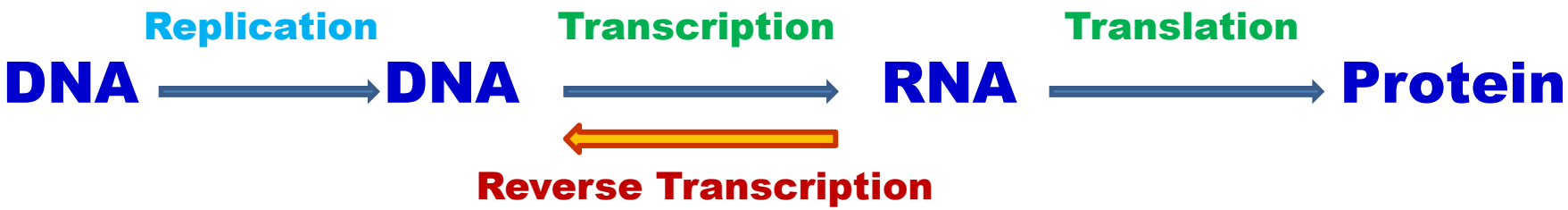
Central Dogma



Transcription

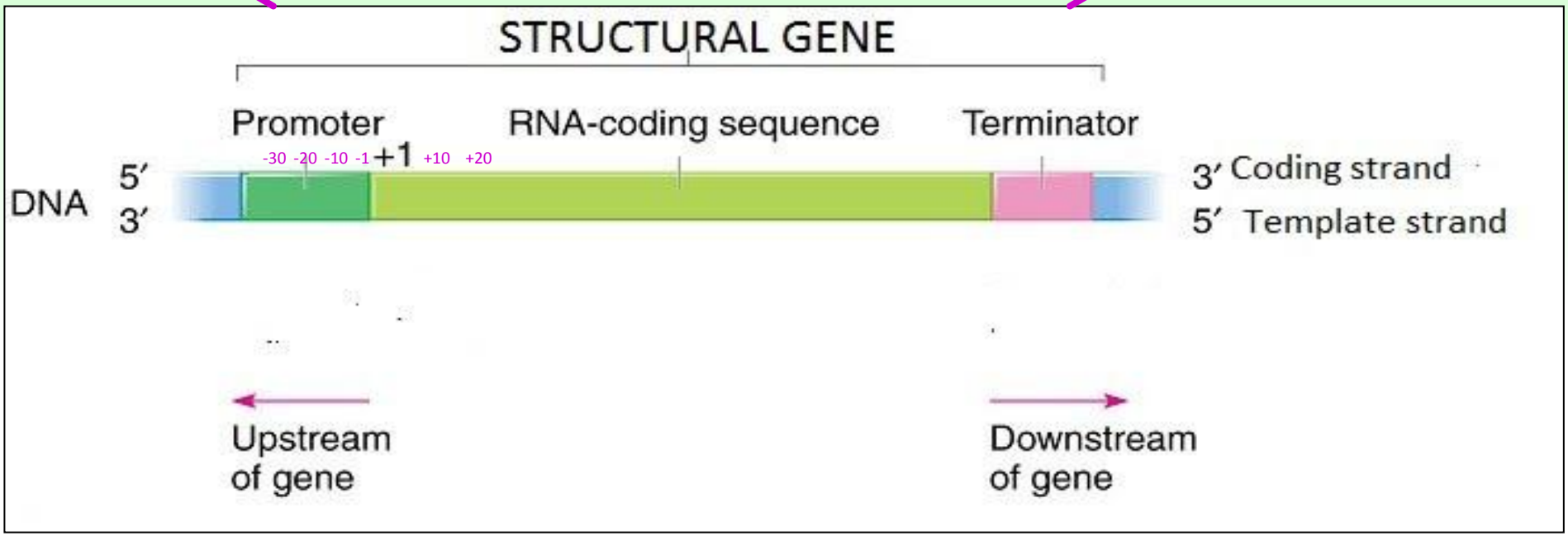
The process

from

DNA → **m-RNA**

Same in both prokaryotes & eukaryotes except some finer details

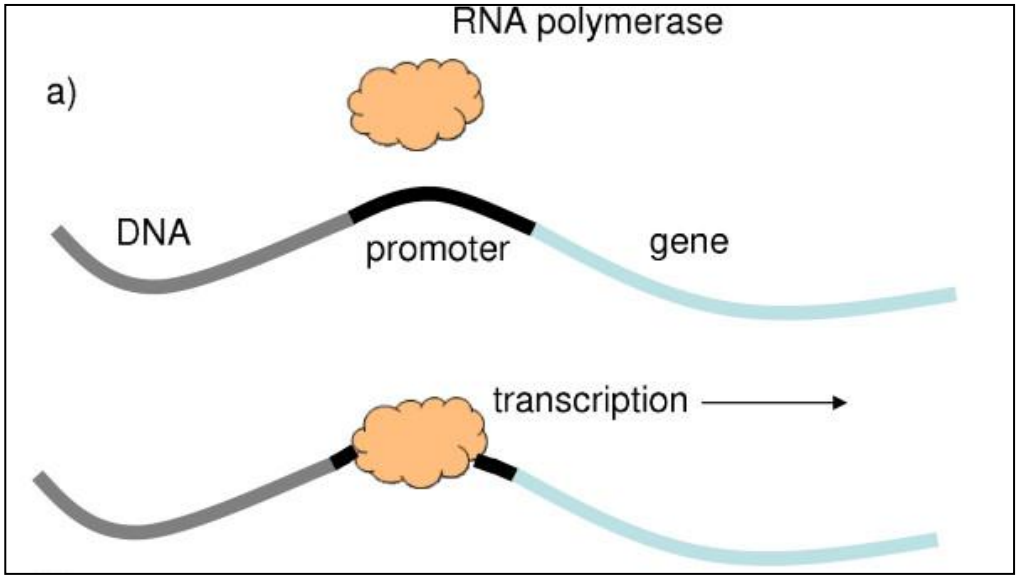
Transcription unit



Enzyme that catalyzes transcription –

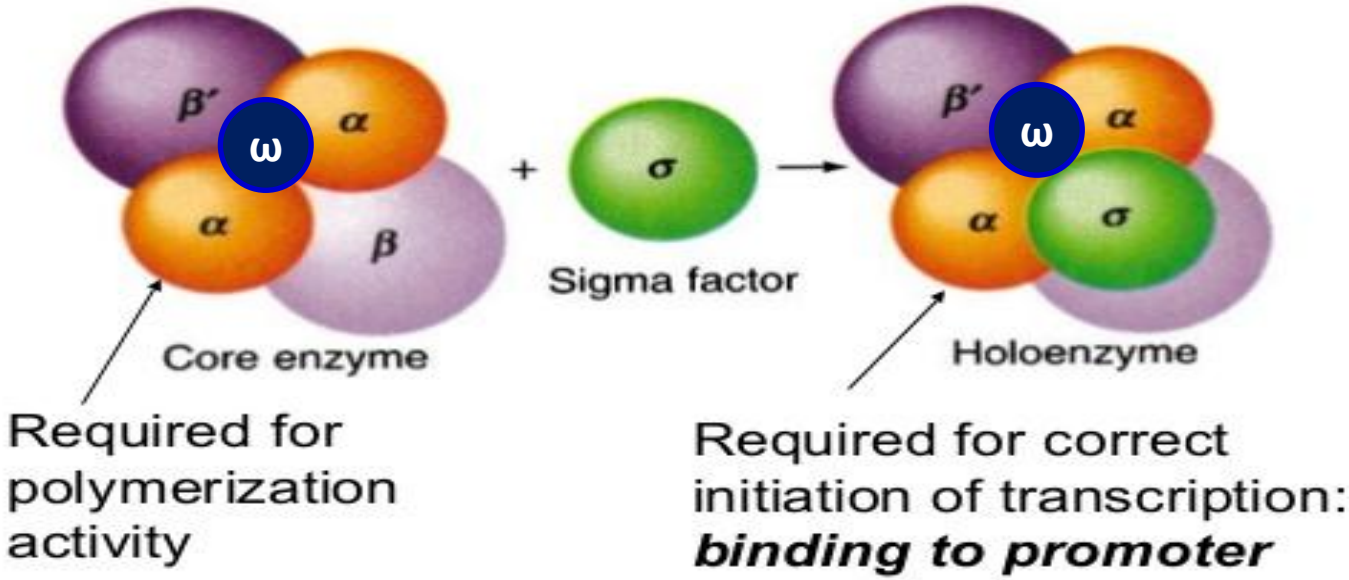
RNA polymerase

which recognizes and binds to discrete regions on the DNA called the promoter



E. coli RNA polymerase

2α, 1β, 1β', 1ω and σ factor



α- assemble of the enzyme molecule, may affect promoter recognition

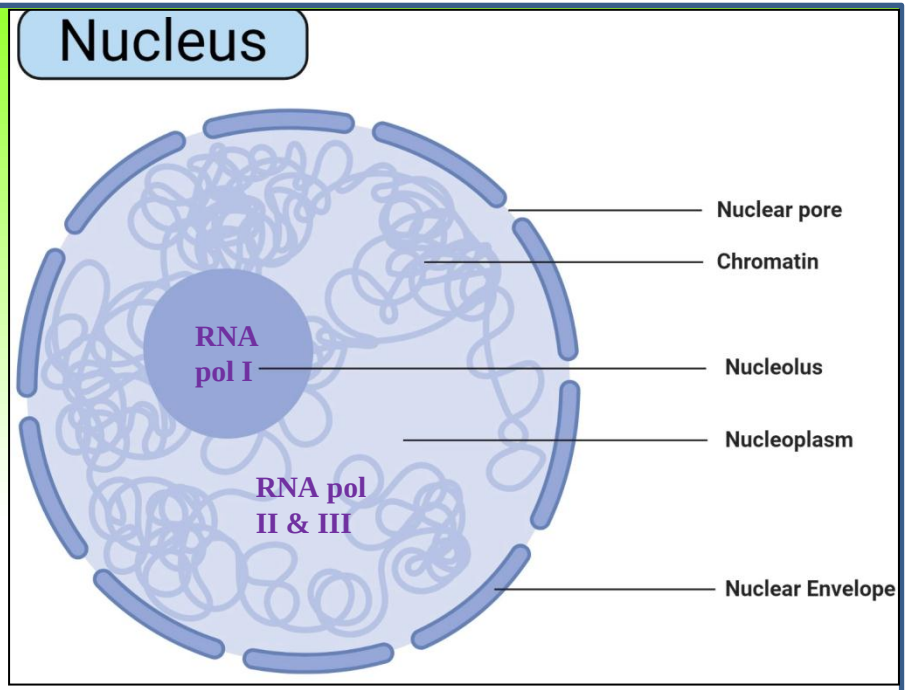
β- involve in catalysis, chain initiation and elongation

β′- binds to DNA template, may require for elongation

ω- role uncertain, may have unidentified regulatory function.

Eukaryotes :

3 different RNA polymerase – I, II, III



RNA polymerase I - catalyzes synthesis of r-RNA] **located in the nucleolus**

RNA polymerase II – transcribed majority of the nuclear structural genes, responsible for pre-m-RNA synthesis

RNA polymerase III - transcribed genes for small nuclear RNA and t-RNA.

present in the nucleoplasm (outside the nucleolus)

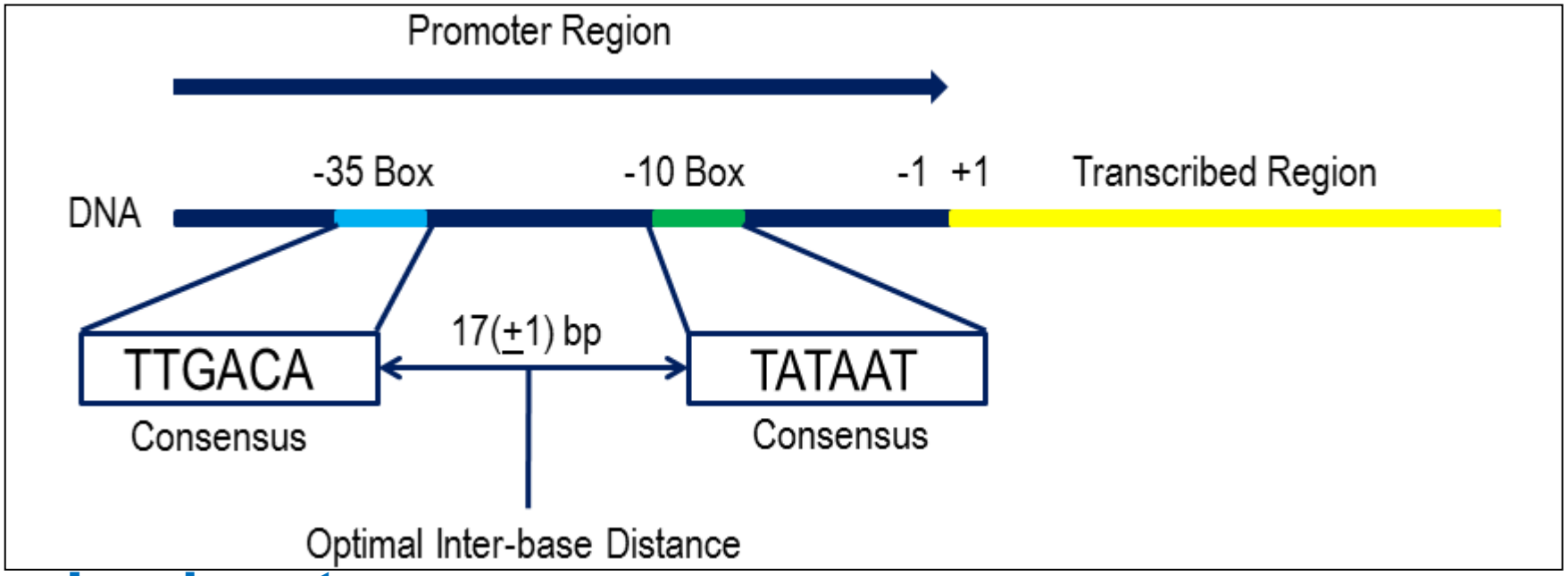
Promoter region

- contains the information
- have some conserved sequences

In prokaryotes –

-10 bp: Pribnow Box - TATAAT

-35 bp: Recognition sequence – TTGACA



In eukaryotes –

-25 bp: TATA Box - TATAAAA

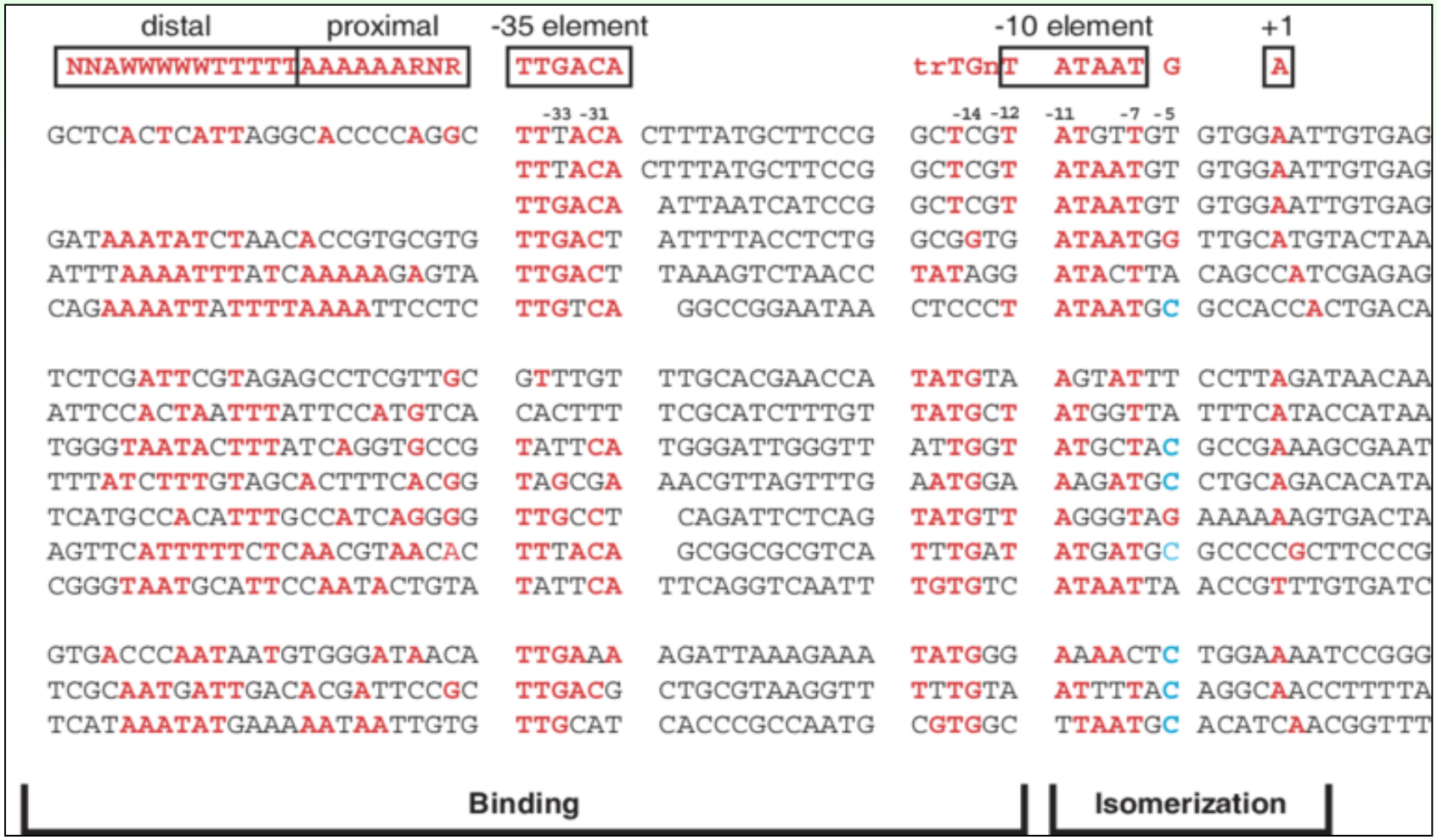
-75 bp: CAAT Box - GGCCAATCT

Variation among consensus sequence has definite significance

Level of mRNA synthesis is controlled by the variation

Some resemble mentioned sequence more closely- strong promoter

Some resemble mentioned sequence less closely- weak promoter



RNA polymerase binds strong promoter with more affinity and weak promoter with less affinity

Efficiency of promoters is inversely proportional to their deviations from consensus sequence

How does the holoenzyme find the promoter?

Binds randomly to any sequence in the genome and then dissociates, then rebinds again to another sequence. Process of association and dissociation continues till the polymerase accidentally encounters a promoter whereby it will form a stable association.

In more than 90% of the transcription unit, the start point is a purine. Very frequently, the start point is the central base in the sequence CAT

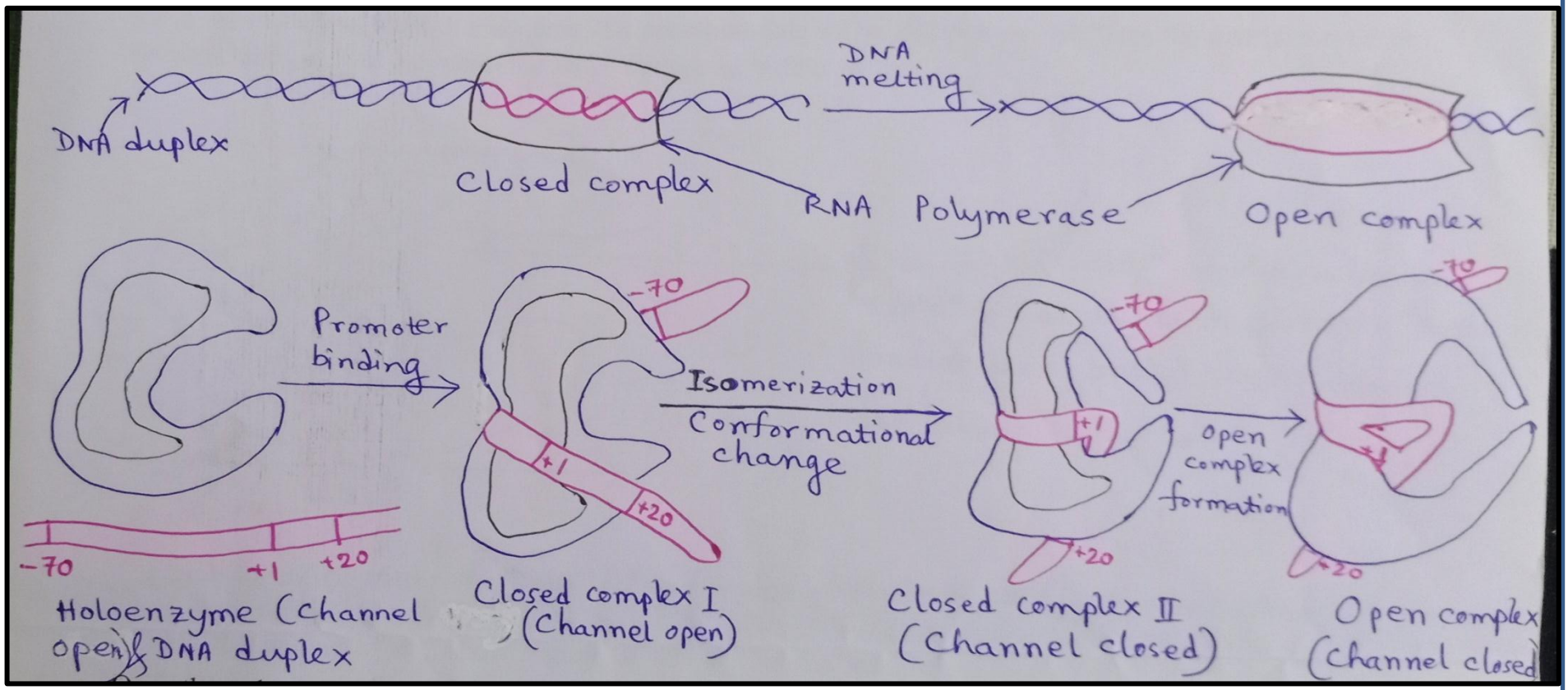
RNA polymerase first forms a closed complex I (60 bp)

Then form a closed complex II (90 bp)

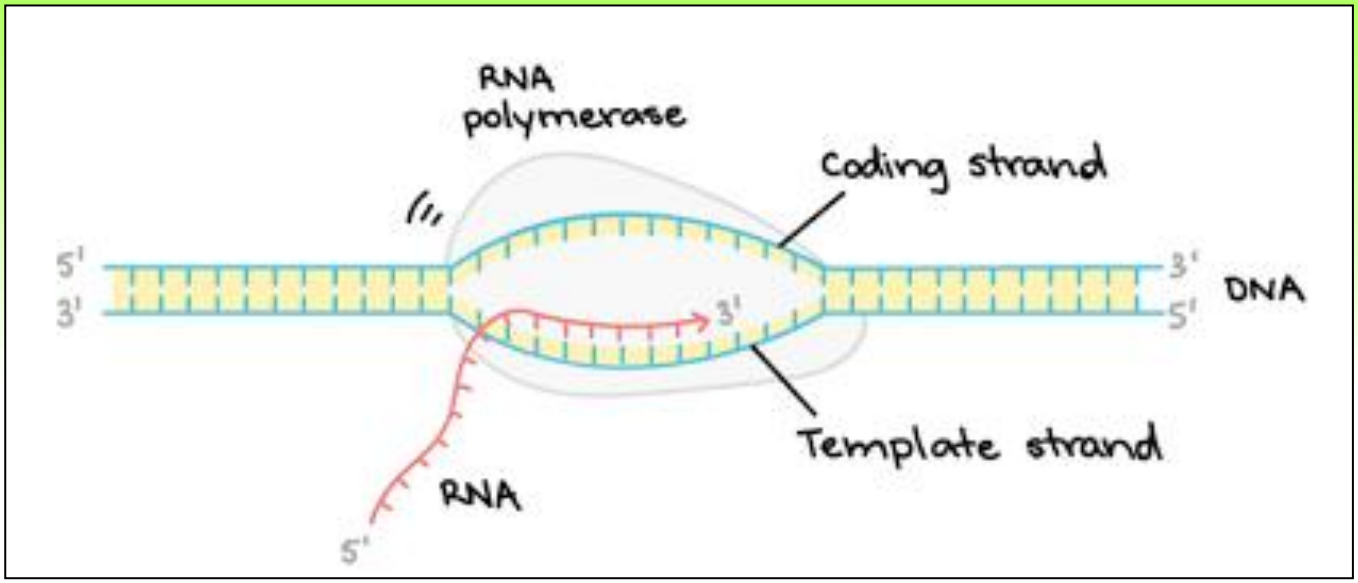
Then holoenzyme actually covers a 75 bp region of DNA extending from -55 to +20

RNA polymerase holoenzyme - dimension of 160A° - cover roughly 50 bp of B-DNA

Closed complex isomerizes to open complex - the region around -10 sequence melts producing single stranded DNA



RNA Polymerase bound to DNA – template strand available for transcription



The structure produced by local melting is called transcription bubble

Within the bubble RNA bases start to incorporate according to the sequence of template and an RNA chain up to nine bases can be generated

Enzyme molecule not move to add nucleotides up to this point

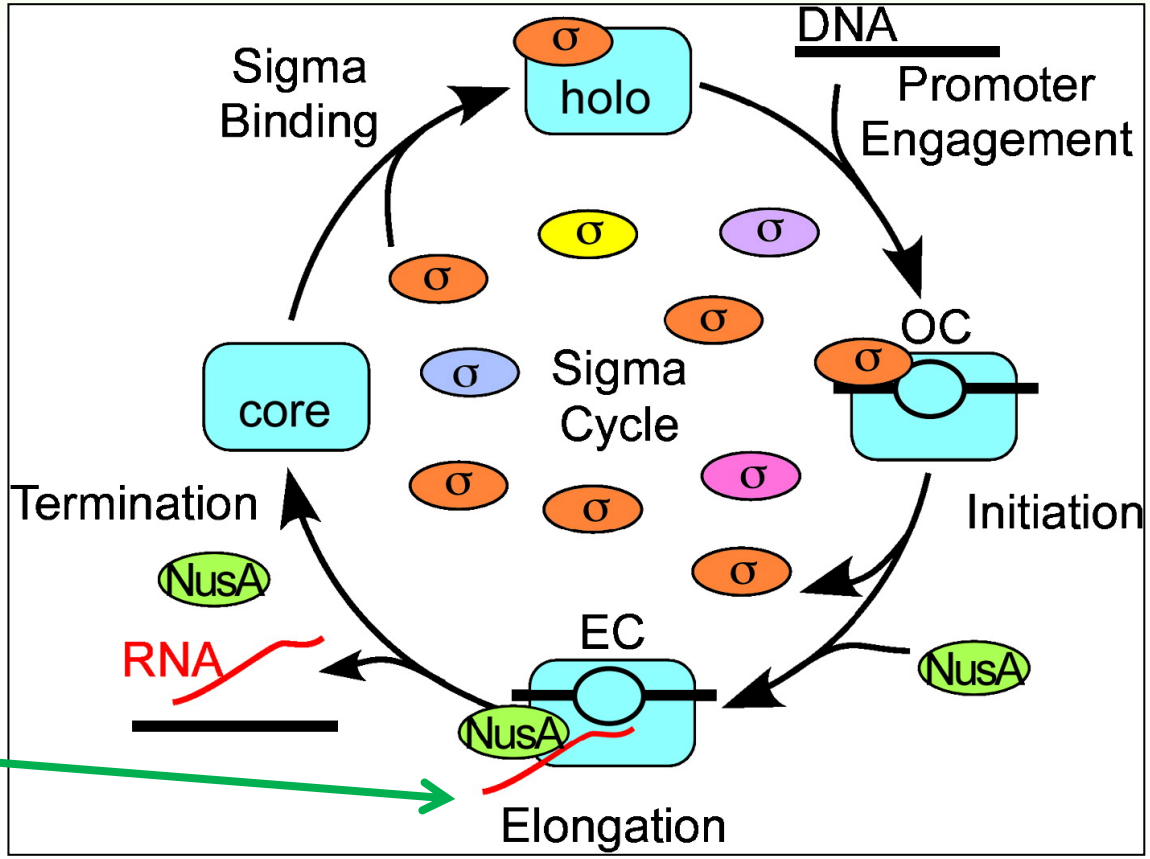
Small RNA chain (called abortive RNA) is released and the enzyme again synthesizes a small RNA which may also be released

Actual initiation takes place after a series of abortive initiation

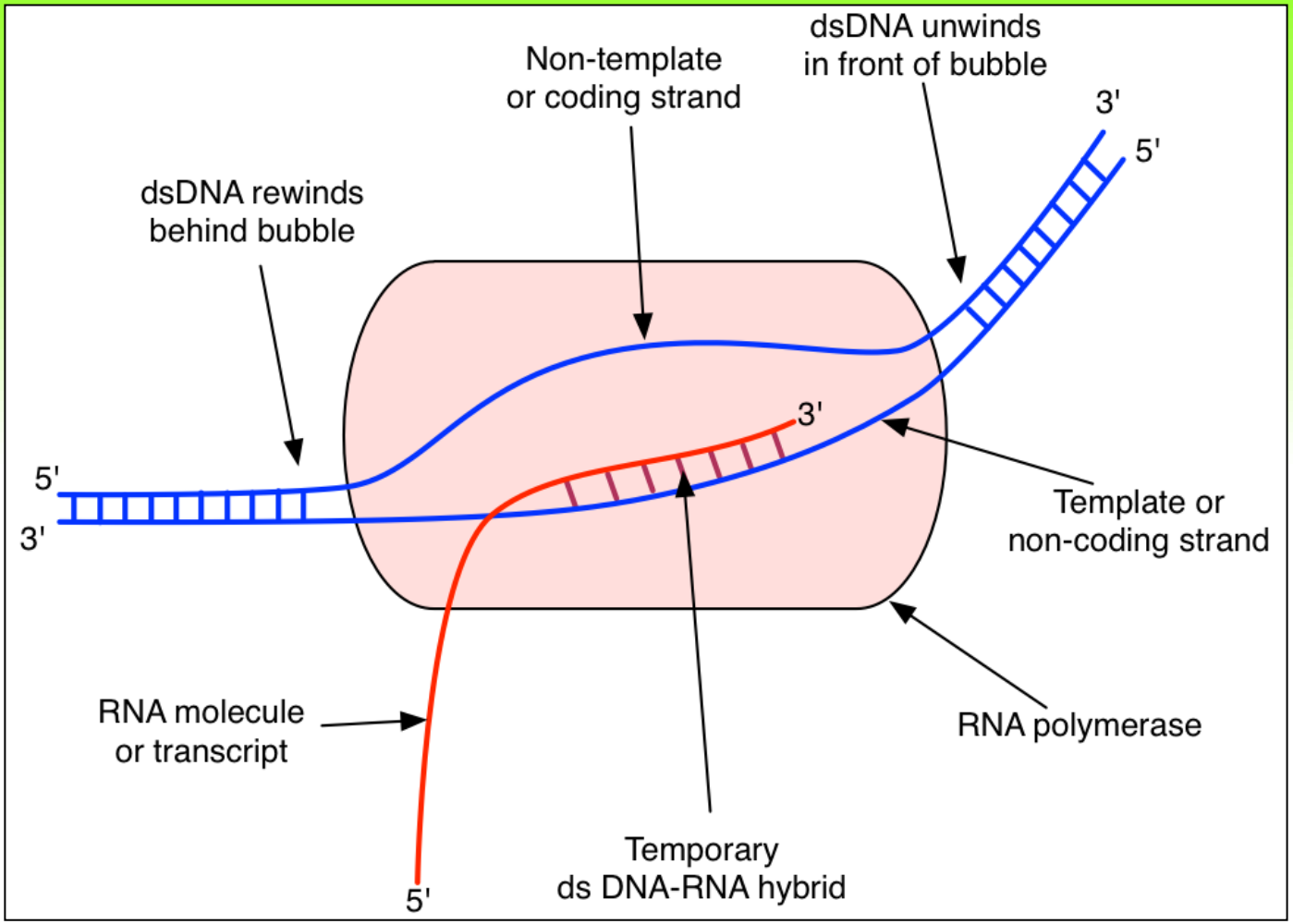
For elongation - sigma factor dissociate

Enzyme molecule escape promoter - move along the template continuing incorporation beyond 9 bp

Dissociation of sigma factor not enough. NusA associates with core enzyme and changes its conformation



Now actual elongation starts



Enzyme moves along DNA during elongation, the bubble also moves along with it, which means RNA polymerase unwind DNA lying ahead and rewinds the DNA leaving behind

Exact structure of bubble and the RNA polymerase holoenzyme is definitely not known

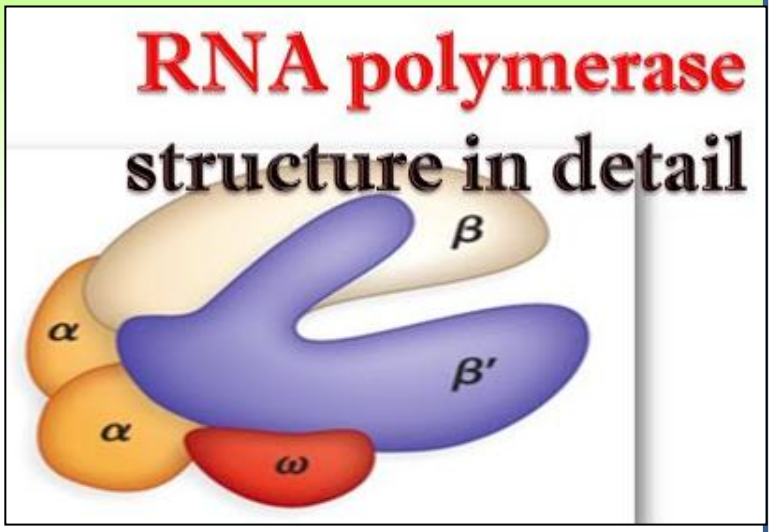
Bubble remains within the womb of the RNA polymerase

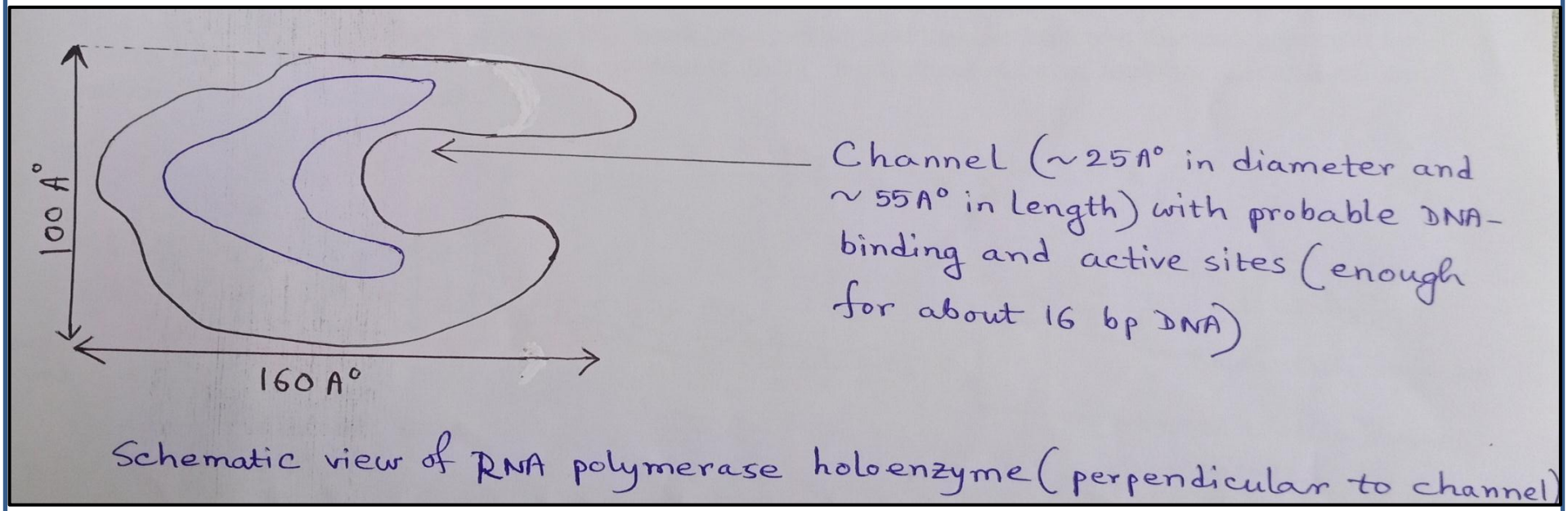
Cylindrical channel (~ 25 A° in diameter and ~55 A° in length), guarded by a thumb like projection

Diameter of a duplex DNA is 20 A°, so this channel can easily fit DNA

Each base pair is 3.4 A° in thickness, then this channel can accommodate 16 bp dsDNA easily

Bubble must be contained within 16 bp.



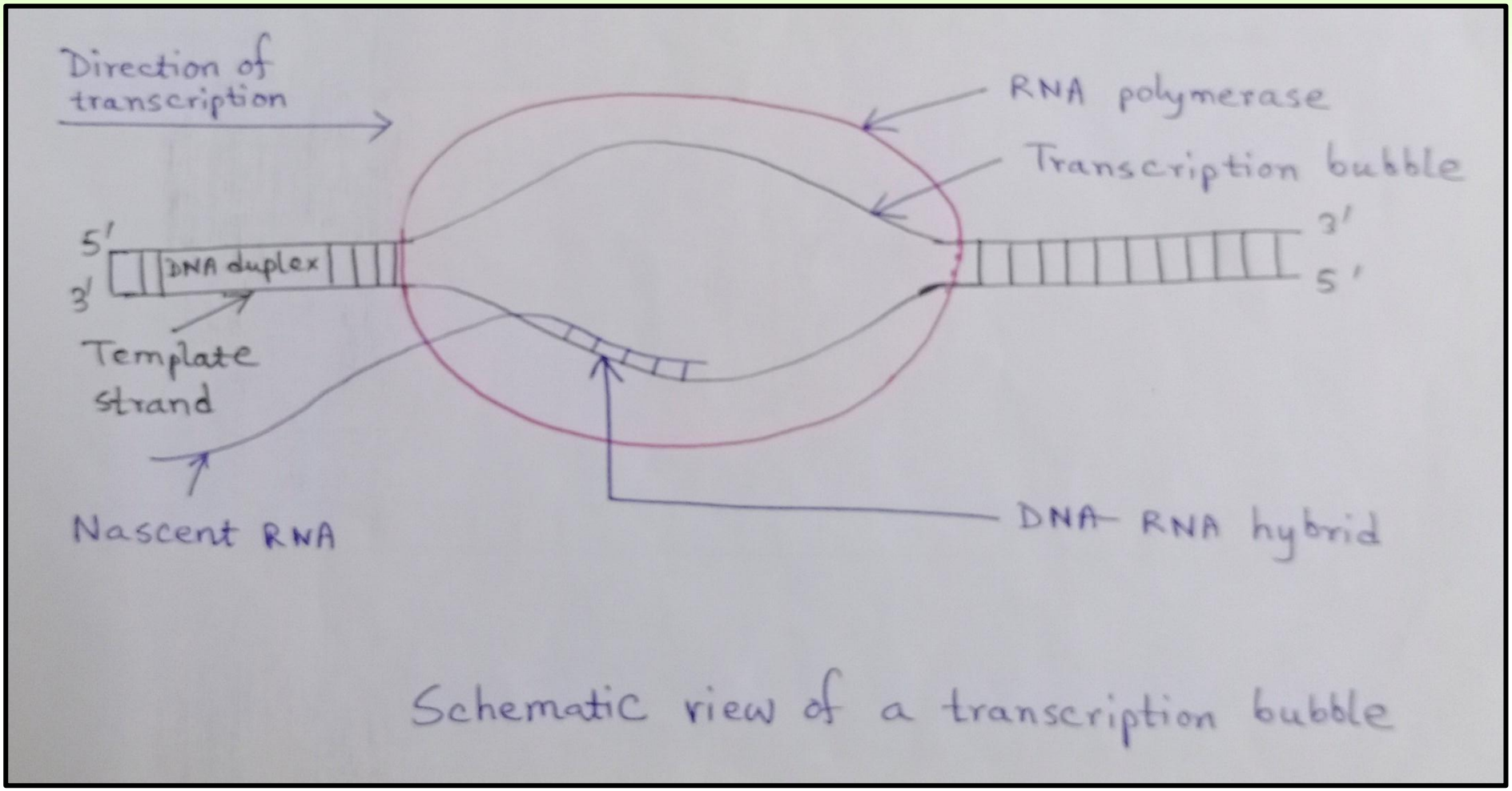


Within the bubble a very short region of DNA-RNA hybrid forms

The hybrid must be considerably shorter than 16 bp

Experiment indicate hybrid may not be larger than 3-4 bp

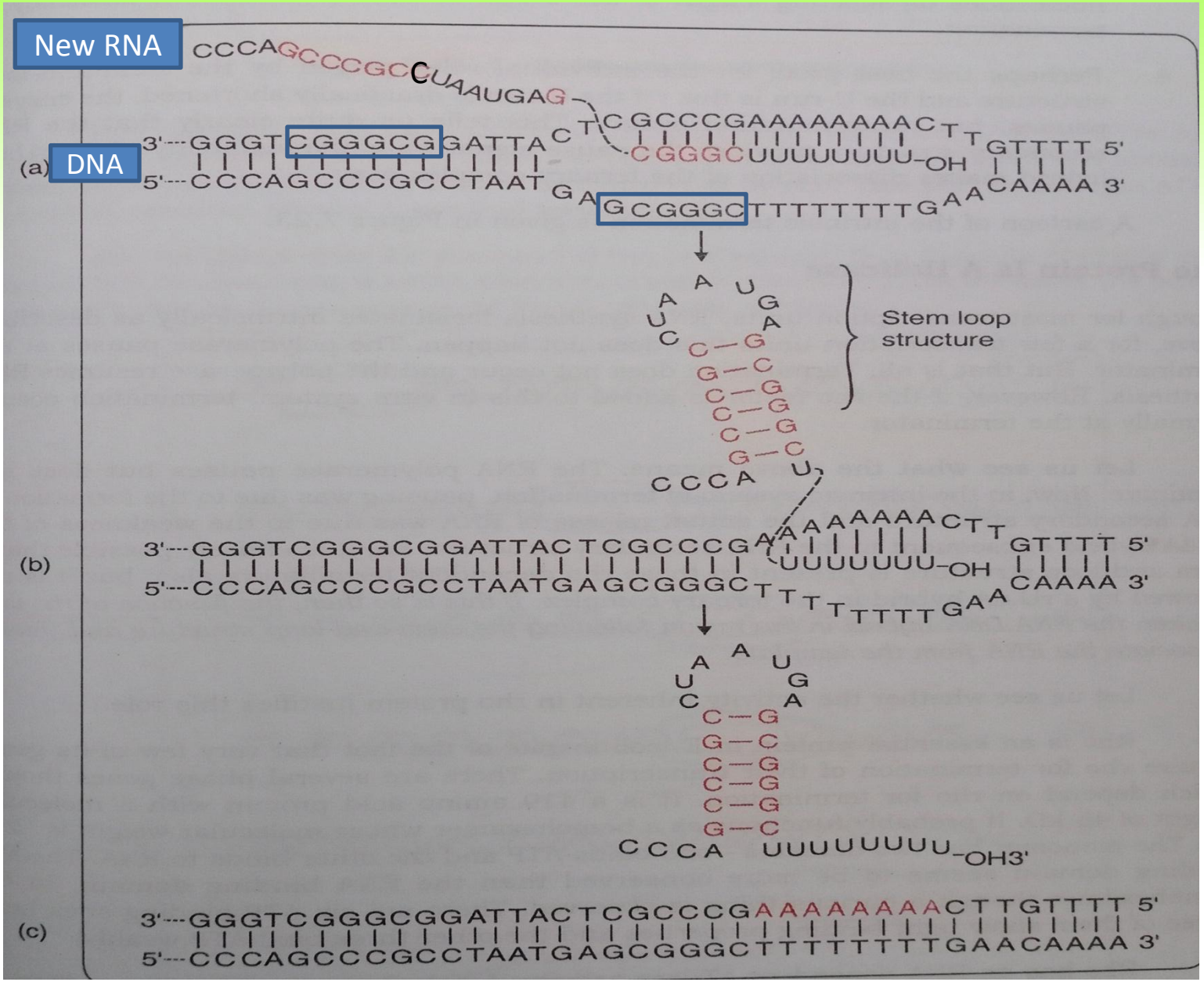
As elongation proceeds, the RNA being synthesized emerges as a free single strand



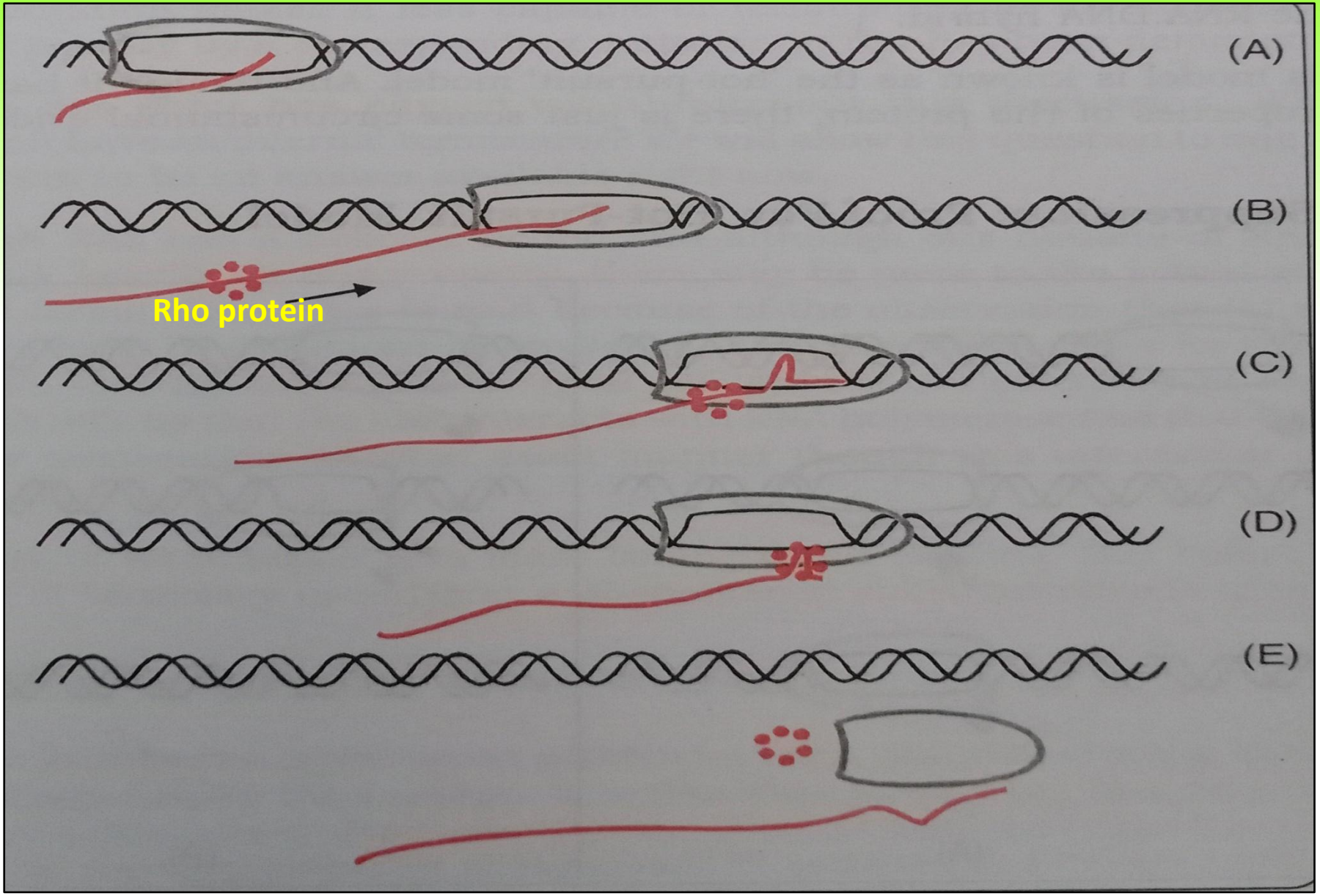
Schematic view of a transcription bubble

Termination – intrinsic and Rho-dependent

Intrinsic method:



Rho dependent method:



Most transcription terminates intrinsically, a few only terminates by rho dependent mode