2: DNA Polymerase, RNA Polymerases, Transcription

Compare and contrast bacterial DNA polymerases and RNA polymerases

Note: ss=single strand ds=double strand P=phosphate

Overview:

DNA polymerases synthesize complementary DNA using a DNA template/guide

______________________________ DNA

E.g., ssDNA template base sequence: A T A G G C

Complementary DNA sequence T A T C C G DNA

synthesized by DNA polymerase

RNA polymerases synthesize complementary RNA sequences using DNA as a template/guide

______________________________ DNA

E.g., ssDNA template base sequence: A T A G G C

Complementary RNA sequence U A U C C G RNA

synthesized by RNA polymerase

Synthesis of DNA and RNA require input of energy, both ATP and charged precursors (see below)
**DNA Polymerase RNA Polymerase**

Template/guide ss DNA ssDNA

Synthesize complementary DNA complementary RNA

Charged precursors deoxyadenosine tri-P = dATP adenosine tri-P = ATP
deyoxymyidine tri-P = dTTP uridine tri-P = UTP
deoxycytodine tri-P = dCTP cytidine tri-P = CTP
deoxyguanosine tri-P = dGTP guanosine tri-P = GTP

primer required? Yes No
proofreading/editing? Yes* No

*DNA polymerase proofreading/editting

Polymerases have a "normal" or "intrinsic" mistake rate of approximately

$10^{-4} - 10^{-5}$ nucleotides (this means the polymerases introduce the incorrect nucleotide every 10,000 to 100,000 nucleotides). DNA polymerases have the ability to "proofread and edit" their mistakes. If they introduce the wrong nucleotide, they can remove or "excise" the wrong nucleotide and try again to make a correct match. This reduces the mistake rate of DNA polymerases to approximately $10^{-9} - 10^{-10}$ (or only one incorrect nucleotide every 1,000,000,000 – 10,000,000,000 nucleotides). RNA polymerase cannot proofread or edit their work so RNA polymerase make many mistakes (one reason many RNA viruses, for example HIV, mutate so rapidly…..more later)

**Transcription Prokaryotic repeated section**

Review flow of information in cell

DNA-------> RNA ---------> Protein

replication transcription translation

I. Genetic Code: one to one relationship between specific codon (specific 3 base sequence) and an amino acid

II. Transcription: use of DNA as template/guide to synthesize complementary RNA. DNA info is rewritten in RNA sequence.

A. First step in gene expression
B. Products of transcription

1. messenger RNA=mRNA: will be translated into specific amino acid sequence of a protein

2. transfer RNA=tRNA: actual “translator” molecule, recognizes both a specific codon and specific amino acid

3. ribosomal RNA=rRNA: combined with ribosomal proteins, will form the ribosome, the “workbench” at which mRNA is translated into a specific amino acid sequence/polypeptide/protein

III. Promoters and RNA polymerases

A. Promoters: specific DNA sequences which signal the “start” points for gene transcription. Sigma factor/subunit of RNA polymerase binds to promoters to initiate transcription

B. RNA polymerases: enzyme complex which recognizes DNA promoters, binds to promoter and synthesizes complementary RNA copy using DNA as template/guide

E. coli RNA Polymerase: 2 subunits, sigma subunit and core

a. sigma subunit/factor= “brains” of RNA polymerase. Travels along DNA until it reaches a promoter, binds promoter

b. core subunit: binds to sigma attached at promoter. “Workhorse” of RNA polymerase, carries out actual RNA synthesis. Requires activated precursors and template strand, DOES NOT REQUIRE PRIMER (compare to DNA Polymerase). Synthesizes RNA in 5’-to->3’, similar to DNA polymerase. No proofreading ability therefore will make more mistakes than DNA Polymerase

c. sigma subunit will drop off after the first few ribonucleotides have been linked together, core continues alone. Note: core would start transcription randomly of DNA without direction of sigma subunit.

Polycistronic mRNA (prok. only)

IV. Termination of transcription

terminators: DNA sequences which signal transcription stop signals. RNA polymerase releases DNA when transcription terminator sequence encountered