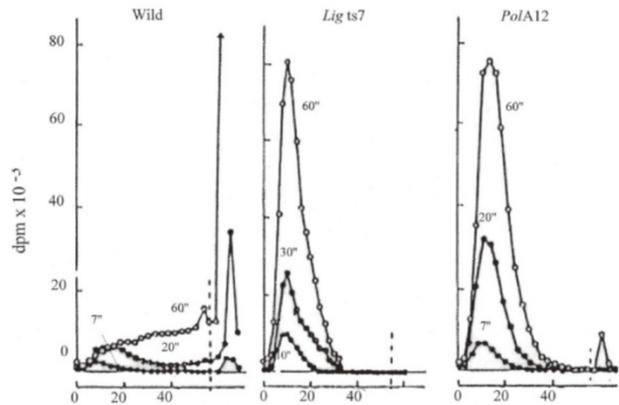


Discussion Problems – Winter 2026 - Week 4 (Polymerases)
Due on February 2 at 11:59pm

Group 1

In this experiment a scientist incubated bacteria with radiolabeled dTTP and after specific times of radiolabeling (indicated by 7", 20" etc..= 7 seconds, 20 seconds), they extracted DNA and measured the radioactivity incorporated in DNA (dpm, y-axis) as a function of the size of the DNA molecules (x-axis, arbitrary units). In the first experiment (left figure) they used a normal bacterial strain (=Wild).

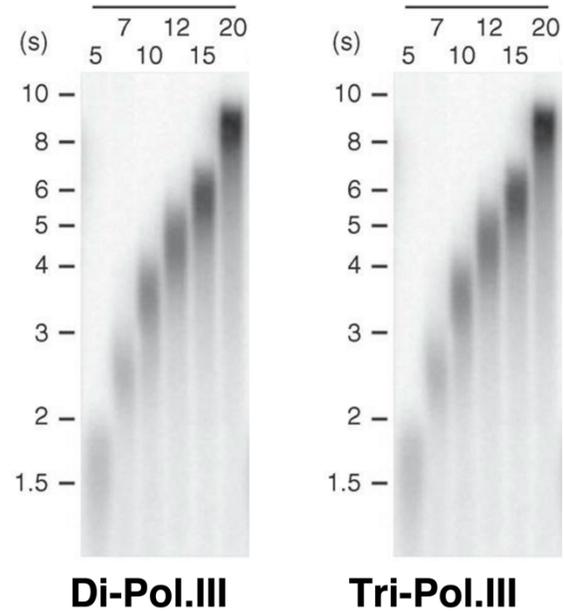


A - Describe the change in size in DNA fragments as a function of time in this strain and explain what the changes in size correspond to.

B - In the second and third experiment they used bacterial strains which contain mutations in gene encoding DNA ligase (Lig ts7) or DNA Polymerase I (PolA12). Describe the differences in DNA synthesis in these strains compared to the wild-type and what this experiment tells us about the roles of DNA ligase and DNA polymerase I in replication.

Group 2

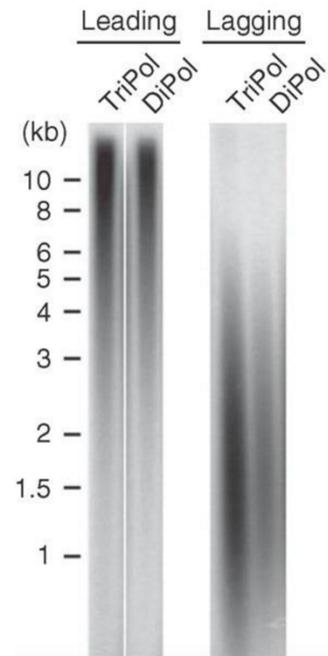
In this experiment, scientists study the properties of bacterial replisomes containing 2 (DiPol.III) or 3 (TriPol.III) DNA polymerases. In a first experiment they incubate each replisome with a primer and a single stranded DNA template in vitro and study DNA polymerization as a function of time. The size of DNA fragments is shown on the y-axis in kilobases, after incubation with the replisomes for 5,7,10,12,15 or 20 seconds (times are shown at the top of each lane).



A- Describe the results observed; what property of Di and Tri-Pol.III replisomes can be concluded from the results of this experiment?

B- Next, researchers specifically analyze the products generated during replication in vivo on the leading and lagging strands with Di or Tri-Pol.III. The size distribution and abundance of the fragments obtained after incubation of Di or Tri-Pol III with leading or lagging strand templates is shown below.

Describe the results obtained in this experiment; based on these results and on the results obtained in the first experiment, compare the ability of Di-Pol.III and Tri-Pol.III replisomes to replicate DNA.

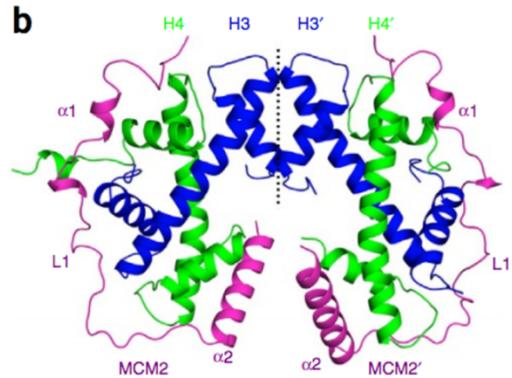


Group 3

Structural Biologists have studied the structure of the MCM2 protein (part of the MCM protein complex present in the replisome in eukaryotes). They have obtained the structure of MCM2 interacting with the H3 and H4 histones as shown in the structure on the right.

H4' means that a second molecule of histone H4 is present. Same for H3 and MCM2.

The formation of the dimer of this structure is an artefact of structure determination. For the purpose of this problem, only consider that one MCM2 interacts with one H3 and one H4.



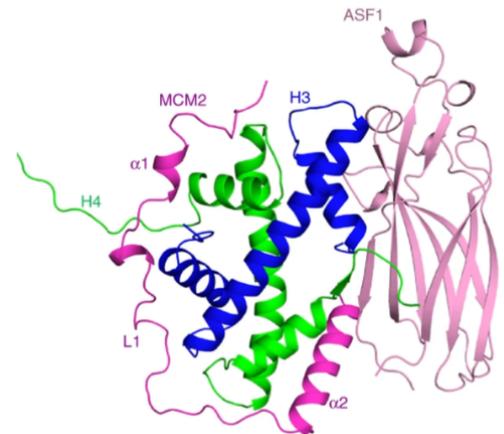
A: What does the dotted line represent?

B: Based on what we covered in class, what is the known function of the MCM complex that MCM2 is involved in?

C: Based on the structure shown above, what other function does this structure suggest for MCM2 during eukaryotic DNA replication?

The researchers have now obtained a second structure of MCM2 bound to histones H3 and H4 in complex with ASF1. The structure is shown on the right.

D: Describe globally the protein-protein interactions that exist in this complex. Does the presence of ASF1 change the structures of H3/H4?



E: Based on the two structures shown and what you know about the role of the MCM complex during replication, propose a more specific role for MCM2 during eukaryotic replication.

Group 4

The purified telomerase enzyme is incubated with a 12nt primer made of telomeric repeats and radio-labeled dNTPs. Two additional proteins, Teb1 and/or CST, are added (+) or omitted (-) from the reaction accordingly. The results are run on an autoradiography gel. Numbers on the right indicate the numbers of nucleotides added to the unextended primer, which is not shown but would be at the bottom of the gel.

A – In the lower part of the gel for lanes 1 or 2, count the number of faint bands between each more intense band. What molecular events related to telomerase activity do the faint and more intense bands represent?

B – What is the effect of the CST protein on telomerase activity especially related to the presence of faint vs. more intense bands? Justify your answer by comparing the patterns observed in lanes 1 and 2.

C - What is the effect of the Teb1 protein on telomerase activity? Justify your answer by comparing the patterns observed in lanes 1 and 3.

D - How does adding both Teb1 and CST affect telomerase activity? Justify your answer in one or two sentences.

