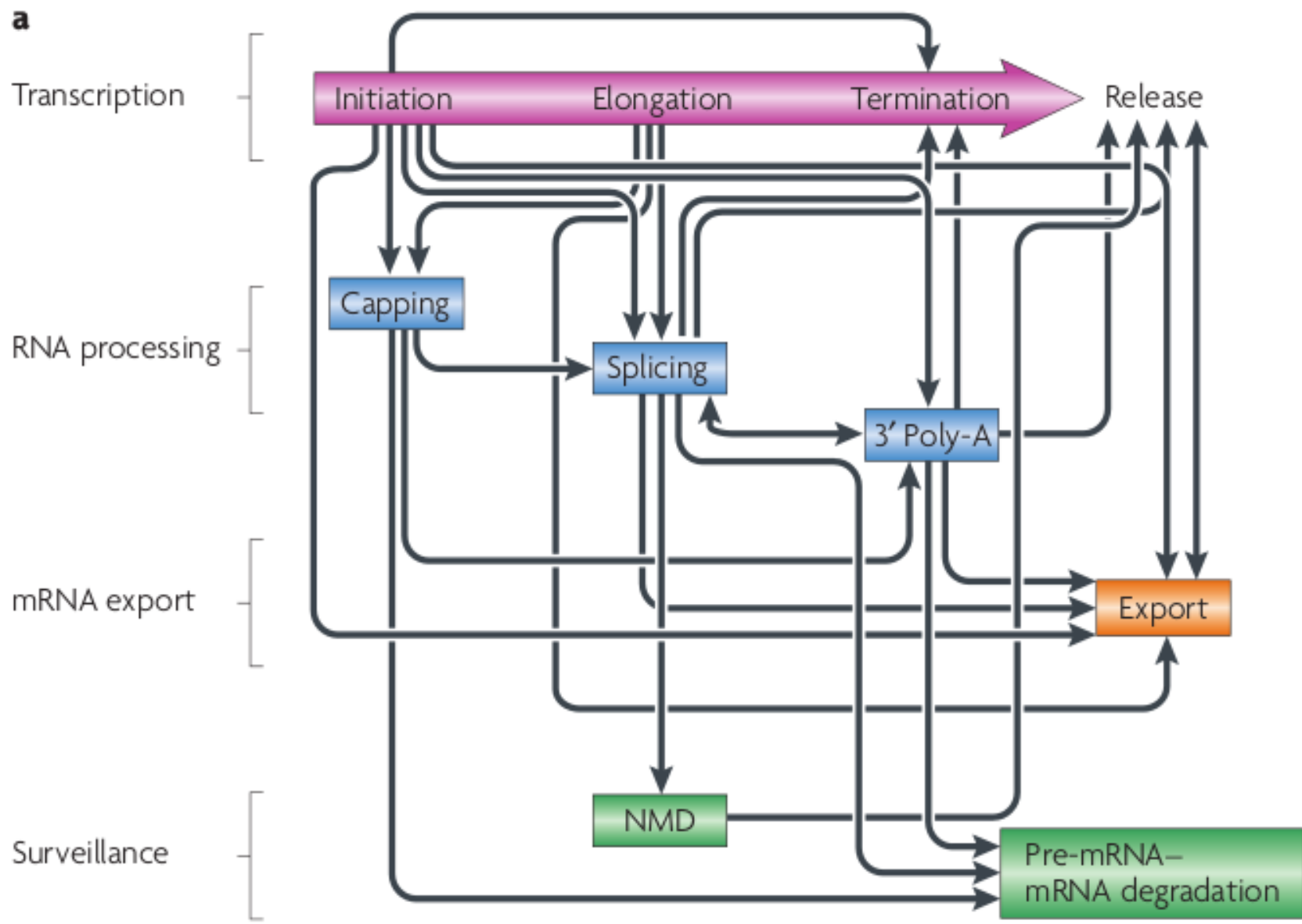


Post-transcriptional regulation

The life and the half-life of RNAs







Capping

Polyadenylation

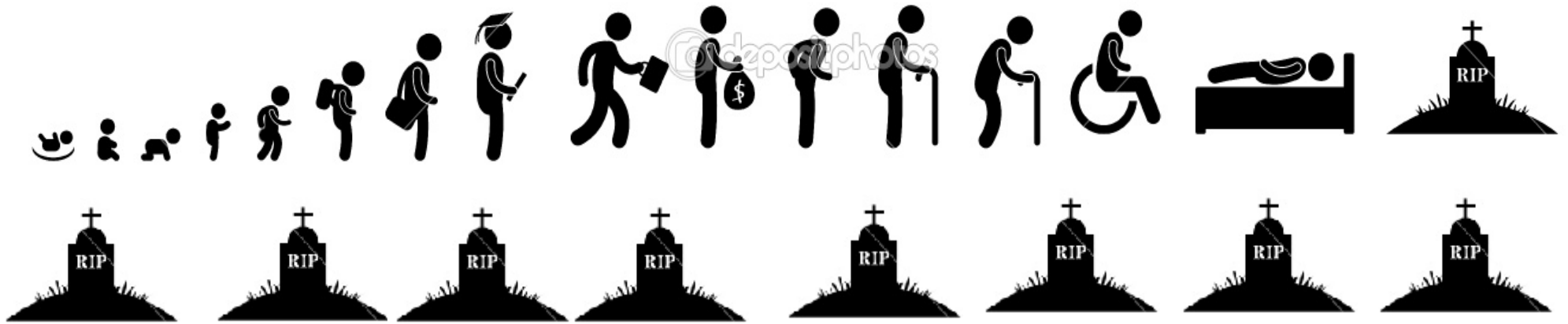
Splicing

Nuclear export

Editing

Quality Control

Degradation



Rest in pieces...

Most basic common feature: ?





Quality control

Superhighways to destruction

Surveillance mechanisms

Non-sense Mediated Decay
Non-Stop decay
No-go decay

Decapping

Deadenylation dependent

Deadenylation independent

Endonuclease mediated

Exonuclease mediated

(5' → 3' or 3' → 5')

Signals that control mRNA decay

AU rich-elements

Proteins

NcRNAs

....

STABILITIES OF NUCLEAR AND MESSENGER RNA MOLECULES IN SEA URCHIN EMBRYOS

BRUCE P. BRANDHORST and TOM HUMPHREYS

From the Department of Biology, Revelle College, University of California at San Diego, La Jolla, California 92037. Dr. Brandhorst's present address is the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80302. Dr. Humphrey's present address is the Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822.

ABSTRACT

The kinetics of accumulation of radioactive adenosine in adenosine triphosphate (ATP) and RNA of nuclear, cytoplasmic, and polysomal fractions of sea urchin embryos were analyzed. 85% of the RNA synthesized decays in the nucleus with an initial half-life of about 7 min. The remaining 15% goes to the cytoplasm, mitochondria, and polysomes, and decays with a quite uniform half-life of about 75 min. The nuclear RNA accounts for one-third and the cytoplasmic RNA accounts for two-thirds of the total RNA which accumulates at steady state in the embryo. The size distribution of the labeled nuclear RNA is very similar to that of long-labeled messenger RNA extracted directly from the cells without a previous cell fractionation.

THE JOURNAL OF CELL BIOLOGY · VOLUME 53, 1972

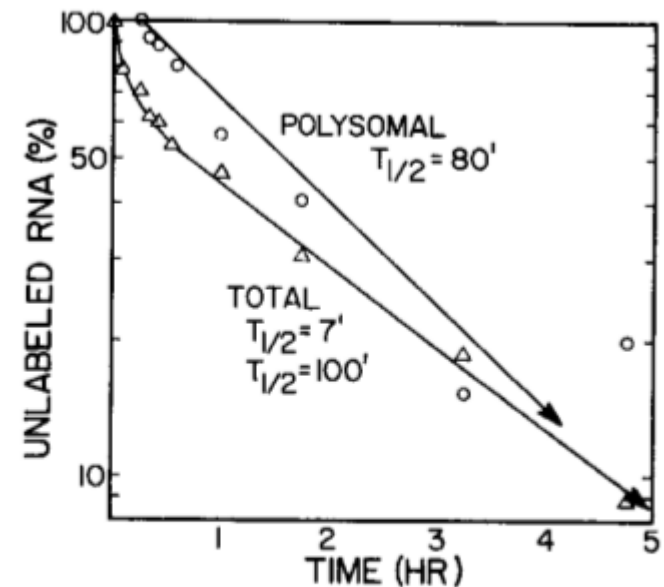
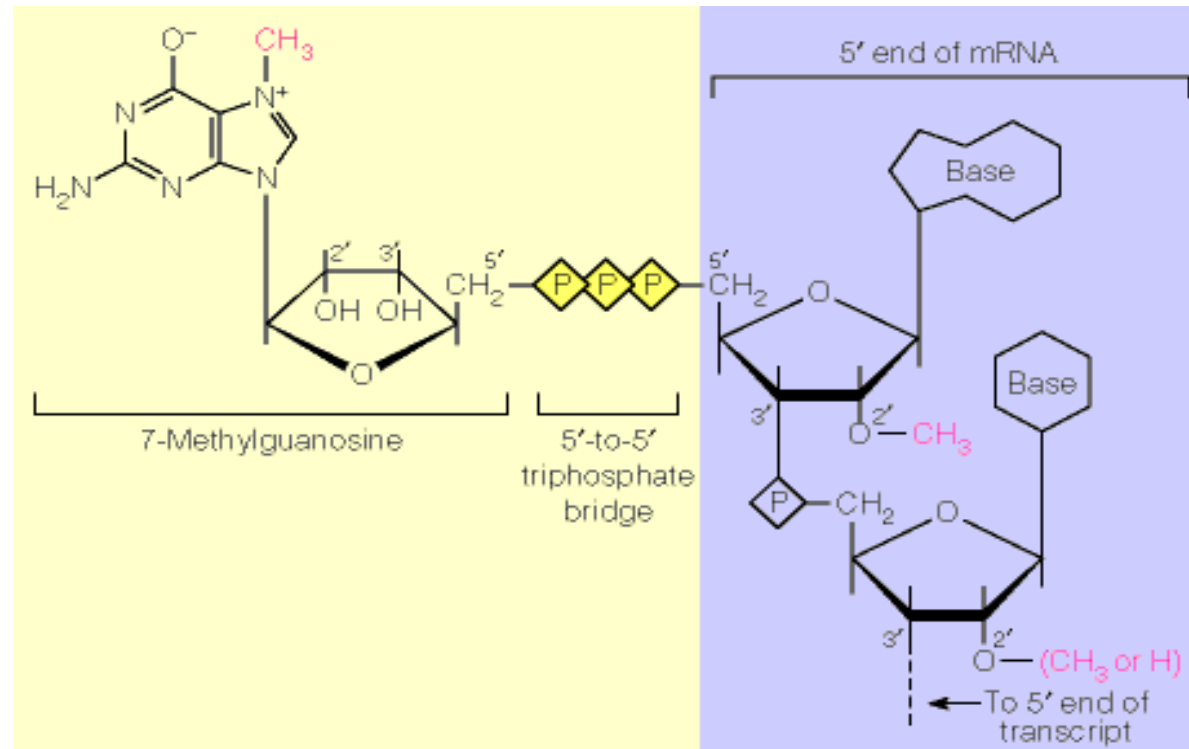


FIGURE 5 First order decay analysis of the molar accumulation of radioactive RNA in whole embryos and polysomes (from data in Fig. 3 c).

Cap Structure



Poly-A tail

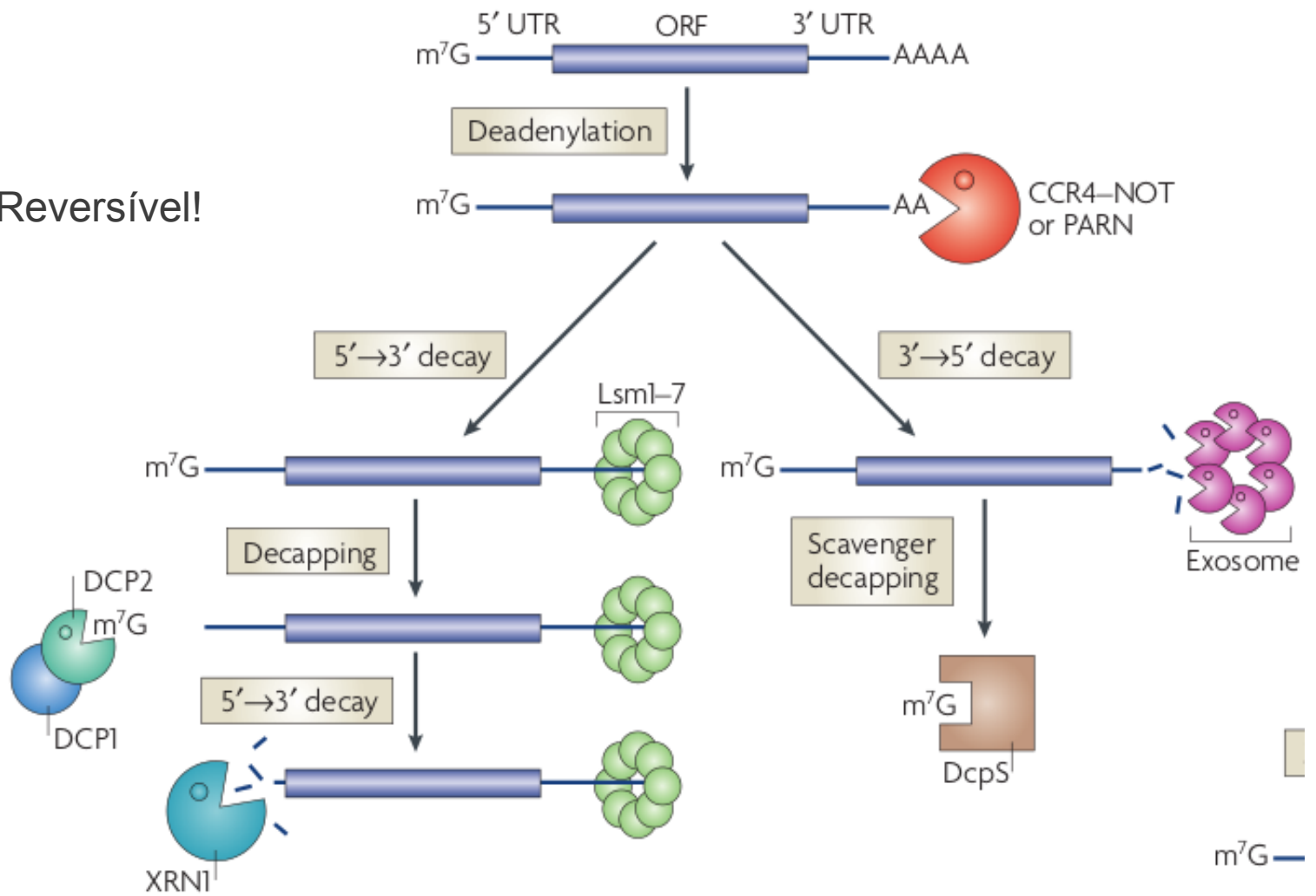
AA

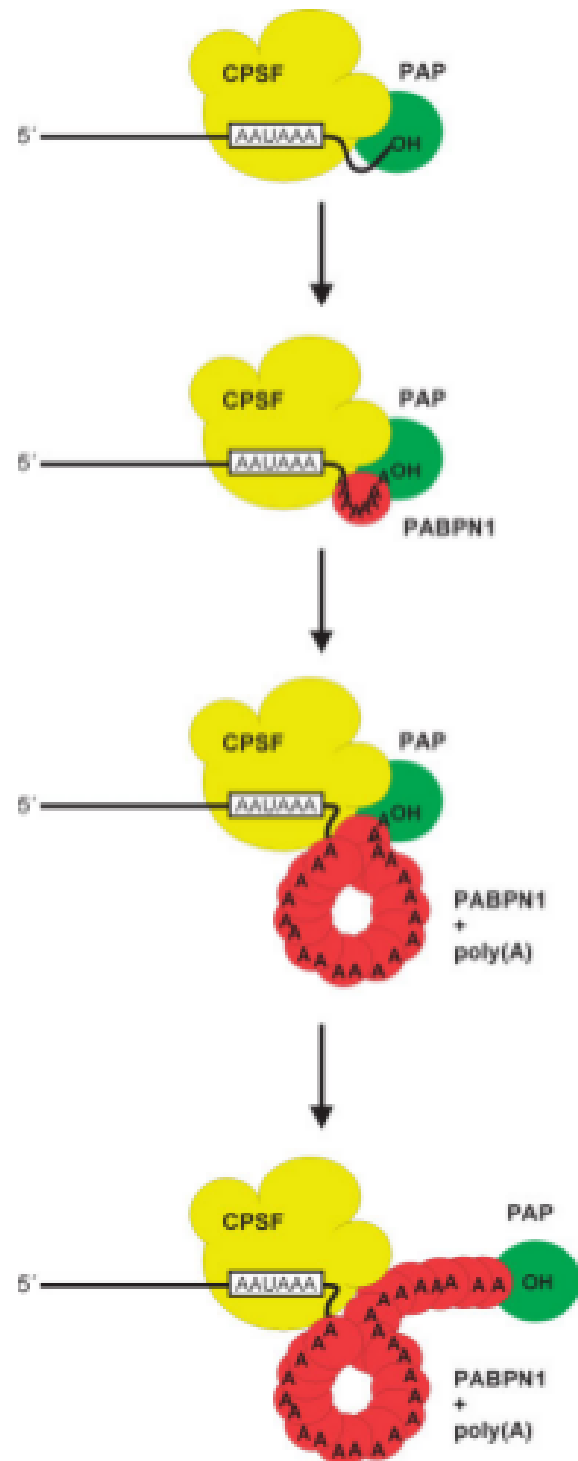
Homopolymeric stretch of ~25-200 adenine nucleotides

a Deadenylation-dependent mRNA decay

b

Reversible!





Eckmann et al, WiresRNA, 2011

Poly-A tail length

Garneau et al, 2007

Decay factor	Protein components	Protein domains	Functions and characteristics	Localization	Other functions
<i>Deadenylation</i>					
CCR4–NOT	Ccr4	3'→5' exonuclease	Main deadenylase in <i>Saccharomyces cerevisiae</i> ; inhibited by PABP	Nucleus; cytoplasm; P bodies	Transcription; protein degradation
	Caf1 (Pop2)	3'→5' exonuclease			
	Caf40 (Rcd1)	Rcd1-like			
	Caf130	Not known			
	Not1	Not known			
	Not2	Not2, Not3 and Not5 share a domain of unknown function			
	Not3	Not2, Not3 and Not5 share a domain of unknown function			
	Not4	Ubiquitin ligase			
	Not5	Not2, Not3 and Not5 share a domain of unknown function			
			Inhibited by PABP		
PAN2–PAN3	PAN2	WD40 repeat	Involved in <u>first phase of poly(A) shortening</u>	Nucleus; cytoplasm	Not known
	PAN3	3'→5' exonuclease			
PARN	PARN	3'→5' exonuclease	Cap-dependent deadenylase activity; inhibited by PABP and nuclear cap-binding complex	Nucleus; cytoplasm	Translation inhibition Embryogenesis in plants Xenopus oocytes maturation

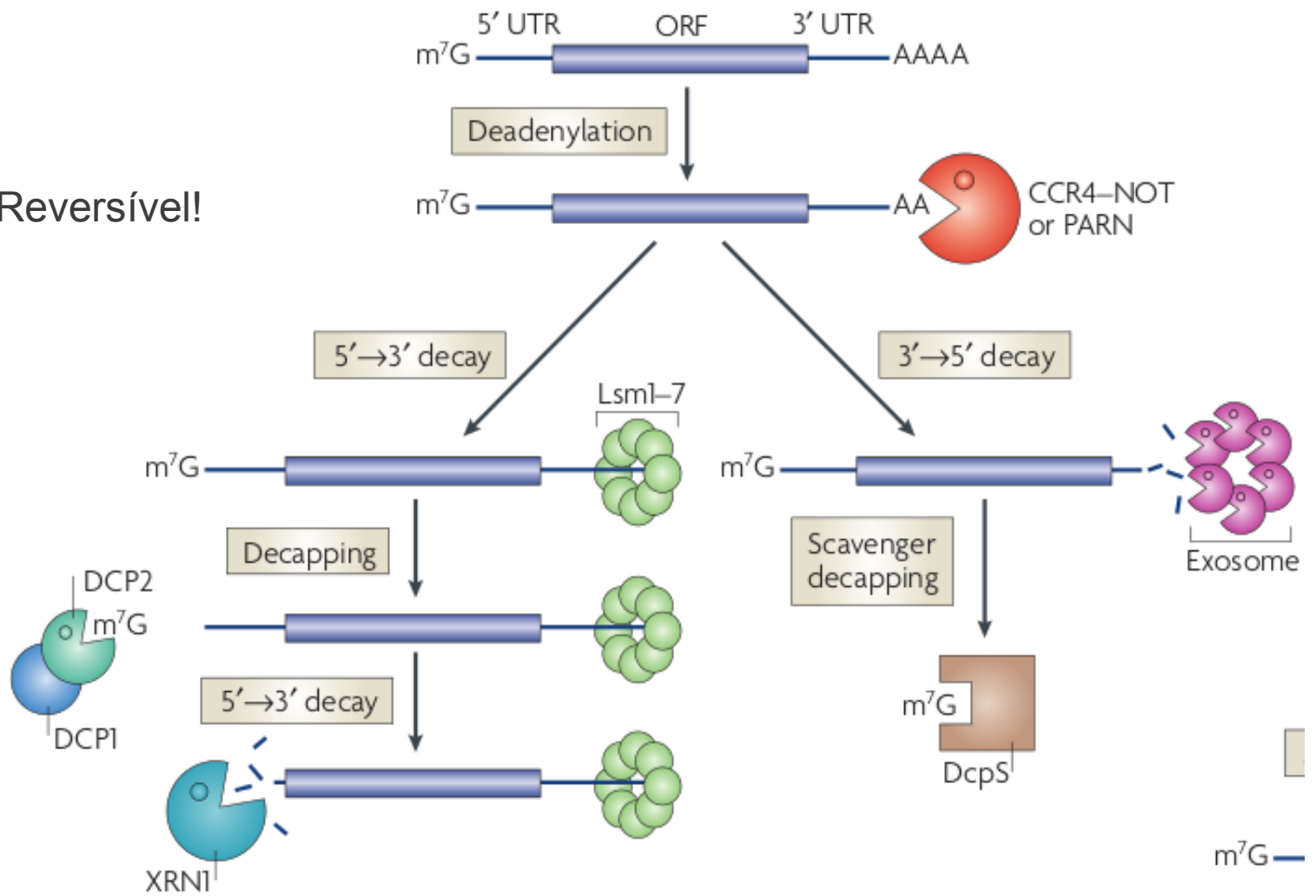
Ribonuclease D activity – 3' → 5'exoribonuclease

How and when/where deadenylation is triggered??? Not sure...

a Deadenylation-dependent mRNA decay

b

Reversible!

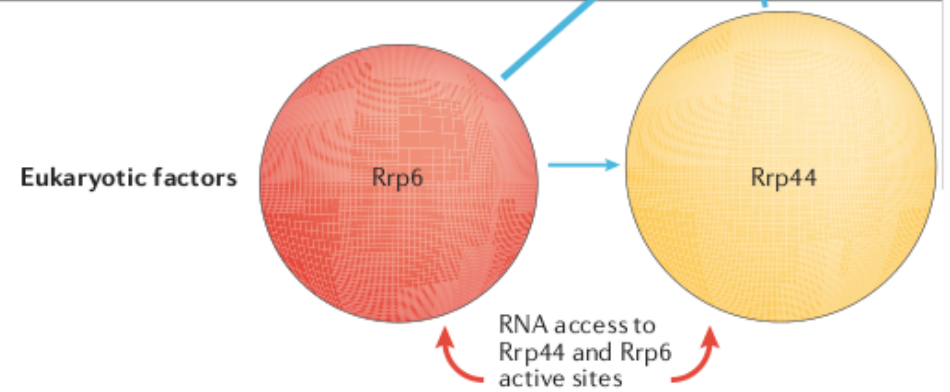
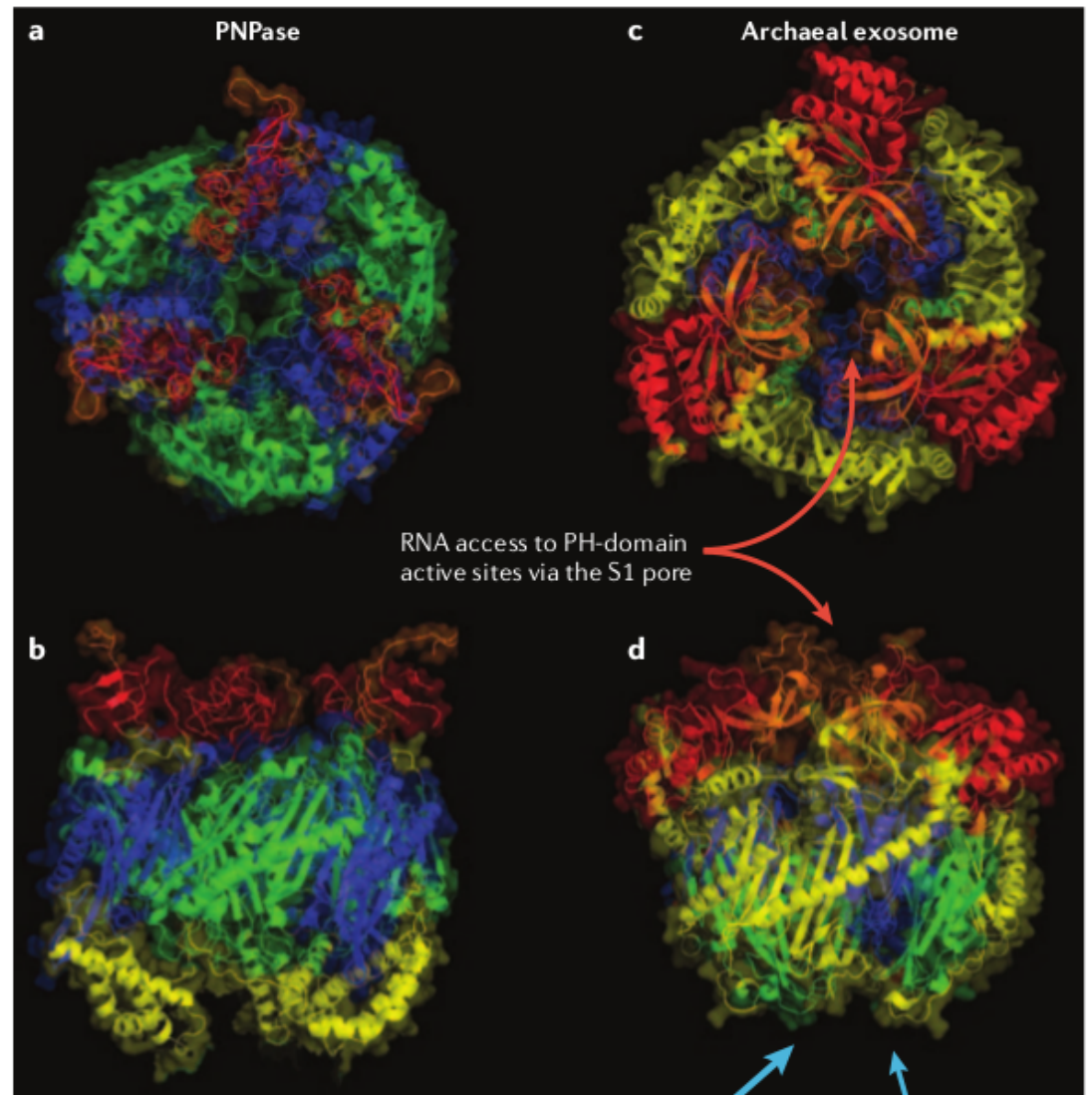


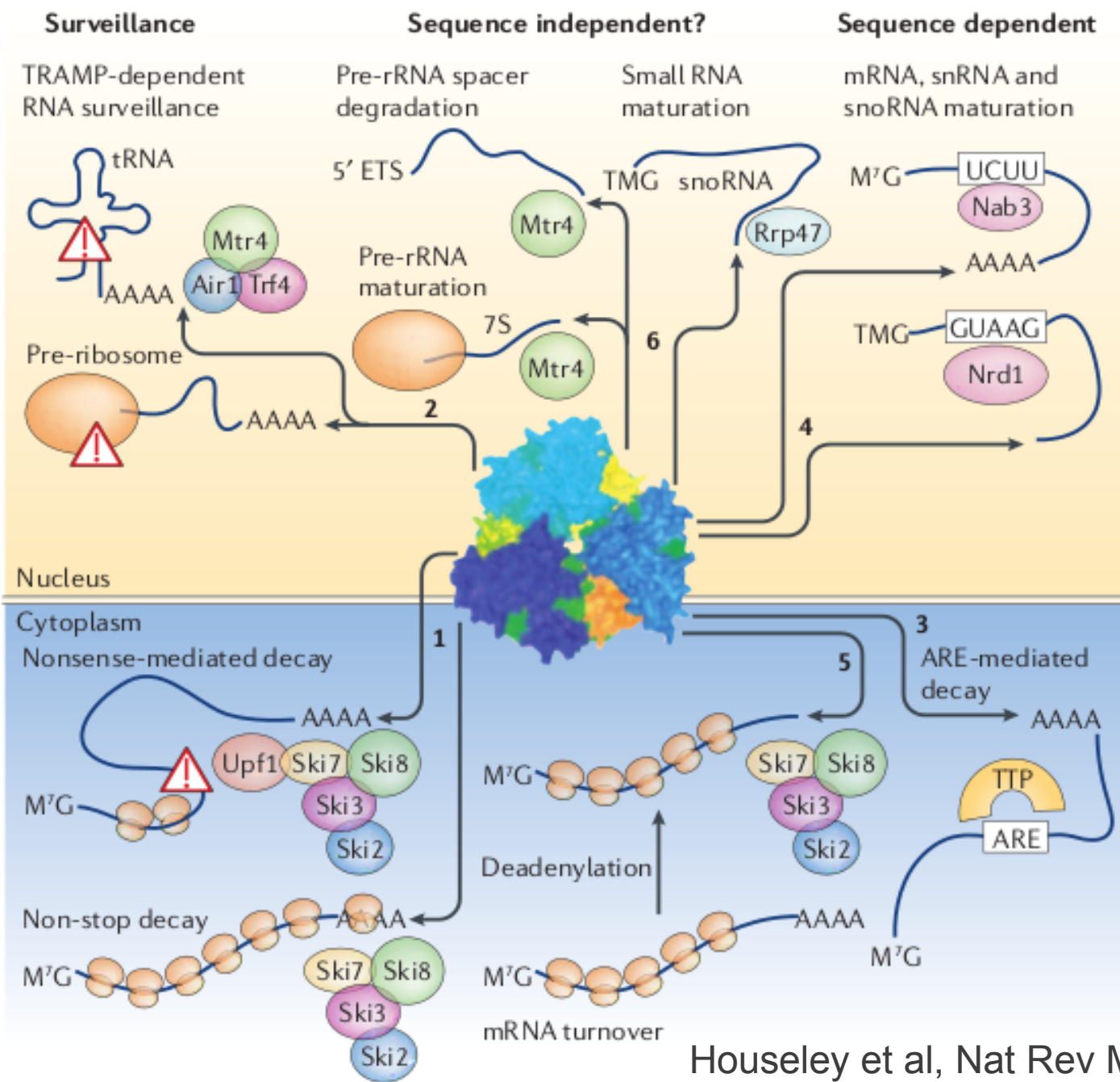
Exosome: 3' → 5'decay

Hydrolytic exonucleases
RNA helicases

RNAse PH domain =
Contributes to catalytic activity

RNA processing



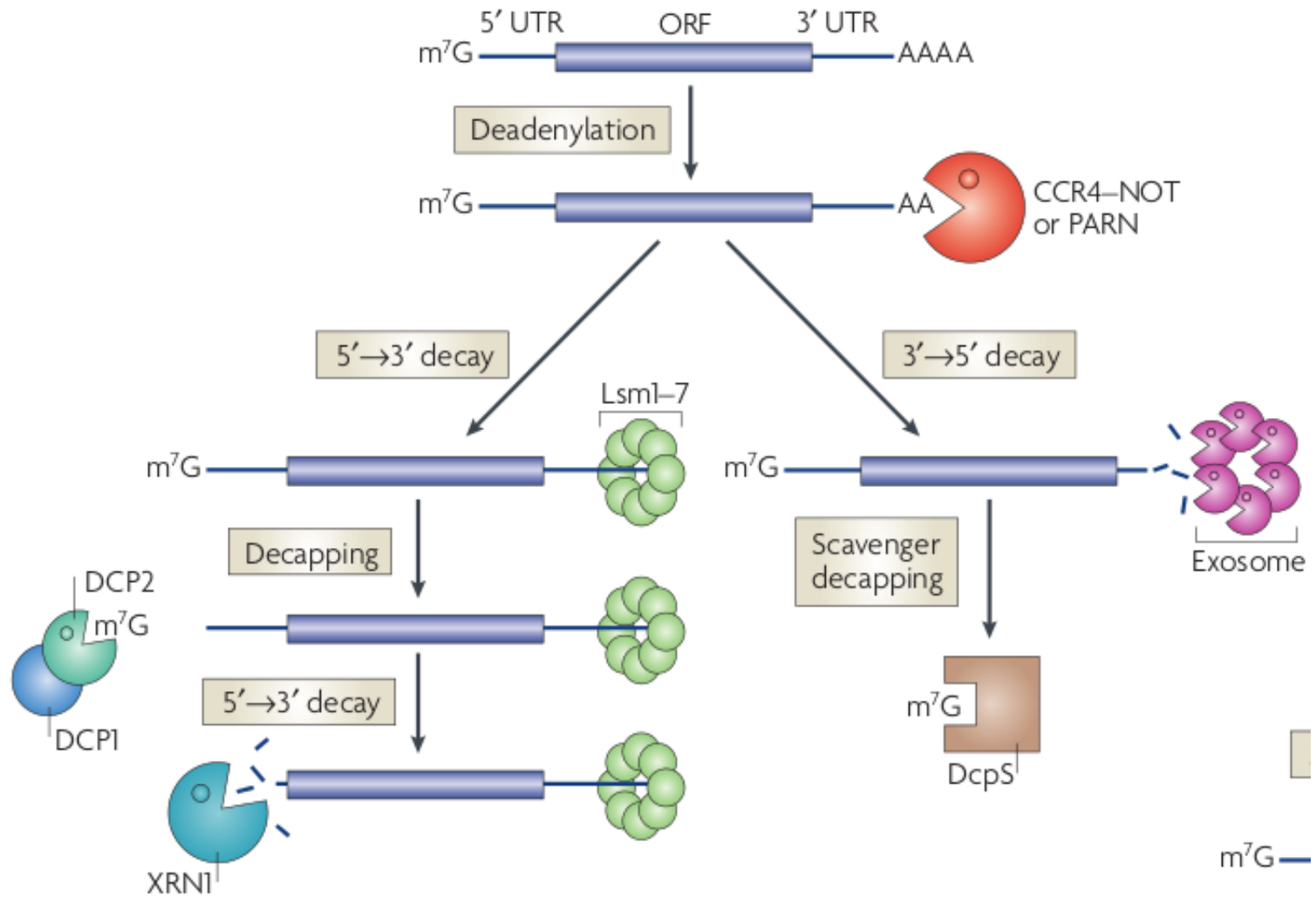


Houseley et al, Nat Rev Mol Cell Biol, 2006

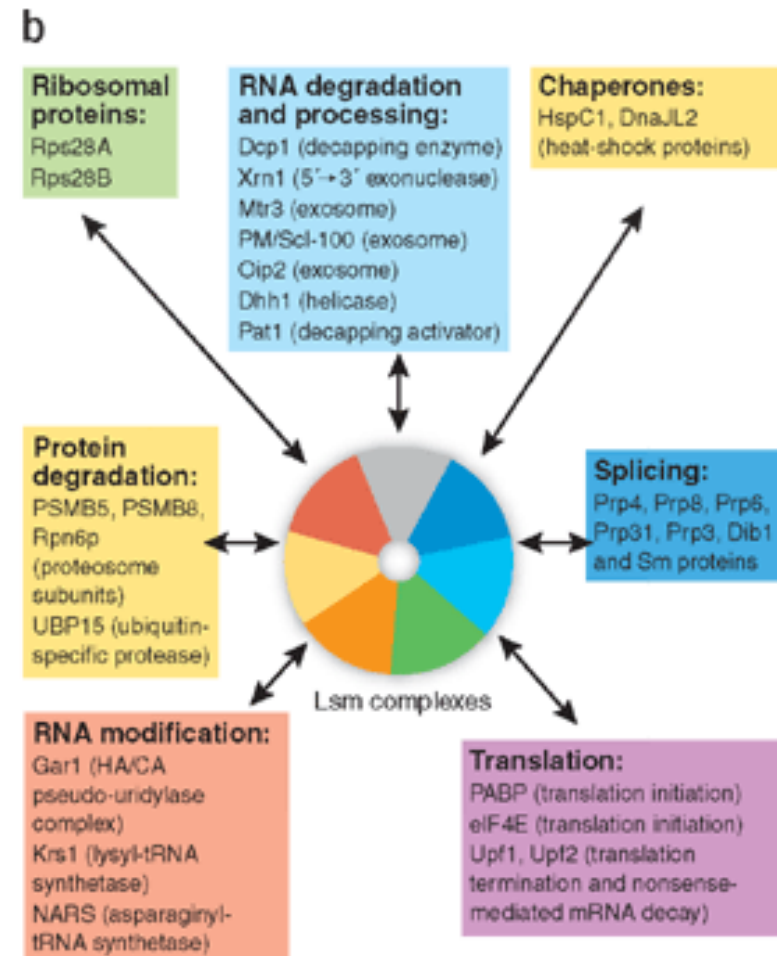
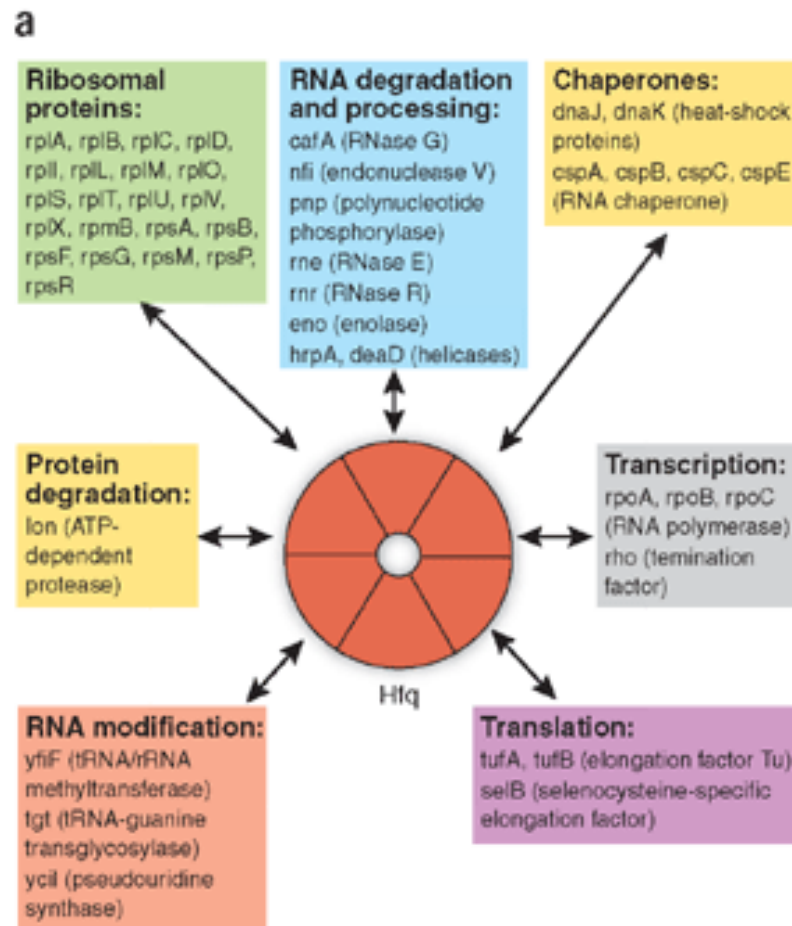
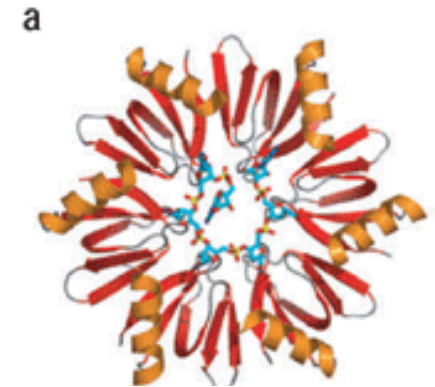
Decapping and 5' → 3' decay

a Deadenylation-dependent mRNA decay

b



Lsm proteins



P bodies : **crossroads of post-transcriptional pathways**

Or GW bodies

Components of the 5' → 3' decay pathway

Translation Initiation

Deadenylation

Decapping

5' → 3' exonucleolytic decay

NMD decay

miRNA decay

Components of exosome?

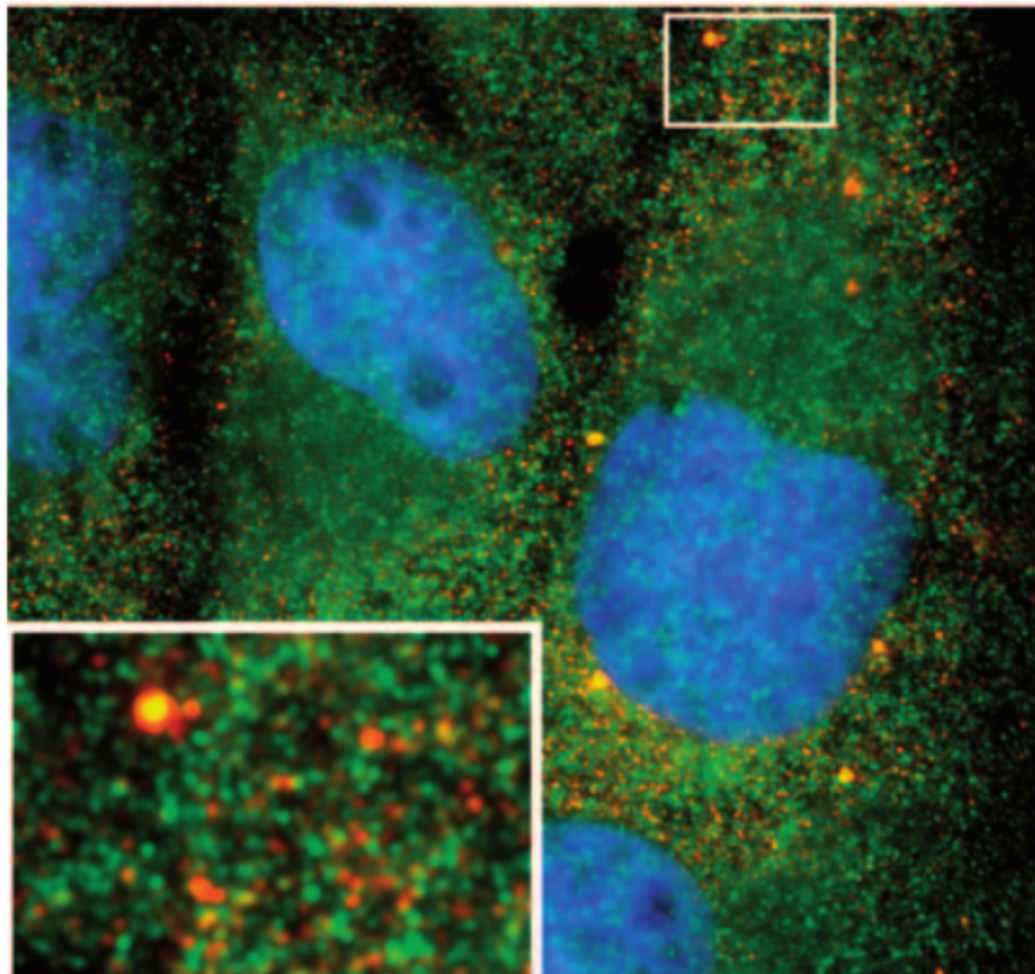
Cellular sites of decay

- although it is unknown what % of mRNA decay actually occur in P bodies

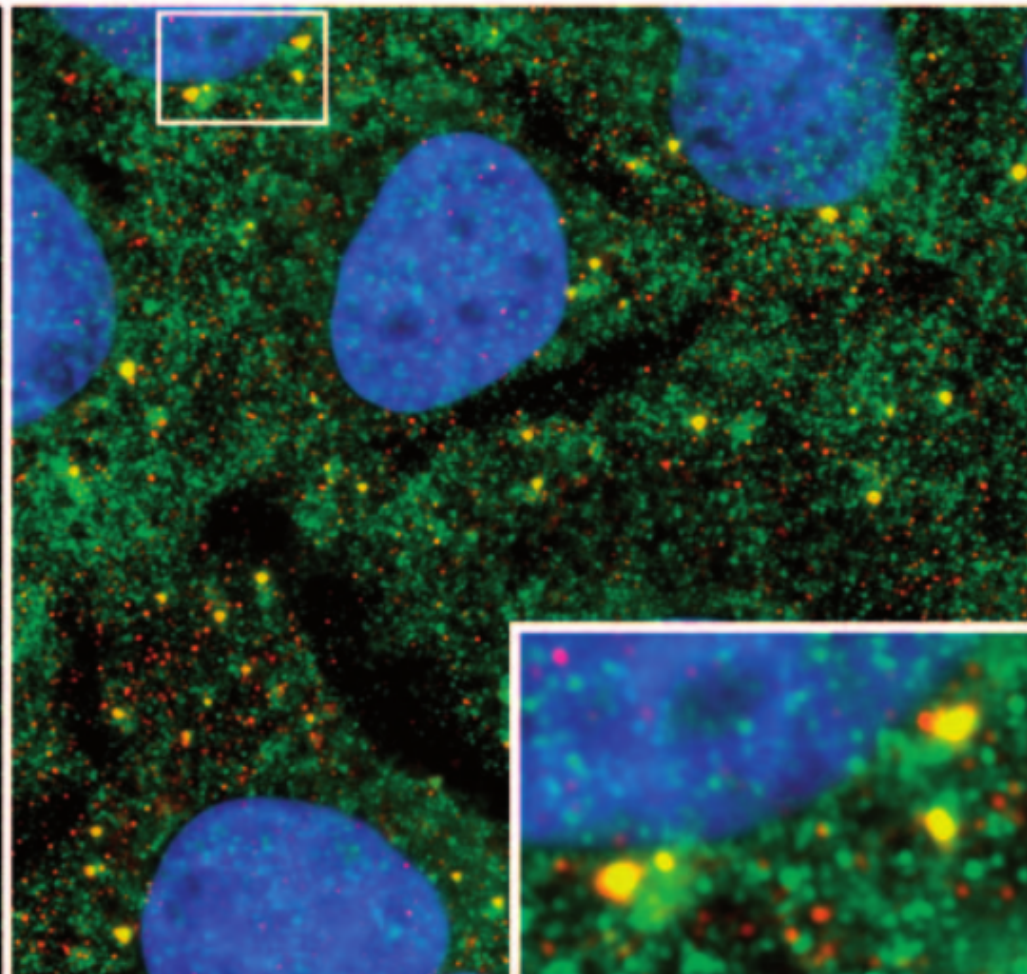
Dynamic structures!

Besides decay – importance of P-bodies??

Normal conditions



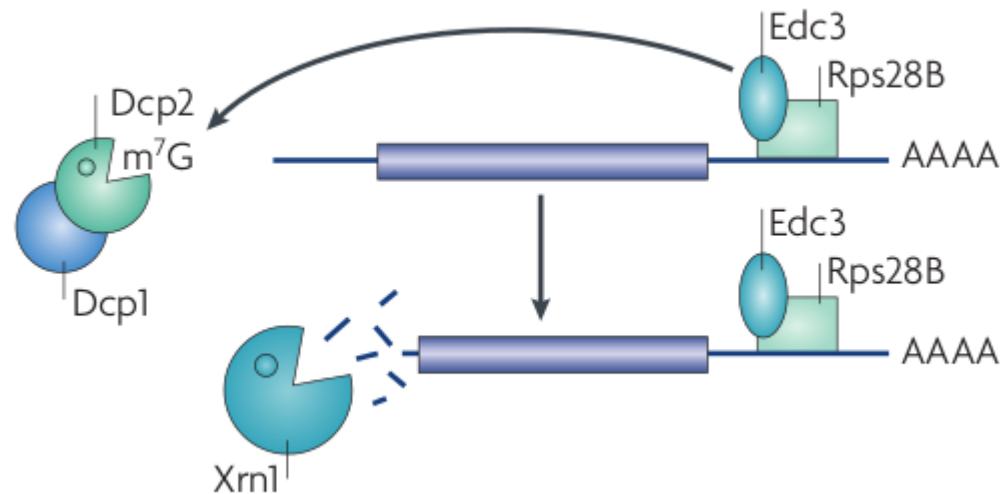
Stress conditions



Lsm – green
XRN1 – red
DNA - blue

Alternative routes to decay

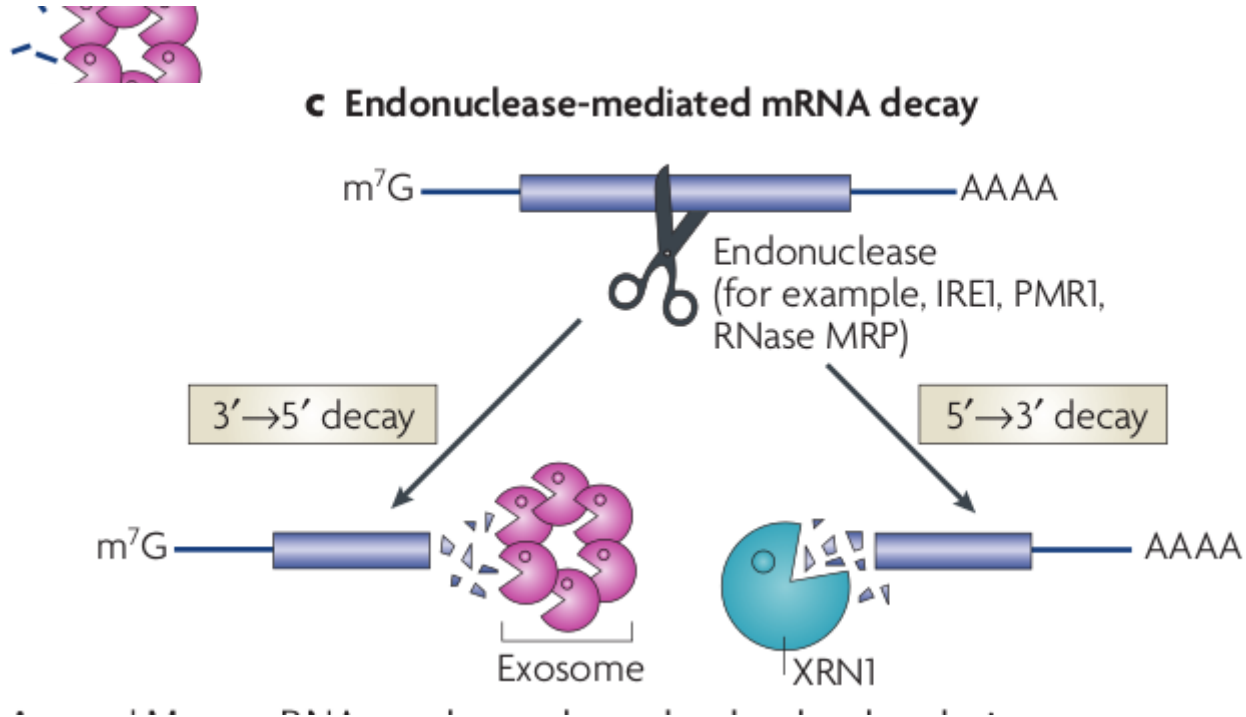
b Deadenylation-independent mRNA decay



Edc3 – enhancer decapping

Rps28 – binds stem loop structure at 3' UTR of its own mRNA

Alternative routes to decay



PMR1 – polysome associated endonuclease – target RNA?
IRE1 -unfolded protein response – ER stress
RNase MRP – rRNA, mitochondrial RNA, cyclin degradation

Highly regulated!!!

mRNA surveillance

Nucleus

What kind of errors would result in nuclear mRNA decay?

Similar to cytoplasm mRNA decay:

5' → 3'

3' → 5'

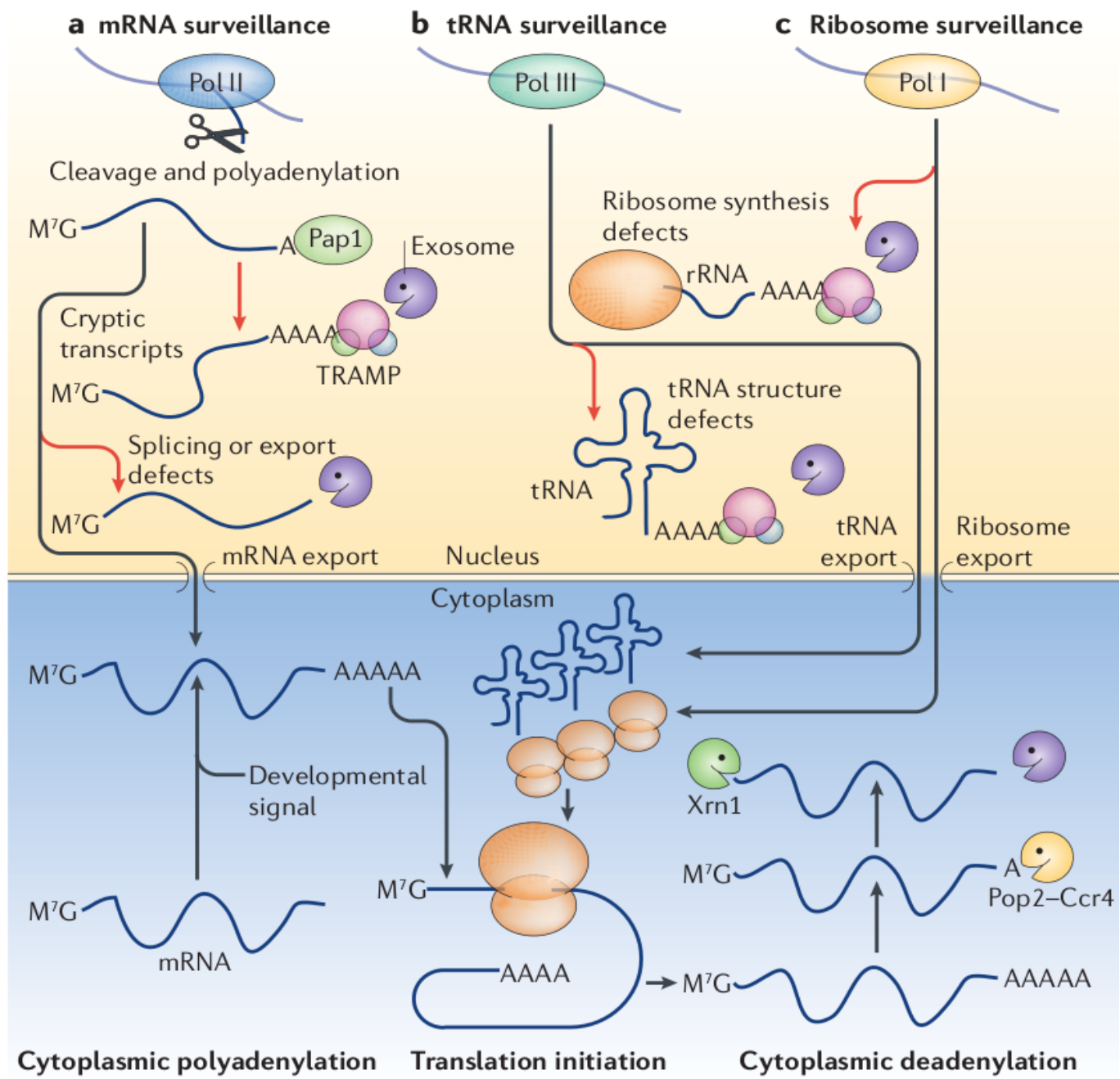
Nuclear Exosome

Lsm

Deadenylation – more processive in the nucleus

REGULATION OF GENE EXPRESSION

TRAMP – polyA polymerase activity → exosome recruitment → rapid decay!



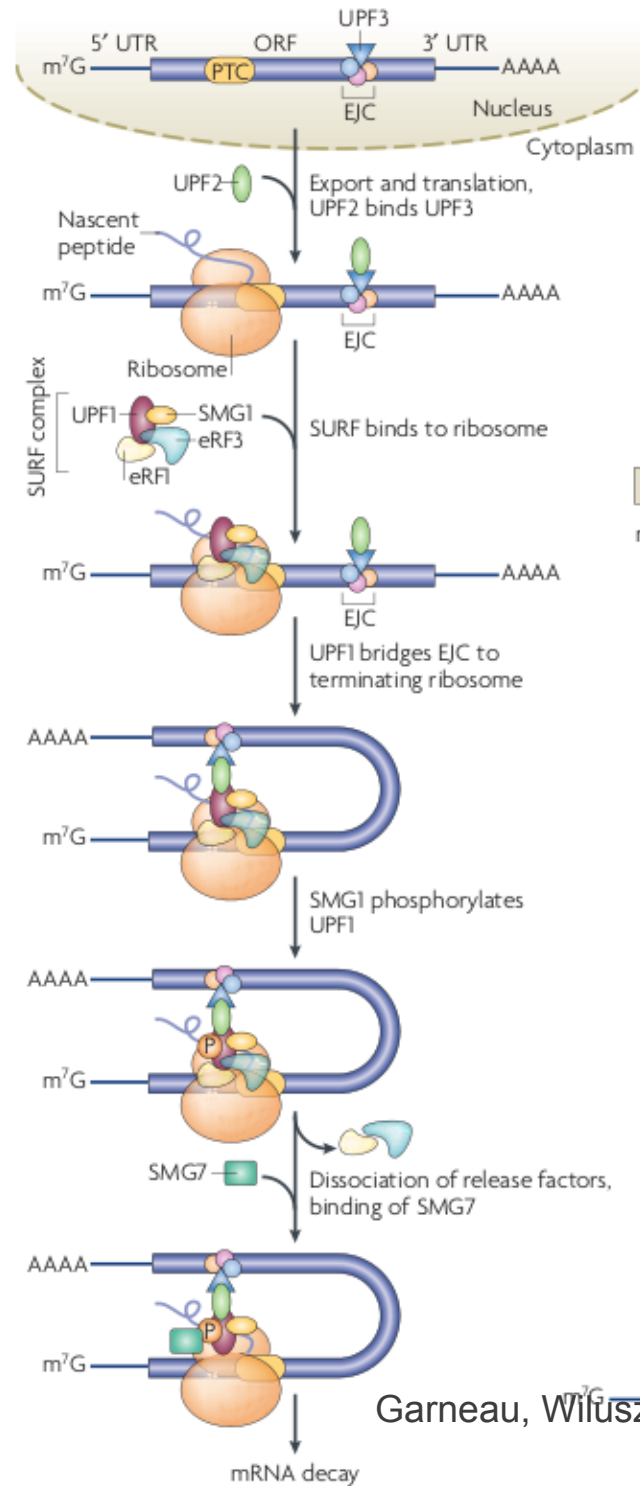
NMD
Nonsense
Mediated
Decay

PTC
Premature
Termination
Codon

EJC
Exon
Junction
Complex

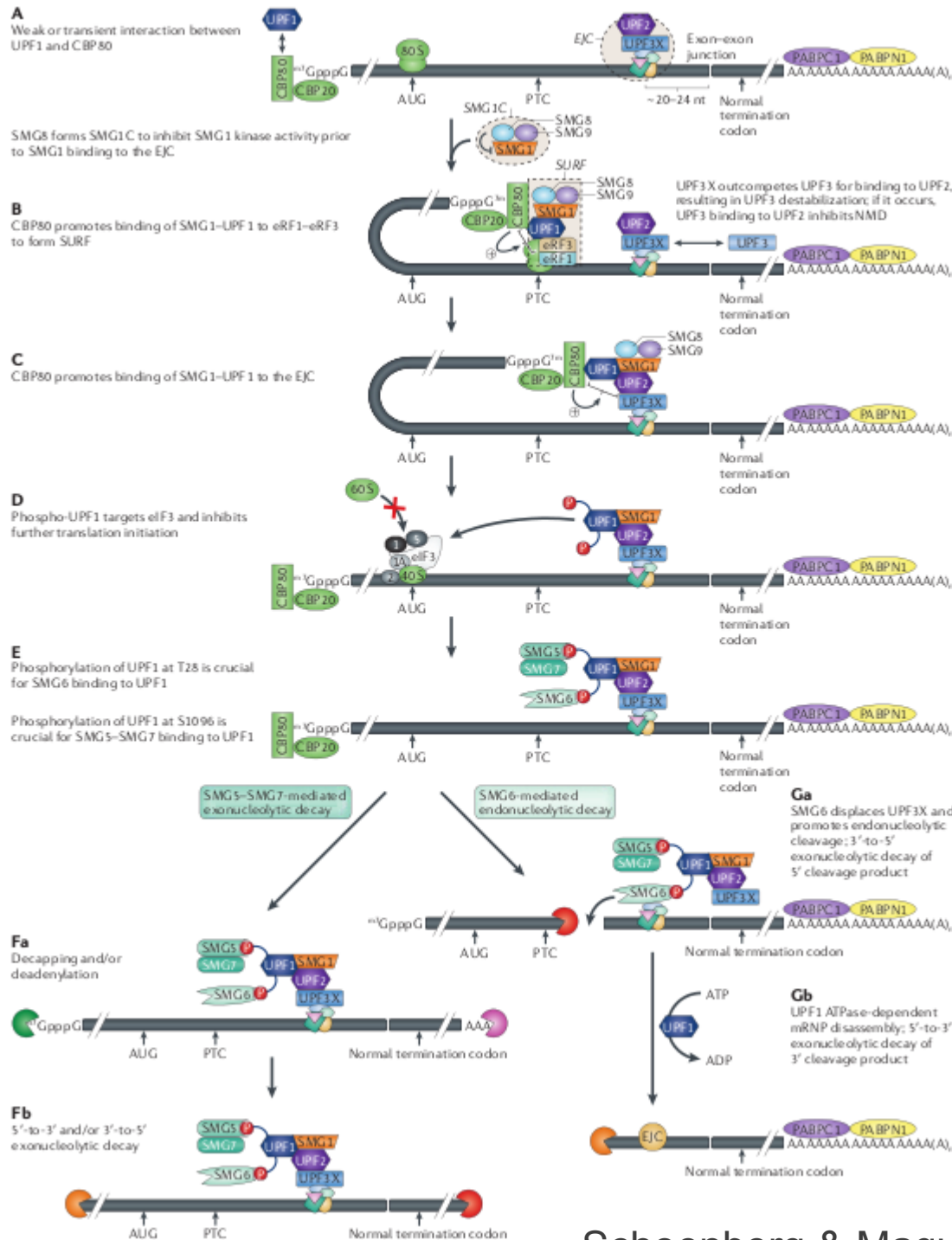
ROLE in
Gene expression
Regulation!

a Recognition of a premature stop codon

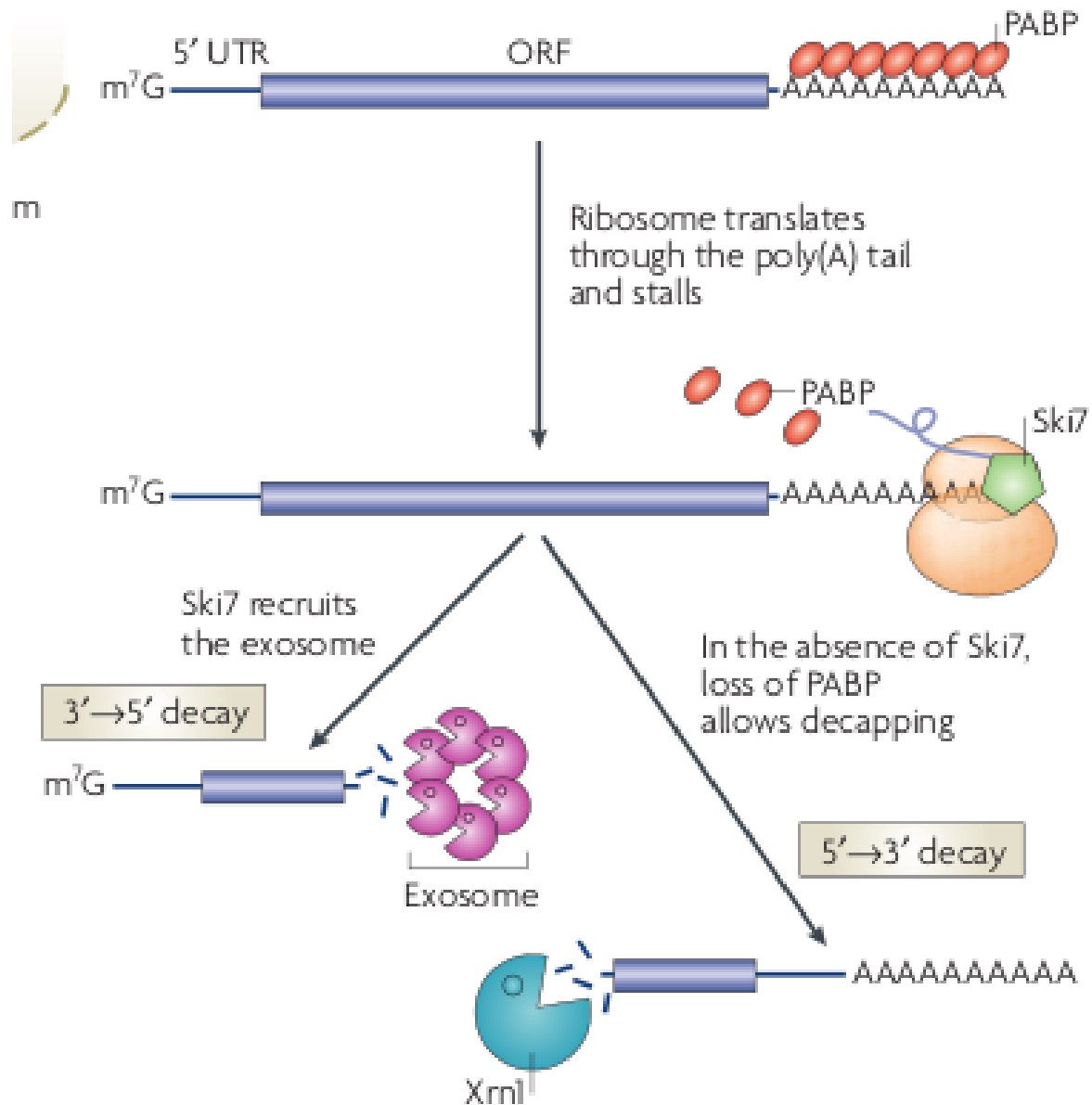


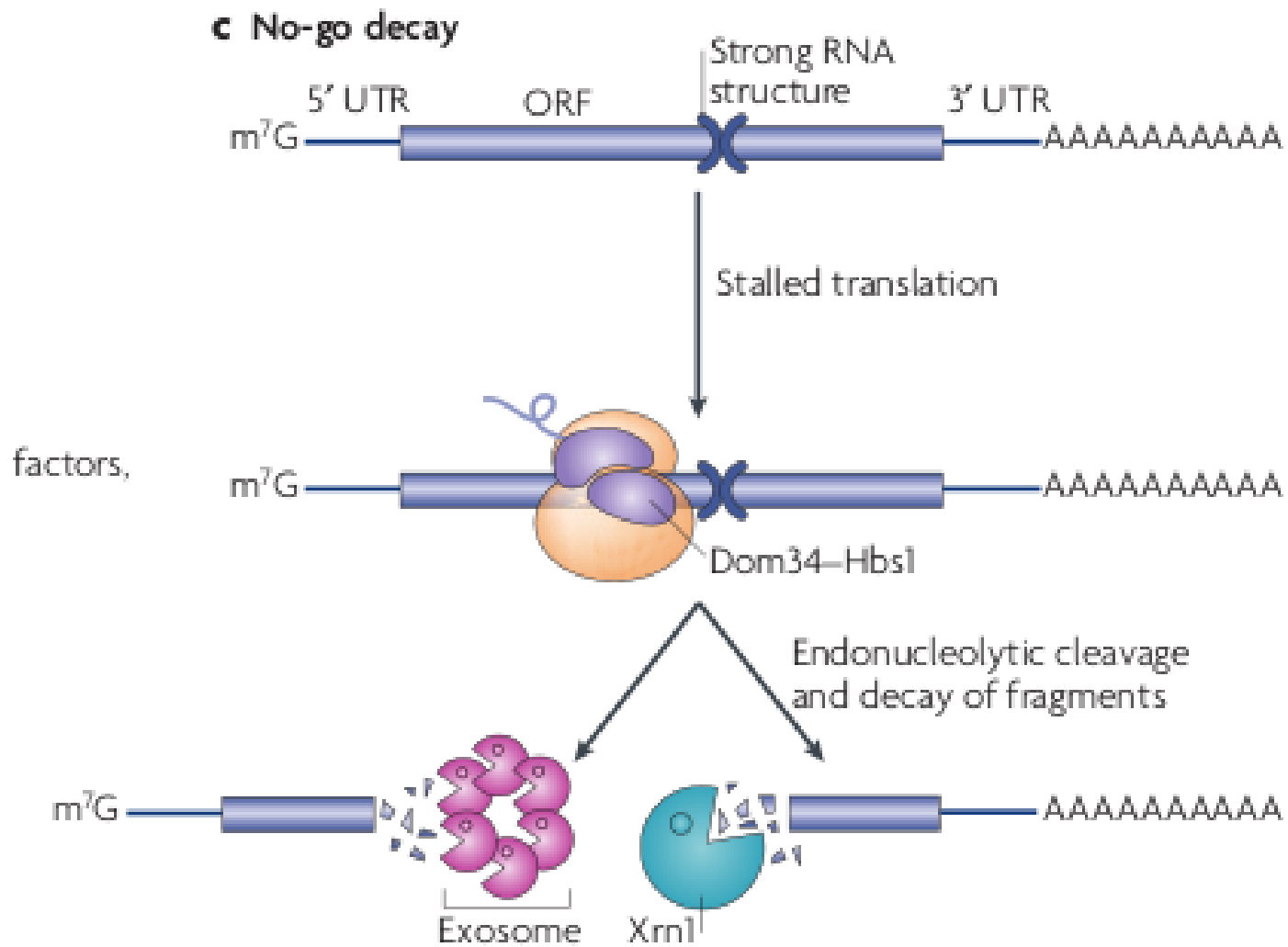
NMD factors
As regulators of
NMD

ALL eukaryotes



b Non-stop decay





40-50% changes in gene expression – at level of mRNA stability

Signals that control mRNA decay

Au -rich elements and their binding proteins (ARE) - 9% cellular mRNA

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Cell, Volume 46, Issue 5, 659-667, 29 August 1986

doi:10.1016/0092-8674(86)90341-7

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Article

A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation

Gray Shaw and Robert Kamen

Genetics Institute, Inc. 87 CambridgePark Drive Cambridge, MA 02140 USA

Abstract

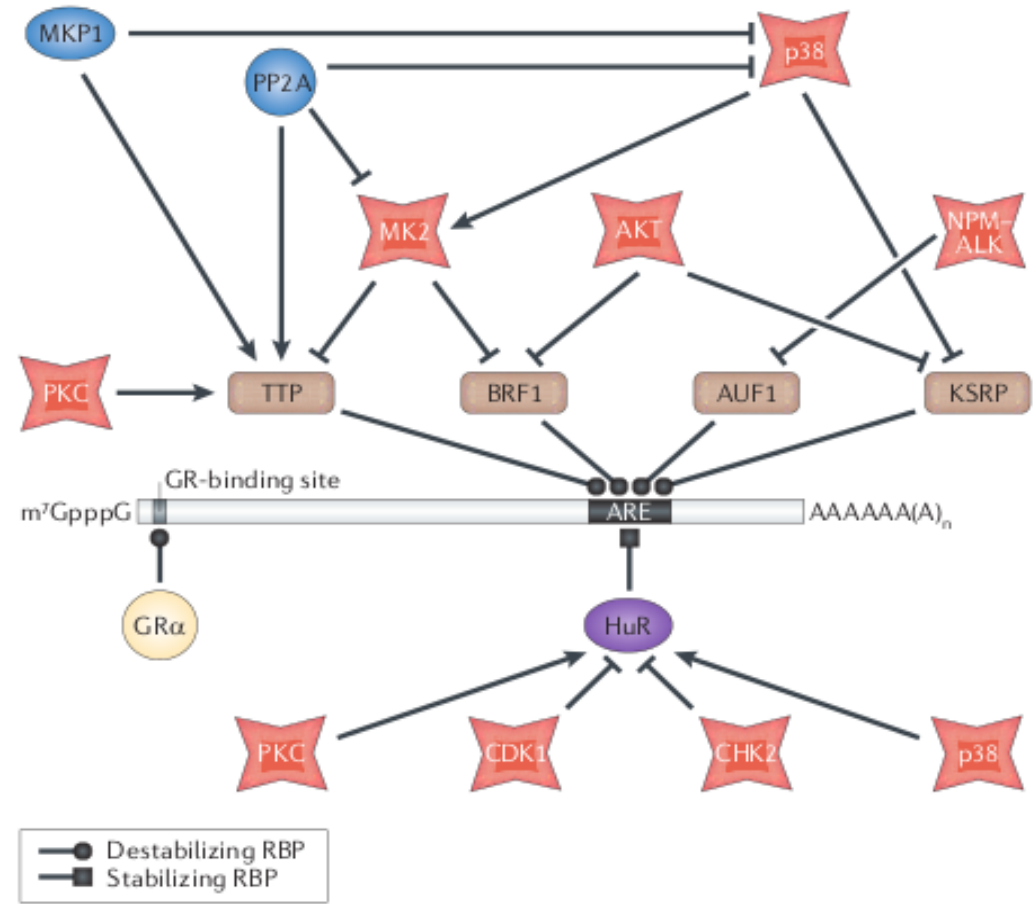
The mRNAs of transiently expressed genes frequently contain an AU-rich sequence in the 3' untranslated region. We introduced a 51 nucleotide AT sequence from a human lymphokine gene, GM-CSF, into the 3' untranslated region of the rabbit β -globin gene. Our experiments demonstrate that this caused the otherwise stable β -globin mRNA to become highly unstable in vivo. The instability conferred by the AU sequence in the mRNA was partially alleviated by treatment of the cells with cycloheximide. We propose that the AU sequences are the recognition signal for an mRNA processing pathway which specifically degrades the mRNAs for certain lymphokines, cytokines, and proto-oncogenes.

Table 2 | **ARE-binding proteins**

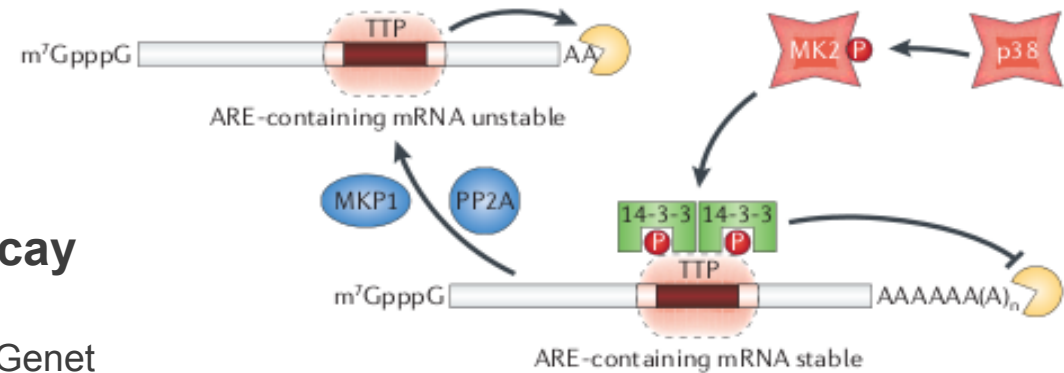
RNA-binding protein	Function	RNA-binding domain	Mode of action	Modifications	Other functions
AUF1 (hnRNP D) and its four splice isoforms (p37, p40, p42, p45)	Usually destabilizing	RRM	Recruit the exosome; remodel mRNA to allow other proteins to bind	Phosphorylation allows isomerization by PIN1 leading 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

RNA binding proteins

Binding modulation in response to signals



b Kinase- and phosphatase-mediated regulation of TTP



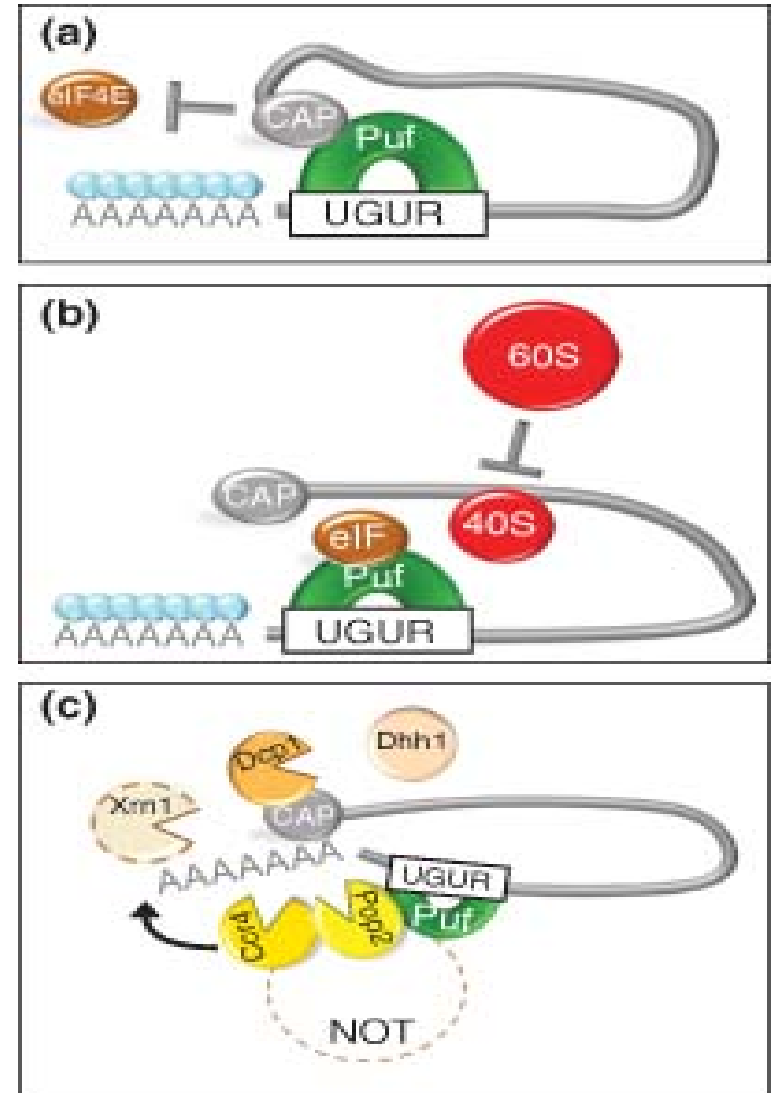
Signals that control mRNA decay

Signals that control mRNA decay

Puf proteins

Recognize UG rich sequences – accelerate decay
Recruits CCR4-NOT deadenylase

Each Puf has functionally related target transcripts



Signals that control mRNA decay

Stabilizing elements

Specific RNA sequences

Stable transcripts – housekeeping role

Pyrimidine rich elements at 3'end

Binds KH domain protein

Helicases

Place markers?

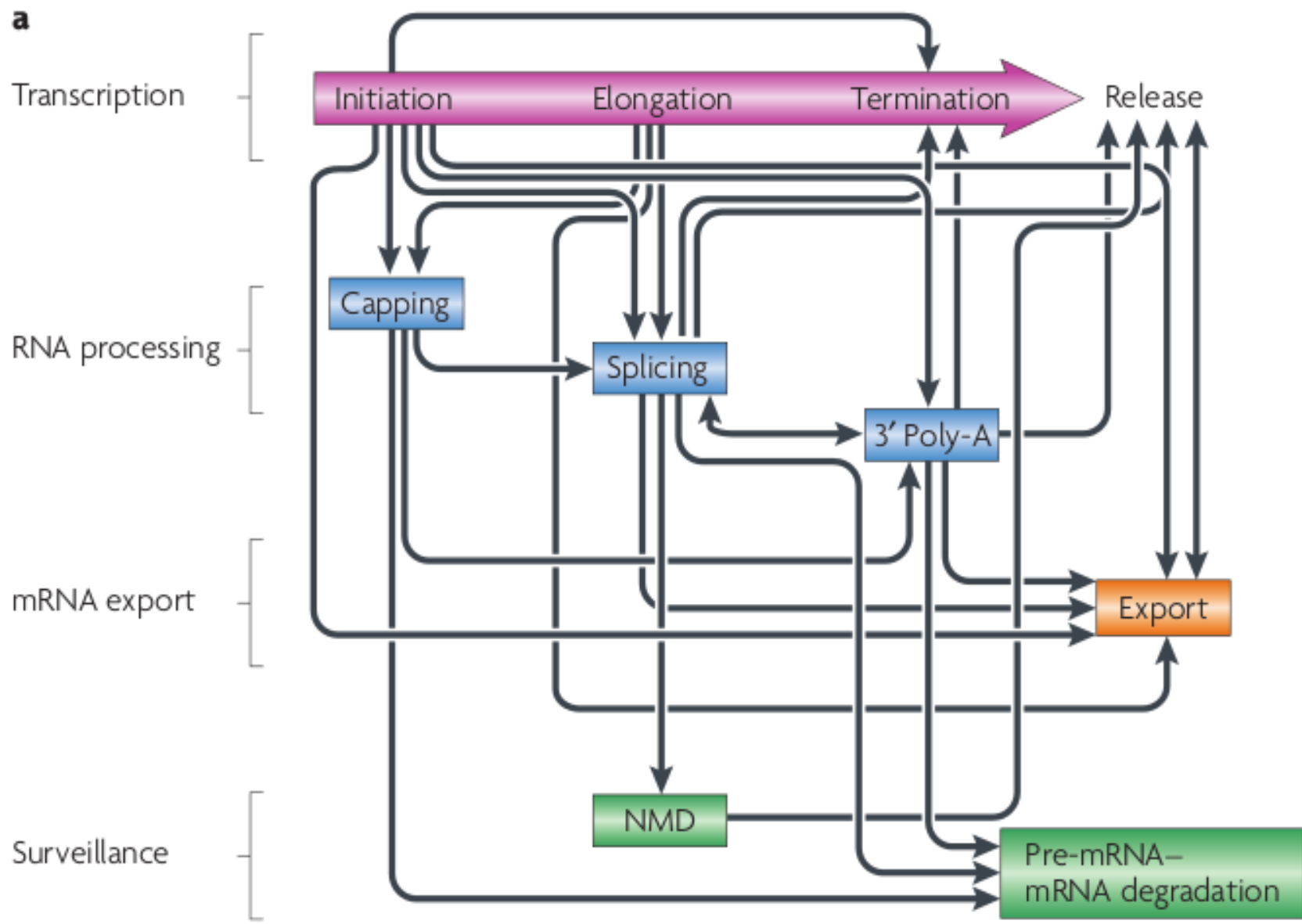
Polymerases

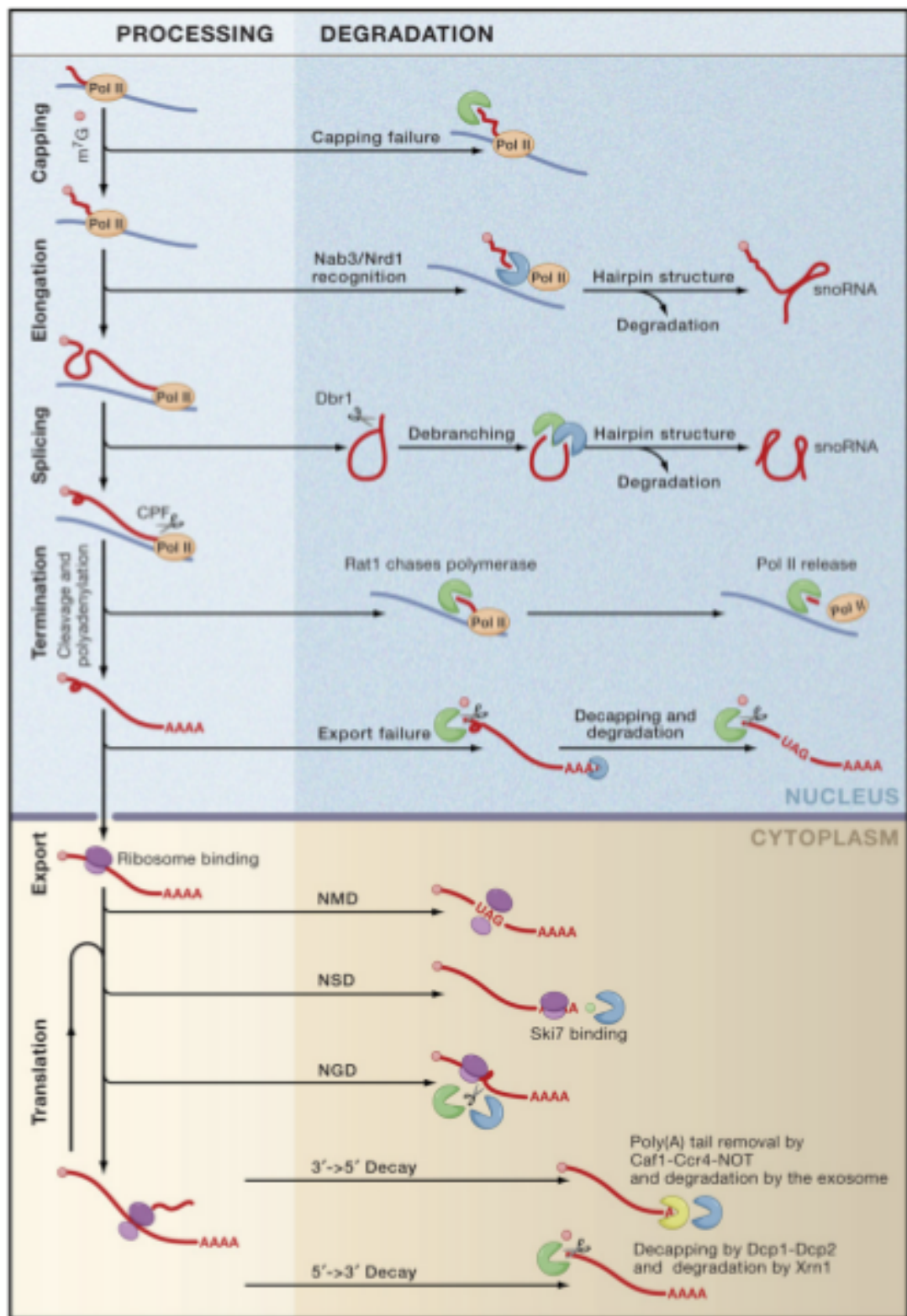
Small RNAs

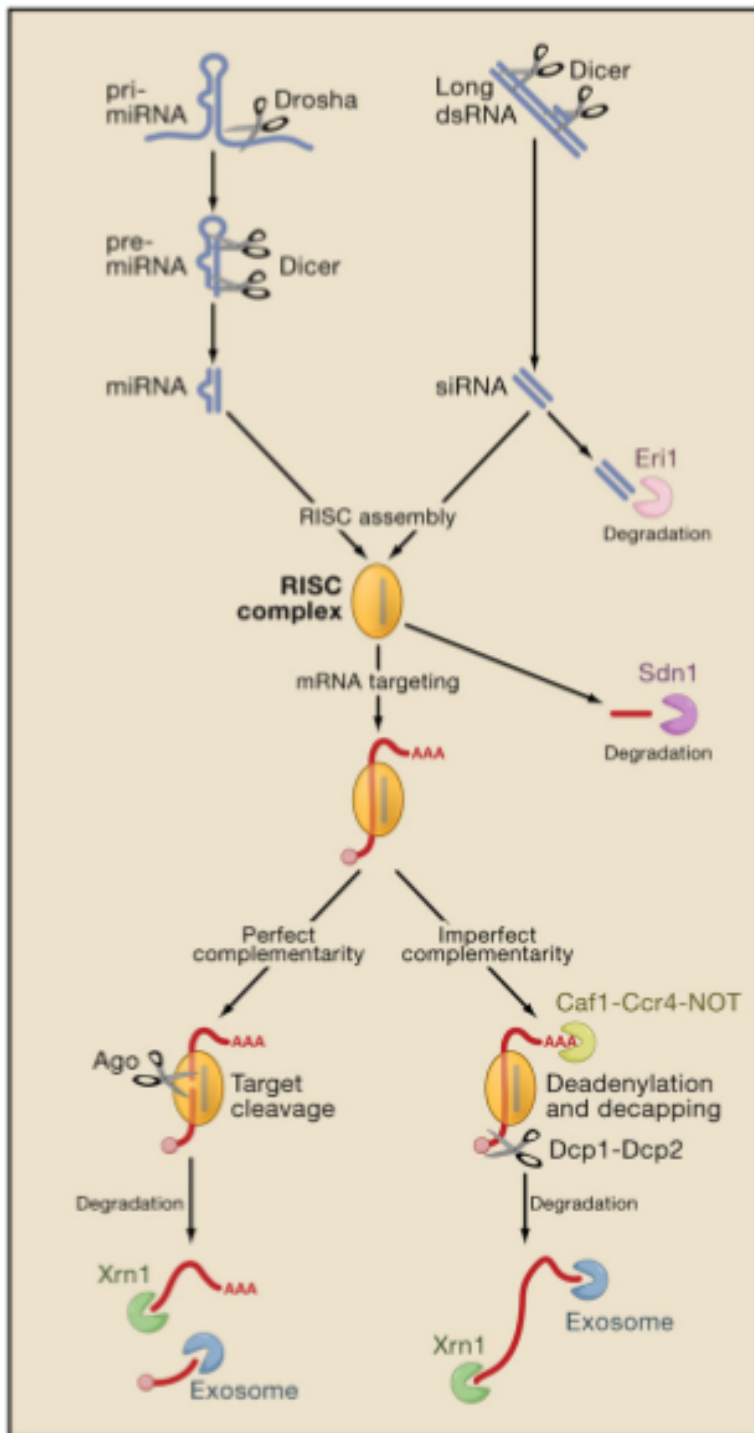
How are ribonucleases regulated?

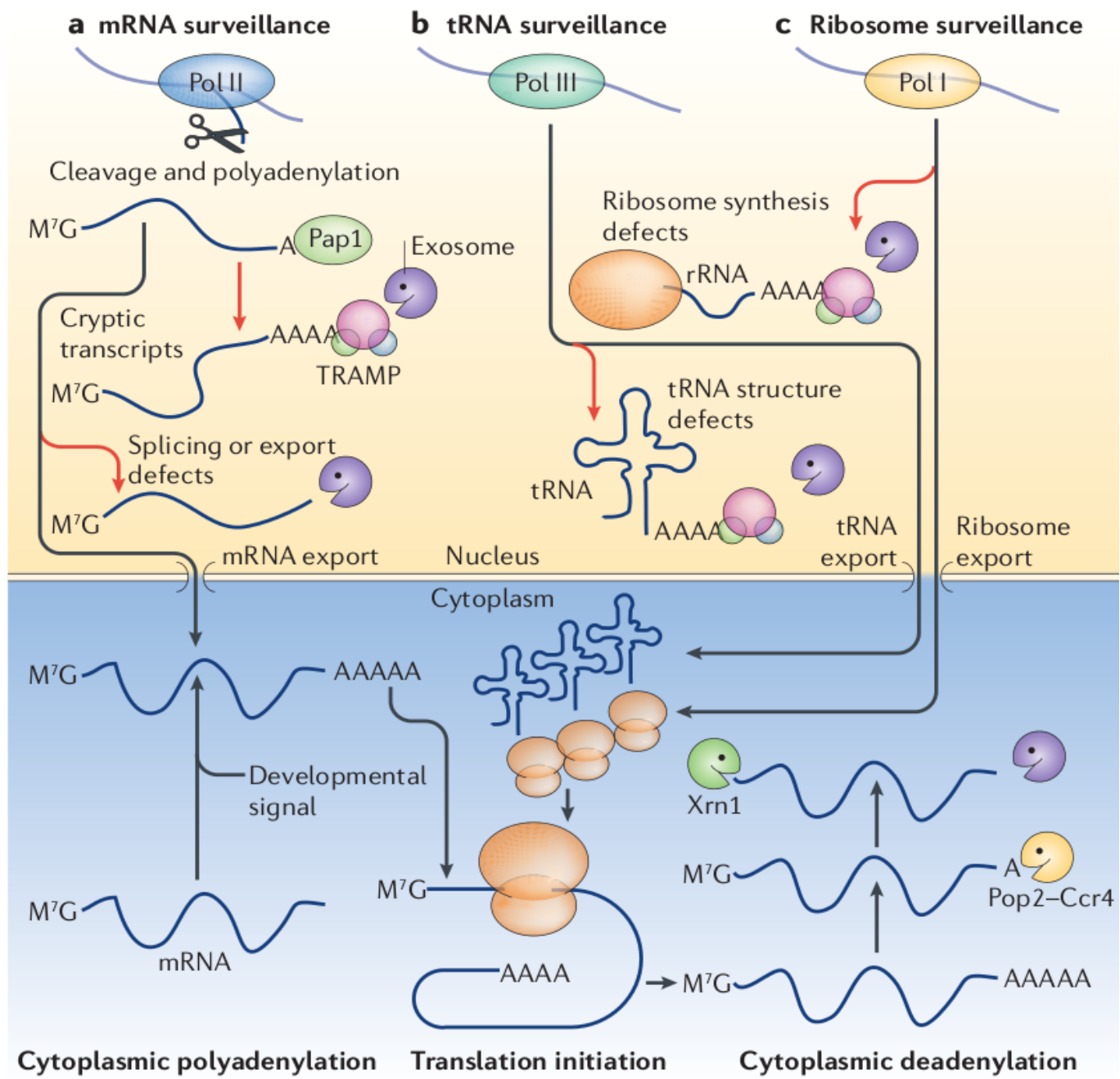
??????????

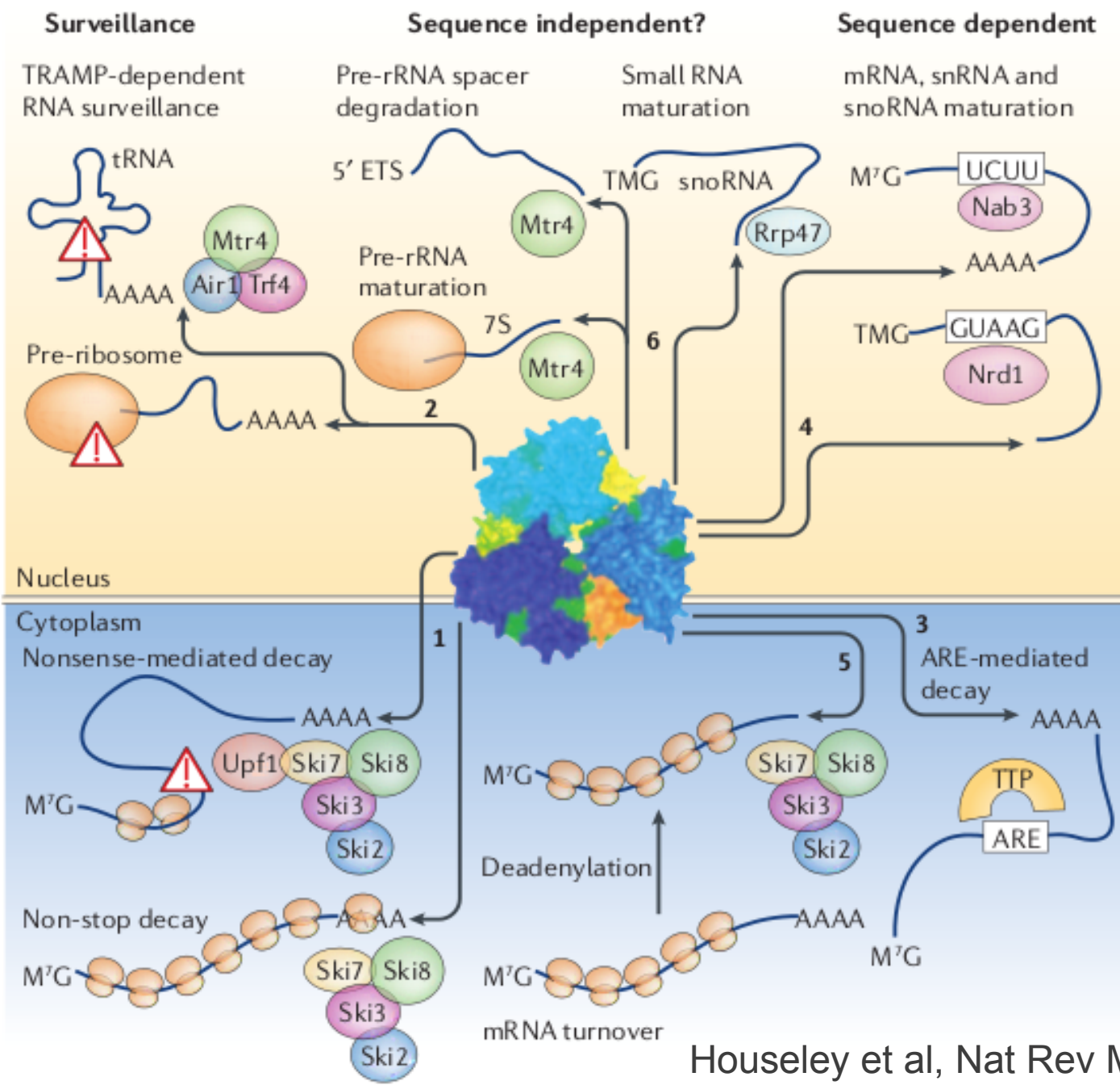
a











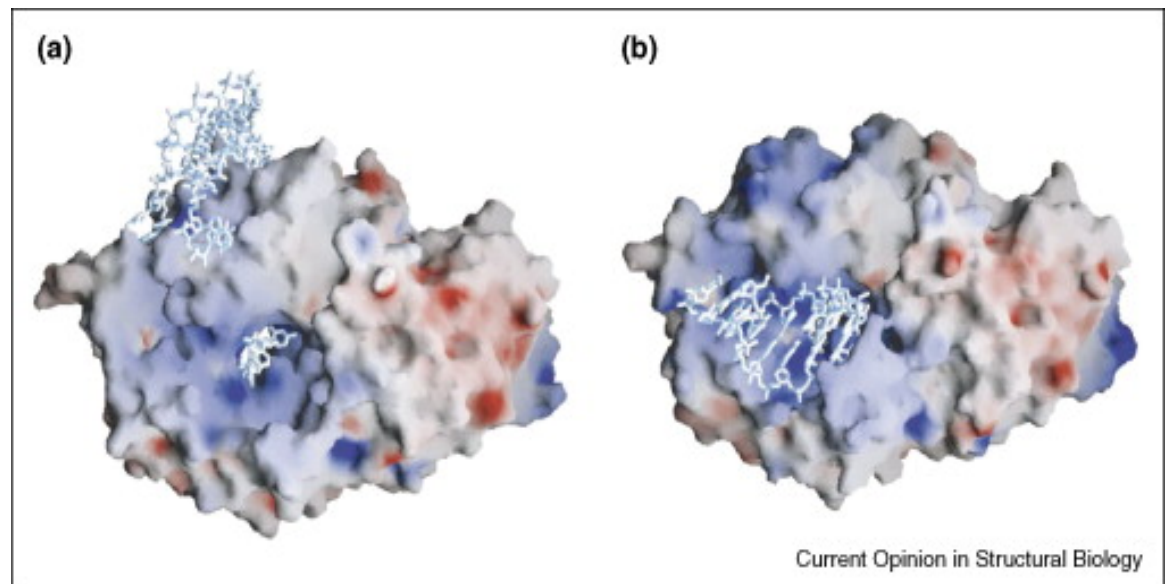
Houseley et al, Nat Rev Mol Cel Biol, 2006

Non-coding RNA quality control

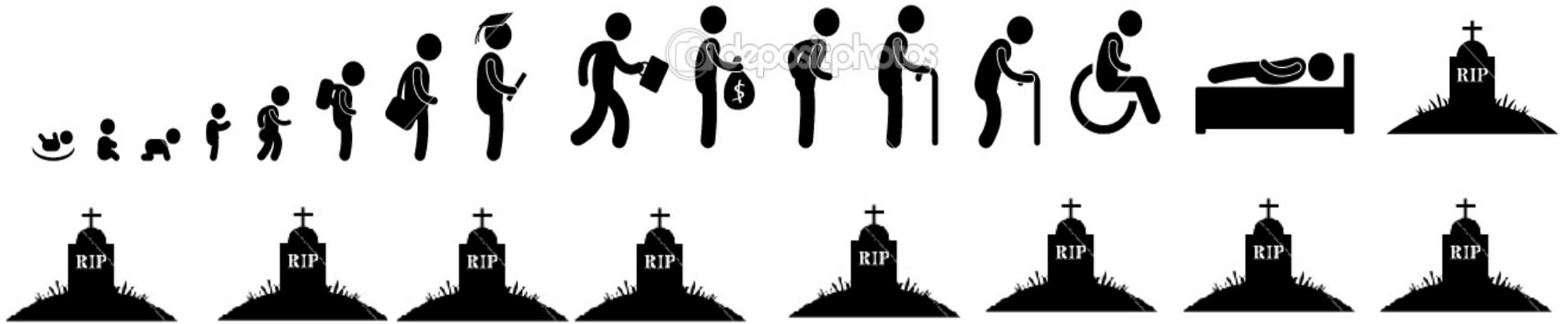
TRAMP/exosome

RNA quality control proteins interact with relatively general RNA structures:
Scavenger pathways

Correctly folded RNAs are sequestered by specific RNA-binding proteins
→ protected from degradation



Ro binds misfolded RNAs that contain both a single-stranded 3' end and helices
Ro may bind RNAs that, because they are misfolded, do not associate with their correct RNA-binding proteins.

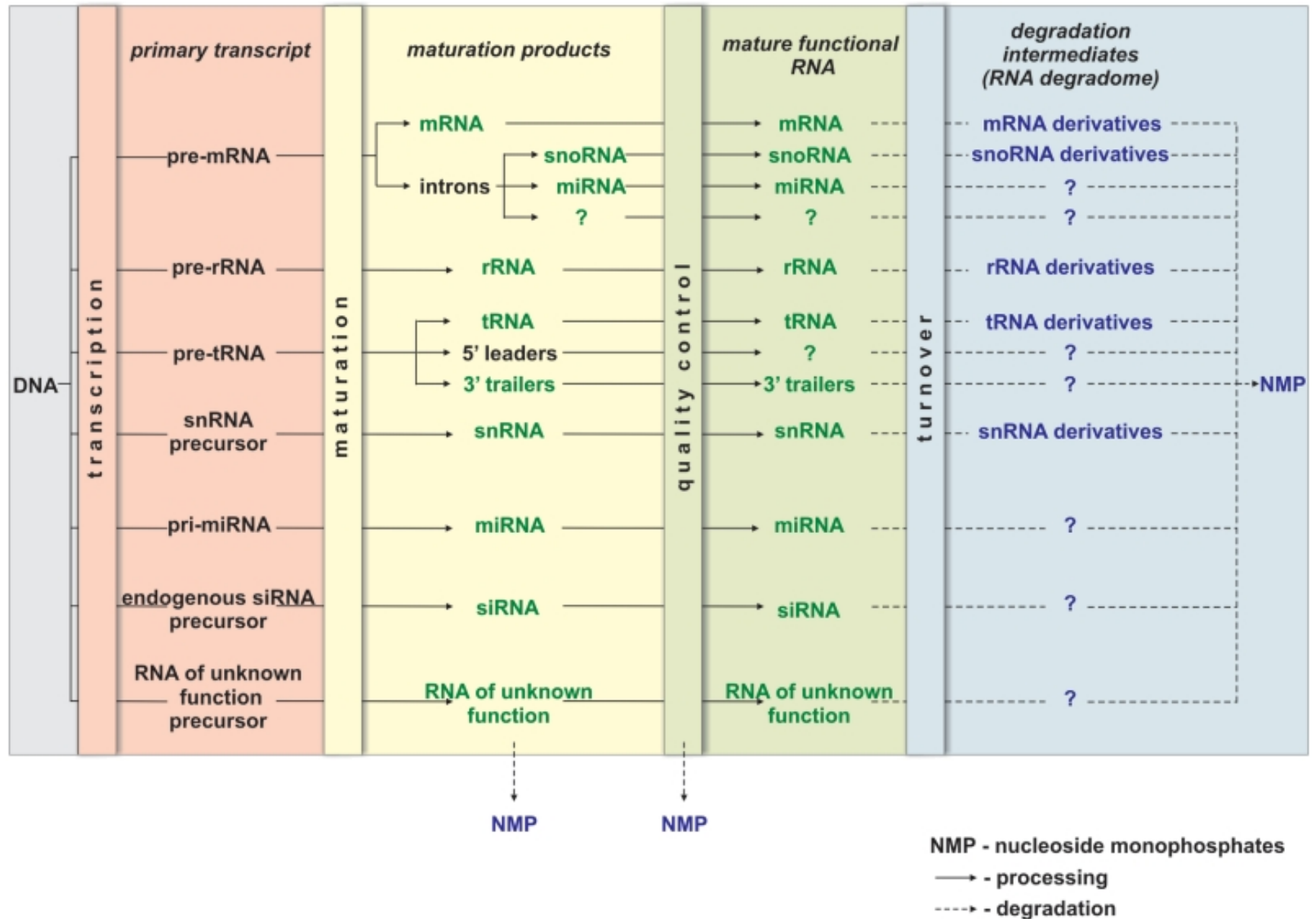


Rest in pieces...

Pieces = not necessarily single nucleotides !!!



RNA degradome



Why is RNA degradation so efficient??

Why is RNA degradation so efficient??

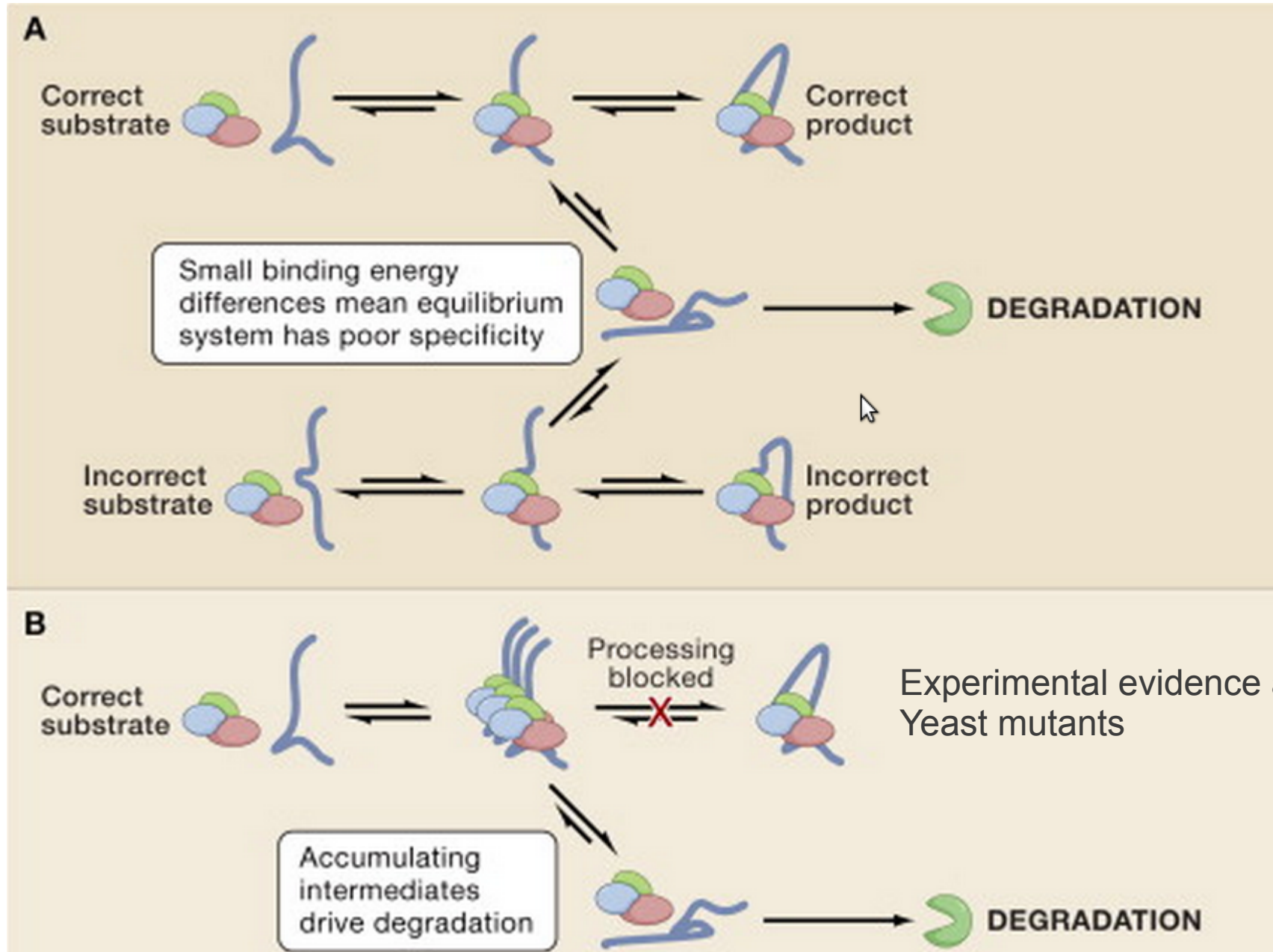
- Prevalence of exonucleases relative to endonucleases
- Role of small RNAs in gene expression
- Selection against accumulation of random RNA fragments
- Hydrolysis is thermodynamically favored (hydrolysis vcs phosphorolysis)
- RNA-DNA hybrids: may interfere with DNA replication
- Sequestration of RNABP
- Viral supression

Most organisms: various extracellular, nonspecific RNAses
5'OH and 3'P

Why is RNA degradation so complicated??

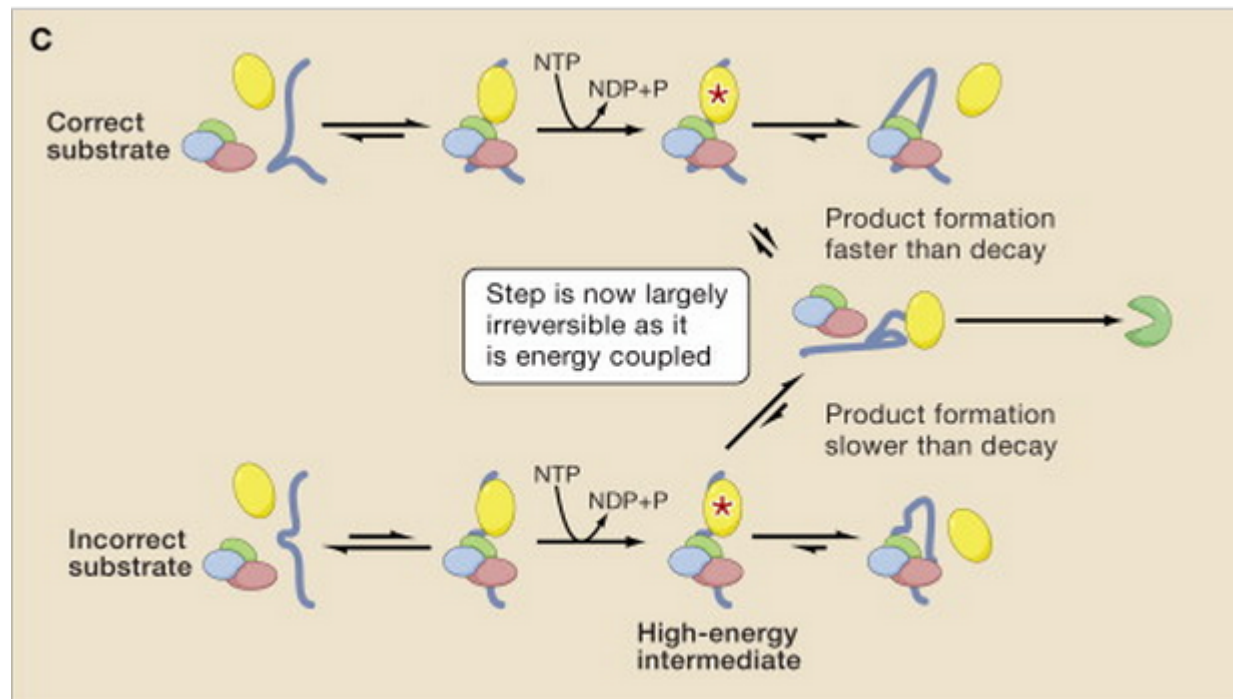
How to distinguish 'normal' vs 'defective'?

Equilibrium model for RNP assembly



Kinetic Proofreading

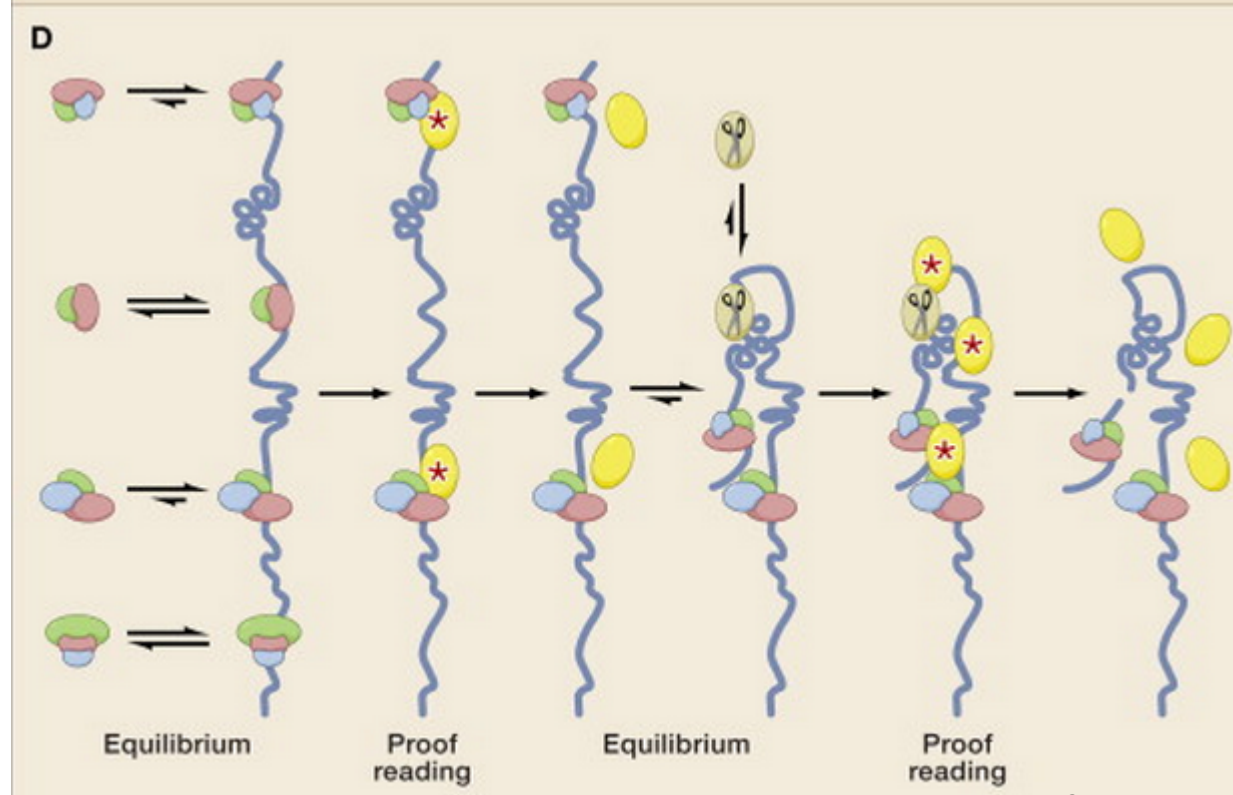
Hopfield, 1974



Kinetic Proofreading In RNP assembly

Ribosome synthesis
 180 proteins
 75 snoRNPs
 79 ribosomal proteins
 7kb pre rRNA

19 RNA helicases
6 GTPases



Bacterial vs Eukaryotic RNA decay