RNA

- Deoxyribose —> Ribose
- Normally single stranded
- RNA sequence complementary to template strand, near identical to coding strand.
- Thymine —> Uracil

RNA polymerase

- High specificity, low fidelity (more error prone than DNA polymerase)
- Prokaryotes have 1, Eukaryotes have 3
- Core structural subunits of prokaryote (conserved in eukaryote):
    - β' subunit: DNA binding
    - β subunit: rNTP binding
    - α subunit *2: scaffolding
    - ω subunit: unknown function
- Eukaryotic RNA polymerase have 10 core subunits

Prokaryotic transcription initiation complex - σ factor

- σ-70 bind to upstream promotor region(-35bp & -10bp), E.coli have σ-70 as primary housekeeping factor
- Region σ1 stabilise dsDNA, and RNA pol III binding
- Region σ2 separates the two strands
- 3.2 region: σ3 and 4 create loop, use it to position incoming rNTP to polymerase active site
- σ1&2 bind at -10bp, σ3&4 bind at -35bp, if -35 bp promotor region absent, then σ35 recognise an extended -10 promotor
- Threre is also an upstram promotor element upstream of -35bp, bound by C-terminal domain of α-subunit.

Eukaryotic transcription start site: core promotor elements

- TFIIB recognition element (BRE): -30~-40bp, TFIIB binding
- TATA box (TA rich sequence): -30~-25bp, TATA-binding protein (TBP) (Main unit of TFIID) binding
- Initiator element (INR): -2~+4bp, TFIID binding (TFIID-associate unit (TAF))
- Motif Ten Element (MTE): 20~30 bp, TFIID binding (TAF)
- Downstream promotor element (DPE): 30~50bp, TFIID binding (TAF)
- Downstram core element (DCE): 30~50bp, TFIID binding (TAF)

Eukaryotic Preinitiation complex Assembly:

- TFIID main unit TBP bind to TATA-box
- TFIID associated factors bind to INR, MTF, DPE, DCE
- TFIIA stabilise TBP binding
- TFIIB bind to BRE, help position Pol II
- TFIIF bring Pol II to bind with DNA
- TFIIE bind to DNA, recruit TFIIH, which uses ATP to unwind DNA, allow preinitiation complex —>open complex

Holoenzyme: additional structures: Activators bind to distal enhancer region, act through the mediator complex

- Mediator (~20 enzymes mediate distal enhancer region to transcription complex)
- Nucleosome modifier (chromatin remodeller)
- Transcriptional activator (Histone acetyltransferase)

Transcription

- Around ~14bp of dsDNA unwinded, transcription bubble forms.
- ~8bp of DNA-RNA hybrid form within the polymerase
- Abortive transcription first, short, repeated failure producing short transcripts
- Polymerase undergoes conformational change close stably around DNA, (passive in Prokaryotes, ATP required in Eukaryotes) Preinitiation complex - Open complex
- Detach from transcription factors, start actual transcription, open complex - Elongation complex

Elongation

- One bp of DNA separate - one rNTP added
- 2 ssDNA kept separate with specific Polymerase regions.
- 20~50 bp per second
- Coiling released by topoisomerases

Prokaryotic termination mechanisms: Intrinsic and Rho-dependent:

- Intrinsic:
    - Inverted repeating sequence create a hairpin structure on the RNA molecule
    - Repeating Adenine on template strand - long U on nascent RNA - weak A-U bonding - RNA released
- Rho-dependent:
    - Rho is a hexameric protein, bind to 5' end of RNA at Rho utilisation site
    - Rho translocate along the RNA to polymerase
    - Transcription stop when hairpin structure formed, Rho catch up
    - Rho pull RNA away from polymerase / induce conformational change - RNA hydrolysis

Eukaryotic termination mechanisms: Allosteric and Torpedo

- Allosteric:
    - Cleavage stimulatory factor (CStF) and Cleavage and polyadenylation specificity factor (CPSF) bind to phosphorylated area on the CTD
    - When poly-A signal present, changes in phosphoylation of CTD on α-subunit, release CStF and CPSF
    - CStF and CPSF transfer to RNA, cleaves
    - Transfer of CStF and CPSF cause Pol conformational change, disengage from DNA
- Torpedo:
    - RNase bind allosterically on RNA Pol II
    - Poly A tail presence cause a change in phosphorylation pattern - RNase move to degrade RNA
    - Transcript protected by poly-A tail and 5' end cap, uncapped nascent RNA from polymerase is degraded.

RNA structure

RNA polymerase characteristic and structure (Pro & Eu)

Transcription start site (Pro + Eu)

Initiation complex (Pro + Eu)

Transcription initiation event

Elongation

Termination mechanism 2 each