# Ch4 - L3.2

RNA stability & decay

## RNA stability

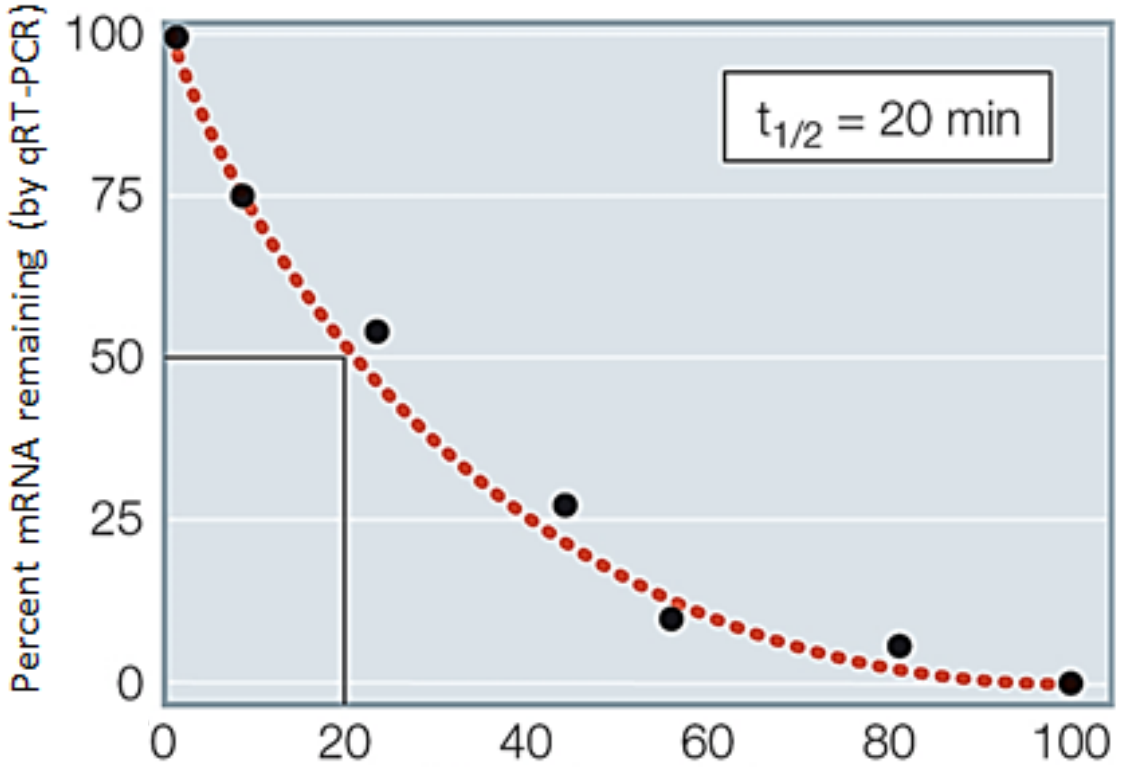
RNAs (both coding ad nonconding) display very **different half-lives**, in a quite wide range, which often are subjected to regulation

Intrinsic factors: *cis* sequences, most often within the 3' UTR

Regulatory factors: RBP or Protein-small RNAs that can induce either stabilization or destabilization.

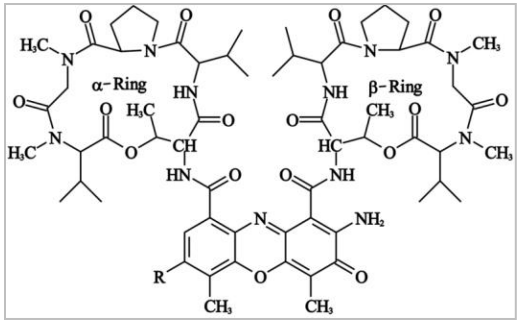
# How to study mRNA stability and decay

## Measuring RNA half-life




- Blocking RNA Polymerase
- Labeling nascent RNA (pulse) and releasing

Cells treated with Actinomycin D

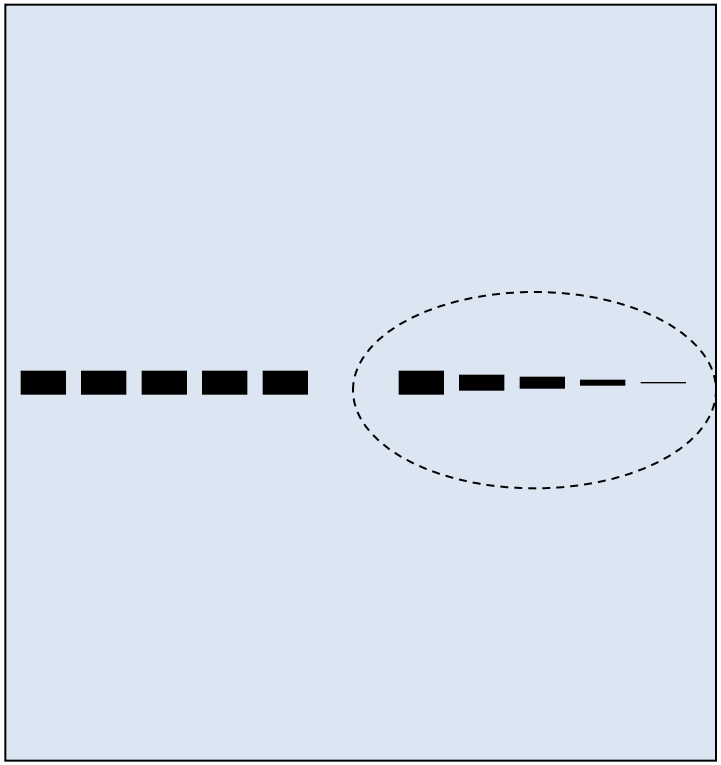



RNA extracted at time intervals

Add radioactive protection probe

RNase (ss)

## Older measure of mRNA half-life on single genes

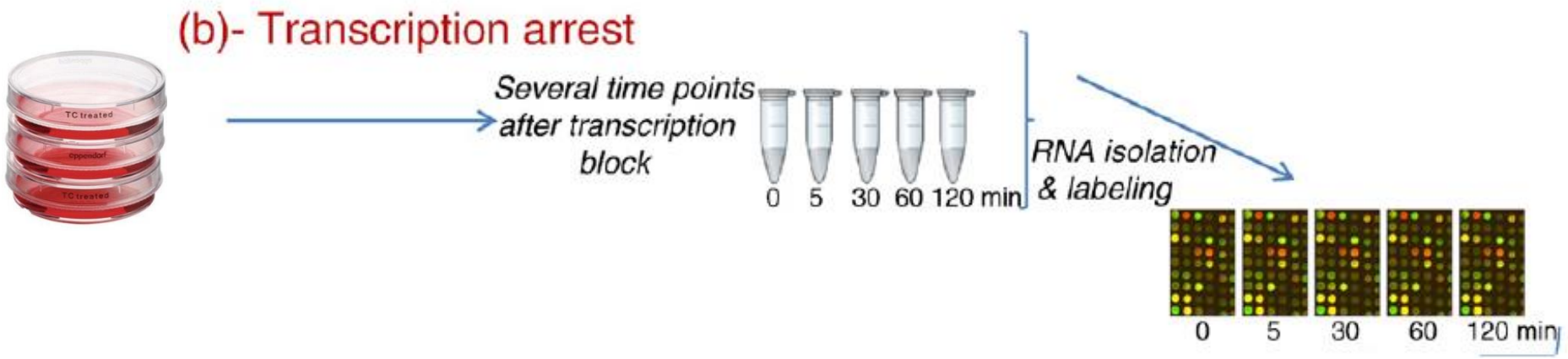


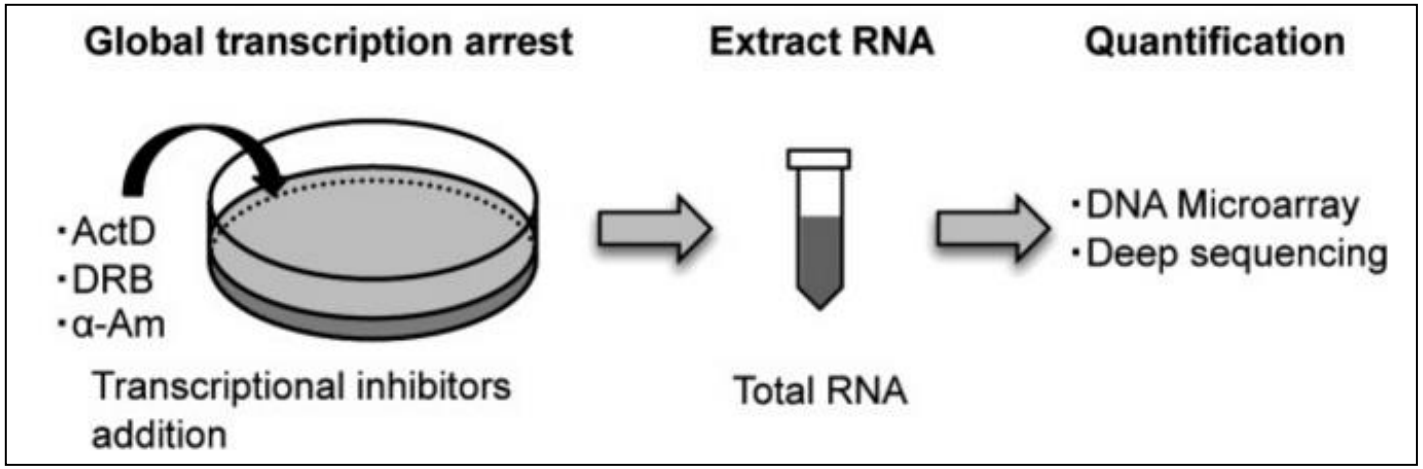

RNase Protection Assay (RPA)

wiki

## Genome-wide

Blockers:
Actinomycin D (ActD),
5,6-dichloro-1–D-ribofuranosyl-benzimidazole (DRB)
α-amanitin (α-Am)

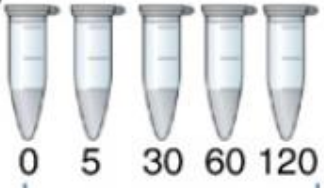


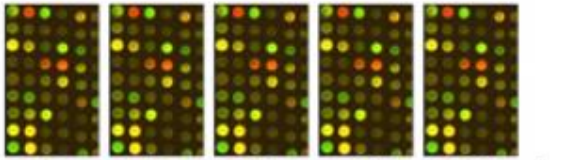
Pulse of modified UTP

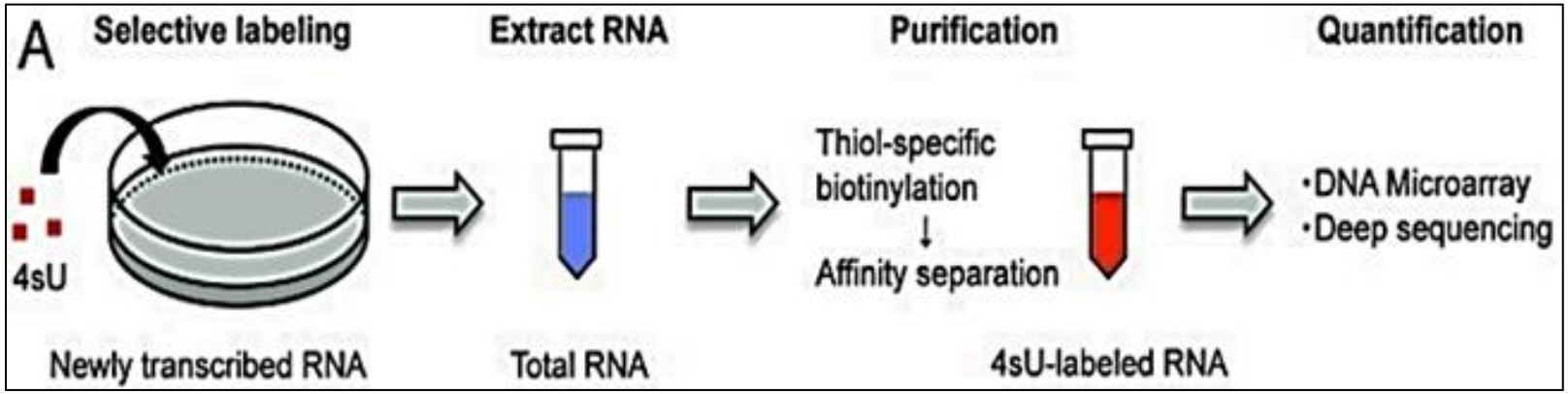

Chase

Several time points after precursor depletion




RNA fractionation & labeling



Letter Genome Res. 2003; 13(8):1863-72.

(First study human cells genome-wide: pre-NGS)

# Decay Rates of Human mRNAs: Correlation With Functional Characteristics and Sequence Attributes

Edward Yang,[1,6] Erik van Nimwegen,[4,6] Mihaela Zavolan,[2] Nikolaus Rajewsky,[5] Mark Schroeder,[2] Marcelo Magnasco,[3] and James E. Darnell Jr.[1,7]

[1]Laboratory of Molecular Cell Biology, [2]Laboratory of Computational Genomics, [3]Laboratory of Mathematical Physics, and [4]Center for the Study of Physics and Biology, The Rockefeller University, New York, New York 10021-6399, USA; [5]Department of Biology and Courant Institute of Mathematical Sciences, New York University, New York, New York 10012, USA

HepG2 cells (human liver carcinoma cell line)

+ primary cells (fibroblasts Bud8)

**Actinomycin 2-3 hours**

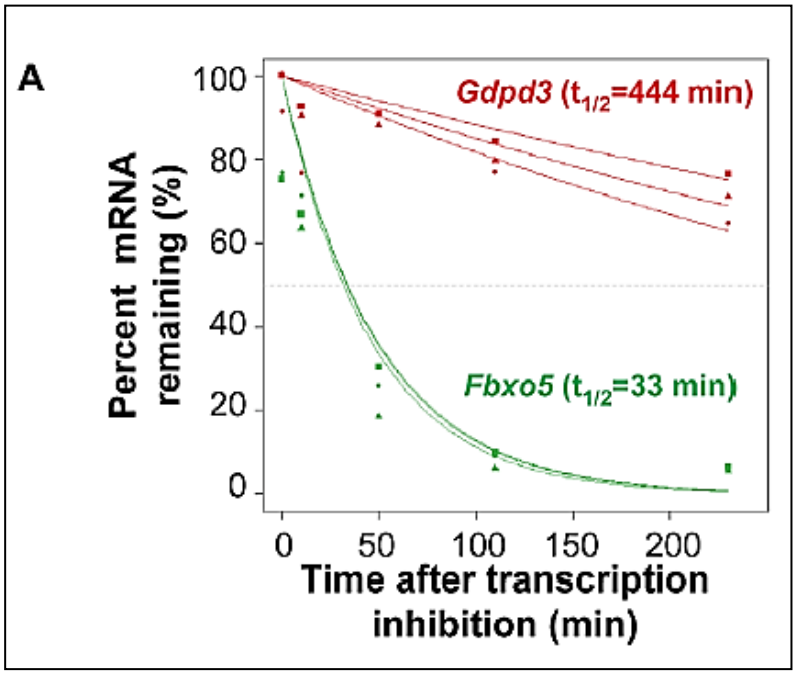
RNA extraction, labelling and → Affymetrix microarrays

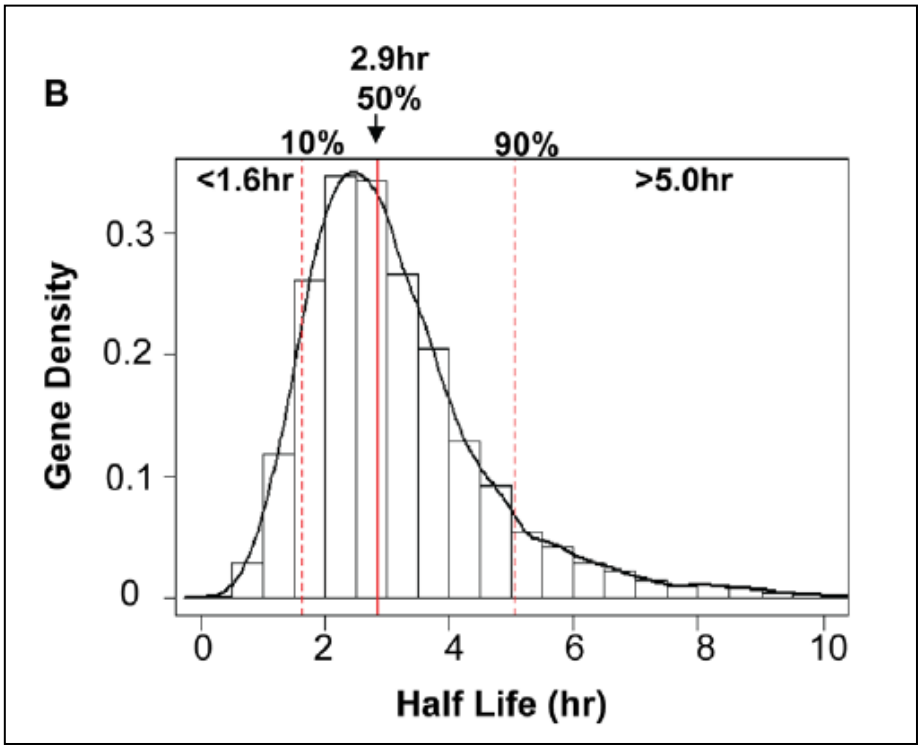
Decay rate estimates for 5,245 genes

The **median half-life** in both cell types is ~10 h, with wide range (0.5 hours up to "days").

**Lee et al., PLOS One, 5:11201 ( 2010 )**

**Mouse myoblasts** in culture treated with actinomycin D
Samples collected at 0, 10, 50, 110, 230 min
Total RNA → labeled → hybridized to Affymetrix Mouse Gene 1.0 arrays.

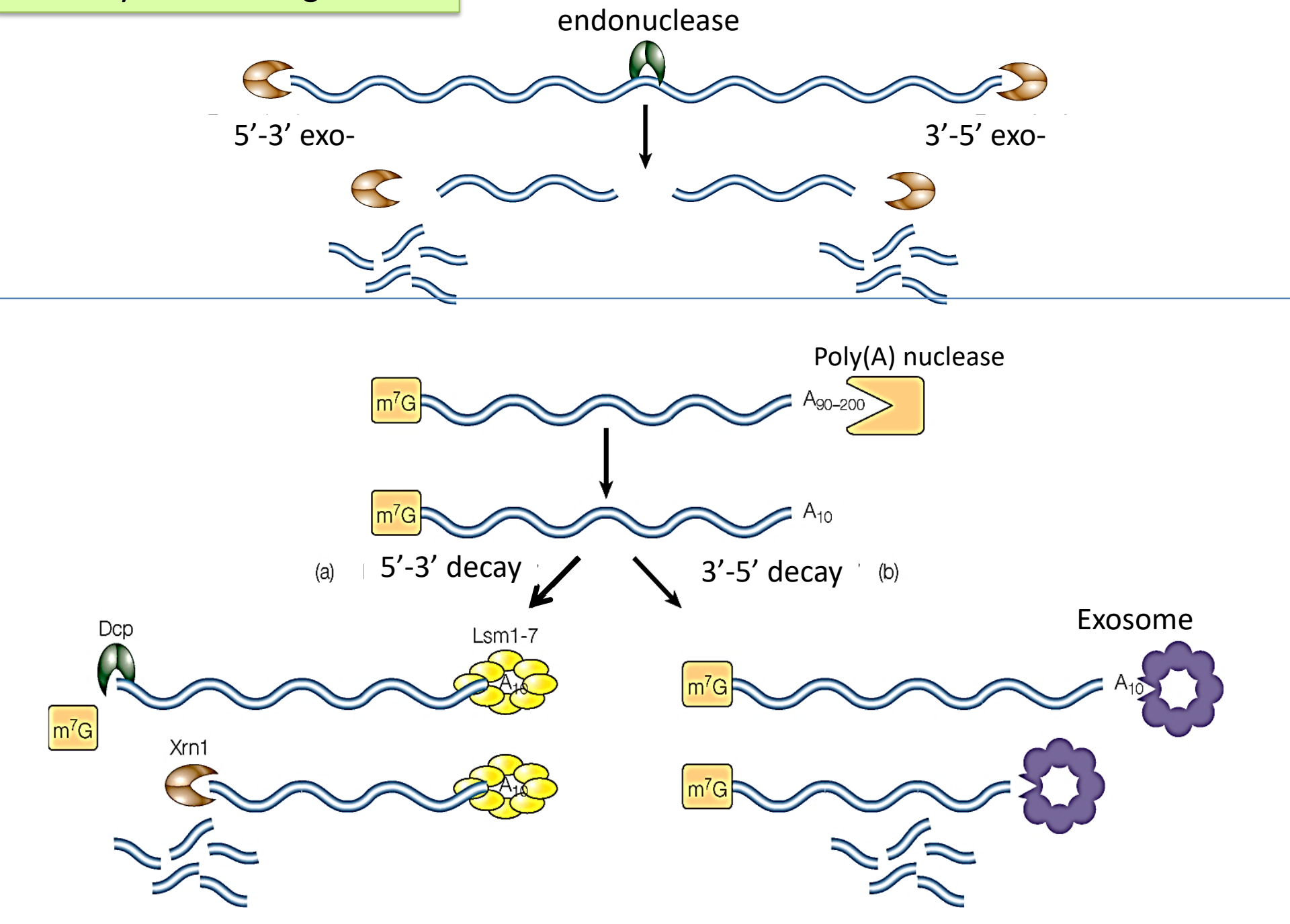



Analysis of mRNA decay rate in C2C12 cells.
(A) Examples of mRNA decay curves at extremes. were derived by the nonlinear least squares method for a long and a short half life mRNA.
(B) Distribution of mRNA half and 90th-percentile values .

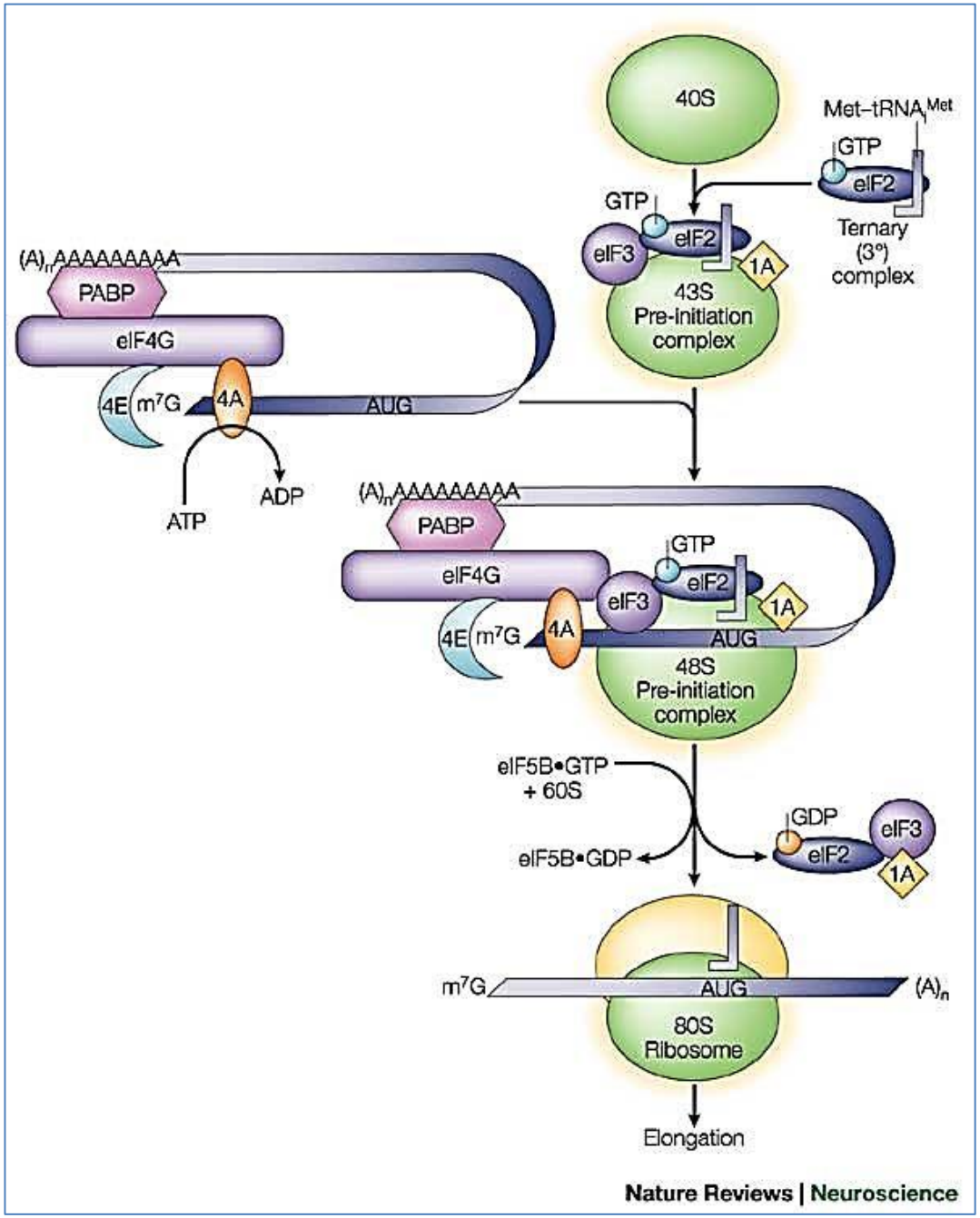
mechanisms

# Pathways to RNA degradation

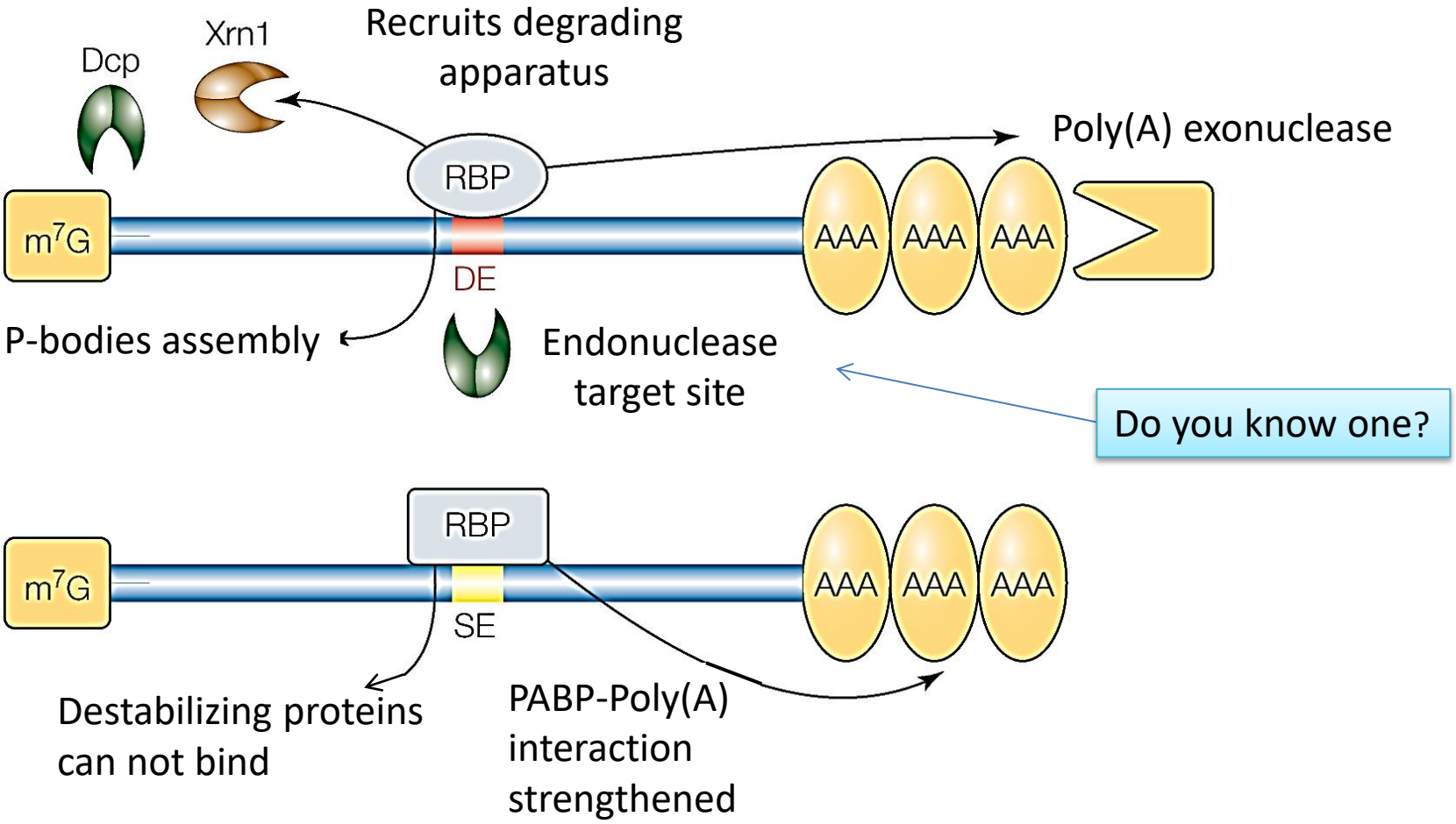


**decapping** or **polyadenylate shortening** strongly impairs translation, since interaction of cap and poly(A) is essential

Degradation and translation are competing events.



## Stabilizing and de-stabilizing sequence motifs (cis-elements)




NOTE: Some cis-elements can function in both pathway, depending which RBP is expressed in specific cell contexts.

**RNA-binding proteins and small RNA**

Motifs imparting shorter or longer half-life may represent:

1 - protein-binding elements for regulatory RBPs

**or**

2 - targets for RNA-RNA interaction

In general, in the latter case, we have miRNA, siRNA or piRNA targets.

Let’s have a look to most common protein regulators: ARE-binding proteins

## Most known regulatory elements: the ARE elements

- are A/U-rich elements found in the **3'-UTR** of some mRNAs encoding cytochines, proto-oncogenes and growth factors
- are defined by their ability to promote rapid deadenylation-dependent mRNA decay
- their sequence requirements are only loosely conserved

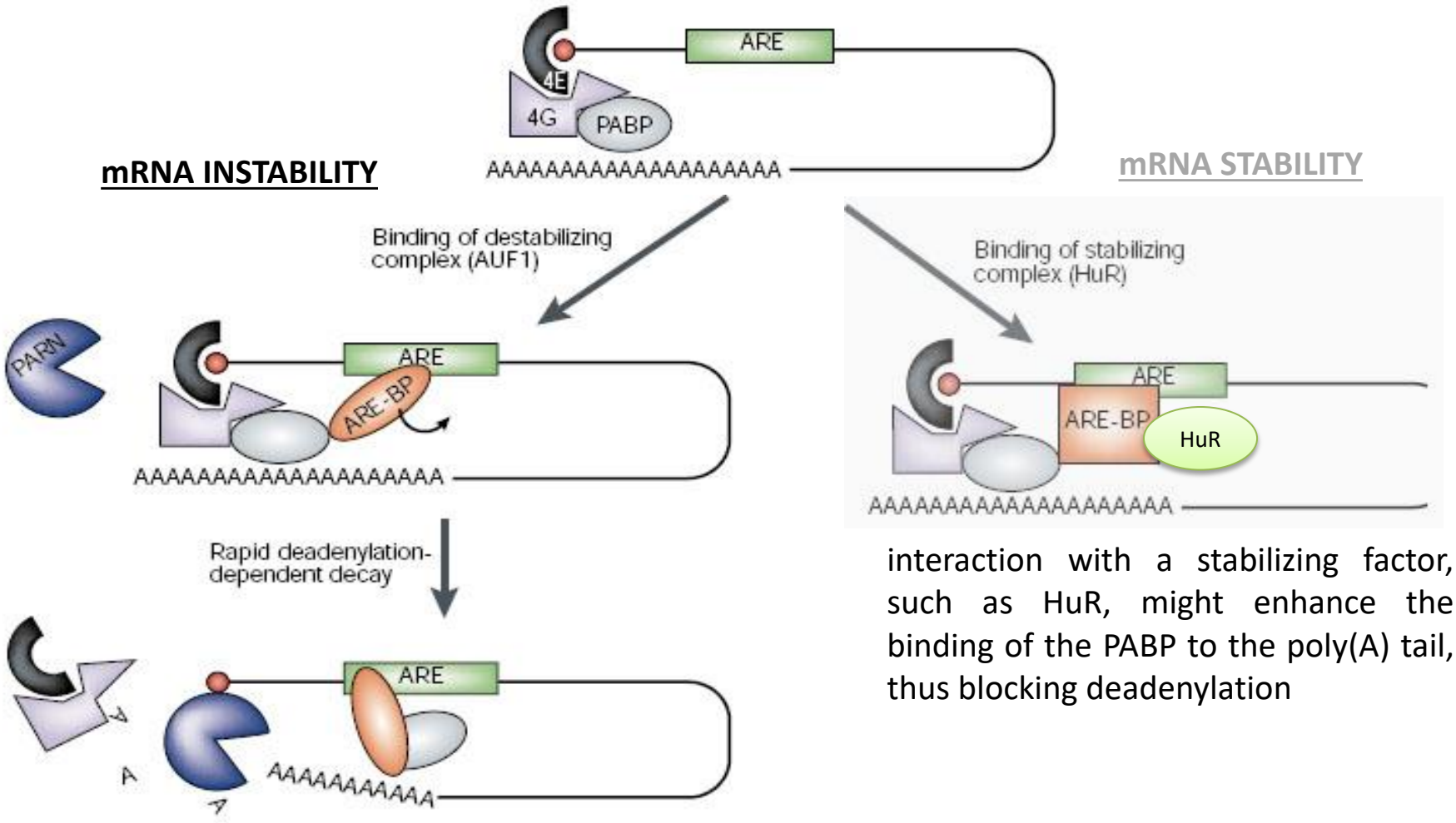
| Group | Motif | Examples |
|---|---|---|
| I | WAUUUAW and a U-rich region | c-fos, c-myc |
| IIA | AUUUAUUUAUUUAUUUAUUUA | GM-CSF, TNF-α |
| IIB | AUUUAUUUAUUUAUUUA | Interferon-α |
| IIC | WAUUUAUUUAUUUAW | cox-2, IL-2, VEGF |
| IID | WWAUUUAUUUAWW | FGF2 |
| IIE | WWWWAUUUAWWWW | u-PA receptor |
| III | U-rich, non-AUUUA | c-jun |

*Wilusz J.C. et al., 2001*

ARE-binding proteins recognize these elements and, in conjunction with other proteins, will guide the mRNA to exosome degradation.

# ARE-binding proteins

**Many ARE-binding protein** have been identified and have either *negative* or in some cases *positive* effect on processes such as stability, translation, subcellular localization of the mRNA




interaction with a stabilizing factor, such as HuR, might enhance the binding of the PABP to the poly(A) tail, thus blocking deadenylation

Destabilizing factor AUF1 might promote rapid deadenylation by reducing the affinity of the poly(A) binding protein (PABP) for the poly(A) tail

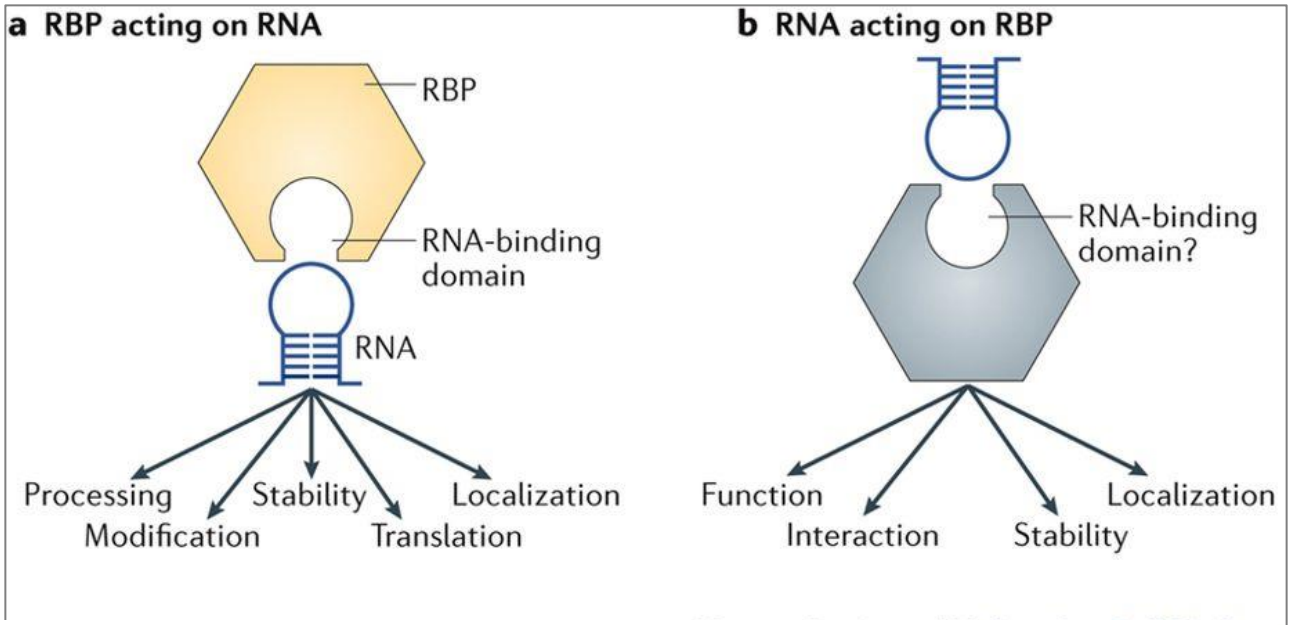
*Wilusz J.C. et al., 2001*

RNA Binding Proteins are a growing class ...

Generic name: RBP (RNA binding Proteins) GO category: RNA-Binding

Recent studies used RIC (RNA Interactome Capture) identified an exceptional number of RBPs (860 from HeLa and 791 from HEK293). Many of these do not carry any of the known domains:

-RRM - RNA Recognition Motif
-KH
-DEAD box helicase
-Zn-fingers motifs



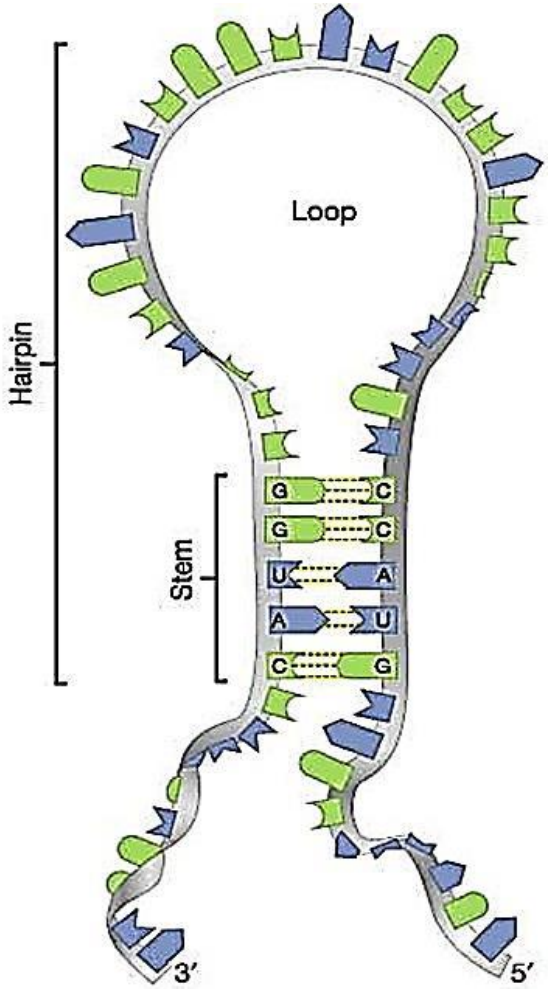

*Form Hentze et al. Nature Reviews Molecular Cell Biology* **volume 19**, pages 327–341 (2018)

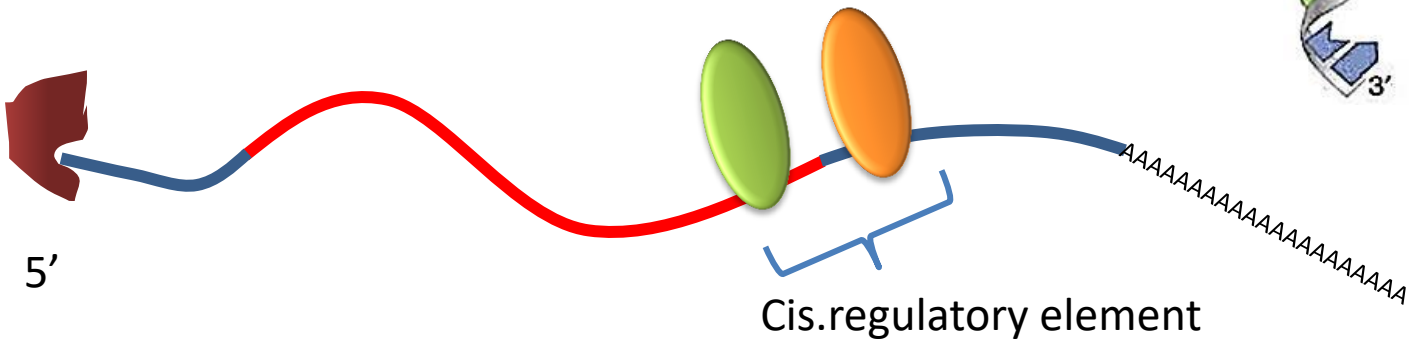
**Identification of RNA binding proteins motifs**

Specificity of RNA binding: both «sequence» and «structure» elements

Problems in predicting regulatory motifs:

- Localization (intron length)
- Sometimes dispersed elements
- Sometimes the structural component prevails upon pure sequence



RBP database: http://cisbp-rna.ccbr.utoronto.ca/index.php

Structure

RRM: Sex-lethal http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=1b7f

RRM: PTB
http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=2adc

KH: NOVA1 http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=1dt4

RNAs intrinsic *cis* regulatory sequences: Destabilizing (DE) or Stabilizing Elements (SE)


Examples of *trans*-acting regulators

| RNA-binding protein | Function | RNA-binding domain | Mode of action | Modifications | Other functions |
|---|---|---|---|---|---|
| AUF1 (hnRNP D) and its four splice isoforms (p37, p40, p42, p45) | Usually destabilizing | RRM | Recruit the exosome; remodel mRNA to allow other proteins to bind | Phosphorylation allows isomerization by PIN1 leading to dissociation from RNA; interacts with 14-3-3 proteins | DNA binding |
| CUG-BP | Destabilizing | RRM | Recruits PARN; modulates ARE function | Phosphorylated by myotonic dystrophy protein kinase | Splicing; translation |
| ELAV proteins, for example, HuR and HuD | Stabilizing | RRM | Compete with destabilizing proteins for ARE-binding; might relocalize mRNAs away from decay machinery | CARM1-mediated methylation reduces stabilizing function | Translation; RNA localization |
| KSRP | Destabilizing | KH domain | Recruits decay enzymes: PARN and the exosome | Phosphorylation by p38-MAPK pathway leads to reduced RNA-binding affinity | Splicing |
| RHAU | Destabilizing | RNA helicase | Recruits decay enzymes: PARN and the exosome | Not known | Not known |
| TIA-1, TIAR | Translational silencing | RRM | Induce aggregation into stress granules | Phosphorylated by FAST | Alternative splicing |
| Tristetraprolin (TTP, TIS11, ZFP36), BRF1 (TIS11B, ZFP36L1), BRF2 (TIS11D, ZFP36L2) | Destabilizing | CCCH-type zinc finger | Recruit decay enzymes: CCR4, DCP1, PM-Scl75, RRP4 | Phosphorylation by p38-MAPK pathway leads to association with 14-3-3 proteins | Transcription |

Garneau *et al. Nature Reviews Molecular Cell Biology* **8**, 113–126 (2007) | doi:10.1038/nrm2104

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Part 3

# RNA interference

From the regulatory point of view, the most interesting class is given by **micro-RNA and other small RNA-guided AGO proteins**

The pathways in which AGO proteins are involved are collectively called ***RNA interference***

**RNA interference, small-interfering RNA, micro-RNA**