

Transcription

Transcription

Transcription- the synthesis of RNA using DNA as a template.

Four stages: Initiation, Elongation, Termination, Post-transcriptional modification

Similarities and Differences from Replication

Similarities

- 5' → 3' direction
- many proteins involved
- initiation, elongation, termination
- transcription bubble
- starts and stops at specific places
- Nt not dNt

Differences

- RNA polymerase instead of DNA polymerase
- no proofreading
- posttranscriptional modification
- 1 strand copied not 2
- not all copied

Terminology of Transcription

(3') ACG GAA TAA TTA TGT TTA TCA CAA TTT AGA (5') + (plus) strand
 template strand

(5') TGC CTT ATT AAT ACA AAT AGT GTT AAA TCT (3') - (minus) strand
 non-template strand
 coding strand

(5') UGC CUU AUU AAU ACA AAU AGU GUU AAA UCU (3') RNA transcript

Upstream 5' end vs. Downstream 3' end

Initiation

Transcription Start Site is Promoter

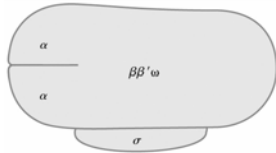
Fig. 26-5 Lehninger POB 3rd Ed. (±UP)

promoter region	-35 region	spacer	-10 region	spacer	RNA start	
<i>rec A</i>	TTGATA	N ₁₇	TATAAT	N ₇	A	
<i>trp</i>	TTGACA	N ₁₇	TTAACT	N ₇	A	
<i>lac</i>	TTTACA	N ₁₇	TATGTT	N ₆	A	
consensus	TTGACA	N ₁₆₋₁₈	TATAAT	N ₆₋₇	A	

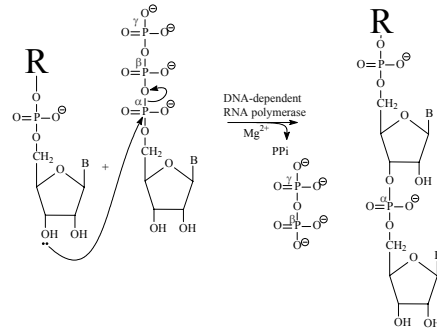
RNA Polymerase DNA-Dependent RNA Polymerase

Fig. 26-4 Lehninger POB 3rd Ed.

- zinc metalloenzyme
- $\alpha\alpha\beta\beta'$ core enzyme (390 kDa)
- with σ subunit, holoenzyme (σ^{70} most common)
- \pm other subunits
- the σ subunit causes specific binding to promoter
- also dependent on NTP's (and Mg^{2+} as usual)
- no requirement for primer
- No proofreading: mistake every 10^4 - 10^5 bases



Elongation

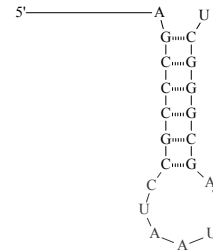


Termination

- Rho (ρ)-dependent vs. ρ -independent

ρ -Independent Termination

- 5'-(N)_n CCCAGCCCCGCCUAAUGAGCGGGCUUU



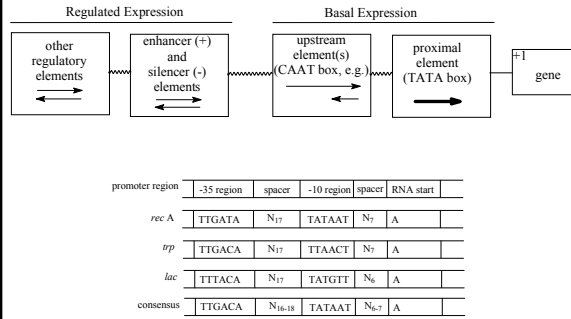
ρ -Dependent Termination

- A hairpin forms
- if protein called ρ is present, polymerase detaches
- Dissociation is somehow coupled to ATP hydrolysis

Comparison with Eukaryotes

<u>Prokaryotes</u>	<u>Eukaryotes</u>
• promoter	promoter + enhancers
• Polymerase	Polymerases I, II and III
• Rho	not Rho
• not as much processing	Processing
• 50-90 nt/sec	Slower

Eukaryote Upstream



Animal RNA Polymerases

Animal DNA-dependent RNA Polymerases

Class	α -amanitin sensitivity	Major Products
I	Insensitive	rRNA
II	Low Conc. (1-10 nM)	hnRNA
III	High conc.	tRNA, 5S RNA and small RNAs

All have in common 2 large subunits and a number of smaller subunits, as well as being zinc metalloenzymes.

Primary Transcript

Primary Transcript- the initial molecule of RNA produced. AKA *hnRNA*

hnRNA-heterogenous nuclear RNA

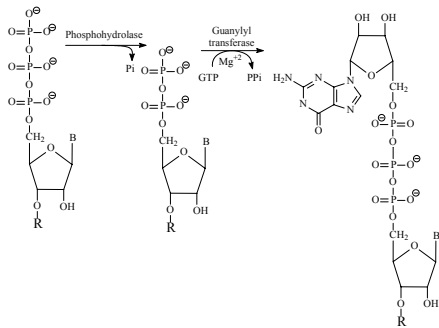
In prokaryotes, DNA \rightarrow RNA \rightarrow protein in cytoplasm concurrently

In eukaryotes nuclear RNA \gg Cp RNA

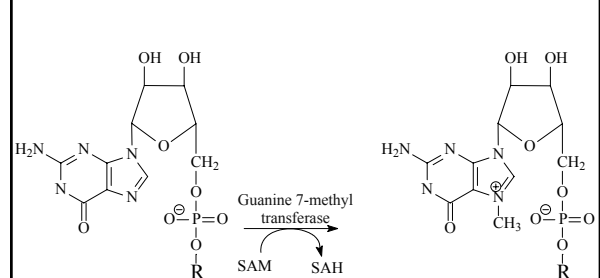
Post-transcriptional Processing of mRNA Occurs in Nucleus

- Cap
 - functions: transport, recognition, prevents exonucleases
- poly A tail
 - functions: prevents exonucleases, recognition, other?
 - Less in prokaryotes
- Introns spliced out- eukaryotes only

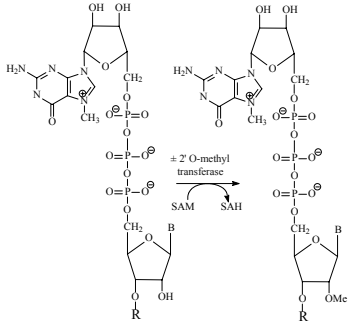
Post-transcriptional Processing of mRNA - Capping



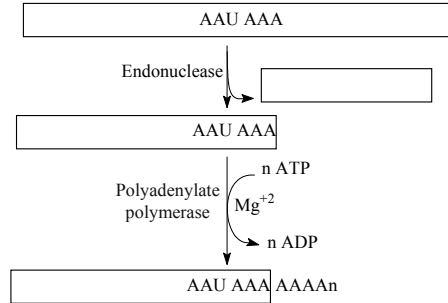
Post-transcriptional Processing of mRNA - Methylating the Cap



Methylation of the 2'-OH of First (and Second) Base Can Also Occur



Post-transcriptional Processing of mRNA - Polyadenylation



Post-transcriptional Processing of mRNA - Splicing out Introns

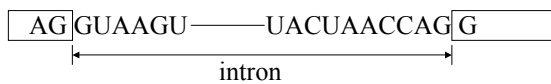
- Complicated
- “snurps” - small nuclear ribonucleoproteins
- snurps 1-6 bind at specific times (no details)
- RNA catalysts
- lariat structure

Intron Groups and Characteristics

Group	Types	Characteristics
I	N, Mw, Chl mRNA & tRNAs	no ATP req'd (ribozyme) GMP, GDP or GTP used
II	N, Chl mRNAs	no ATP, lariat (ribozyme)
*III	N mRNAs	Lariat structure (details following)
IV	tRNA	ATP required, endonuclease

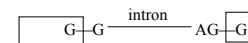
*largest class

Group II Intron Consensus Sequence



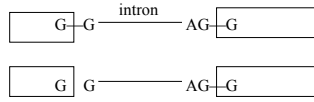
Group II Intron Splicing

- U1 base-pairs first by 5' end of intron



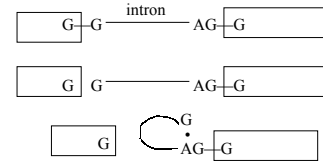
Group II Intron Splicing

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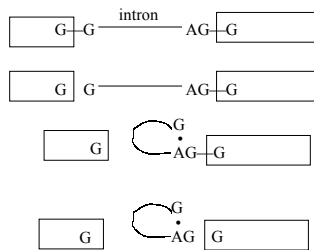
Group II Intron Splicing

- U1 base-pairs first by 5' end of intron
- U2 binds *branch site* and directs U1 binding there



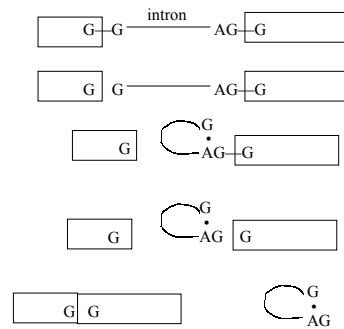
Group II Intron Splicing

- U1 base-pairs first by 5' end of intron
- U2 binds *branch site* and directs U1 binding there
- U5 and U4/U6 then bind
- U4 released
- U2/U6 causes cleavage?

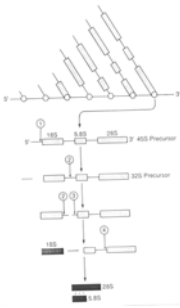


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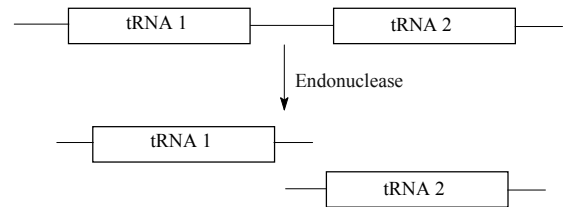
Processing of rRNA Like Fig. 26-23



- RNA synthesized longer than needed (*polyenes*)
- structural portions methylated
- non-methylated parts degraded
- RNA associates with protein
- final shape globular

Processing of tRNA

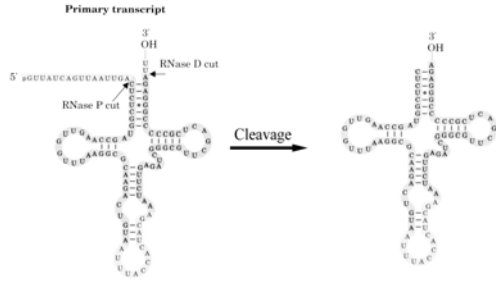
- Synthesized longer than needed...



Processing of tRNA

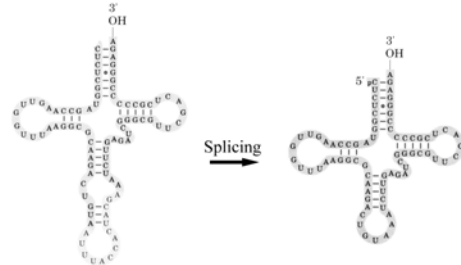
Fig. 26-25 Lehninger POB 3rd Ed.

Synthesized longer than needed... trimmed...



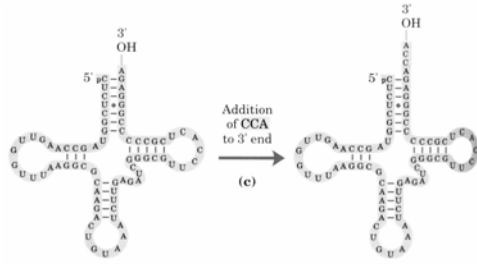
Processing of tRNA

Synthesized longer than needed... trimmed... spliced...



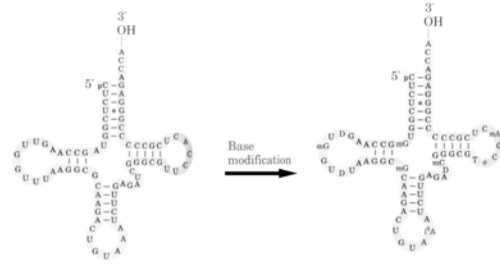
Processing of tRNA

Synthesized longer than needed... trimmed... spliced... CCA added...



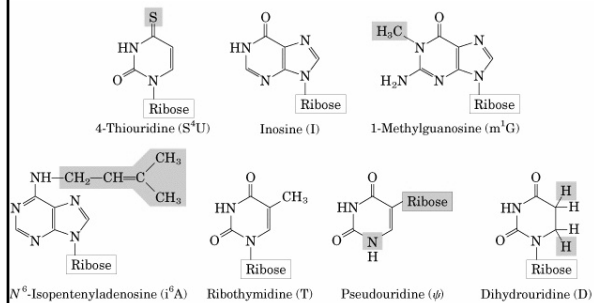
Processing of tRNA

Synthesized longer than needed... trimmed... spliced... CCA added... and bases modified.



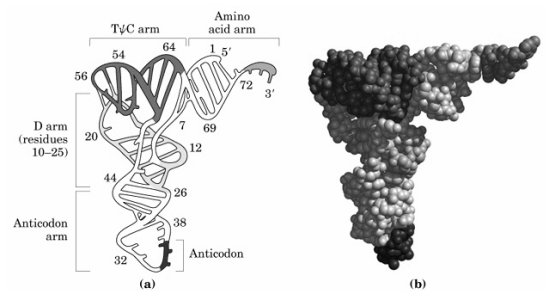
Base Modification

Fig. 26-26 Lehninger POB 3rd Ed.



Final Structure "Cloverleaf"

Fig. 27-15 Lehninger POB 3rd Ed.



Advanced Topics

- Regulation of mRNA half-life
- Alternative splicing

mRNA Half-life

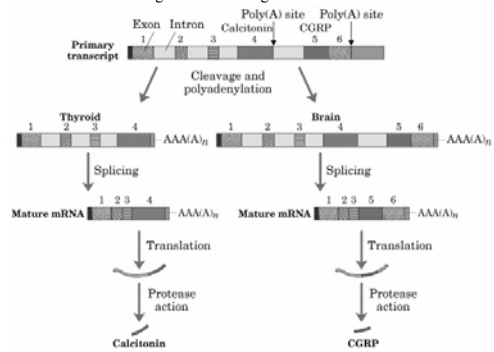
- $t_{1/2}$ seconds if seldom needed
- $t_{1/2}$ several cell generations (i.e. ~48-72 h) for housekeeping gene
- \approx avg 3 h in eukaryotes
- \approx avg 1.5 min in bacteria

mRNA degrades by RNase

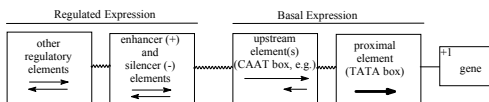
- Exonuclease
- Major p/w:
 - Uses 5'3'
 - Deadenylate
 - Decap
 - Degrade
 - 3' sequence inhibits

Alternative Splicing

Fig. 26-22 Lehninger POB 3rd Ed.



Eukaryote Upstream



- Promoter tells *where* to start
- Boxes tell *how often* to start
- TATA box has many proteins associated: TBP, TFII₁, TAF's

Boxes and Factors

Harpers Review of Biochemistry

Table 39-3. Three classes of transcription factors in class II genes.

General Mechanisms	Specific Components
Basal components	TBP, TFIIA, B, E, G, F, H, and J
Coactivators	TAFs
Activators	SP1, ATF, CTF, AP1, etc.

- Cis- vs. Trans-acting factors

Element	Consensus Sequence	Factor
TATA box	TATAAA	TBP
CAAT box	CCAATC	C/EBP α , NF-Y α
GC box	GGGCGG	Sp 1
	CAACTGAC	Myo D
	TTCGGA/CN ₁ GCCAA	NF1 α
Sp octamer	ATGCAAAAT	Oct-1, 2, 4, 6 α
SP 1	TGAG/CTGAAA	Jun, Fos, ATF α
Serum response	GATGCCCATTA	SRF
Heat shock	(NGAAN) ₂	HSF

Enhancers Work Upstream, Downstream or in the Middle of a Gene

- They also work forwards or backwards
- Possible ways of working
 - Different transcription factors
 - Order of binding (differing concentrations)
 - Affinity of transcription factors