

Transcription in Prokaryotes

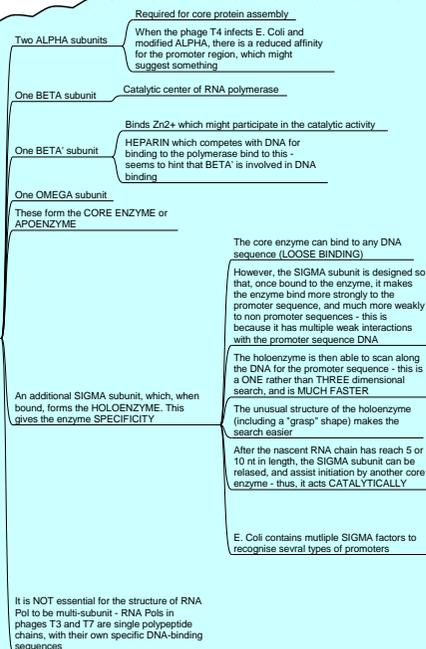
Introduction

TRANSCRIPTION is the enzymic synthesis of RNA on a DNA template

All RNA in cells is synthesised by a single type of DNA dependent RNA polymerase, which

- Requires double-stranded DNA as a template
- Uses dNTP precursors (ie: UTP instead of TTP)
- Does NOT need a primer
- Synthesizes in the 5' > 3' direction, as usual

The structure of E. Coli RNA polymerase is as follows



Since this is so essential and there is only one RNA Pol, it is a target for several antibiotics

- RIFAMPICIN - stops initiation of RNA synthesis by preventing the formation of the first few bonds
- ACTINOMYSIN D - intercalates into DNA thereby preventing the polymerase to bind to it

Only one of the two strands of DNA becomes transcribed as RNA

- The RNA is a DIRECT COPY of the SENSE STRAND
- The other, ANTISENSE STRAND is the TEMPLATE

The process

Therefore, often promoted by NEGATIVE SUPERCOILING

However, not always - promoters for enzyme subunits of DNA GYRASE are INHIBITED by negative supercoiling - perhaps an elegant feedback loop

The RNA polymerase docks with the promoter - a "CLOSED-PROMOTER COMPLEX" is formed

About 15-17 bp of the duplex are unwound to form an "OPEN PROMOTER COMPLEX"

1 Initiation

The first nine bases are added without enzyme movement along the DNA, and abortions are common at this stage

Adding ribonucleotides at the 3'-end of the growing RNA [ie: synthesis in 5' > 3']

At the same time, it's moving in a 3' > 5' direction along the ANTISENSE strand

When initiation has succeeded, the enzyme release the SIGMA factor

The RNA polymerase moves down the DNA

As it moves, it locally unwinds DNA and re-winds it behind

2 Elongation

Termination occurs at a specific DNA sequence called the TERMINATOR

These cause the polymerase to pause and cease transcription

These are often GC rich, since G-C base pairs are more stable than A-U ones

This sequence is often followed by four or more U residues, which pair only weakly with the antisense strand - this favours the release of the complex

These sequences often contain self-complementary regions which can form a STEM-LOOP or HAIRPIN secondary structure in the RNA product

They use an ACCESSORY FACTOR, the RHO PROTEIN to MEDIATE TERMINATION

It binds to a stretch of 72 nt on RNA which has already been transcribed

Uses ATP to "move along" the RNA, "following" the RNA polymerase

At the terminator (eg: weak hairpin), RNA Pol pauses

RHO catches up, and enables transcription to terminate

RHO is a HEXAMERIC PROTEIN

Sometimes, "RHO DEPENDENT TERMINATORS" are hairpin structures lacking the subsequent stretch of Us

RHO is an ESSENTIAL PROTEIN, but there are few RHO dependent terminators

Most such terminators are found in PHAGES

Some terminator sequences do not form strong hairpins

3 Termination

Promoters

Some mutations are located just "upstream" of the coding region of the protein

This must be the promoting region

Label target DNA at ONE end (with 32P, say)

Incubate with RNA polymerase preparation

Incubate with low conc DNase (so each molecule is cut once only)

The DNA will have been cut EVERYWHERE, except at the point where the polymerase binds

Therefore, the end that was labelled will now have ALL lengths, except for the one involving a cut at the Pol binding site

Resolve sample by gel electrophoresis

Genetic approach

Footprinting

Identifying promoters

Mutagenesis studies have shown that only very short conserved sequences are critical for promoter function

Alignment of DNA sequences corresponding to this protected region shows two stretches in which the sequence is well conserved, relative to "+1", the start of transcription

"TTGACAT" at -35

"TATAAT" at -10

Appears to be the sequence at which DNA unwinding is initiated

Separated by 5-8 bp from start site - sequence doesn't matter, but distance does

This is for SIGMA70, the most common in E. Coli

The closer to the consensus that a promoter is, the greater the level of gene expression - there is considerable variation in sequence between different promoters, which may vary in efficiency by up to 1000 fold

The first nt to be transcribed is almost always a PUERINE

Footprinting and DNase protection studies show that RNA Pol typically protects a stretch of ~60 bp of DNA

Post-transcriptional RNA modification

DNA cleavage
In E. Coli, the 16S, 23S and 5S rRNA are synthesized as one long transcript
It needs to be processed before yielding functional RNA
Accomplished through action of RNasell

Addition of non-coded nucleotides
In tRNA, E. Coli have the trinucleotide sequence -CCA added at the 3' end
The sequence is not coded, but added post-transcriptionally

Chemical alteration of bases
About 10% of the bases in tRNA are chemically modified

mRNA, however, cannot be modified in prokaryotes
Transcription and translation are not spatially separated by a nuclear membrane
Therefore, translation often starts before transcription is finished - no time for modification!
Also, prokaryotic mRNA is rapidly degraded