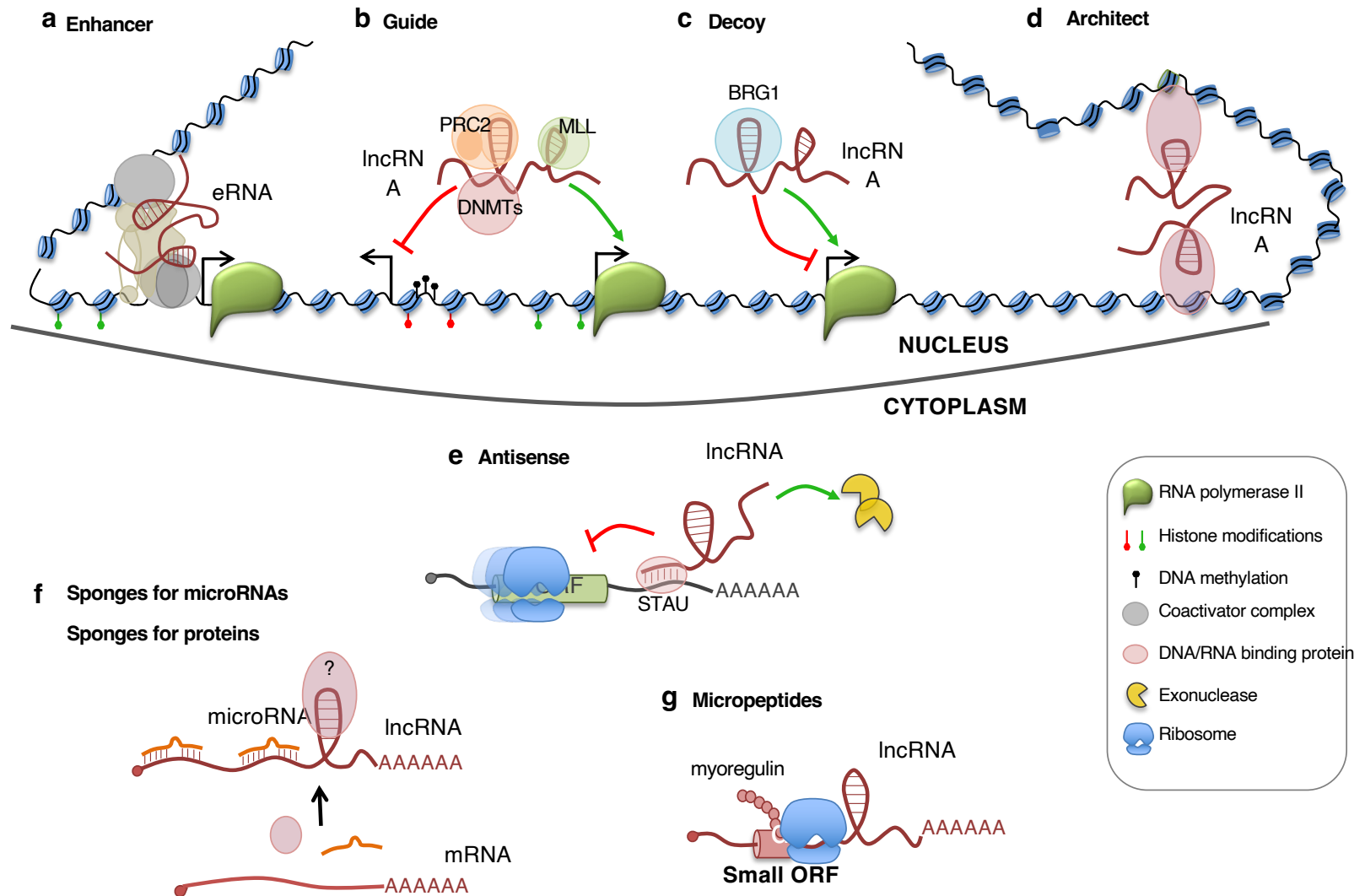
c snoRNA host gene



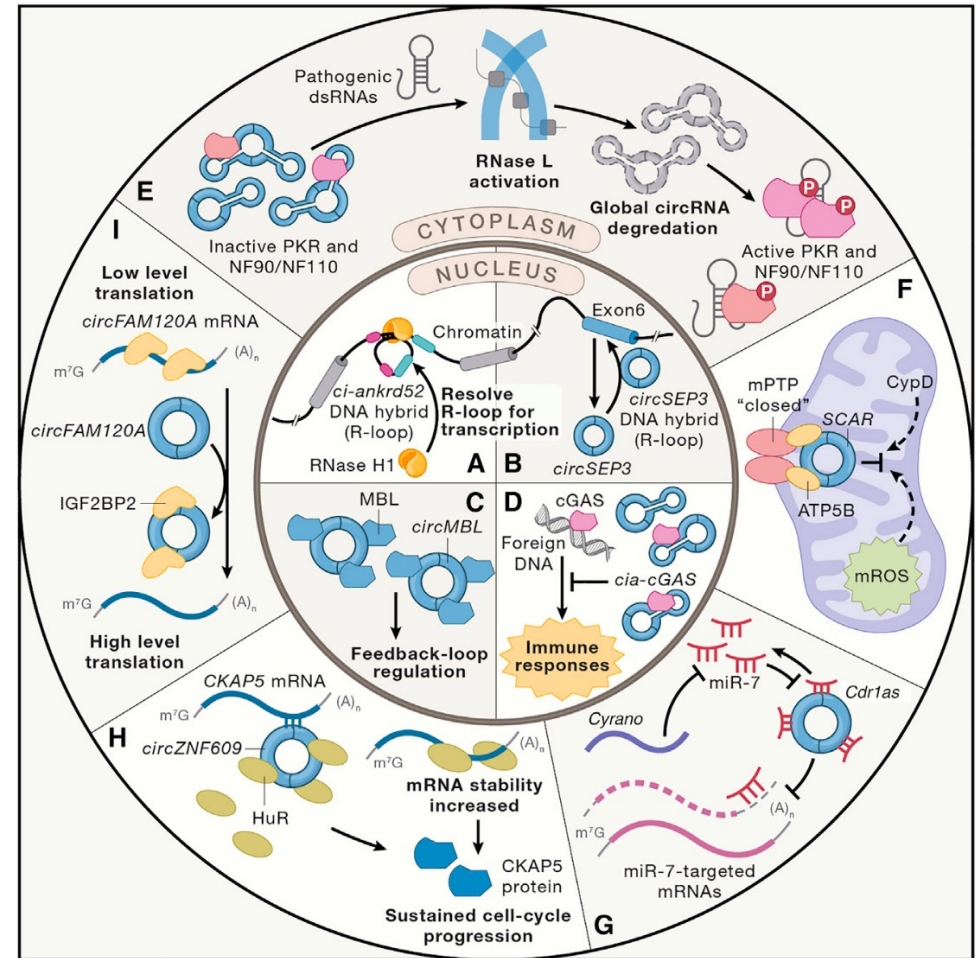
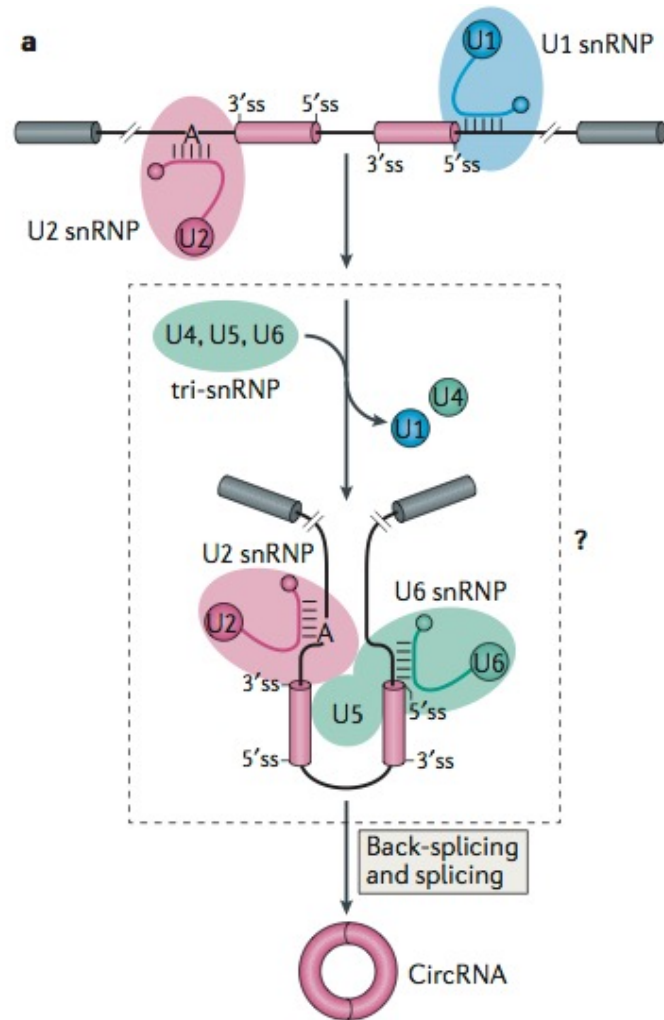
siRNA, microRNA e piwiRNA



lncRNA



circRNA

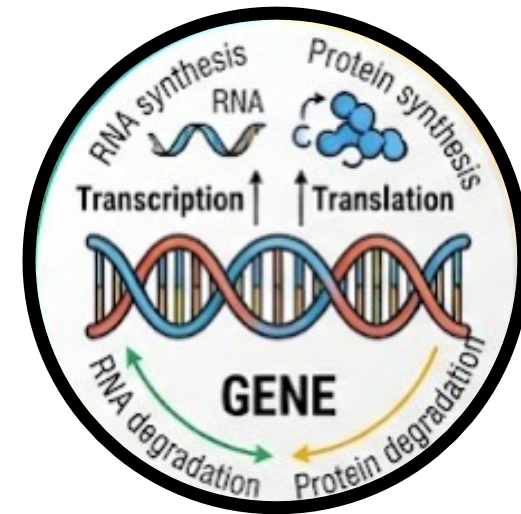


Studying Gene Expression

- RNA analysis (all RNAs)
- Protein analysis (mRNAs)
- Analysis of transcriptional (all RNA) and translational regulation (mRNAs)
- Functional analysis for RNA and proteins (e.g., localization, interactions)
- Analysis of chemical modifications (all RNAs)

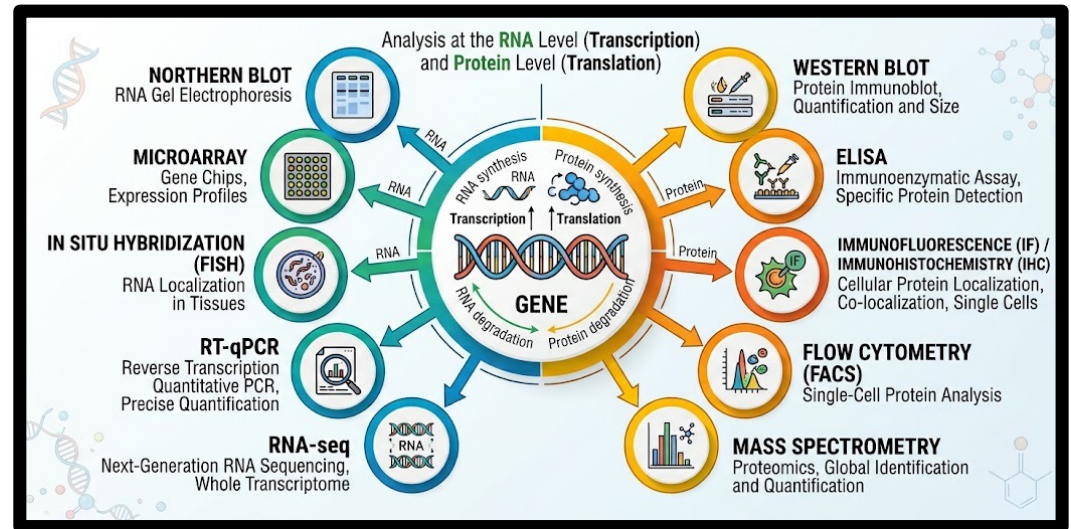
How is gene expression measured?

Measuring a Desk vs. Measuring a Gene



Images by Gemini

Measuring a Desk vs. Measuring a Gene



Measuring a Desk vs. Measuring a Gene

	<u>DESK</u>	<u>GENE EXPRESSION</u>
What is measured?	Fixed physical dimensions (length, width, height)	Amount of generated gene products (RNA or proteins)
Nature of the data	Static: Dimensions do not change from minute to minute.	Dynamic: Expression varies based on time, tissue type, and environmental stimuli.
Targeted measurement	Tape measure: Precise measurement of a single dimension.	RT-qPCR / Western Blot: Relative quantification of a single target RNA or protein*.
Global measurement	Maps and measures all furniture simultaneously.	Genome-wide profiling of all transcripts (Transcriptome) or proteins (Proteome).

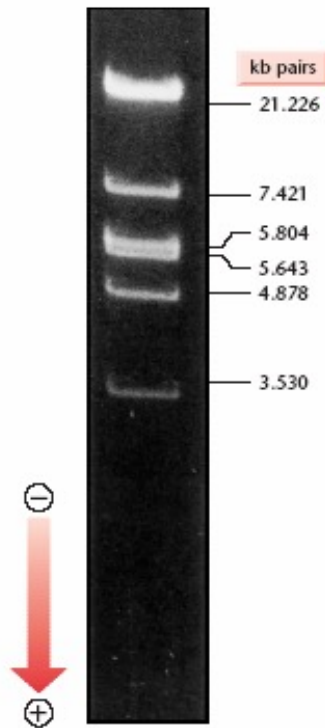
*Samples are almost always compared against a control condition, calculating the fold change in expression.

Methods for Studying RNA

- **RNA Electrophoresis** – quality and processing
- **Northern Blot** – processing and gene expression
- **Real-Time PCR (RT-qPCR)** – gene expression
- **Electrophoretic Mobility Shift Assay (EMSA)** – RNA/protein interactions *in vitro*
- **Immunoprecipitation or Pull-down** – RNA/protein interactions *in vivo*

RNA Electrophoresis

Electrophoresis is a biochemical technique used to separate charged molecules based on their molecular weight. Electrophoretic separations are carried out through a gel matrix.

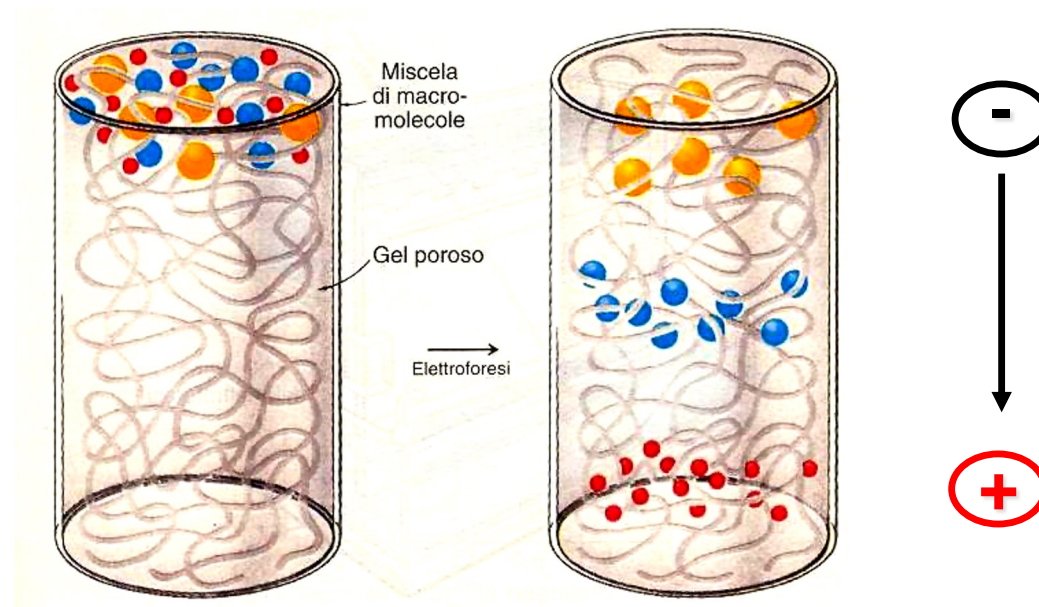


✓ **agarose** (resolution range: 0.5–20 kb)

✓ **polyacrylamide** (resolution range: 10–500 bp)

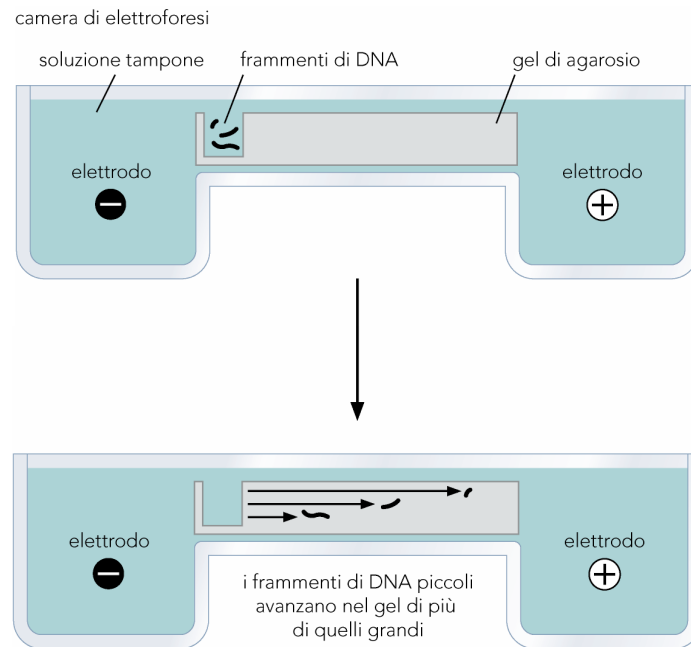
RNA Electrophoresis

For linear RNA fragments (under denaturing conditions), migration distance is inversely proportional to molecular size (which corresponds to its length in bases).

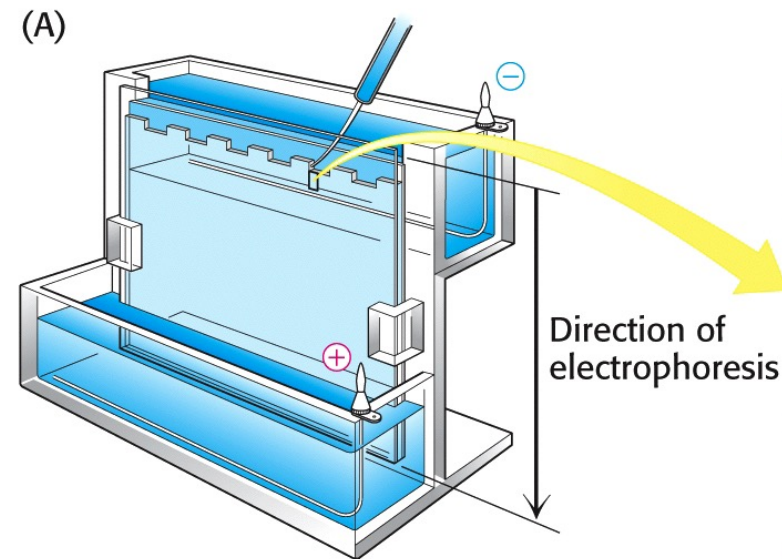


RNA Electrophoresis

✓ Horizontal gel agarose



✓ Vertical gel acrylamide

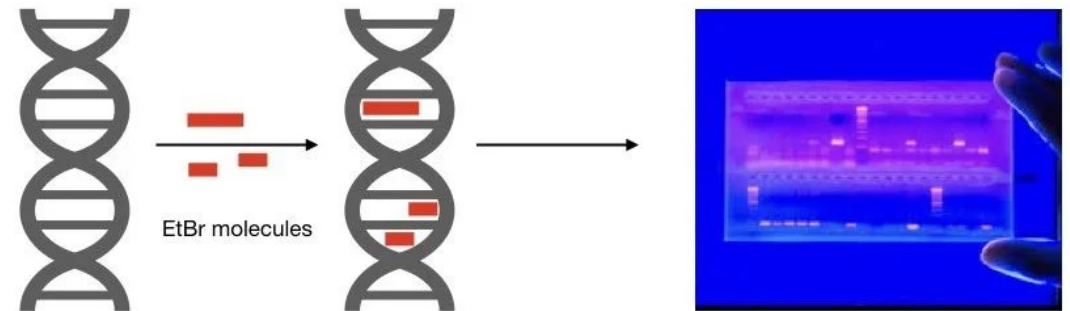


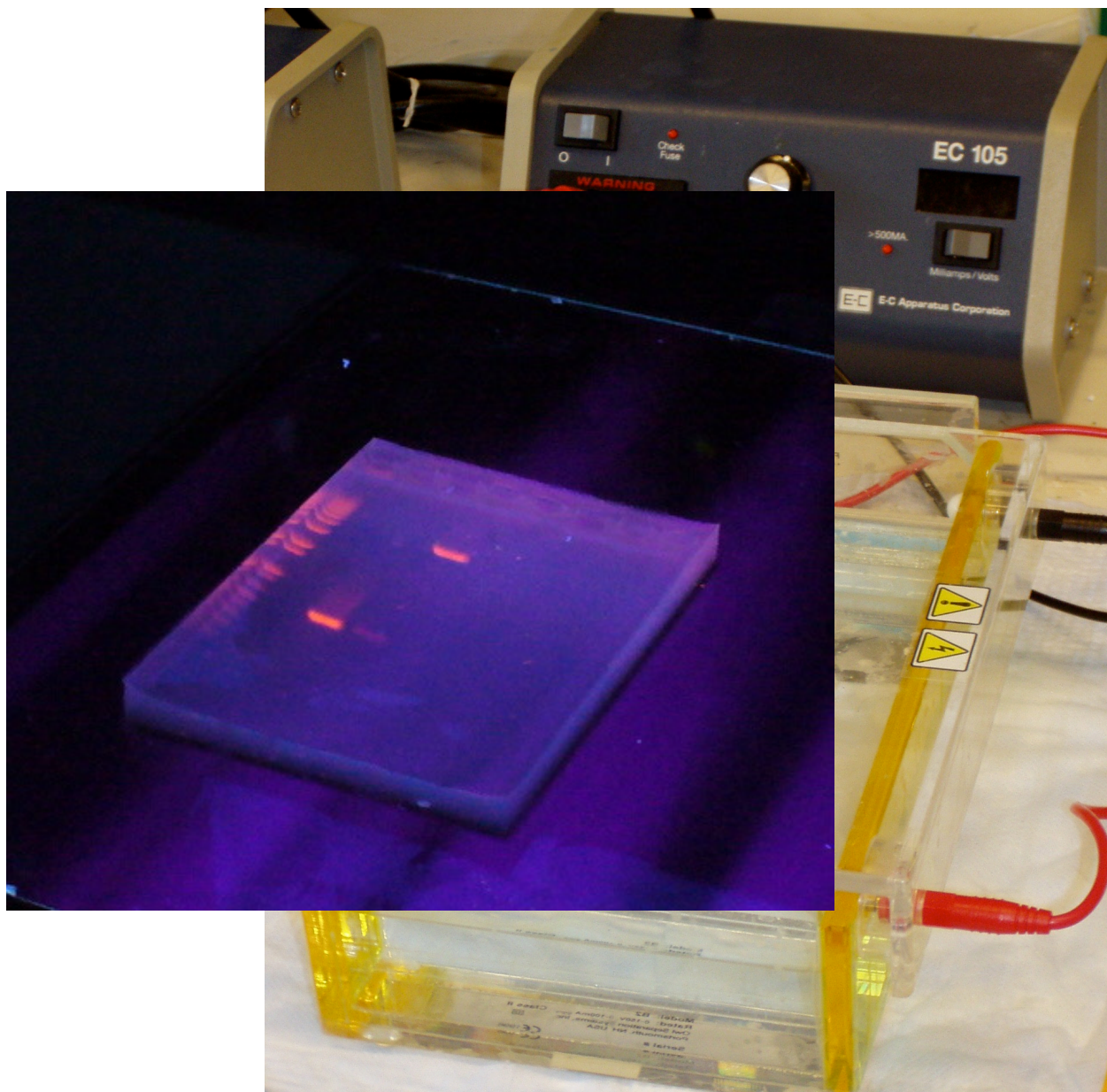
RNA Electrophoresis

Samples are loaded in a denaturing loading buffer containing formaldehyde and/or formamide and ethidium bromide. Prior to loading onto the gel, samples are incubated at high temperature (65–95°C) to promote denaturation.

Ethidium bromide (EtBr):

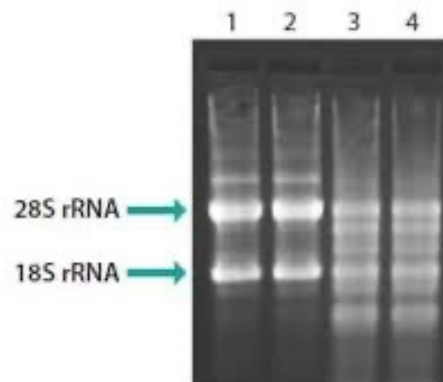
A fluorescent intercalating dye used to visualize DNA and RNA fragments in agarose gel electrophoresis. EtBr absorbs UV light at 300 nm, emitting a yellow-orange signal. It is useful for both visualizing and quantifying the sample, as the fluorescence intensity is directly proportional to the amount of sample



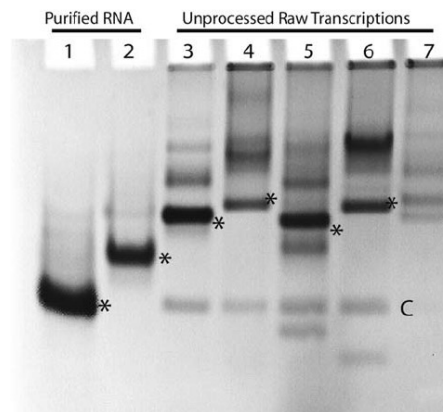


RNA Electrophoresis

RNA Integrity

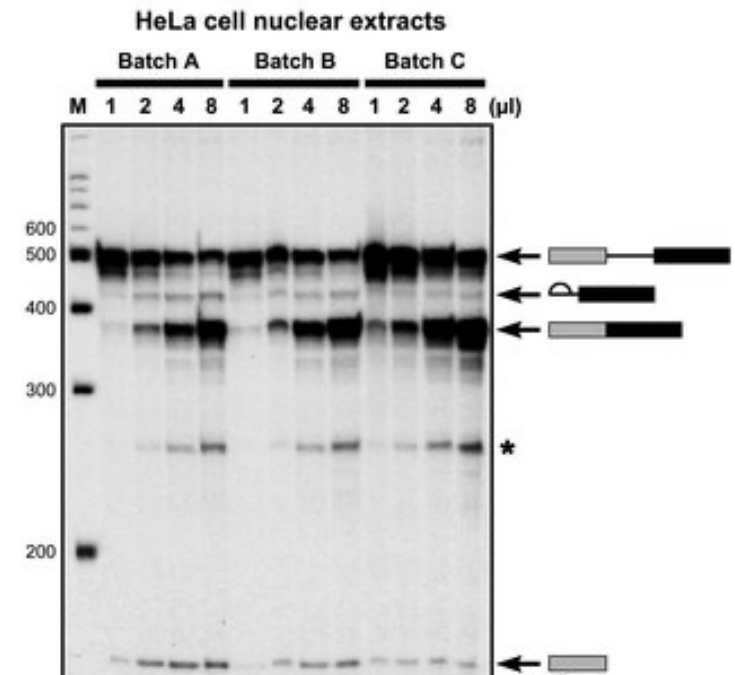


Total RNA
(agarose gel)



In vitro transcribed RNA
(acrylamide gel)

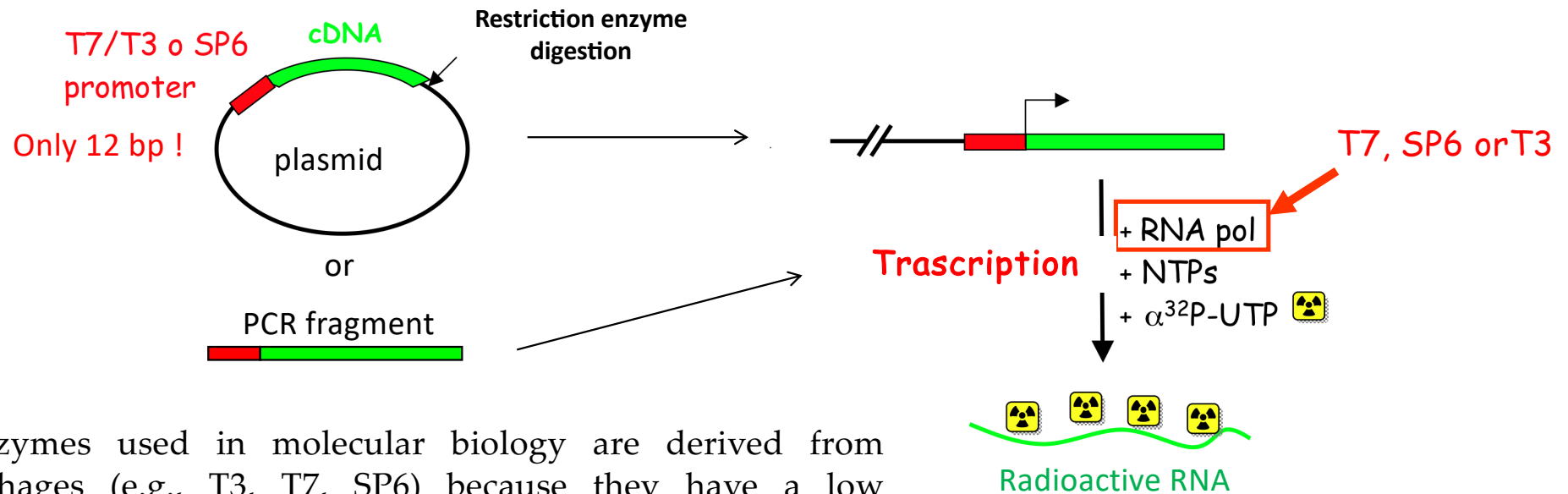
RNA processing



In vitro transcribed RNA
+
protein extract
(acrylamide gel)

How to generate RNA molecules *in vitro*

In vitro transcription:



Most enzymes used in molecular biology are derived from bacteriophages (e.g., T3, T7, SP6) because they have a low molecular weight and are monomeric.

Transcription termination is achieved by allowing the RNA polymerase to 'run off' (or 'fall off') the template, which must therefore be linear.

Chemical Synthesis: Oligonucleotide synthesizers can produce RNA molecules up to approximately 200 nt.

Non-Radioactive Tracers

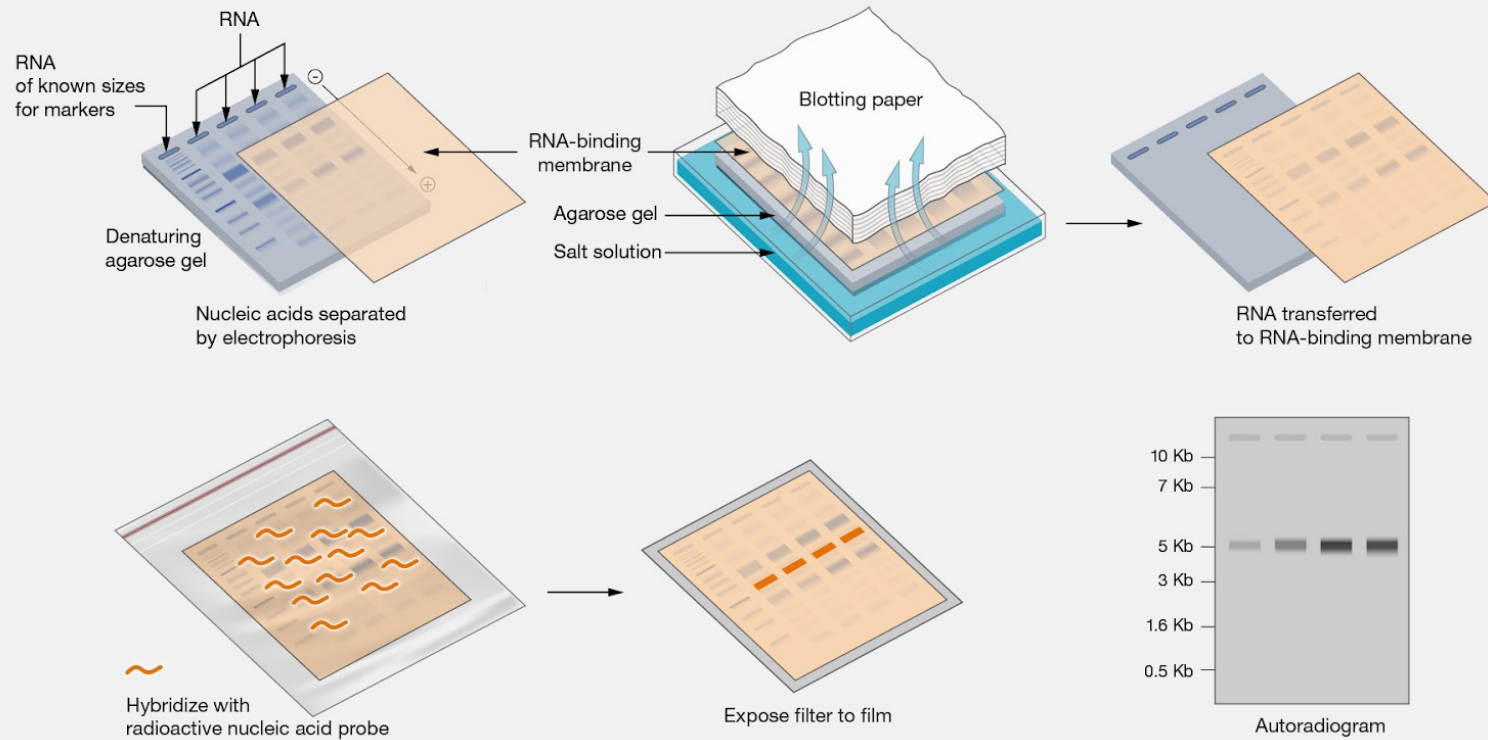
In certain types of experiments, non-radioactive tracers are used in place of radioactive nucleotides:

Fluorochromes (fluorescein, rhodamine), visualized via fluorescence microscopy, gel scanners, or fluorometers.

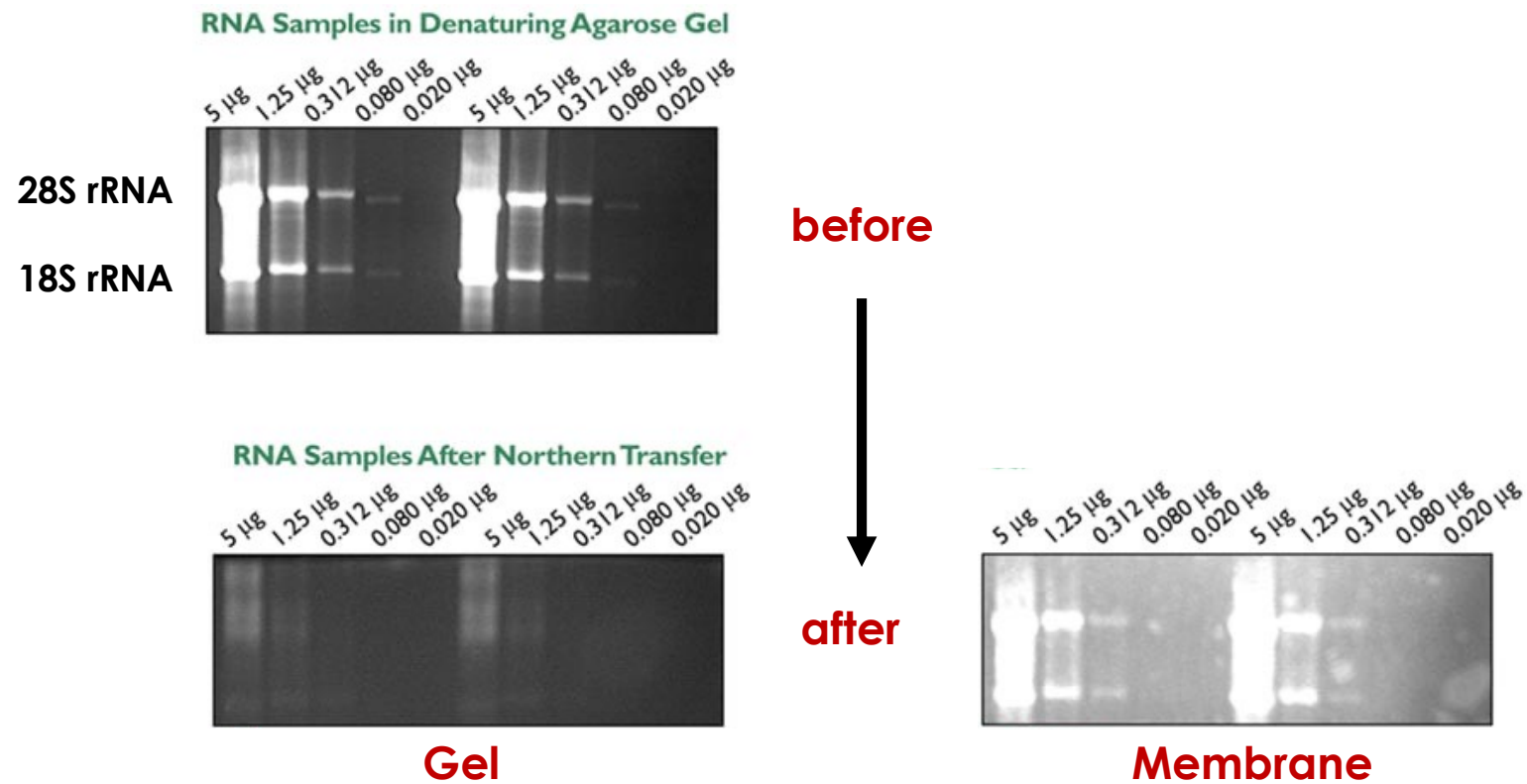
Digoxigenin (DIG), recognized by specific anti-DIG antibodies conjugated to chemiluminescent, chromogenic, or fluorescent indicators.

Biotin, relies on strong biotin–streptavidin interaction coupled to reporter enzymes (e.g., HRP, AP) or fluorophores. Also utilized for RNA pull-down assays (affinity purification).

Northern blot



Northern blot

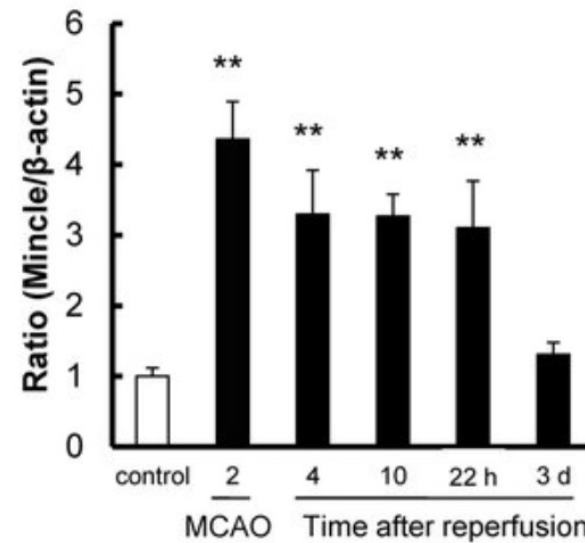
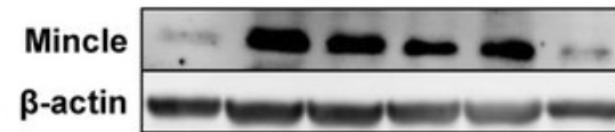


Applications

1. RNA Expression Analysis

Provides several key pieces of information:

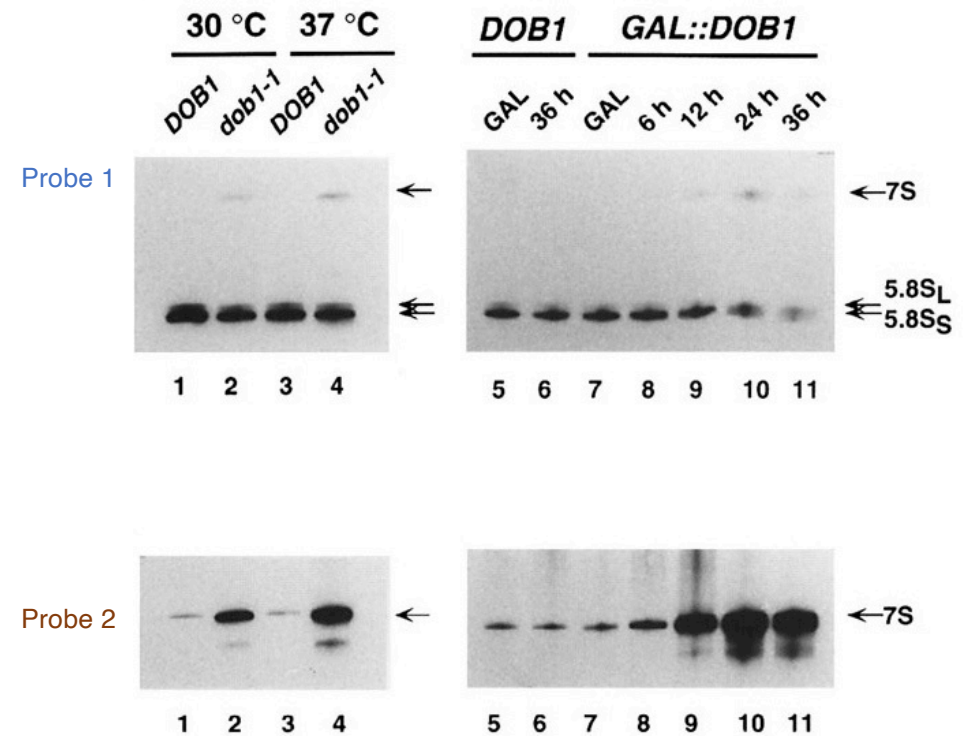
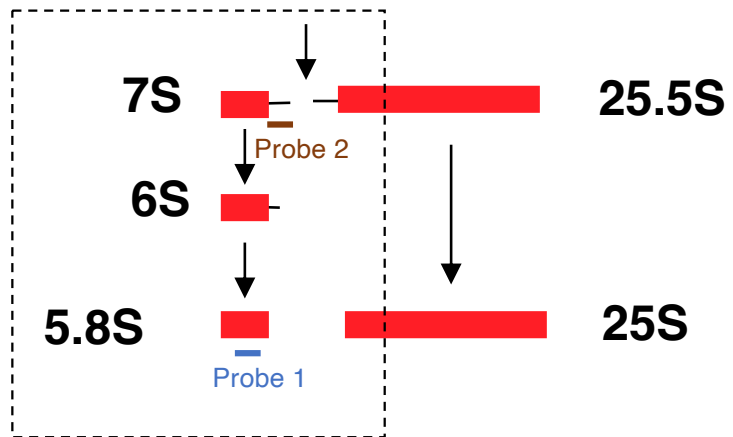
1. Expression levels (relative to a control/reference gene)
2. Length of the RNA



largely superseded by real-time PCR for expression quantification

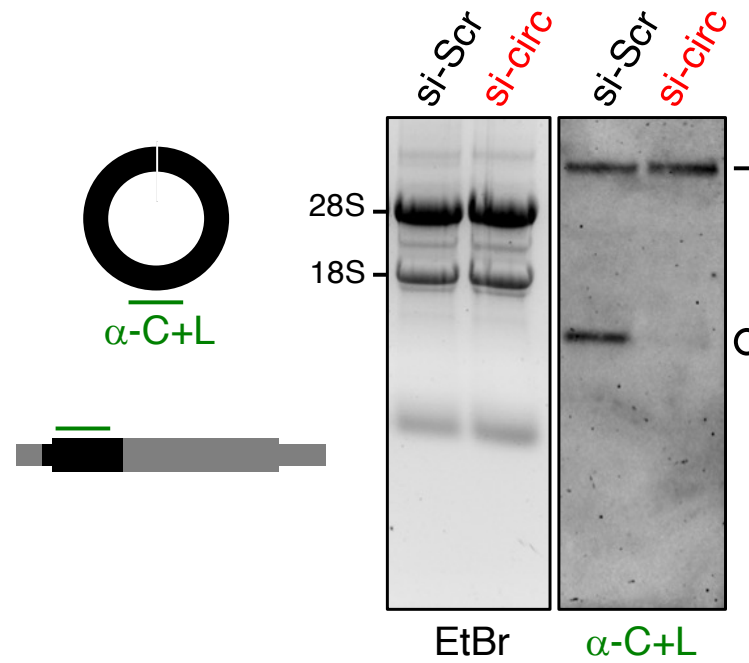
Applications

2. RNA processing



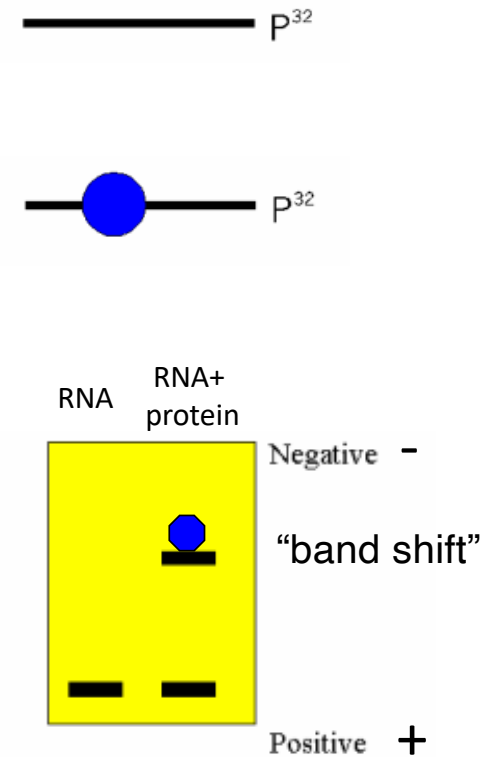
Applications

3. circRNA



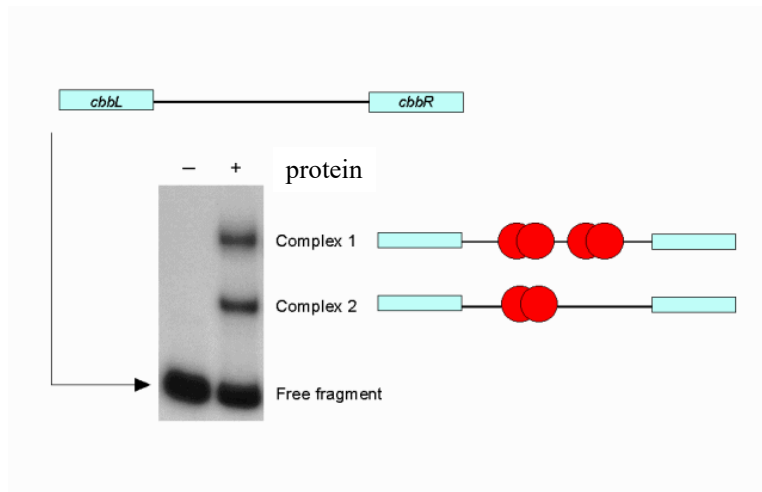
Electrophoretic mobility shift assay (EMSA)

- RNA transcription *in vitro*
- Incubation with proteins or protein extract
- RNA–protein complexes are resolved on **native** polyacrylamide gels.

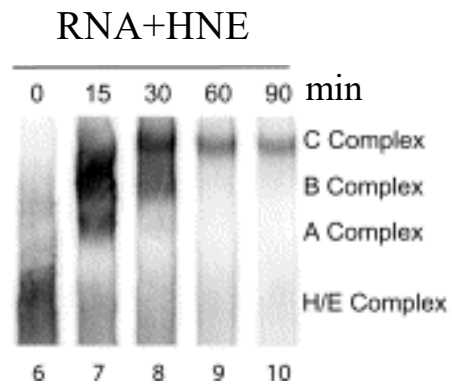


Electrophoretic mobility shift assay (EMSA)

RNA/protein complexes



Splicing complexes



HNE: HeLa Nuclear Extract

