7. RNA TURNOVER

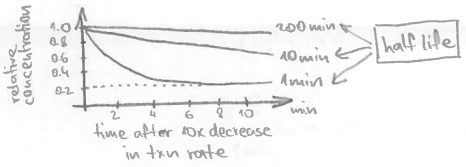
7.1. INTRODUCTION TO RNA TURNOVER

- ch.7: primarily about unregulated turnover
  - unmodulated turnover
  - each RNA has its own rate of degradation
  - regulation ← rate
    - usually on synthesis, not degradation
    - there are some genes regulated at the point of degradation

unregulated ⇒ does not change in response to events

7.2. RNA DECAY

transcription → mRNA abundance ← decay ⇐ rate of decay has strong effect on rate of change of RNA concentration ⇒



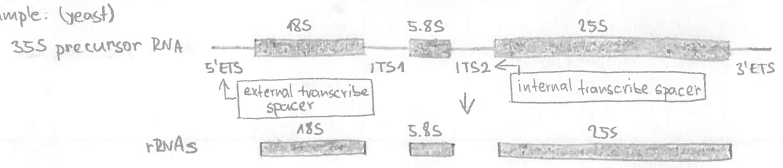
half life

	avg. mRNA half-life	cell division time	ratio
human	~8-10h	~24h	~0.33-0.42
yeast	~20 min	~90 min	~0.22

↑ "similar"

mRNAs encoding proteins whose levels must change rapidly in response to environmental signals have short half-lifes.

- essential for RNA quality control: ribosome makes mistake on average in ~ 10^4 aas incorporated ⇒ can cause stoppage of tnl
  - translation dependent mechanisms sense mRNA defects leading to decay
  - defects in processing, folding, or assembly w/ proteins for non-coding RNA lead to decay
- example: (yeast)



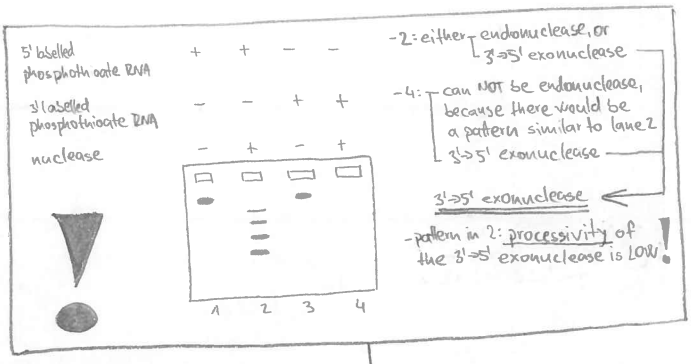
6858 nt ⇐ ~2000 ribosomes produced per minute in rapidly growing yeast cell

5357 nt ⇐ ~3'000'000 nucleotides of extra RNA from precursor RNA must be degraded to mononucleotides ((6858-5357)×2000)

- ETS/ITS RNA must be degraded
  - accumulates rapidly
  - competes w/ other RNAs ⇒ interferes w/ processes happening in cell
  - not degrading lethal to the cell

7.3. RNA HALF-LIVES

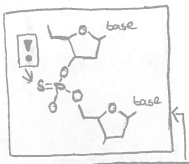
- class-specific
  - mRNAs
    - intermediate half-lives
    - most dynamic range
    - can be regulated
  - stable RNAs (tRNAs, rRNAs, snoRNAs, splicing RNA, ...)
    - usually highly folded or tightly bound to a protein
    - half-lifes on the order of many cell divisions (typically not measureable)
    - degraded rapidly only if ← deffective, or cell in starvation conditions
  - "junk RNA" (introns after excision, anti-sense transcripts, ETS/ITS from precursor rRNA)
    - rapid turnover (typically < 1min.) ⇐ measurable by Gro-seq, NET-seq !



7.4. RIBONUCLEASES AND IN VITRO NUCLEASE ASSAY

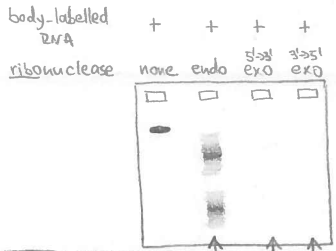
- classes of RNA nucleases
  - endonucleases
  - 5'⇒3' exonucleases
  - 3'⇒5' exonucleases
    - highly processive
    - do not degrade RNA in nucleotide-by-nucleotide fashion ⇒ products are oligonucleotides
    - types
      - hydrolytic: use H_2O to attack phosphodiester bond, release mono-phosphate (rNMP) ⇐
      - use P_i as nucleophile, release di-phosphate (rNDP) ⇐ easier to regenerate than

oligonucleotidase degrades these



- same as DNA, except it has S instead of one O in phosphates
- RNA nucleases can NOT cut it !

- process
  - body labeled RNA (RNA Pol + labelled nucleotides)
  - incubate w/ extract/fraction/purified enzyme
  - gel electrophoresis & autoradiogram

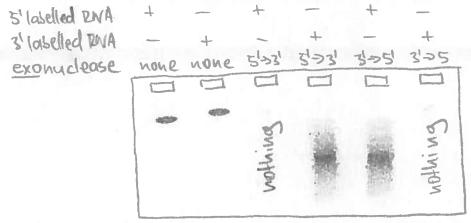


different-sized fragments (depending on endonuclease specificity - if any)

exonucleases act very rapidly ⇒ in order to see something, very short time-points have to be taken

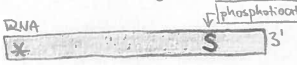
there still is NO distinction b/w 5'⇒3' & 3'⇒5' !!!

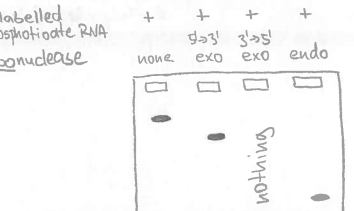
- variation: use 5'/3' end-labelled RNA
  - 5' ← cut off 5' triphosphate, use kinase to add labelled triphosphate
  - 3' ← use poly(A) polymerase to add labelled As at 3' end (trickier than)



need to stop after short time so that RNAs are not yet completely digested

- variation: phosphothioate
  - difficult → synthetize, use RNA ligase to add it to 5'/3' end



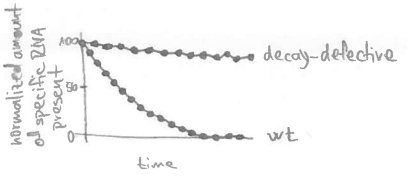


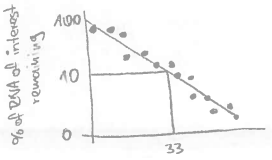
no need to stop, phosphothioate will do the job

7.5. In Vivo Half-Life Assay

- process
  - inhibit transcription (t=0) — need to synthetize artificial constructs (more difficult)
    - abruptly
      - promoter shut-off, or ←
      - RNA Pol III ts mutant, or ← — possible side-effects: all txn stops, temperature (in)stability
      - drug (thiolutin, α-amanitin, actinomycin D)
  - isolate RNA from cells over timecourse
    - phenol extraction & ethanol precipitation
  - detect the RNA of interest
    - either: qRT-PCR (single gene); RNA-seq (genome wide)

! by turning off all txn, also genes producing RNA-degrading proteins are turned off !





$m = \frac{\log(10) - \log(100)}{33 - 0} = \frac{-1}{33} = -0.03$

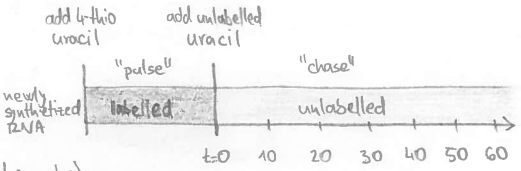
$k = 0.03 \cdot 2.3 = 0.069$

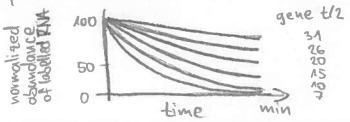
$t_{1/2} = \frac{0.69}{0.069} = 10h$

  - calculate decay rate and half life
    - quantify % of remaining RNA at each time-point ($t_0 = 100\%$)
    - plot a semi-log plot and calculate
      - slope: $m = \frac{\log(y_2) - \log(y_1)}{x_2 - x_1}$
      - decay-rate: $k = -m \cdot \ln(10)$
      - half-life: $t_{1/2} = \frac{\ln(2)}{k}$

7.6. RNA Pulse-Chase Assay

- 4-thio uracil – can link to biotin
- process
  - grow cells in 4-thio uracil environment for several generations
  - add excess of unlabeled uracil
  - take time points
  - isolate RNA
  - spike in 4-thio uracil labelled RNA control (baseline for normalization of individual samples)
  - biotinilate total RNA (biotin will link only w/ 4-thio uracil labelled RNA)
  - enrich for mRNA (vast majority of RNA in cell is rRNA)
  - streptavidin-select 4-thio uracil labelled RNA
  - RNA-seq





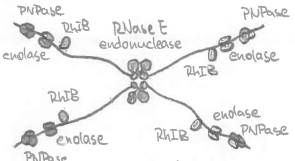
7.7. Protection from Bacterial mRNA Decay

- 5' end
  - protected by 5' triphosphate
    - only mRNAs that still retain their first nucleotide have 5' triphosphate
    - any RNA that is cut will lack 5' triphosphate
  - in some cases protected also by 2° structure at 5' end (very long half-life mRNAs)
- 3' end – typically protected by 2° structure — see 7.28.2x pg. 4.1
  - intrinsic txn termination: the GC-rich hairpin
  - rho-dependent txn termination: a hairpin that forms immediately after txn (independent of txn termination)

! in 3'-UTR ↑

if it occured BEFORE stop-codon it would have been unwound during tnl

7.8. Bacterial RNA Degradosome



- RNA degradosome: multi-enzyme complex
  - functional
    - endonuclease – RNase E
    - 3'→5' exonuclease – polynucleotide phosphorylase (PNPase)
      - phosphorylitic exonuclease (see ch. 7.4, pg. 7.1)
        - as opposed to hydrolysis, phosphorolysis is not so much favourable => can run in both directions !!!
          - exonuclease 3'→5'
          - polymerase ← ATP is by far most abundant nucleotide in cells => will synthetize poly(A) tail
    - 3'→5' RNA helicase – RhlB ← not required in vitro
    - enolase – not clear what is its purpose here – wild guess: measuring metabolic rate (?!??)

7.9. Bacterial mRNA Decay Mechanisms

2. RNase E cuts mRNA internally
   - prefers to cleave AU-rich RNAs
   - blocked by 2° structures (important for 5'/3'-UTRs, not so much in coding regions) ← ribosome removes 2° structures during tnl
   - favors cleavage of RNAs w/ 5' monophosphate ← ! uncut RNAs have 5' triphosphate (see ch. 7.7) !
   - degradation of resulting fragments ← creates its own ideal substrate (by cutting – exposes 5' monophosphate)
     - 3'→5': PNPase / RhlB
     - 5'→3': E. coli: repeated cutting by RNase E (there is no 3'→5' exonuclease in E. coli)
       - most bacteria: RNase J – 3'→5' hydrolytic exonuclease

↑ direct access mechanism — 2
↓ 5' end-dependent mechanism — 1
see (ch. 7.12)

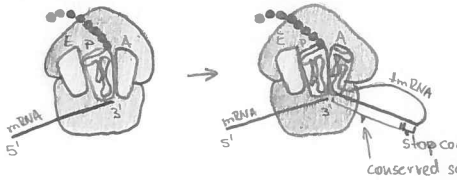
1. 5' triphosphate is cleaved to monophosphate by RppH (RNA pyrophosphohydrolase): UNprotects 5' end of the mRNA
   - cleaves off pyrophosphate
   - one of two mechanisms
     - degrade from 5' end by RNase J ← can NOT act on triphosphate ! ← most organisms
     - degradation by RNaseE (see above: direct access mechanism) ← E. coli

7.10. Bacterial Decay of RNA with Secondary Structure

- 2° structure
  - in 3'UTR (protecting mRNA from decay)
  - internal structures
- PNPase – phosphorylitic exonuclease => adding/removing nucleotides approx. equally favourable
  - if can't remove nucleotides (because of 2° structure), some (poly(A) tail) will be added instead & retry removing them through 2° structure — "running start"
    - ATP is by far most abundant in cells

7.11. Bacterial Rescue of Stalled Ribosomes

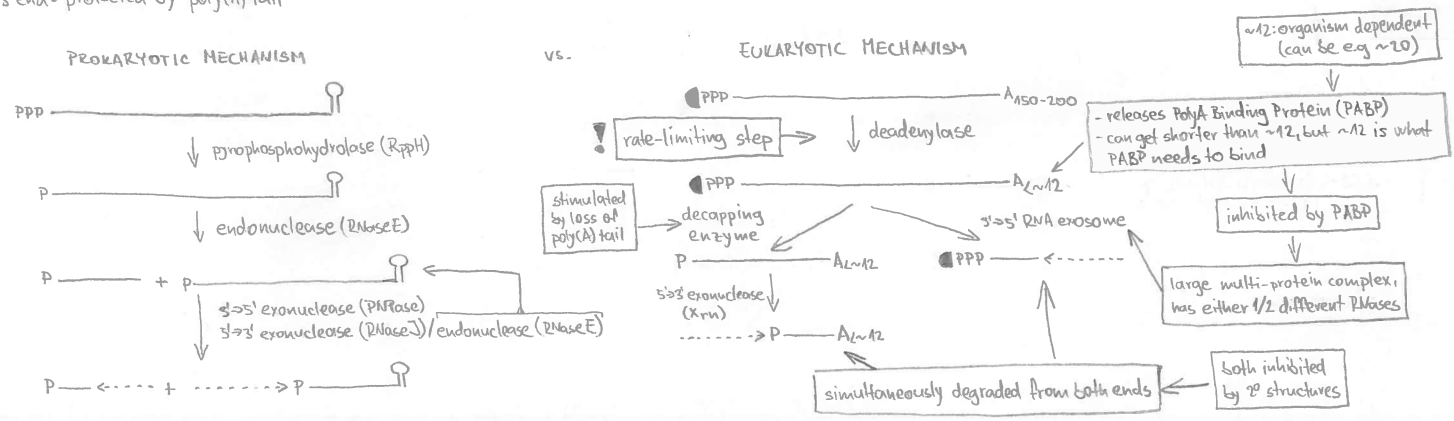
- mRNA decay inevitably captures some ribosomes still translating, reaching cleaved end of mRNA before reaching end codon
  - solved by special tmRNA (called SsrA in bacteria)
    - tRNA: alanine tRNA
    - mRNA: functions as mRNA surrogate
      - can be loaded only if there is no real mRNA occupying A site
      - will displace remaining "real" mRNA, making it available for digestion (unprotected by ribosome)
      - there still can be 1 or 2 nucleotides left in A site, but not more



- AlaAsnAspGluAsnTyrAlaLeuAlaAla
- recognized by ClpXP protease
- incomplete protein immediately degraded

7.12. Eukaryotic mRNA Decay

- 5' end - protected by 5' cap
- 3' end - protected by poly(A) tail



7.13. Eukaryotic Deadenylases

- CCR4-Not - completely inhibited by PABP (PolyA Binding Protein)
- Pan2/Pan3 - stimulated by PABP: part of constitutive turnover rate (acts on all mRNAs as soon as they are made)
- PARN
  - inhibited by PABP
  - stimulated by 5' cap
- each deadenylase stimulated either by
  - PABP
  - 5' cap structure

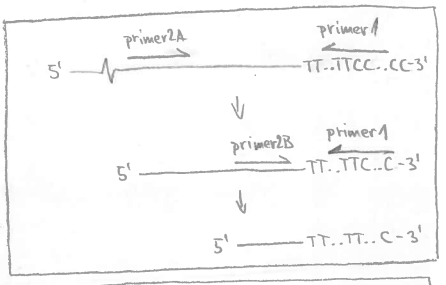
exonucleases specific for A

- mostly recruited (used e.g. for microRNAs)
- mostly constitutive ← also recruited as part of regulation in some cases

typical RNA degradation regulation:
mRNA — RNA-binding protein — deadenylase

7.14. Measuring PolyA Tail Length

- process
  - isolate mRNA
    - e.g. using column w/ oligo-DT (poly-deoxyT): mRNAs will bind by their polyA tails
  - extend polyA tails w/ polyG
    - using PolyA Polymerase & GTP: PPP—AAAAAAA → PPP—AAAAAAAGGGGGGG
    - no A must be present!
  - make cDNA
    - using 3'-TT..TTCC..CC-5' primer
  - PCR
    - using primers
      - 3'-TT..TTCC..C-5' ← the other one will match all mRNAs
      - primer specific to given gene: only this primer has specificity for particular mRNA
      - use two different primers to solve the lower PCR specificity problem
  - measure length of polyA tail (TT..TT sequence of cDNA)
    - compare sizes
    - sequence



both primers should be close enough to beginning of polyA tail ← reason: processivity; time: need to make it in time inside PCR cycle

7.15. Eukaryotic RNA Decapping

see ch.7.4, pg 7.1 → even oligoribonuclease would not be able to degrade capped oligos

- DCP1/DCP2 - typically acts on full-length mRNAs to make them susceptible to Xrn1
- DCPS - "scavenging" decapping enzyme: works on short oligos that are still capped (e.g. leftovers from exosome-only mRNA degradation)
- PolyA Binding Protein forms closed loop w/ EIF4G, stabilizing interaction of EIF4G & EIF4E w/ the 5' cap (we think this, but do not know for sure) ← see ch 3.6 pg 3.2
- deadenylation → no PABP
  - no stabilization of EIF4G & EIF4E — EIF4E sterically inhibits decapping — tul↑ dcp↓: tul↓ dcp↑ — see ch 3.6 pg 3.2
  - recruitment of proteins to 3' end of RNA
    - another theory: stimulates decapping
    - Pat1, Lsm1-7, Dhh1
      - inhibit tul initiation (recruitment of 48S PIC)
      - recruit decapping enzyme & stimulate DCP2 → DCP2 removes 5' cap

7.16. Eukaryotic Ribosome Synthesis and Decay

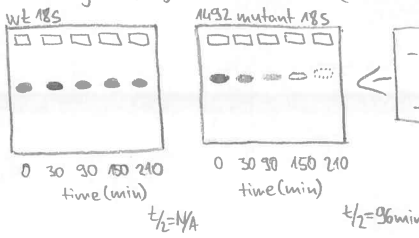
- degradation of misfolded/misassembled rRNA in nucleus/(nucleolus)
  - polyA Polymerase in a complex called TRAMP (Trf4/Air2/Mtr4p Polyadenylation complex) adds polyA to 3' end of rRNA
  - RNA exosome degrades rRNA
- degradation of rRNA in cytoplasm
  - ubiquitinylation of ribosomal proteins and their degradation by proteosome
  - unfold rRNA degraded by RNA exosome (TRAMP?)

responsible for rRNA folding: degradation ⇒ rRNA unfolds

3' end needs to be accessible
usually (not always) true in misfolded ribosomes

mutation ⇒ non-functional ribosome

- ribosome
  - wt
  - 1492 mutant: 1492 & 1493 - adenines that make H-bonds w/ minor groove
- northern blot



$t_{1/2}$ = N/A

$t_{1/2}$ = 96 min

- ribosomal DNA: clusters of repeats, spread throughout chromosomes
- nucleolus: part of nucleus, where ribosomal DNA is being transcribed (recruits proteins & self-assembles around txbed DNA ?)

7.17. Eukaryotic tRNA Decay

- inappropriately modified tRNAs polyadenylated by Trf4 ⇒ degraded by RNA exosome
  - post-txn modifications of tRNA
  - Trf4 part of TRAMP (see ch. 7.16)

seems to be common theme for to-be degraded RNA in both eukaryotes and prokaryotes

added Poly(A) tail not long enough for PABP to bind
- recruits Pat1/Lsm1/Dhh1 that stimulate decapping (see ch. 7.15, pg 7.3)

!