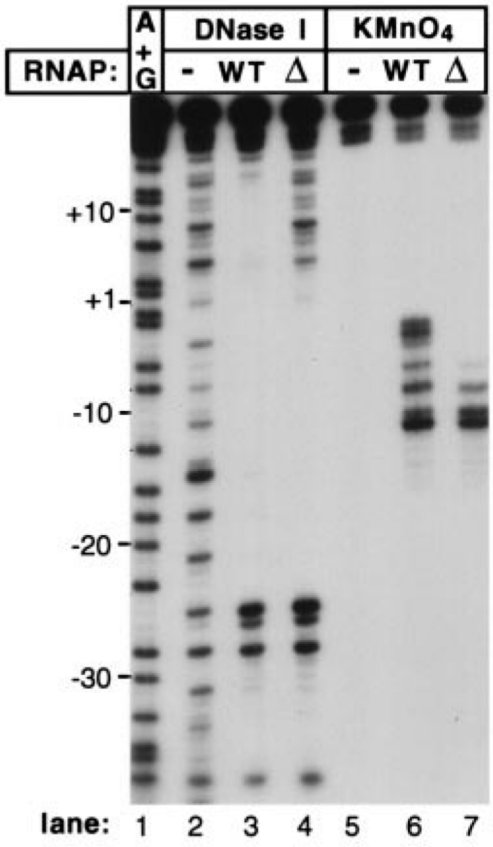
Discussion Worksheet –Week 8 – Winter 2026
Bacterial Transcription
Due on February 23 at 11:59pm

Group 1



Researchers have purified a version of E.coli RNA polymerase (RNAP) where amino acids 186 to 433 of the β subunit have been deleted (hereby indicated as Δ). They perform footprinting experiments in the presence of σ70 using a DNA containing a promoter sequence and in the absence of rNTPs. They compare the footprinting patterns obtained with the normal RNAP (shown as WT, lanes 3 and 6) or with the Δ RNAP. Footprinting is performed with either DNAse I (lanes 2,3,4) or with KMnO4 (lanes 5,6,7). Lanes 2 and 5 do not contain RNAP (-). Lane 1 is a sequencing marker showing the location of A and Gs. The transcription initiation site is indicated as (+1). Numbering of the base pairs on the DNA is according to the standard numbering seen in class.

A -What information is provided by the addition of DNAse I or KMnO4 in these experiments?

B – describing the patterns obtained by DNAse I and KMnO4 footprinting for the normal RNAP(WT) and the mutant RNAP (Δ).

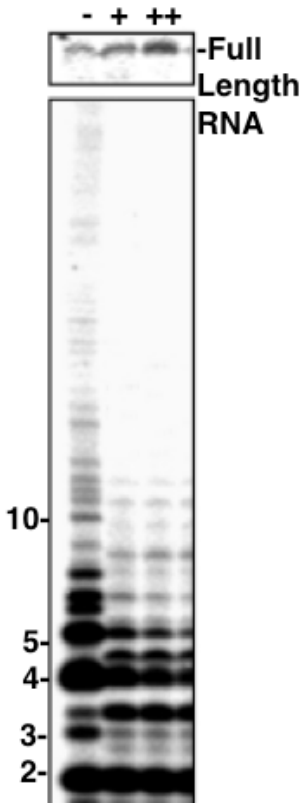
C - Use this information to propose a function for the amino acids that were deleted from the β subunit in the Δ mutant.

Group 2

Protein X has been identified as an important positive regulator of the yfg gene in bacteria.

Experiments in cells have shown that in the absence of X, the yfg gene is expressed at low levels.

To understand the function of X in the regulation of yfg, researchers isolate the DNA of the yfg gene with its promoter, and add the RNA polymerase holoenzyme containing σ70, as well as radiolabeled NTPs, in the absence (-; first lane) or presence of 10 and 50nm of protein X (+ and ++; second and third lanes). After incubation, radiolabeled molecules are fractionated by electrophoresis and the result shown on the right.



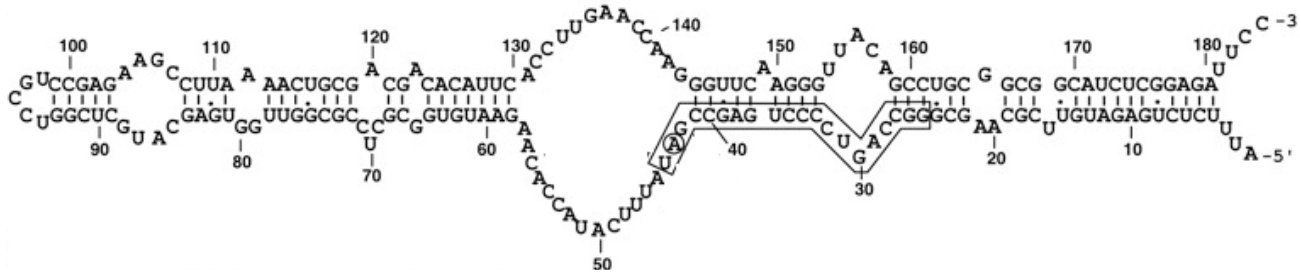
A- Describe the results obtained in the absence of protein X (lane -)

B- Based on the results obtained in the absence (-) or presence (lanes +,++) of protein X, what is the impact of this protein on transcription?

C- Based on these results propose a mechanism to explain how Protein X positively regulate the expression of the yfg gene]

Group 3

The M6 RNA is produced at high levels in bacteria grown in stationary phase. The structure of this RNA is shown below.



Experiments have shown that expression of the M6 RNA specifically inhibits transcription of genes having a promoter recognized by σ70. However there is no effect on the transcription of genes having a promoter recognized by σ54 or σ32.

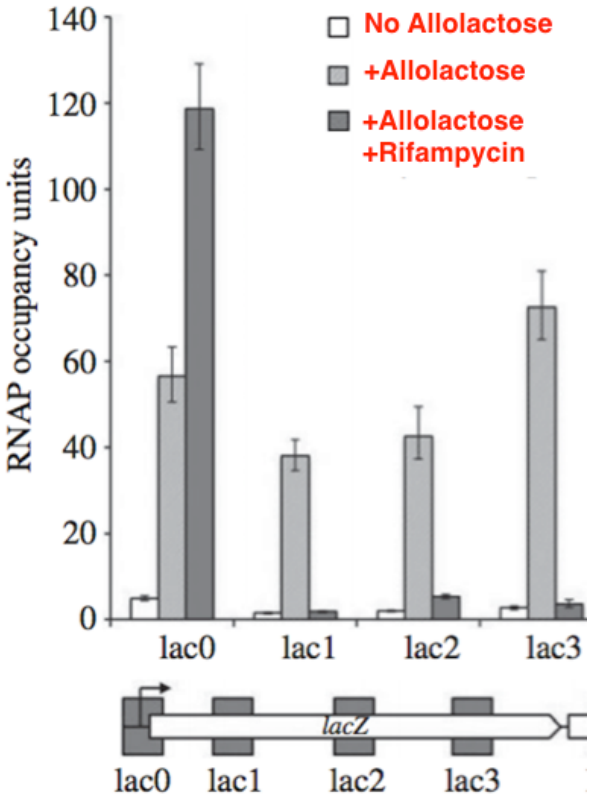
Further experiments show that during stationary phase, small RNA molecules are produced, which are complementary to the sequence that is boxed in the secondary structure.

Propose a mechanism by which the M6 RNA can inhibit σ70-dependent transcription. Make sure that you base your answer on a specific biochemical mechanism that takes into account the observations listed above.

Group 4

A “chromatin” immunoprecipitation experiment is performed to measure RNA polymerase occupancy on different regions of the Lac Operon. Lac0 corresponds to the Lac Promoter/Transcription start site. Lac1,2,3 correspond to different segments of the LacZ region.

RNAP occupancy is measured by ChIP in the absence of allolactose (white bars), presence of allolactose (light gray), and presence of both allolactose and the antibiotic Rifampicin (dark gray) and plotted in bar graphs for each region.



A – Describe the effect of allolactose on the occupancy of RNA polymerase in the Lac Operon. Explain the molecular mechanism that results in this effect.

B- Based on this result, would you predict that the culture conditions used for this experiment include glucose or not? Explain your answer using what you know about regulation of the Lac Operon.

C- Describe the impact of adding Rifampicin on RNAP occupancy when allolactose is present. Based on this result, propose a possible biochemical mechanism by which Rifampicin impacts bacterial transcription and why this is an antibiotic.