

- 1  **Chapter 13**  
Transcription
- 2  **Primeval world**
  - ▶ 3.5-4 billion years ago
  - ▶ Ribozymes
    - ▶ Catalytic RNA
    - ▶
    - ▶ Central Dogma
    - ▶
- 3  **13.1 RNA**
  - ▶ Ribose sugars
    - ▶ Free hydroxyl on 2' carbon
    - ▶ Less stable than deoxyribose
  - ▶ Uracil
    - ▶ Replaces thymine
  - ▶ Single stranded
    - ▶ Can form secondary structures on itself
    - ▶ Structure determines function
- 4 
  - ▶ Types
    - ▶ rRNA
      - ▶ Ribosomal
    - ▶ mRNA
      - ▶ Messenger
    - ▶ tRNA
      - ▶ Transfer
    - ▶ Others
      - ▶ eukaryotic
  - ▶
- 5  **13.2 Transcription**
  - ▶ Similar to replication
  - ▶ Individual genes as needed
  - ▶ Components
    - ▶ DNA template
    - ▶ Substrates
    - ▶ Transcription apparatus
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  - ▶ DNA to RNA
  - ▶ Template strand
    - ▶ Coding
  - ▶ Non-template strand
    - ▶ Non-coding (not used)
- 7 
  - ▶ Transcription unit
    - ▶ Promoter
    - ▶ RNA-coding region
    - ▶ Terminator
    - ▶
    - ▶
    - ▶

- ▶
- ▶
- ▶

- ▶ Location terminology
  - ▶ Downstream are positive
    - First transcribed nucleotide +1
  - ▶ Upstream are negative

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- ▶ Ribonucleoside triphosphates
  - ▶ Added to 3'OH of RNA molecule
  - ▶ Antiparallel
  - ▶ complementary
  - ▶ No primers needed

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### **Transcription apparatus**

- ▶ Prokaryotic
  - ▶ holoenzyme
    - ▶ RNA polymerase
      - Multiple subunits
    - ▶ Sigma factor
      - Binds to promoter
      - Multiple types
- ▶ Eukaryotic
  - ▶ Many types of RNA polymerase
    - ▶ Many subunits
    - ▶ Multiple accessory proteins

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### **13.3 Process of bacterial transcription**

- ▶ Initiation
- ▶ Elongation
- ▶ Termination

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#### **Initiation**

- ▶ Promoter recognition
  - ▶ -10 consensus sequence
    - ▶ TATAAT
  - ▶ -35
    - ▶ TTGACA
  - ▶ Sigma factor
    - ▶ Some contain upstream elements
      - ▶ -40 to -60

- ▶

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- ▶ Formation of transcription bubble
  - ▶ Core RNA polymerase
    - ▶ Covers -50 to +20
    - ▶ Conformational change tightly binds & unwinds DNA
      - Beginning within -10 sequence
      - End at ~+2
      -

- ▶

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- ▶ First rNTP bonds
  - ▶ template strand
    - ▶ Determined by orientation & spacing of consensus
    - ▶ Initiation at +1
  - ▶ No primers
    - ▶ Initial nucleotide retains 5' phosphates
  - ▶ Abortive attempts
    - ▶ 2-6 nucleotides
- ▶ Escape from promoter
  - ▶ 9-12 nucleotides

14 15 **Elongation**

- ▶ RNA polymerase
  - ▶ Conformational change releases polymerase from consensus
  - ▶ Sigma is released
  - ▶ 40 nucleotides /second
  - ▶ Transcription bubble
    - ▶ ~18 nucleotides
    - ▶ ~ 8 RNA-DNA pairs
  - ▶ Exonuclease activity
- ▶ Topoisomerase
  - ▶ Relieves stress of moving transcription bubble
- ▶ Transitory pauses

16 **Termination**

- ▶ Terminators
  - ▶ Transcribed before affecting elongation
- ▶ Rho-independent terminators
  - ▶ Terminate without rho factor protein
  - ▶ Inverted repeats cause hairpin formation
    - ▶ Followed by string of uracil
  - ▶ Hairpin destabilizes the A-U pairs
  - ▶ RNA separates from template
- ▶
- ▶
- ▶

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- ▶ Rho-dependent terminators
  - ▶ Terminate only with rho factor protein
  - ▶ DNA sequence stalls polymerase
  - ▶ No upstream secondary structures
    - ▶ Allows rho to bind
  - ▶ Rho catches polymerase at stall site
    - ▶ Helicase activity separates RNA from template
- ▶
- ▶ Polycistronic RNA
  - ▶ Multiple genes
- ▶

18 **Eukaryotic Transcription**

- ▶ Similar to prokaryotic
  - ▶ Initiation, elongation, termination
  - ▶
- ▶ Three different polymerases
  - ▶ Each recognizes different promoters
  - ▶ Each have a different set of associated proteins
- ▶ Chromatin structure
  - ▶ remodeled prior to transcription

#### 19 **Promoters**

- ▶ General transcription factors bind
  - ▶ Recruit correct promoter
    - ▶ Basal transcription apparatus
  - ▶ Transcriptional activator proteins
    - ▶ Stimulate assembly
- ▶

#### 20

- ▶ RNA polymerase II
  - ▶ Proteins
  - ▶ Core promoter
    - ▶ Recognizes TATA Box
      - TATAAA
      - -25 to -30 upstream
    - ▶ Additional sequences
  - ▶ Regulatory Promoter
    - ▶ Immediately upstream of core promoter
    - ▶ Affect rate of transcription initiation
  - ▶ Enhancers
    - ▶ Distant location
- ▶ Polymerase I & III
  - ▶ Functional RNA
  - ▶ Contain internal promoters
    - ▶ Downstream of start site

#### 21 **Initiation**

- ▶ Basal transcription apparatus
  - ▶ RNA polymerase
  - ▶ Transcription factors
    - ▶ TFII
      - A, B, D, E, F, H
  - ▶ Mediator proteins
  - ▶ TFIID subunit (TBP)
    - ▶ Recognizes TATA box
    - ▶ Binds to minor groove
    - ▶ Bends DNA
- ▶ Polymerase & additional factors bind
  - ▶ 11-15 bp open

#### 22 **Elongation**

- ▶ Promoter escape
  - ▶ ~ 30 bp of RNA

- ▶ Many transcription factors remain at start site
- ▶ Elongation
  - ▶ Transcription bubble
    - ▶ ~15 nucleotides
    - ▶ ~ 8 RNA-DNA pairs
    - ▶ DNA at right angle in polymerase

- ▶
- ▶

23  **Termination**

- ▶ Different for each polymerase
  - ▶ Pol I
    - ▶ Factor similar to rho
    - ▶ Binds to DNA downstream not RNA
  - ▶ Pol III
    - ▶ Terminator sequence
      - String of uracil in RNA
  - ▶ Pol II
    - ▶ Continues hundreds –thousands past coding sequence
    - ▶ Pre-mRNA cleaved from polymerase
    - ▶ Remaining 5'end recognized by enzyme Rat1
      - Degrades RNA 5'-3'
      - Terminates transcription when it catches polymerase