

nucleotide & nucleic acids

nucleotide	sugar + N base + phosphate backbone
nucleoside	^ - phosphate
nucleotide functions	energy for metabolism (ATP) enzyme cofactors (NAD+) signal transduction (cAMP)
nucleic acid functions	storage of DNA transmission of DNA processing of ribozymes protein synthesis regulation of expression

DNA & RNA are polymers of nucleotide subunits which are linked by phosphodiester bonds

DNA structure

two chains of nucleotides coiled around each other in a right-handed double helix
sugar-phosphate backbones of two strands spiral around outside of helix
N bases extend into centre at right angles to the acids of helix
adenine forms 2 H bonds with thymine
cytosine forms 3 H bonds with guanine
opposite polarity of two strands

RNA types

rRNA	80%	120 -5070 nucleotides
tRNA	15%	75 nucleotides
mRNA	varies	varies

RNA molecules

mRNA	intermediates that carry genetic information from DNA to ribosomes
tRNA	adaptors between amino acids & codons in mRNA
rRNA	structural & catalytic components of ribosomes
siRNA	RNA interference
long non-coding RNA	transcription
microRNAs	RNA interference
ribozymes	RNA enzymes



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siRNA -> ribozymes are regulatory

mRNA carries instructions for building a protein, eukaryotic mRNA is capped, polyA tail is not coding & is added after transcription to stabilise mRNA - its removal degrades RNA & inhibits translation

transfer RNA is an intermediary between nucleic acid & protein worlds, acts as a translator

assembly of RNA polymerase	initiated by interaction of TATA-binding protein (TBP) with the promoter, two TF's bind (IIA & IIB) ,TFIIE and TFIIH bind: TFIIF binds to RNA Pol and targets it to the promoter, TFIIE thought to be involved in DNA melting, Helicase activity in TFIIH unwinds DNA at the promoter
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transcription in eukaryotes (cont)

RNA strand initiation & promoter clearance	Kinase activity in TFIIH phosphorylates the polymerase allowing the latter to escape the promoter, Initially 60-70 RNA nucleotides are synthesised, Then TFIIIE & TFIIH are released
elongation, termination & release	TFIIF remains attached to RNA Pol II, Elongation factors help efficiency, EF stop pausing and regulate post-transcriptional processing, phosphate removed at termination

RNA processing

Almost all newly synthesised RNA molecules (primary transcripts) are processed to some degree in eukaryotic cells

The 5'-end is capped with methylguanosine

Introns are spliced out

Poly-A tail is built at the 3' end - it probably protects 3' end from enzymatic destruction. However some bacteria acquire Poly A tails but these promote destruction.

capping of 5' of mRNA

protects mRNA from 5'exonuclease degradation

Cap is 7-methylguanosine linked to 5' end of mRNA

Formed by condensation of GTP with 5' end of mRNA

Guanine is then methylated

Occurs early in transcription

capping enzymes are tethered to the c-terminal domain of polymerase II

placing poly (A) tail on mRNA

Pol II synthesises RNA up to and beyond the highly conserved seq: (5')AAUAAA

An endonuclease cleavage signal seq is bound by an enzyme complex

The RNA is cleaved by the endonuclease at a point 10-30 nucleotides 3' to (downstream of) the sequence AAUAAA

The polyadenylate polymerase synthesises a poly(A) tail

transcription & RNA processing

the central dogma of biology is that information stored in DNA is transferred to RNA molecules during transcription & to proteins during translation. information stored in the nucleotide sequences of genes is translated into the aa seqs of proteins through unstable intermediaries (mRNAs). the mRNA codons on mRNA are translated into an aa seq by the ribosomes



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transcription & RNA processing (cont)

in eukaryotes, the primary transcript is pre-mRNA. it is modified at both ends & introns are removed to produce mRNA. it is then exported to cytoplasm for translation by ribosomes.

in RNA synthesis, the precursors are ribonucleoside triphosphates, only 1 strand of DNA is used as template & RNA chains can be initiated de novo (without primer). RNA molecule will be complementary to DNA antisense (template) strand & identical to DNA sense (non-template) strand). catalysed by RNA polymerases & proceeds in 5' to 3' direction

transcription vs DNA replication

RNA does not remain H-bonded to DNA post-synthesis

RNA molecules are selective copies of shorter DNA segments

both employ polymerases - to make phosphodiester linkages

DNA is unwound ahead of synthesis

similar building blocks

RNA polymerase does not need a primer

DNA in a human chromosome can be up to 250 million bases whilst most RNA molecules are a few thousand bases in length.

RNA polymerase makes an error 1×10^4 nucleotides compared to 1×10^7 for DNA polymerase. As RNA is temporary it is not so critical. Still RNA polymerase has a proof reading mechanism.

the 'transcription bubble' - because of unwinding & rewinding there are positive supercoils ahead of the bubble & negative behind. topoisomerases deal with positive supercoils & regulate negative ones.

transcription in prokaryotes

stages 1. RNA chain initiation, 2. RNA chain elongation, 3. RNA chain termination

e.coli RNA polymerase core enzyme = $\alpha_2\text{-}\beta\text{-}\beta'\text{-}\omega$

holoenzyme = $\alpha_2\text{-}\beta\text{-}\beta'\text{-}\omega\text{-}\sigma$

α = assembly of the tetrameric core

β = ribonucleoside triphosphate binding site

β' = DNA template binding region

σ = initiation of transcription

promoters must be >12 bp in e.coli to avoid occurrence by chance, have only small conservation in sequence

Startpoint, -10 sequence (Pribnow box), -35 sequence and, the 17 nucl spacer seq between -10 & -35 seqs

70 bases in length before start point & 30 after.

transcription unit numbering initiation site is +1



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transcription in prokaryotes (cont)

Bases preceding the initiation site are given minus (-) prefixes and are referred to as upstream sequences

Bases following the initiation site are given plus (+) prefixes and are referred to as downstream sequences

1. binding & initiation Binding of RNA polymerase holoenzyme to a promoter region in DNA

Localised unwinding of both DNA strands (around -10 region) by RNA polymerase to provide a single-stranded template

Formation of phosphodiester bonds between the first few ribonucleotides in the nascent RNA chain

Conformational change in enzyme, promoter is cleared - sigma factor released

Nus A protein binds instead, ready for elongation - 'antitermination complex'

sigma cycle RNA polymerase, guided by a bound sigma subunit, binds to DNA at a promoter sequence. Once RNA synthesis is initiated, the sigma subunit dissociates stochastically and is replaced by NusA. When RNA polymerase reaches a terminator sequence, RNA synthesis halts, NusA dissociates from the polymerase, and the RNA polymerase dissociates from the DNA. The free polymerase can, in principle, bind any sigma subunit. The type bound determines the promoter to which the RNA polymerase will bind in the next round of synthesis.

2. elongation RNA polymerase is bound to DNA & is covalently extending the RNA chain, moves downstream.

Transcription bubble is about 18 nucleotides pairs and about 40 bases are added per second. Only about 3 bases are base paired at any moment in time.



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transcription in prokaryotes (cont)

3. termination	RNA polymerase transcribes until it meets a terminator, Transcription then stops & the RNA product disassociates from the DNA template, Many terminators are hairpin forming sequences
	Rho-dependent terminators — require a protein factor
	Rho-independent terminators — do not require protein factor
rho-independent termination	G-C rich stem, 7-9 bases after loop is U-run, U-DNA pairing is very weak allows dissociation
rho-dependent termination	(rho factor) 46-kD protein, active as a hexamer, Seqs for the few Rho terminators are 50-90bp, Rho binds to RNA
	(hot pursuit model) - It binds to RNA tail and moves along transcript until it catches the polymerase, Rho has helicase activity causing RNA-DNA to separate

introns

- non-coding seqs located between coding sequences
- removed from the pre-mRNA and are not present in the mRNA
- Exons (both coding and non-coding sequences) are composed of the seqs that remain in the mature mRNA after splicing
- Introns are variable in size and may be very large



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