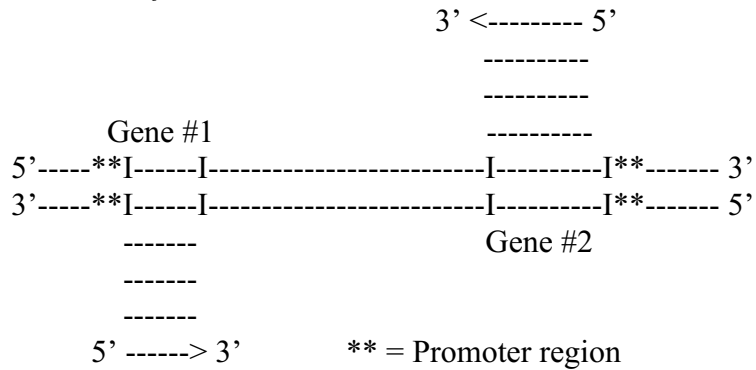


Transcription – RNA Synthesis

Direction: RNA formed 5' to 3'
Enzyme: RNA polymerase
Start: Promoter region
Template: 1 strand / gene
Product: Multiple RNA copies of gene
Timing: Most genes not continuously transcribed
Result: Specialized cells contain different mRNAs

Overview of Process:



Close View of Transcription:

Time 0:

Transcription bubble:	~ 20 bp	RNA/DNA hybrid:	~12 bp
RNA polymerase binds:	~50 bp	Rate of movement:	~ 50 bp/sec

< 1 Second Later:

RNA Polymerase

Large, multimeric enzyme

Catalyzes RNA synthesis from DNA template

*Prokaryotes: Same enzyme for mRNA, tRNA, rRNA
Sigma factor: Confers binding specificity*

*Eukaryotes: Different enzymes for different RNAs
I: rRNAs; II: mRNAs; III: tRNAs
Differ in sensitivity to alpha amanitin
Toxin present in some mushrooms*

Other proteins bind to promoter before RNA polymerase

*Binding required for proper gene function
Example: TATA binding protein
Recognizes short nucleotide sequence in promoter
Facilitates RNA polymerase binding*

Promoters:

*Sequence of DNA just “upstream” of gene
Vary in size, activity, and nucleotide sequence
Typically 50-100 bp long
Contain conserved (consensus) sequences*

Examples: TATA and CAT boxes

*Found in prokaryotic and eukaryotic promoters
Binding sites for proteins that regulate transcription*

Promoters vary in strength (RNA polymerase binding)

Strong Promoters:

*Efficient RNA polymerase binding
High level of transcription
Abundant protein (usually)*

Weak promoters:

*Inefficient RNA polymerase binding
Low level of transcription
Minimal protein (usually)*

Regulation of RNA Polymerase Binding:

Regulatory DNA Sequences:

Usually located upstream of promoter

*Examples: Enhancers, Signal response elements
Often called “boxes”*

*Function: Binding sites for transcription factors
Facilitate RNA polymerase binding*

Transcription Factors:

DNA binding proteins

Recognize and bind to regulatory DNA sequences

Proteins have conserved motifs to enable binding

Examples: Zinc fingers and Leucine zippers

*Cellular signals often regulate presence / activity of
transcription factors via signal transduction pathway*

*Disruption of these pathways may cause uncontrolled
cell growth (tumors)*

Termination of Transcription in Prokaryotes:

*Determined by: Terminator sequences in DNA
Shape of RNA transcript (3')
Associated proteins (e.g. rho)
Stability of RNA:DNA duplex*

Many terminators cause hairpin loop in RNA (3')

RNA pairs back on itself in hairpin loop

Product of palindromic DNA sequence

Palindromes: Read same forwards and backwards

*WAS IT A CAT I SAW
ABLE WAS I ERE I SAW ELBA*

*DNA: 5' --- GGTACC--- 3'
3' --- CCATGG--- 5'*

*Hairpin destabilizes RNA:DNA duplex
RNA polymerase and RNA transcript released*

Prokaryotic mRNAs:

Unstable in cell: Half life < 15 minutes
Need constant transcription for steady supply of protein

Some mRNAs are monocistronic: Copy of 1 gene

Others are polycistronic: Copy of >1 adjacent gene

These are products of gene clusters (operons)
Operons common in prokaryotes; rare in eukaryotes
Clusters of bacterial genes, coordinately transcribed,
that code for proteins with related functions

Monocistronic mRNAs in Prokaryotes:

Coding region defined by start and stop codons
Untranslated regions at 5' and 3' ends
Translation often begins before transcription ends
Processes not separated by nuclear membrane

Drawing of Prokaryotic mRNA:**Simultaneous Transcription / Translation:**

Eukaryotic mRNAs:

Not exact copy of gene; introns removed by splicing

Several post-transcriptional modifications to RNA

Added by enzymes; not coded in genes

Methyl (G) “cap” at 5’ end of RNA

Unusual S-P-S linkage (5’ – 5’)

May facilitate ribosome binding

Poly (A) “tail” at 3’ end of RNA

50-200 ‘A’s added to RNA

Present in most eukaryotic mRNAs

May increase mRNA stability

Drawing of Eukaryotic mRNA:

RNA Splicing in Eukaryotes:

Removal of introns from hnRNA

Introns discovered by:

Visualization of mRNA / DNA hybrids in E.M.

Intron regions fail to hybridize with mRNA

Form large ss loops visible under microscope

Comparison of mRNA / DNA nucleotide sequences

Intron sequences missing from mRNA

Allows precise definition of intron / exon borders

Several different mechanisms of splicing discovered:

Group I Introns: rRNA Transcripts in Protozoans

Intron provides catalytic activity for own removal

Self-splicing introns (ribozymes) in Tetrahymena

Discovery of process resulted in Nobel Prize

Group II Introns: Some RNAs in Organelles

*Process discovered in mitochondria and chloroplasts
Self splicing but different mechanism from Group I*

*Group III : Removal of Introns from hnRNA
Regulated by spliceosome in nucleus*

Spliceosome: *Large complex of RNA and proteins
Equal size to large subunit of ribosome
snRNAs and proteins form snRNPs
snRNPs recognize intron boundaries
Intermediate lariat (loop) structure*

Spliceosome : Splicing :::: Ribosome : Translation

Differential Splicing: *Characteristic of some genes*

*Different mRNAs produced from same hnRNA
Occurs in different tissues of multicellular organism
Specific intron removed in one tissue, not another
Consequence: Different proteins from same gene
Rare but includes some important regulatory proteins*

RNA Editing: *Bizarre example of RNA processing*

*Post-transcriptional modification of RNA sequence
Common in plant mitochondria and chloroplasts
Some changes are substitutions (nucleotide changed)
Other changes are insertions/deletions of nucleotides
Changes must be made before translation*