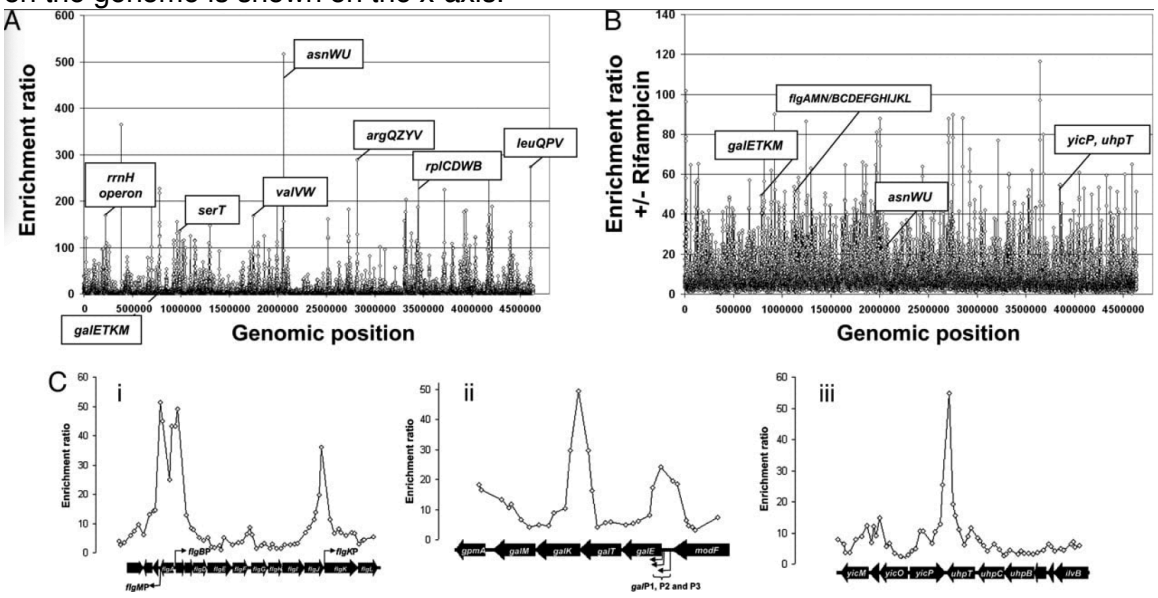
Discussion Worksheet — Winter 2026 –Week 9
Due on March 2 at 11:59pm

Group 1.

An experiment to study RNA Polymerase's association with select regions on an E. Coli gene was conducted using the ChIP methodology (chromatin immunoprecipitation) explained in class in the presence or absence an antibiotic called rifampicin. The enrichment ratio, which shows relative abundance, is shown on the y-axis. The position on the genome is shown on the x-axis.



A - Figure A is labeled with several boxes, which represent the locations of genes that are essential to cell processes. Explain why these peaks are high relative to the other locations.

B – By comparing the data in A (without rifampicin) to the data in B (with rifampicin), what is the overall impact of this drug on transcription?

C - Figure C is an enlarged version of certain regions in B. Based on the data given in C, which step of the transcription cycle is inhibited by rifampicin?

D - How would the data in C look in the absence of the inhibitor? Explain in 1-2 sentences, and sketch peaks onto the graphs and label them "not inhibited."

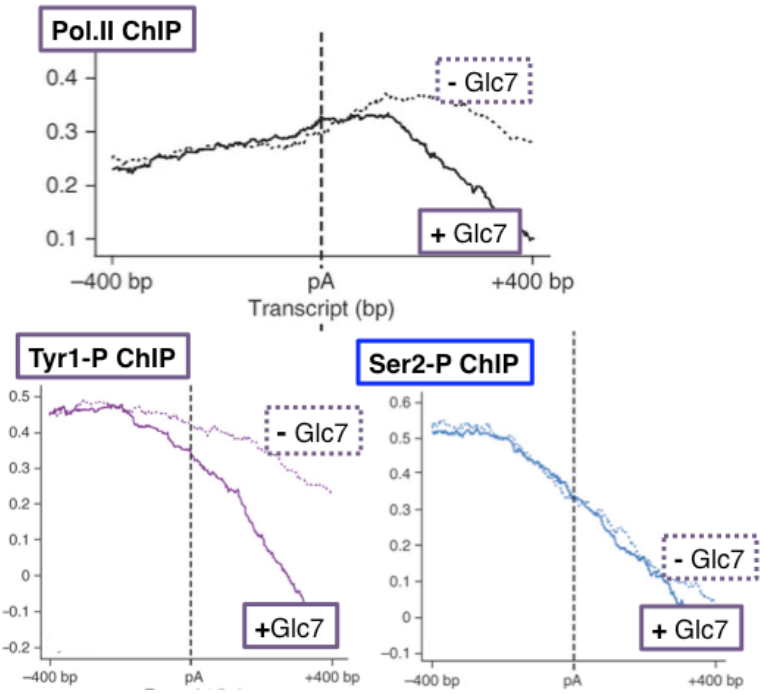
Group 2.

Glc7 is a protein that interacts with RNA Polymerase II. Researchers have studied the distribution of Pol.II by chromatin immunoprecipitation (ChIP) in the presence or absence of Glc7, upstream and downstream the site of cleavage and polyadenylation of a gene (pA) (for this problem, assume that the pA site indicates the site of termination by RNA Polymerase II). They also perform ChIP with antibodies recognizing the C-Terminal Domain (CTD) phosphorylated at Tyrosine 1 (Tyr1-P ChIP) or at Serine 2 (Ser2-P) of the YSPTSPS repeats (bottom two graphs) in the presence (+) or absence (-) of Glc7.

A-Describe the Pol.II ChIP signal on the top panel in the presence of Glc7 and explain the change in signal after the poly(A) site.

B- Based on the results shown on the top panel, what is the effect of inactivating Glc7 on the distribution of Pol.II on the gene? What does this indicate regarding the effect of Glc7 on RNA polymerase II?

C - Describe the effects of inactivating Glc7 on the distribution of the CTD modifications of Pol.II observed by ChIP



D - Based on these results, propose a biochemical activity for Glc7 and explain how Glc7 and CTD phosphorylation at different residues of the CTD controls transcription by RNA polymerase II.

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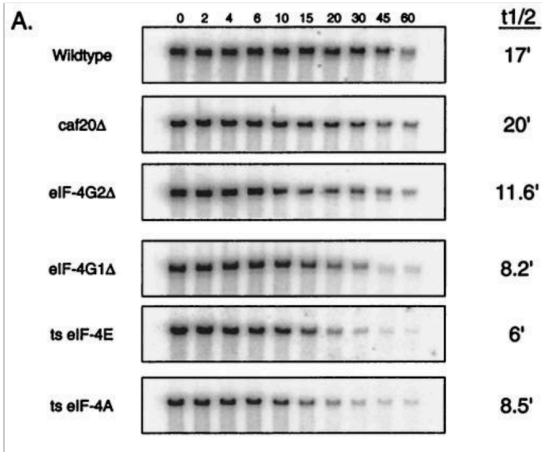
Group 3.

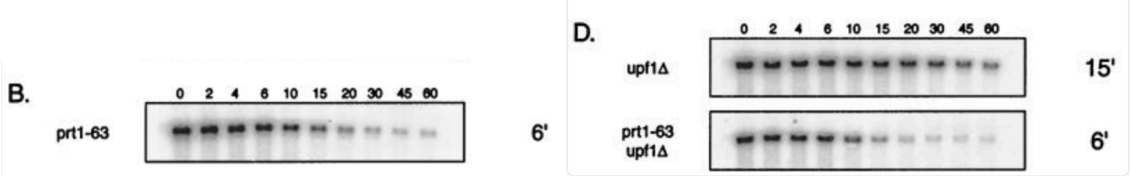
Researchers set up an experiment to test whether mutations in proteins involved in translation initiation led to higher rates of mRNA decay. To test this, the genes encoding several components of eIF-4F cap binding complex were mutated ("ts") or deleted (Δ), and the resulting half-life of the full-length mRNA was measured by taking samples of the reaction at various time points (measured in minutes, labeled at the top of each lane). Each gel represents a mutation to a different gene, which is labeled to the left of each plate, with "Wildtype" acting as the control condition with no mutations. The portion of the gels containing the full-length mRNA is shown in figure A. The half-life ($t_{1/2}$) of each condition is shown to the right of each gel.



A – Based on the data in Figure A, which mutation led to the greatest increase in the rate of decay? Explain your answer.

B – Why would mutation/deletion of this protein promote mRNA decay?

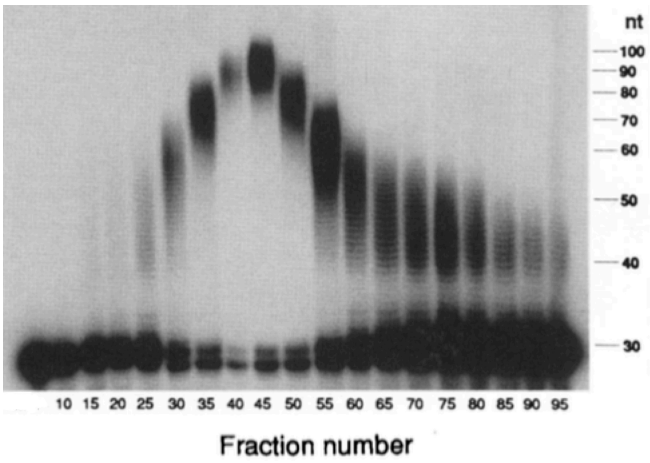
In addition to eIF-4F, several other proteins are involved in translation initiation and regulation. PRT1, a component of the eIF3 translation initiation complex, directly interacts with both the eIF-4F complex and the mRNA. UPF1 is a protein that regulates mRNA by degrading RNA that contain premature stop codons. The same experiment was carried out with mutations to PRT1 (and therefore mutations to the eIF-3 complex) and UPF1, where the genes prt1-63 and upf1Δ encode for PRT1 and UPF1 respectively. The data is shown in Figures B and D.



C – Compare the effects of the mutations to PRT1 and UPF1. What can be determined about the involvement of these proteins in this degradation process based on the data shown in figures B and D?

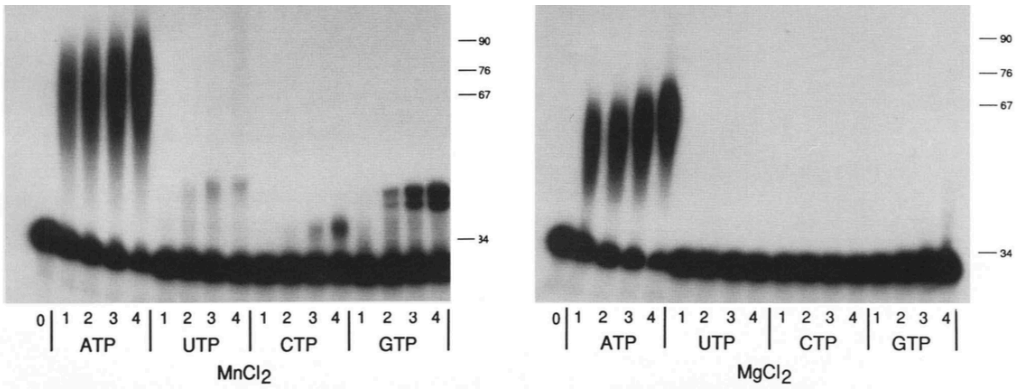
Group 4.

An extract prepared from eukaryotic nuclei is loaded on a column that fractionates macromolecular complexes. Each fraction collected by elution from the column is incubated with a 30 nucleotide long radiolabeled RNA substrate, ATP and Mg^{++}.
After incubation, the RNAs are purified such that the proteins are no longer associated with them and the RNAs are fractionated according to size on a gel and visualized by autoradiography. The size of the products is indicated on the right. The first lane corresponds to the unreacted RNA substrate; numbers on the x-axis indicate the fractions numbers.



A – Based on the results obtained and on the size of the RNA products, predict and explain what protein(s) and biochemical activities are contained in fractions 35 through 55, and in fractions 65 through 80.

B – Fraction #45 is now incubated with the same RNA, but in the presence of either $MgCl_2$ or $MnCl_2$, and with ATP, UTP, CTP or GTP. The reaction products are shown on the right. 1,2,3 and 4 refer to different time points (15, 30, 60, and 120 sec, respectively).



Describe the results obtained with the two different metal ions. Based on these results, propose a function for Mg^{++} in the biochemical activity present in fraction#45.