Macromolecule turnover

- rapid response to changing environment

- removal of damaged macromolecules

Fundamental aspects of RNA turnover

enzymes - nucleases (endo, exo)

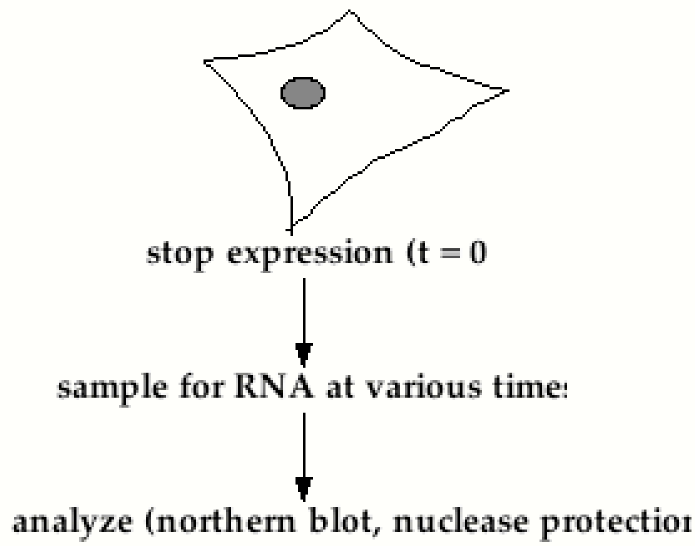
cofactors - RNA binding proteins?

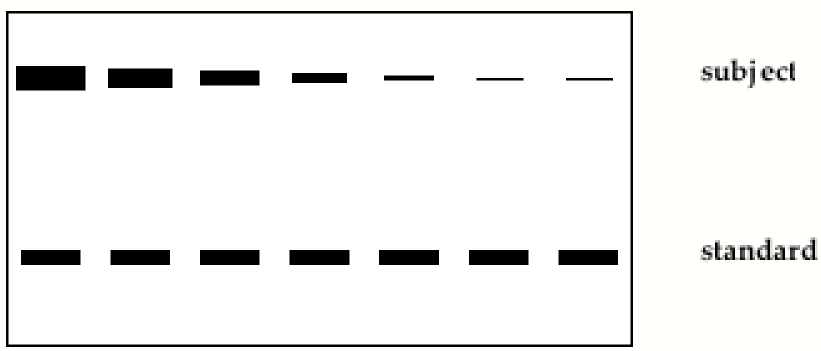
specificity - RNA or RNP?, nucelotide sequence, RNA structure?

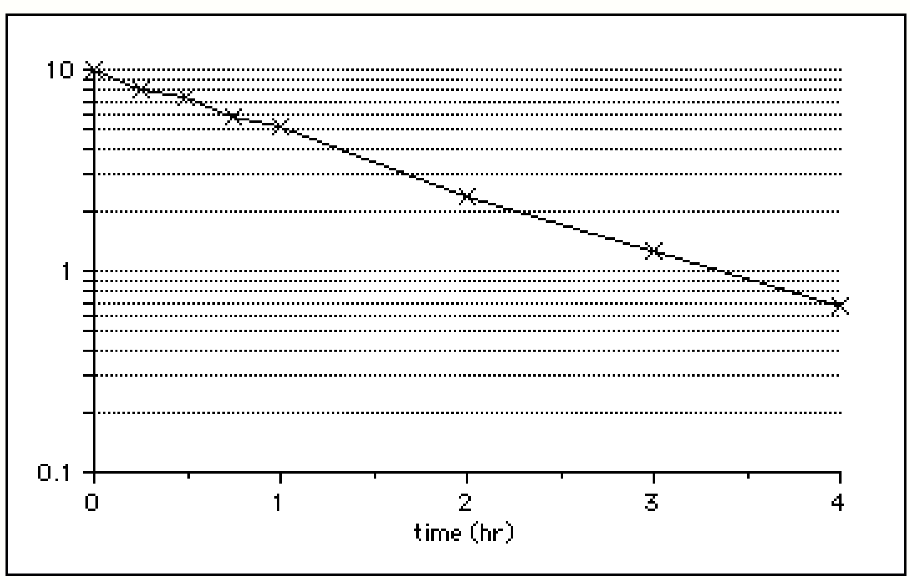
regulation - ??

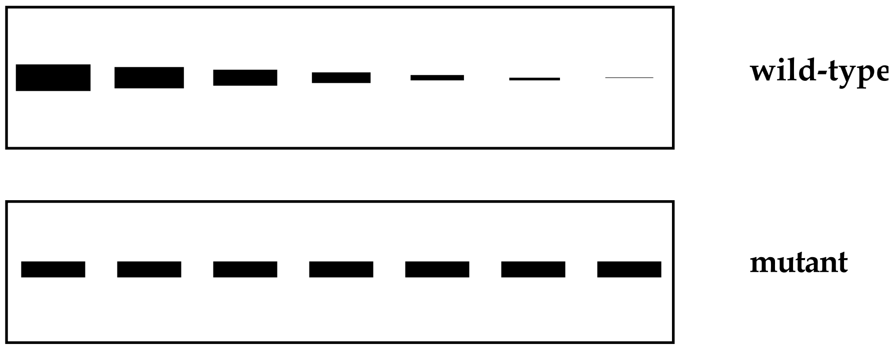
localization - ribosome-associated, nuclear?

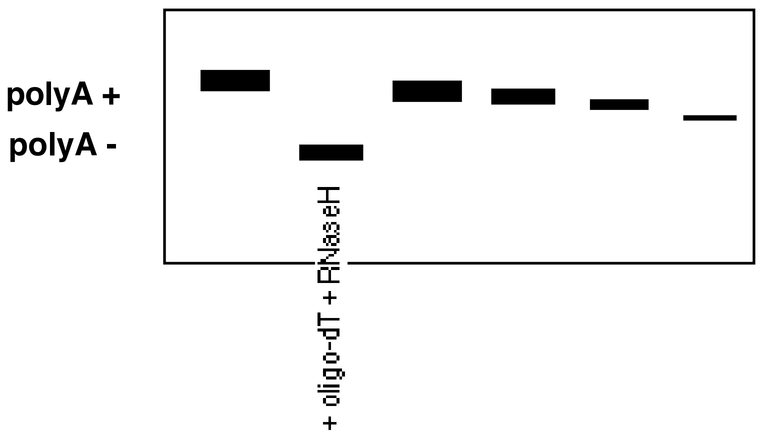
interdependence with other systems - translation, RNA processing

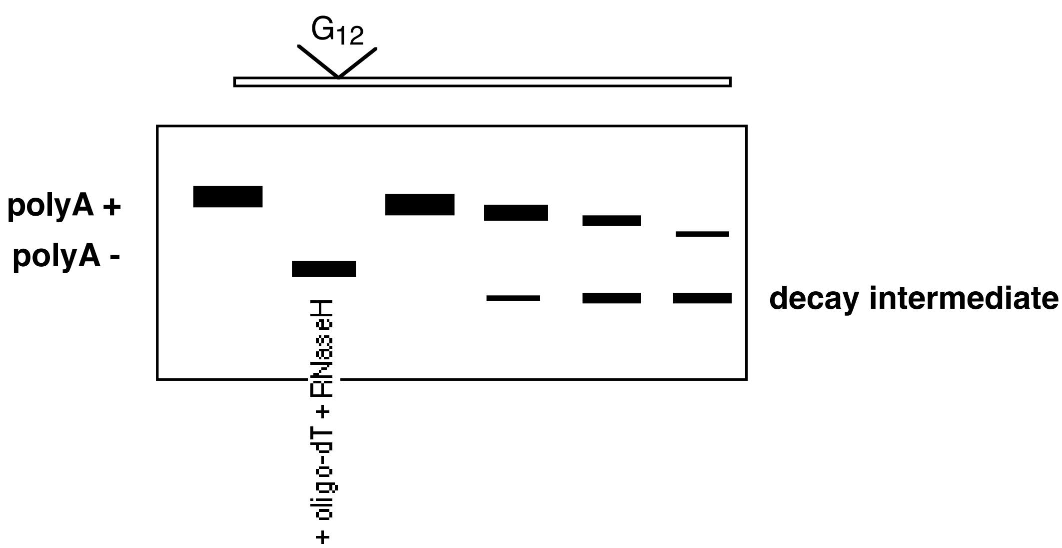

stop expression (t = 0
sample for RNA at various time
analyze (northern blot, nuclease protectio


subject
standard

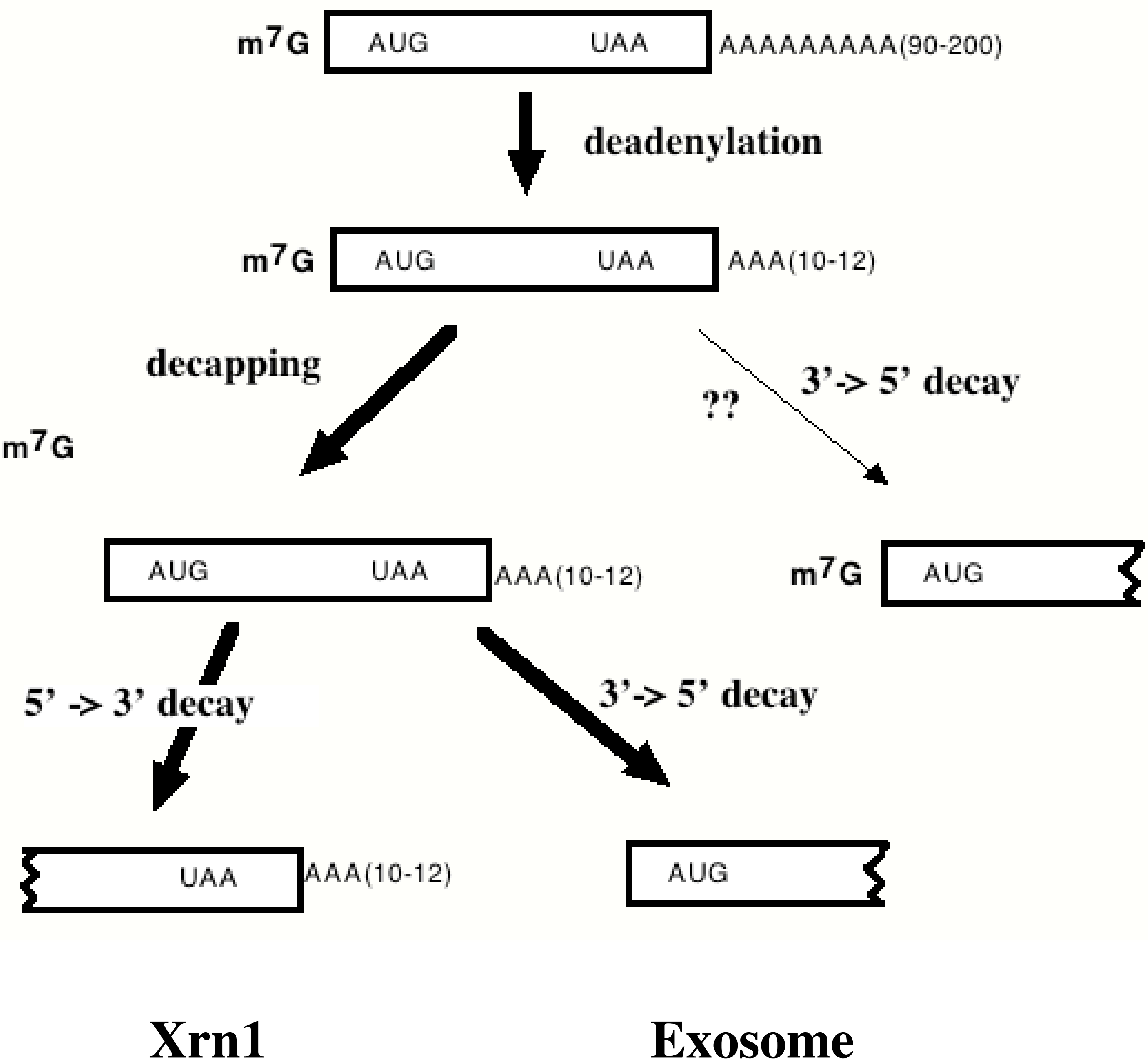

10
1
0.1
0
1
2
3
4
time (hr)


wild-type
mutant

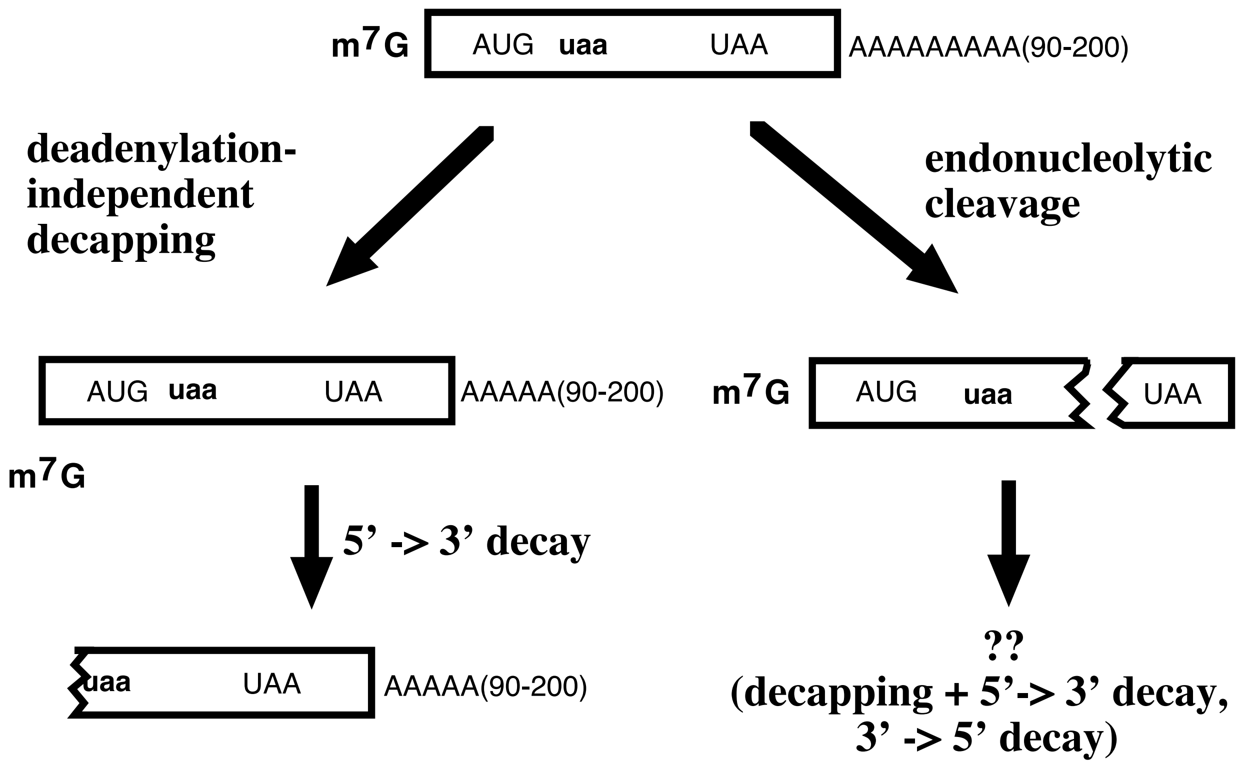

polyA +
polyA -
+ oligo-dT + RNaseH


G12
polyA +
polyA -
+ oligo-dT + RNaseH
decay intermediate

Deadenylation-dependent mRNA decay



Deadenylation-independent mRNA decay



Mechanisms involved in mRNA turnover

1. Poly(A) shortening

Appears to be a rate-determining step for mRNA turnover: in yeast and animals, unstable mRNAs shorten their poly(A) more rapidly than longer mRNAs

Involves a nuclease that is specific for poly(A)

> **PAN: poly(A) nuclease, requires PAB**
> **CCR4-Not complex: multifunctional complex, may link deadenylation with other processes**

The mammalian PAN nuclease also requires that the mRNA be capped -> competition between translation and mRNA turnover may involve interactions with the 5’ and 3’ ends of the mRNA

Regulation is not understood

2. mRNA decapping

Occurs on mRNAs with shortened poly(A) tails

Removes the 5’-cap, which may have a direct “protective” effect against exonucleases

3. 5’->3’ exonucleases

Xrn1p in yeast

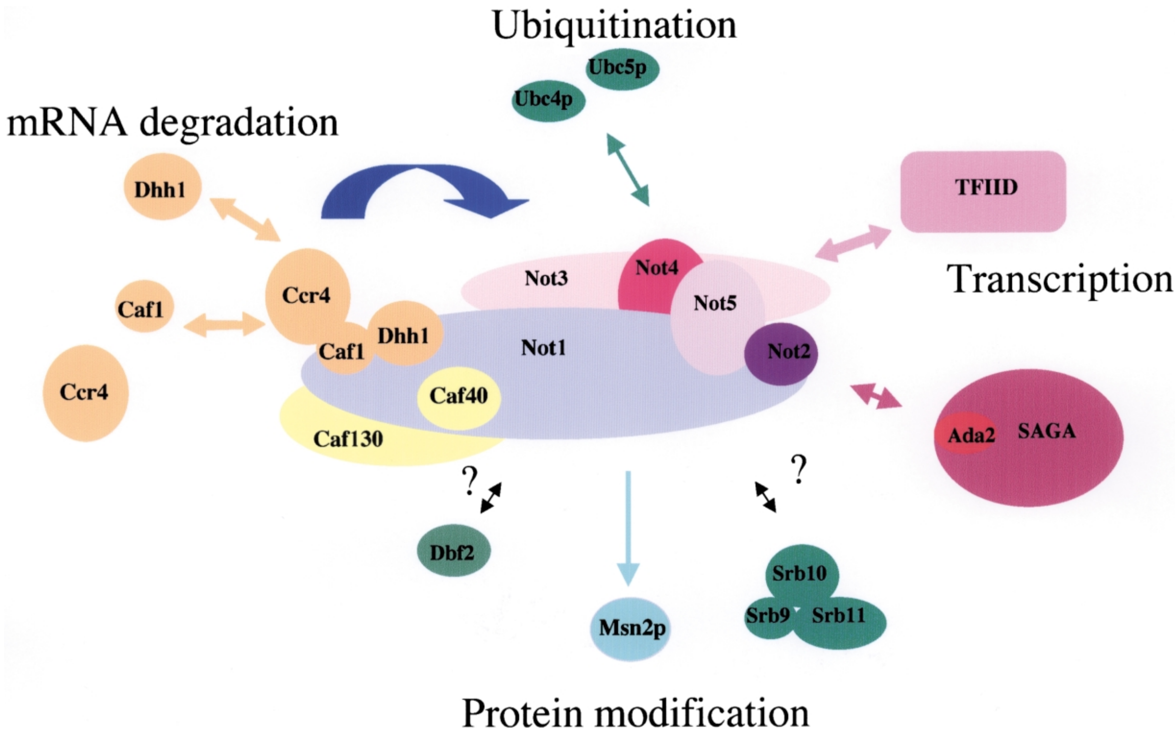
4. 3’->5’ exonucleases

A complex known as the exosome

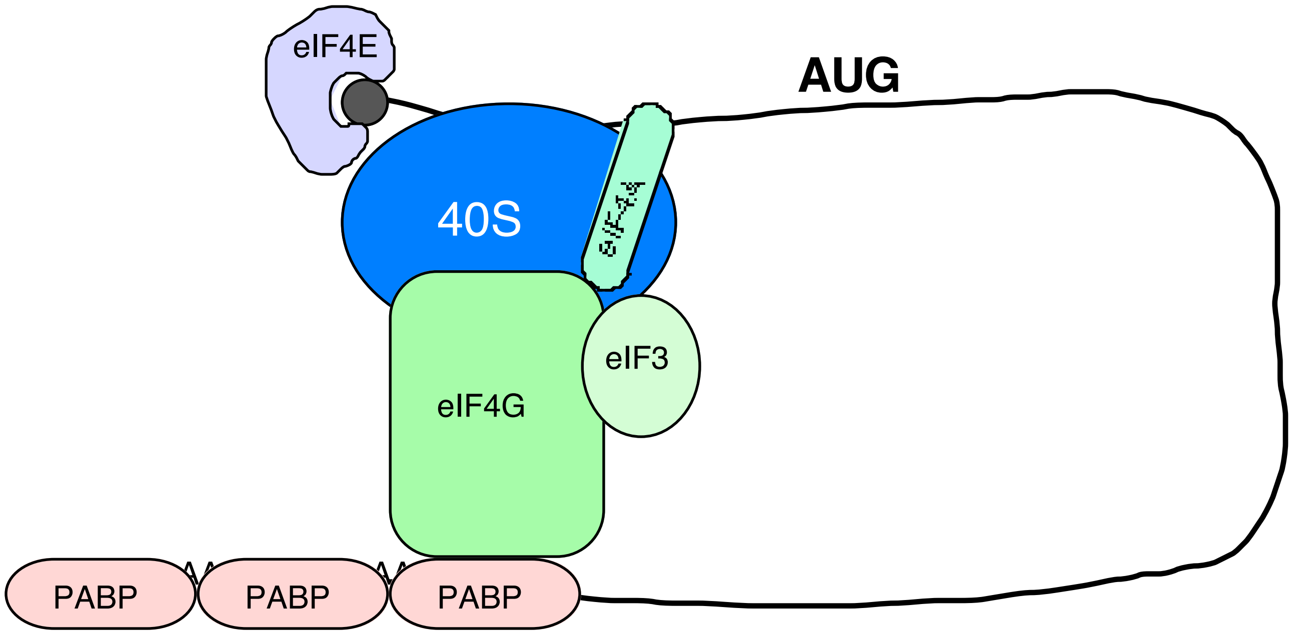
Analogous in function and subunit composition to bacterial mRNA turnover machinery - the degradosome

The CCR4-NOT Complex - links between many processes

M.A. Collart / Gene 313 (2003) 1–16



Structure of the translation initiation complex



Efficient recruitment of the 40S ribosomal subunit requires interactions at the 5' (cap binding complex) and 3' (poly[A] binding protein) ends of the mRNA

Initiation can be modulated by modifications (e.g., phosphorylation) of various initiation factors and by the association of the mRNA with proteins that alter the functioning of the initiation complex

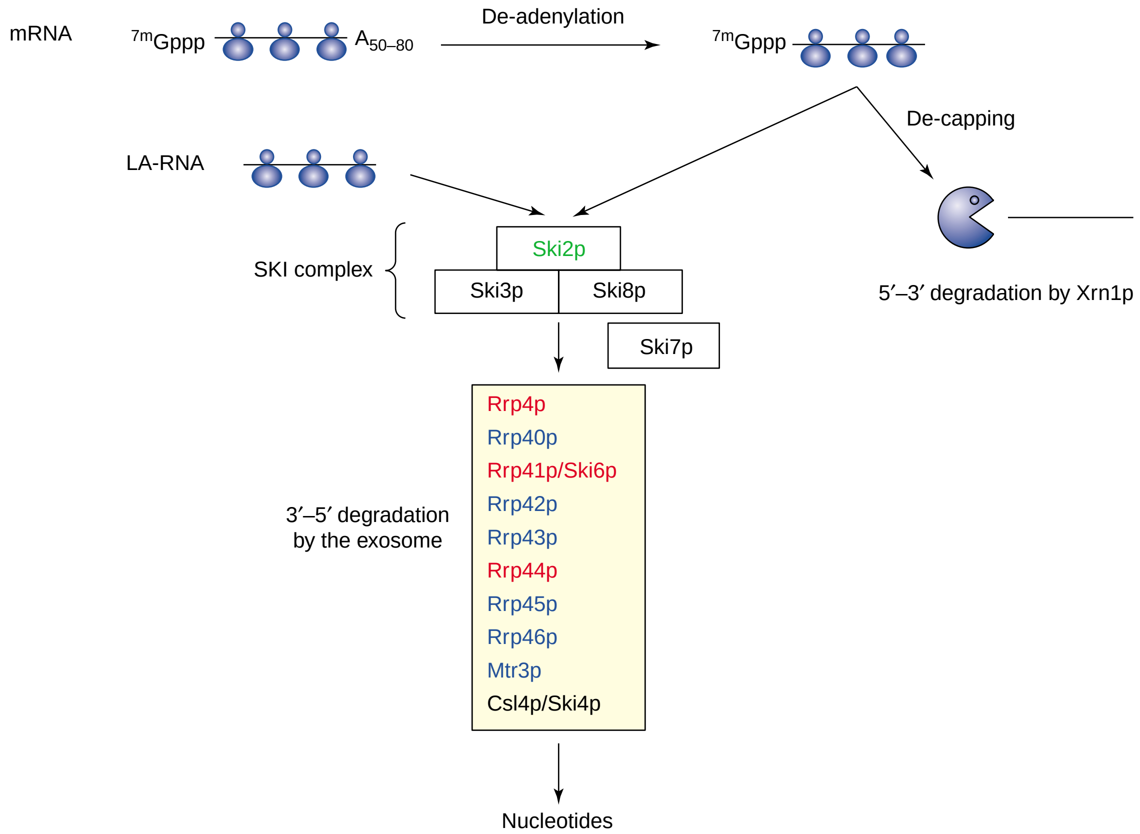
Provides conceptual links between poly(A), decapping, turnover, and translation

Xrn1

- 5'-3' exonuclease
- Cytoplasmic
- Related to nuclear Xrn2

Exosome

- RNA degrading complex
- Consists of several 3'-5' exonucleases
- Phosphorolytic - uses phosphate to cleave phosphodiester bond; related to polynucleotide phosphorylase
- Hydrolytic- uses water to cleave phosphodiester bond



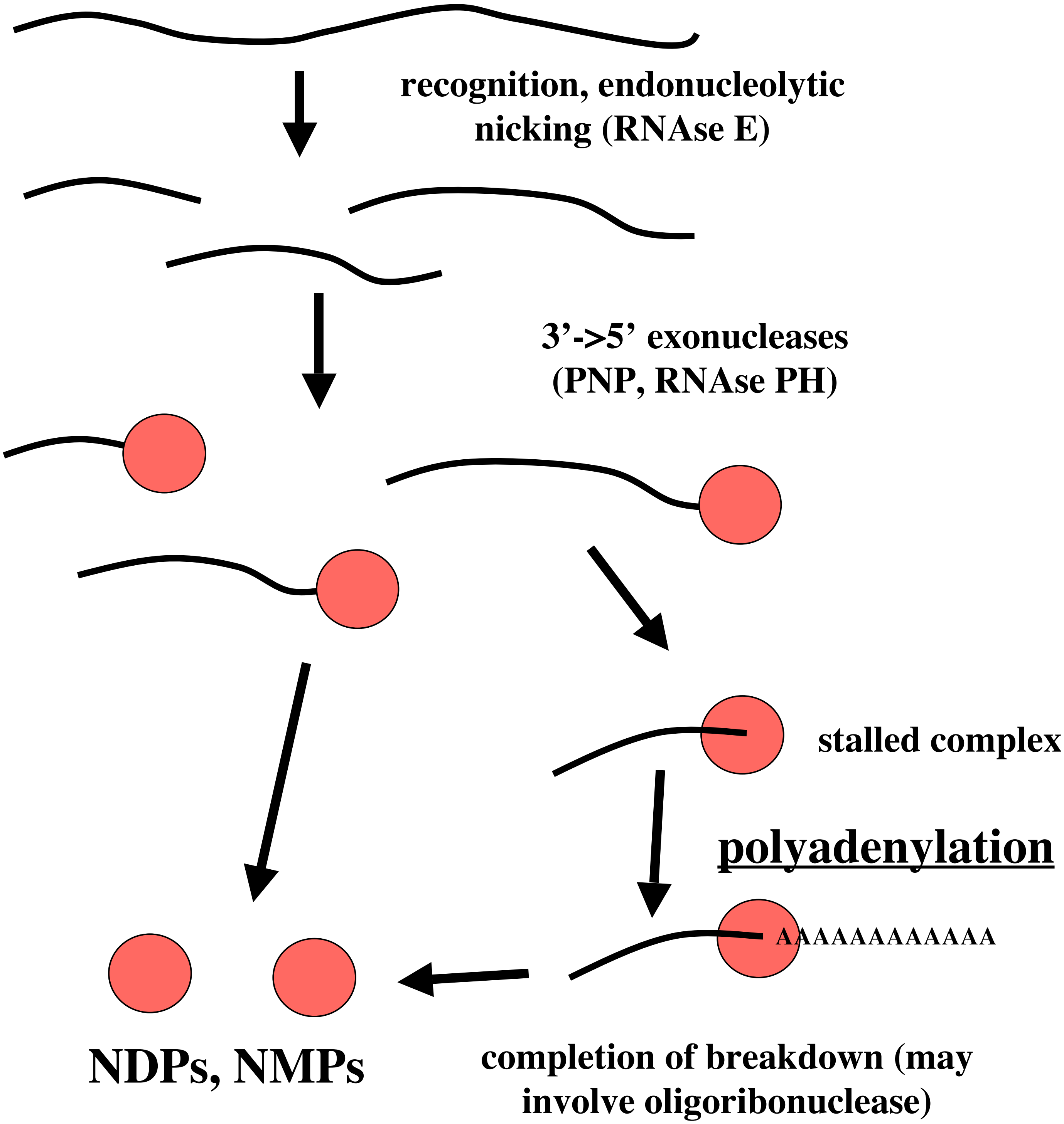
TRENDS in Cell Biology

Escherichia coli homologue	Archaeal exosome subunit	Yeast exosome subunit	Description of yeast component(s)
PNPase*	Rrp41*	Rrp41* Rrp46 Mtr3	All components are part of the hexameric ring structure, and have similarity to archaeal Rrp41. Each is likely to posses a catalytic RNase PH domain
	Rrp42	Rrp42 Rrp43 Rrp45	All components are part of the hexameric ring structure, and have similarity to archaeal Rrp42. Their PH domains are probably catalytically inactive
	Rrp4 or Csl4	Rrp4* Rrp40	Both components are part of the 'S1 pore' structure. Rrp4 and Rrp40 have S1 and KH RNA-binding domains. These domains are also present in PNPase
		Csl4	Archaeal Csl4 has an S1 domain and a zinc-ribbon domain. Yeast Csl4 shares a similar fold structure
None	None	Rrp44*	Homologous to *E. coli* RNase R (RNase II family)
		Rrp6*	Similar to *E. coli* RNase T/D family. Present only in the nuclear exosome
		Rrp47	Possible RNA-binding protein. Present only in the nuclear exosome
		Ski7	Putative GTPase. Present only in the cytoplasmic exosome

* Proteins for which *in vitro* exonuclease activity has been reported. Mtr, mRNA transport regulator; PNPase, polynucleotide phosphorylase; Rrp, ribosomal RNA-processing protein.

Houseley *et al.* *Nature Reviews Molecular Cell Biology* **7**, 529–539 (July 2006) | doi:10.1038/nrm1964

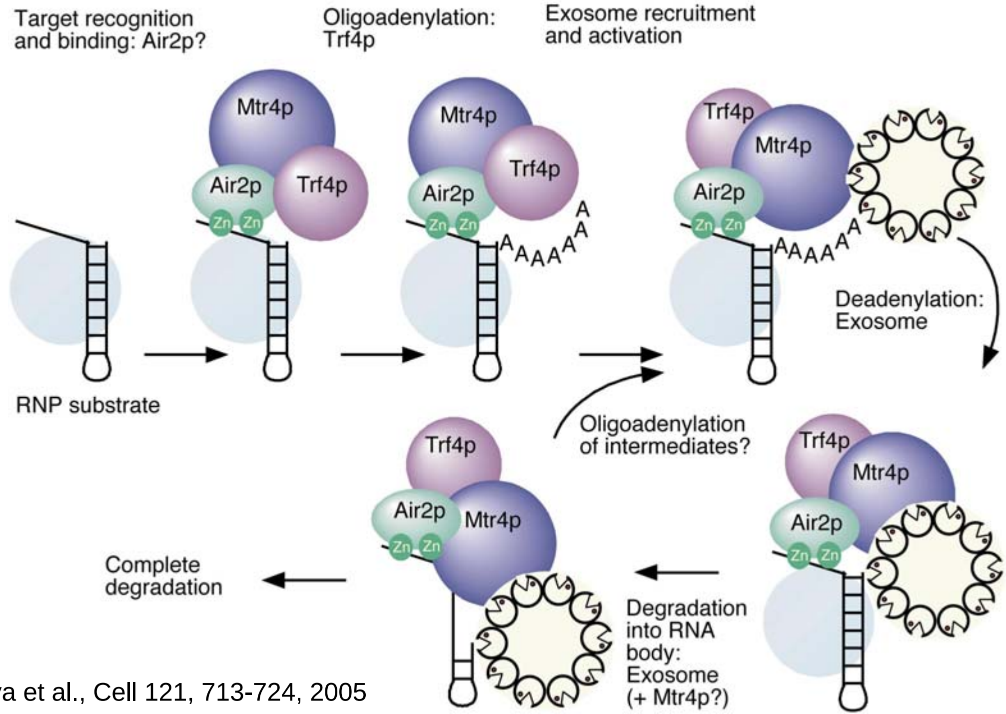
nature REVIEWS MOLECULAR CELL BIOLOGY



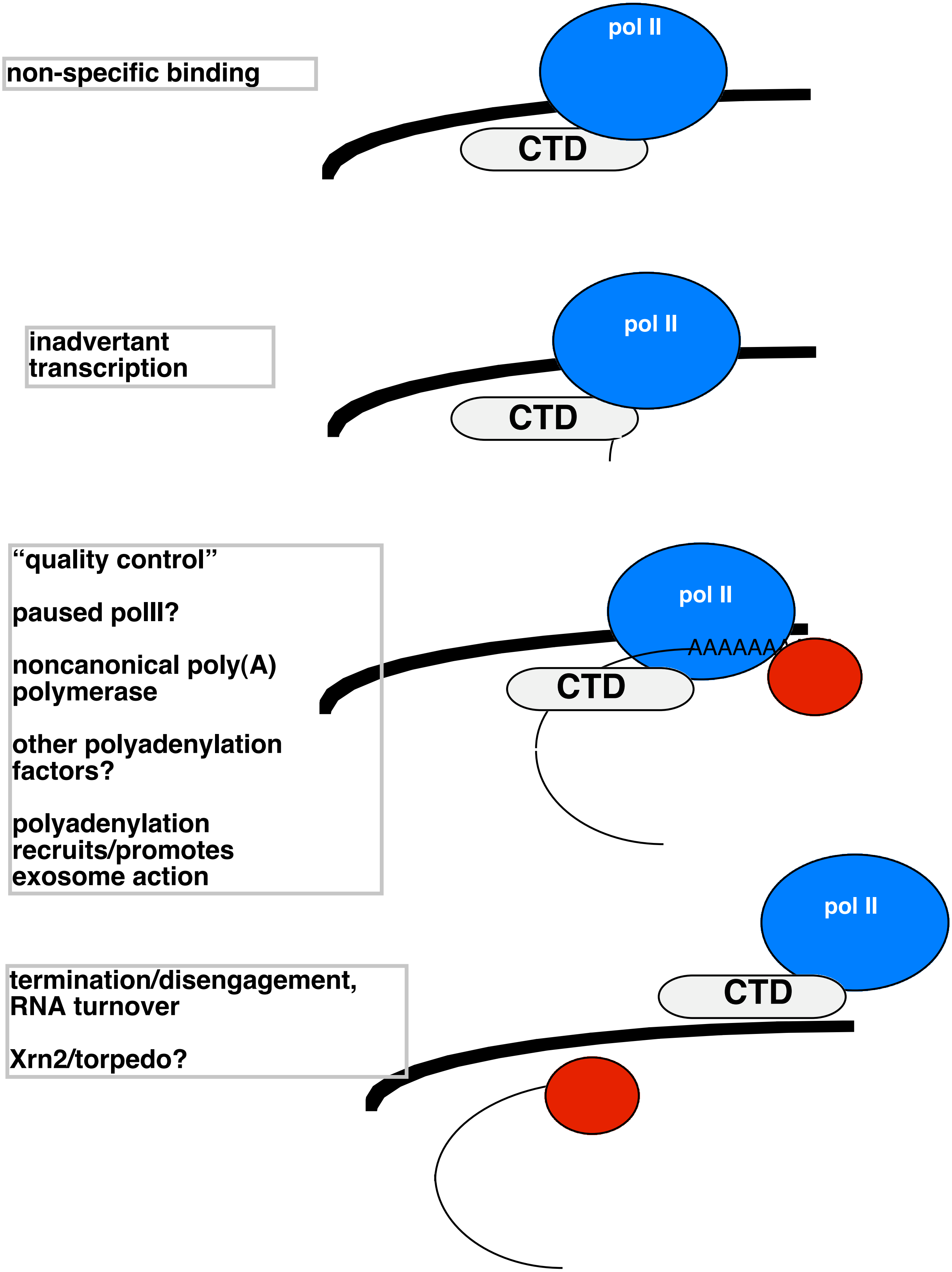
Coburn, G. A. and Mackie, G. A. (1998) Reconstitution of the degradation of the mRNA for ribosomal protein S20 with purified enzymes. J. Mol. Biol. 279, 1061-1074.

Blum, E., Carpousis, A. J., and Higgins, C. F. (1999) Polyadenylation Promotes Degradation of 3*-Structured RNA by the Escherichia coli mRNA Degradosome in Vitro. J. Biol. Chem. 274, 4009-4016.

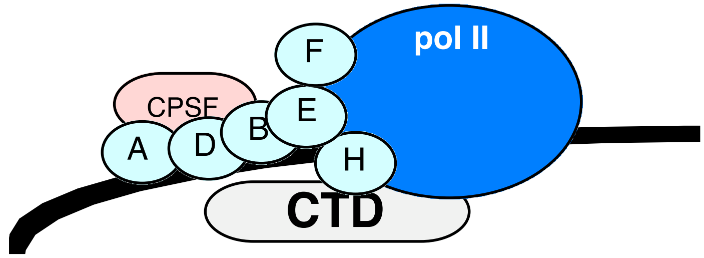
Figure 7. Model for the Roles of the TRAMP Complex in RNA Degradation



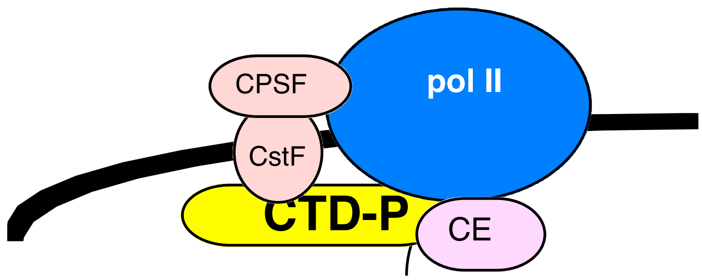
from Lacava et al., Cell 121, 713-724, 2005


non-specific binding
pol II
CTD
inadvertant
transcription
pol II
CTD
“quality control”
paused polII?
noncanonical poly(A)
polymerase
other polyadenylation
factors?
polyadenylation
recruits/promotes
exosome action
pol II
AAAAAA
CTD
pol II
termination/disengagement,
RNA turnover
Xrn2/torpedo?
CTD

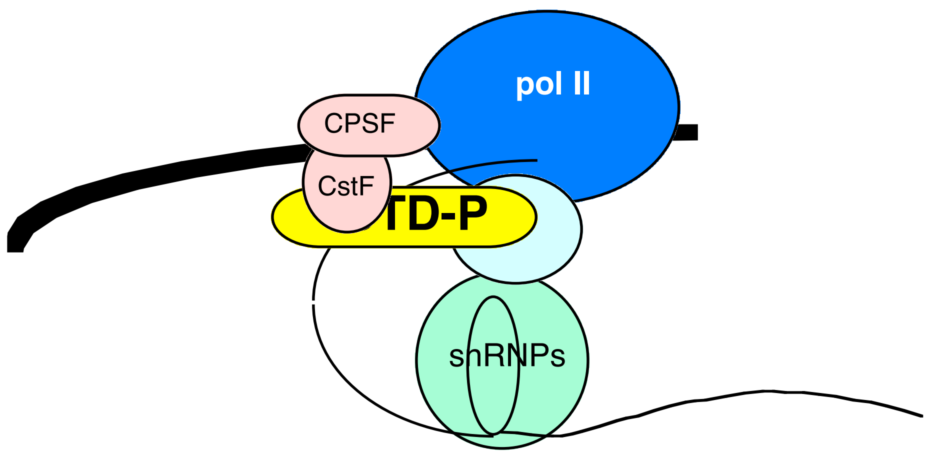
pre-initiation complex



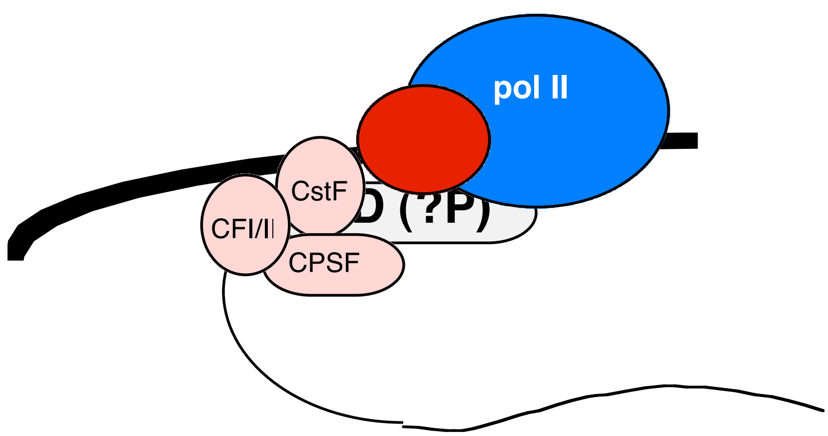
capping (30 nts)



elongation (splicing)



termination/
polyadenylation



Elements that destabilize mRNAs

Best known examples - AUUUA (found in animals, works in plants), DST elements (plant-specific)

Usually situated in the 3'-UTR

Accelerate poly(A) shortening

Mode of action not understood

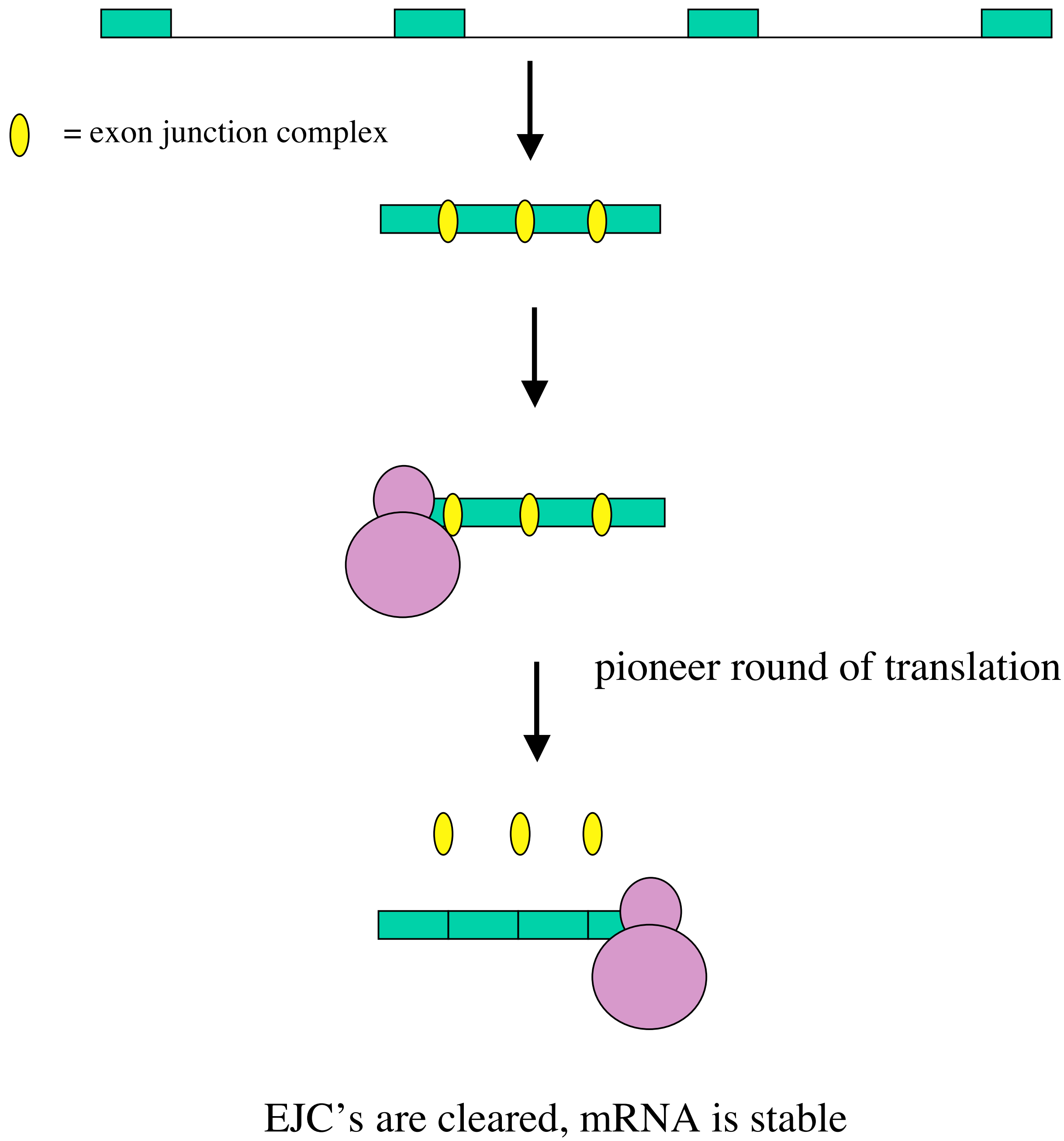
Questions:

Recruitment of deadenylases?

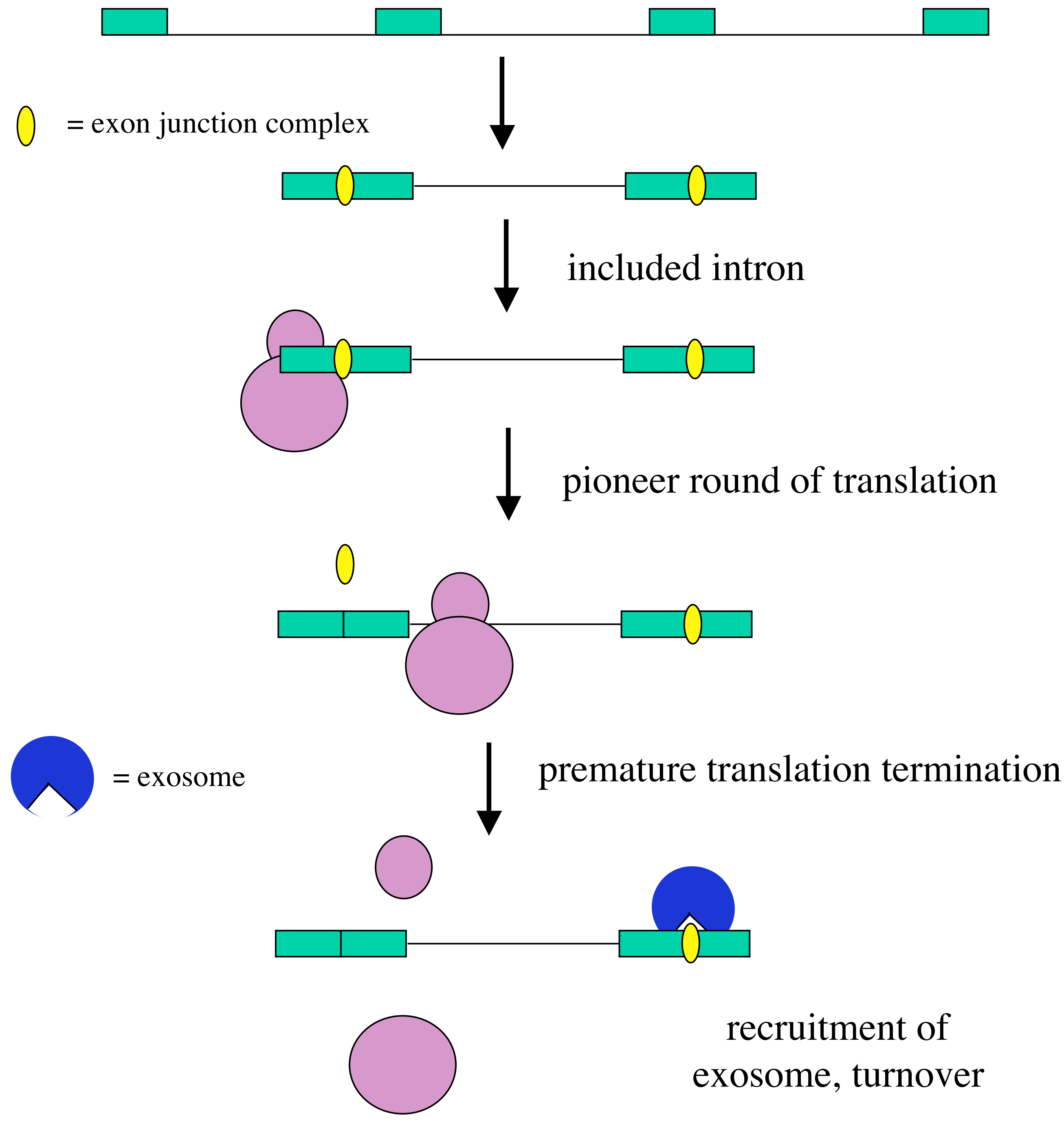
Inhibition of the formation of the translation initiation complex?

Links with protein turnover (one or more subunits of the proteasome bind AUUUA, have nuclease activities)?

Exon junction complexes, premature stop codons, mis-splicing, and RNA turnover



Exon junction complexes, premature stop codons, mis-splicing, and RNA turnover



Exon junction complexes, premature stop codons, mis-splicing, and RNA turnover