Week 8
Genetics: BIO-2306

The concepts this resource covers are the topics typically covered during this week of the semester. If you do not see the topics your particular section of class is learning this week, please take a look at other weekly resources listed on our website for additional topics throughout the semester.

We also invite you to look at the group tutoring chart on our website to see if this course has a group tutoring session offered this semester.

If you have any questions about these study guides, group tutoring sessions, private 30 minute tutoring appointments, the Baylor Tutoring YouTube channel or any tutoring services we offer, please visit our website www.baylor.edu/tutoring or call our drop in center during open business hours. M-Th 9am-8pm on class days 254-710-4135.

Keywords: Transcription, Ribonucleic Acid (RNA), Promoter, Polymerase

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Topic of the Week: ***Transcription in Prokaryotes vs In Eukaryotes*** (13.3-4)

Prokaryotic:

Initiation

1. Promoter Recognition:
    a. The core enzyme of ***RNA-pol*** binds to the **σ factor** to form the ***RNA-pol holoenzyme***. This allows the polymerase to bind
        i. Bacterial ***RNA-pol*** binds to *consensus sequences* at **TTGACA** (-35) and **TATAAT**(-10)
2. Formation of **transcription bubble**: binding ***RNA-pol holoenzyme*** begins to unravel DNA (**FYI:** the **β'** subunit contains the active site)
3. Synthesize first bonds between rNTPs (**note:** the first nucleotide keeps all *3 phosphates*)
4. Escape of Transcription apparatus from promoter: ***RNA-polymerase*** undergoes a change in shape that causes it to *release* **σ** and 'escape' the promoter to move downstream, synthesizing RNA

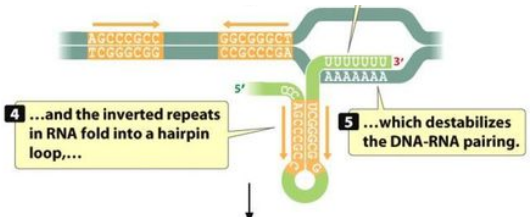
Elongation

RNA-pol acts as a helicase to unwind **downstream** DNA and rewind **upstream** DNA; it also adds rNTPs complementary to the **template/non-coding** strand in the **5' → 3'** direction

Termination (once *RNA-pol* reaches the terminator)

Rho-Dependent Termination: a protein (***rho***) binds to the **rut** site on the RNA transcript (a consensus sequence). The protein travels up (5' → 3') toward *RNA-pol* and uses helicase activity to unwind the new RNA from the DNA template

Rho-Independent Termination: **inverted repeats** and/or **poly-uracil stretches** of RNA are transcribed and form hairpins and destabilize connection of RNA transcript to the DNA template (ie the transcript loses affinity for DNA and falls off)



Eukaryotic: https://www.youtube.com/watch?v=WsofH466lqk

Initiation

Promoters:

Core Promoter: stretch containing (-25) **TATA** consensus sequence where the *basal transcription apparatus* (**BTA** aka *holoenzyme*) binds:

BTA: the complex that makes up ***RNA-pol-II*** at the promoter

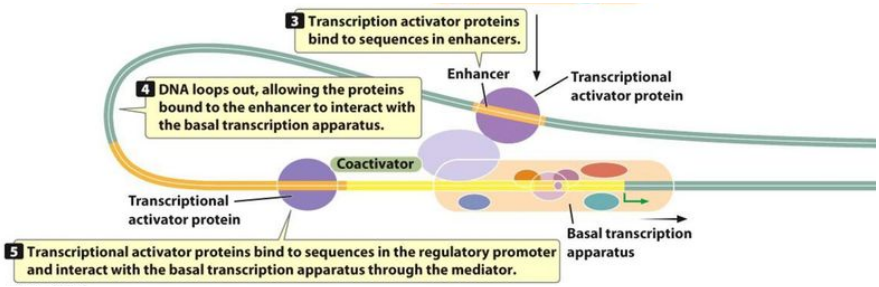
TFII-D (protein complex containing specific protein **TBP**) binds to the **TATA box**; other TF's (**TFII-A,B,E,F,H**) bind and help position the active site of ***RNA-pol-II*** over (+1) the transcription start site

Mediator: a protein chain on the **BTA** which will interact with **TAP's** at enhancers or regulatory sites

Regulatory Promoter: a stretch of DNA upstream of core promoter where **TAP's** bind:

Coactivator: a protein which connects a **TAP** at the *regulatory promoter* to the **mediator** on the **BTA**

Enhancer: a stretch of DNA <u>far</u> upstream from the core promoter and influences transcription ***directly*** through **TAP**- **mediator** interaction



Above: the basal transcriptional apparatus (BTA/*holoenzyme*) and regulatory sites of a eukaryotic transcription unit

Elongation (Same basic steps as bacterial Elongation)

Termination (once *RNA-pol* reaches the terminator)

Proteins cleave the **3'** end of the RNA transcript <u>while transcription is still happening</u>; **Rat-1** (an RNA degrading protein) bind sto the **5'** end of the cut and "chews" its way up the excess RNA until it reaches ***RNA-pol-II*** and causes it to leave the DNA

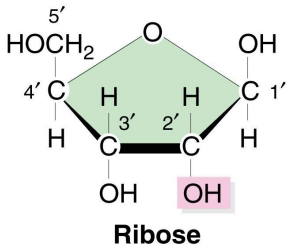
****recall:** polymerases add NTPs to a free **3'-OH** to form a ***phosphodiester bond*** with the **5'-OPO_3^{2-}** of the NTP****

Highlight #1: Types of RNA (13.1//14.3-5 → see also: table 13.2)

RNA: nucleic acid with ribose sugar base which may be single stranded linear, or form complex 3D or double stranded folds

mRNA: the type of RNA which encodes the information needed to translate proteins (**Nucleus** [euk] **and cytosol**)

pre-mRNA: the *primary transcript* in eukaryotes which is processed to form mRNA (**nucleus** [euk])



Ribose

tRNA: type of RNA which *transfers* specific amino acids to ribosome for translation; specific binding of tRNA *anticodon* complementary to an RNA *codon* (**cytosol** *only*)

rRNA: type of RNA which makes up ribosomal structure and may have catalytic activity(**cytosol and the Rough ER** [euk])

snRNA: process pre-mRNA to create mature mRNA(**nucleus** [euk])

snoRNA: RNA molecules which help process and shape rRNA (**nucleolus** [euk])

miRNA:(**Nucleus and cytosol:** prevents translation by binding to a *specific* (complementary) stretch of forigen nucleic acid [euk])

siRNA:(**Nucleus and cytosol:** assists in the degradation of [euk])

crRNA: protects bacterial cells from ***specific stretches*** of forigen DNA through association with a ***Cas*** protein (**cytosol** [prok])

CHECK YOUR LEARNING

Concept Check: *(Answers found on the last page)*

1. Suppose a molecule irreversibly binds to and changes the shape of the prokaryotic **β'** subunit (the active site containing one). Which of these would you NOT expect to happen?
    a. Due to the shape change, Sigma factor may not be able to bind
    b. RNA polymerase may not bind to the promoter
    c. dNTPs will be added to the growing transcript
    d. The protein product of the transcription will not be synthesized
2. Where must a TAP bind that interacts directly with the Eukaryotic holoenzyme?
    a. Core promoter
    b. TATA box
    c. Enhancer site
    d. Regulatory Promoter
3. Which of these correctly orders events in Prokaryotic transcription?
    a. Recognition of the start site, bonding of the holoenzyme, elongation, laying the first rNTP, escaping the promoter
    b. Laying the first rNTP, escaping the promoter, bonding of the holoenzyme, elongation, recognition of the start site
    c. Escaping the promoter, bonding of the holoenzyme, elongation, laying the first rNTP, recognition of the start site
    d. Recognition of the start site, bonding of the holoenzyme, laying the first rNTP, escaping the promoter, elongation
4. Where do you find *snoRNA* in a prokaryotic cell?
    a. In the nucleolus
    b. You will not find *snoRNA* in a prokaryote
    c. Next to crRNA
    d. In the nucleoid
5. The following is a *template/non-coding* strand of DNA. What is the mRNA transcript created by ***RNA-pol-II***?
    5'-ATTGCATGATACA-3'
    a. 5'-TUUGCAUGAUACA -3'
    b. 5'-UAACGUACUAUGU -3'
    c. 5'- UGUAUCAUGCAAU-3'
    d. 5'- UAATAGCAUACGU-3'
    e. None of these; ***RNA-pol-II*** is an RNA-dependent RNA-polymerase (*RdRp*), so DNA would not be transcribed
6. TFs are different from TAPs in that:
    a. TFs bind to the core promoters but TAPs bind to regulatory or enhancer sites
    b. TAPs have a stronger affinity for DNA than TFs

c. TFs have a longer protein sequence that TAPs
d. TAPs will bind to both the regulatory and core promoters

7. T/F The *RNA-pol holoenzyme* in both eukaryotes and prokaryotes binds and only physically spans one base of RNA in length.

8. The DNA of the *coding* strand is complementary to the _____, but is 'identical' to the _____ (with the exception of T's and U's).
a. RNA transcript; *template strand*
b. *Template strand*; RNA transcript
c. *Non-template strand;* RNA transcript
d. *Non-template strand*; *non-coding strand*

9. What allows TFII-D to find its binding site on the promoter?
a. TBP allows it to find and bind the TATA box
b. TBP allows it to find the local TAPs to bind to the core promoter
c. TFII-D binds to TFII-B and binds to the promoter
d. TFII-D has molecular recognition that help it find its binding site on the regulatory promoter

10. If a mutation removed the ability of a coactivator to interact with a mediator, which would be the most likely response?
a. TFs bond to the enhancer would be unable to interact with the BTA
b. TAPs bound at the regulatory promoter could not increase the speed of initiation
c. The speed of transcription would be significantly increased
d. TAPs bound to the core promoter would not interact with the mediator

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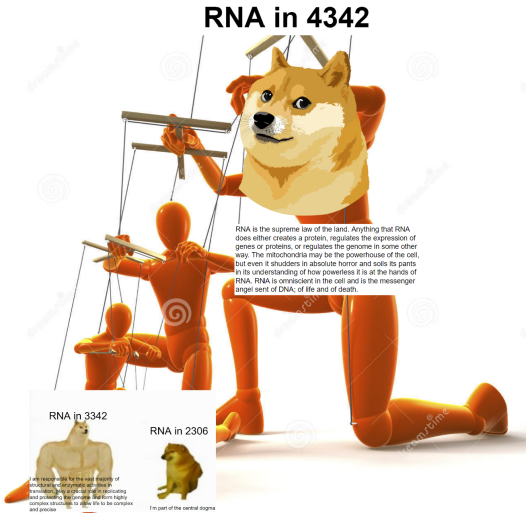
THINGS YOU MAY STRUGGLE WITH:

1. "Pol" = polymerase; "TF" = transcription faction; "TAP" = transcriptional activator protein; "TBP" = TATA binding protein; "BTA" means basal transcriptional apparatus; (-X) = x-number of bases *upstream*; (+X) = x-number of bases *downstream*;

2. There are many types of eukaryotic **RNA-pol**, but we look at ***RNA-pol-II*** because it synthesizes pre-mRNA, so it is best suited for the overview of the central dogma (see **table 13.3**)

3. The "*template strand*" is the strand directly complementary base pairs with the RNA transcript as the process of transcription is happening. Because the *template* is complementary to the transcript, the DNA code of this strand is not the same as the RNA sequence. However, the other DNA strand (the *non-template* strand) is the same sequence as the RNA transcript (with the substitution of $T_{DNA} \rightarrow U_{RNA}$). Thus, the *non-template* is also called the **coding** strand.

4. The active form of an enzyme is called the *holoenzyme*; you may remember this by thinking of it as the "**whole**" enzyme (containing the core protein and any other factors that are added to make it active.



CONGRATS: You made it to the end of the resource! Thanks for checking out these weekly resources! Don't forget to check out our website for group tutoring times, video tutorials and lots of other resources: www.baylor.edu/tutoring!

Answers to check your learning questions are below!

Answers:

1. C. → we are looking at RNA transcription from DNA, not DNA replication so we won't be adding dNTPs even if the active site **wasn't** inhibited
2. C.
3. D.
4. B. → prokaryotes do not have a nucleus [nor a nucleolus within], so thus their rRNA is not processed by *snoRNA*
5. C.
6. A.
7. F. → a holoenzyme will be pretty big and may span a good length of DNA (even though the catalytic activity of the active site only occurs at *one base* at a time
8. B.
9. A.
10. B.