Using a Viral Protein to Study DNA Replication

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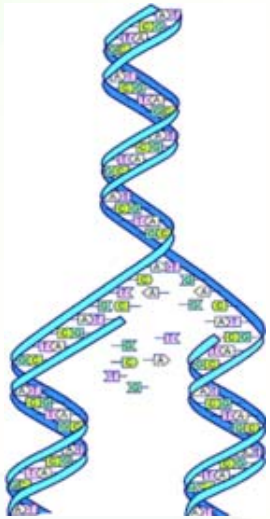
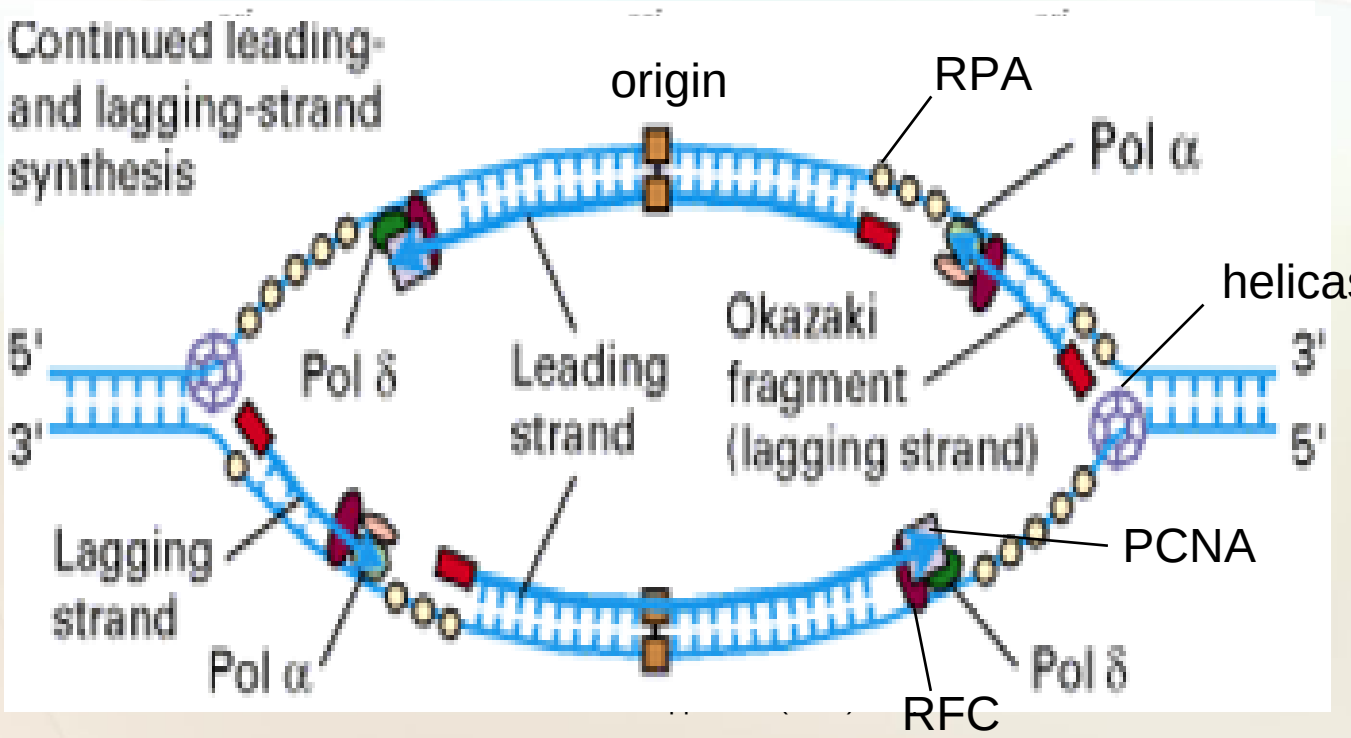
Eukaryotic DNA Replication: A well understood process?

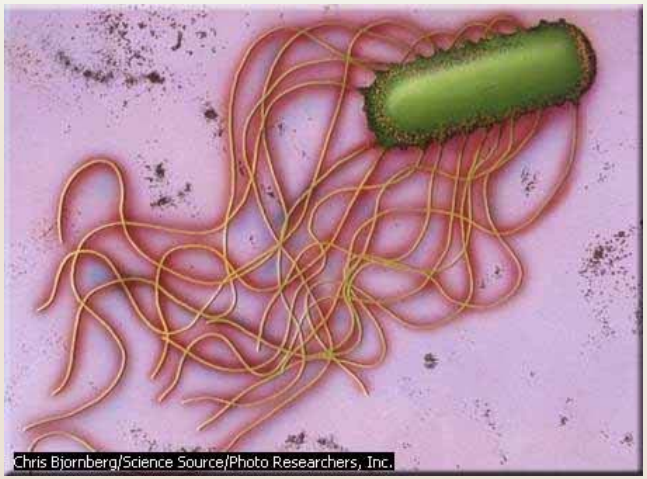


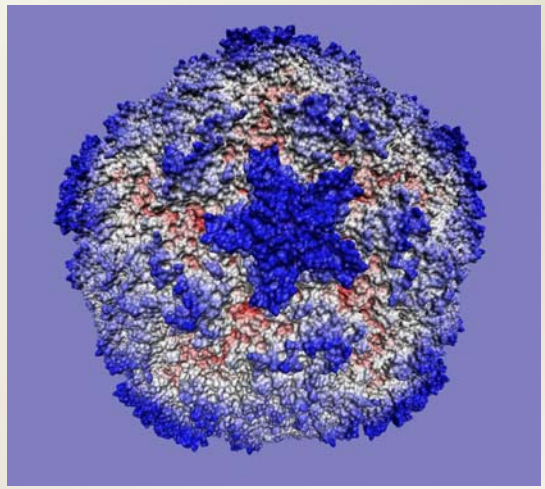


Molecular Cell Biology. 4th ed. (2000) Lodish.

How can we overcome the problem of studying complex systems?

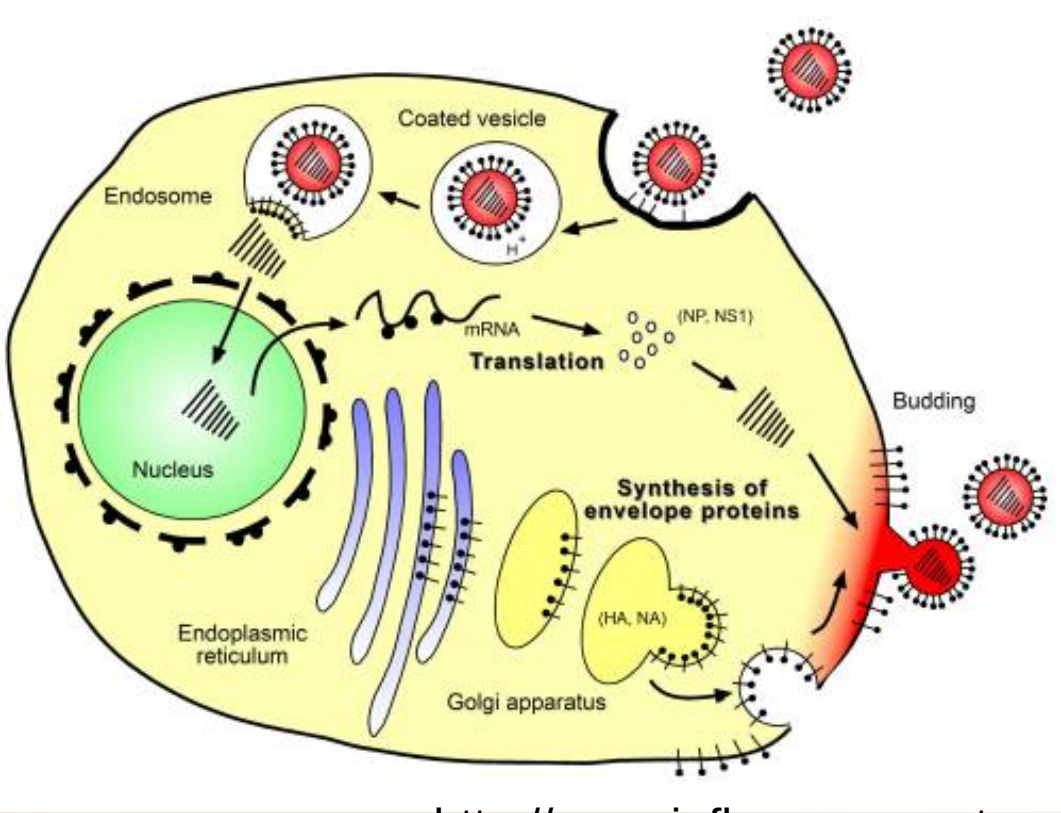
- **Modeling: Use a simplified system to study a complicated process**
- **Examples of models:**
  - **Animal models to study human body systems**
  - **Bacterial and viral models to study eukaryotic cell processes**





Why use viruses to study DNA replication?

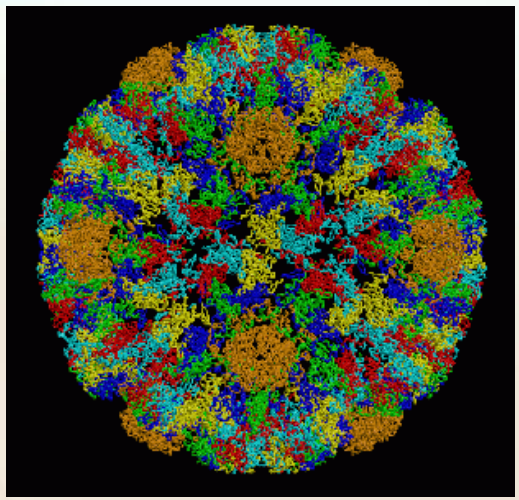
- **Viruses can’t replicate their DNA alone, they rely on infecting other cells**
- **Viruses use cell proteins for replication**



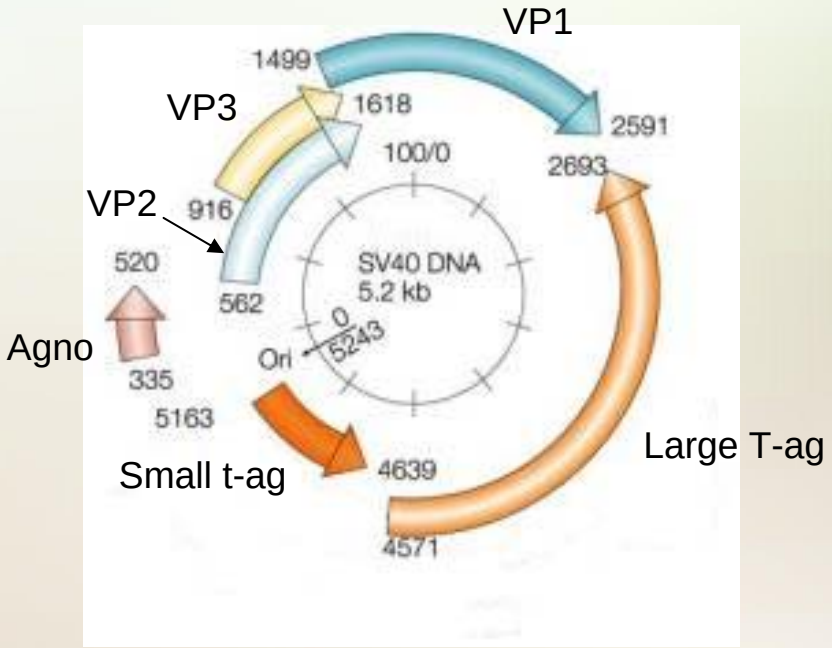
http://www.influenzareport.com/ir/pathogen.htm

What's special about Simian Virus 40?

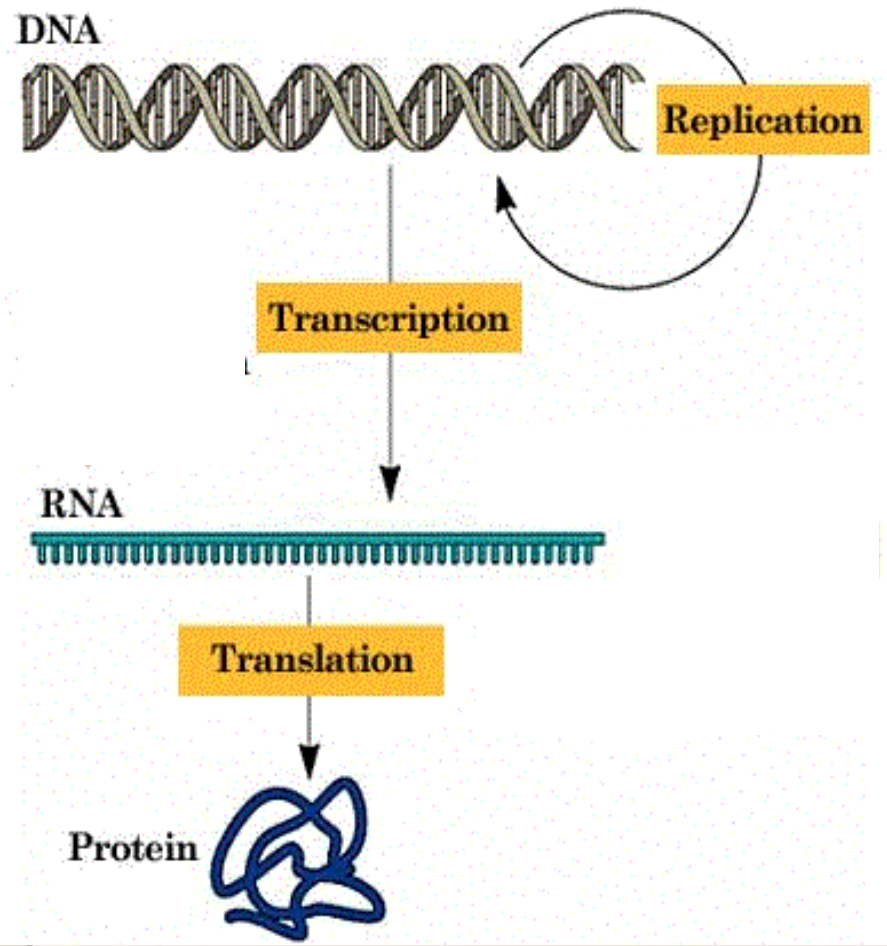
- **Structure of SV40 chromosome comparable to eukaryotic DNA**
- **Single, well-defined origin of replication**
- **T-ag is the only viral protein required**



www.protein.osaka-u.ac.jp



How do we use T-ag?

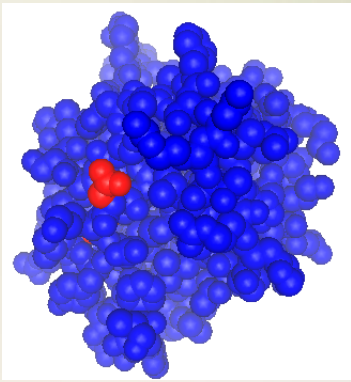


Making mutations in the DNA sequence that encodes T-ag

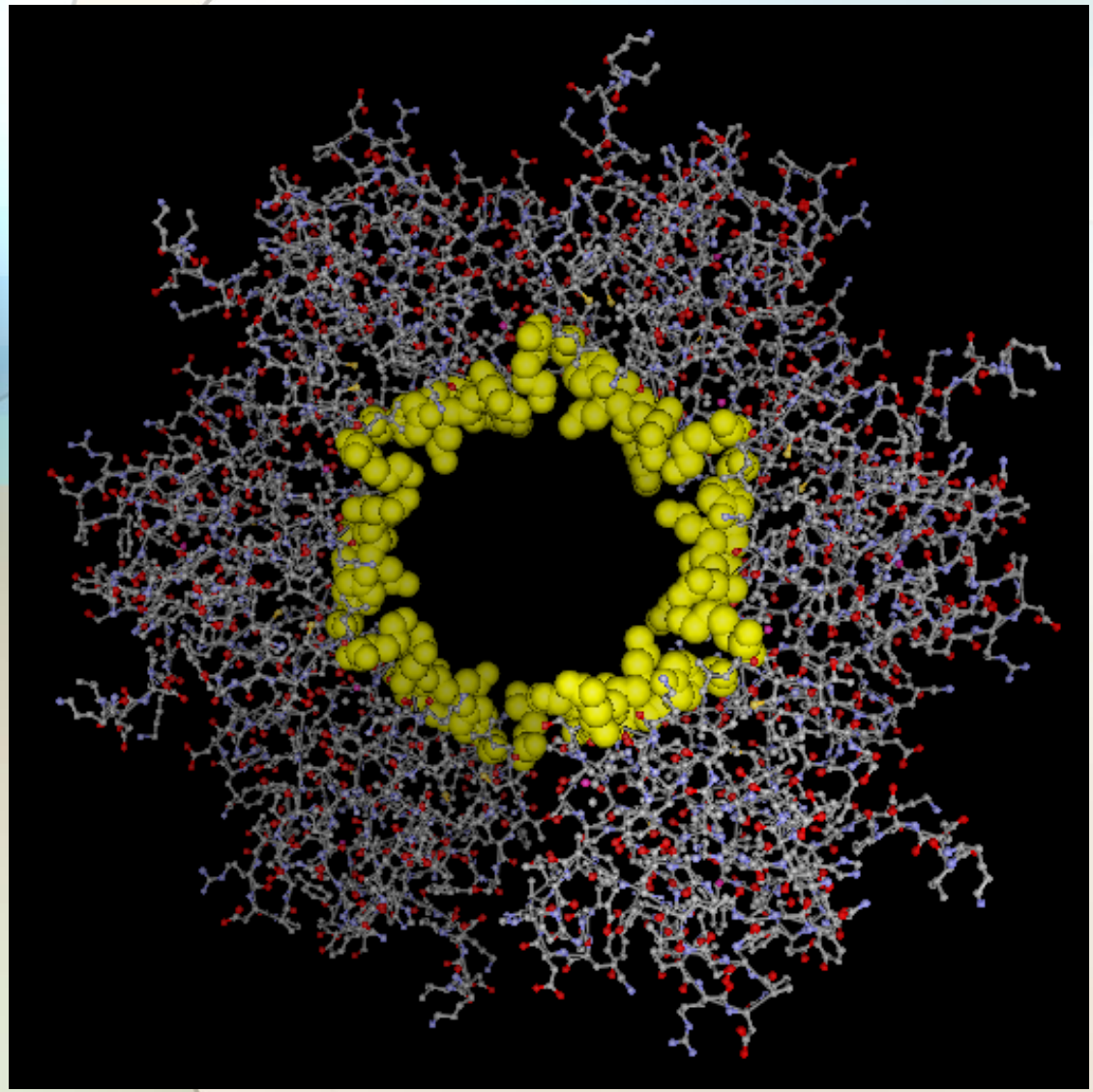
ACTGTTACG
TGACAATGC

Results in

Mutated T-ag protein

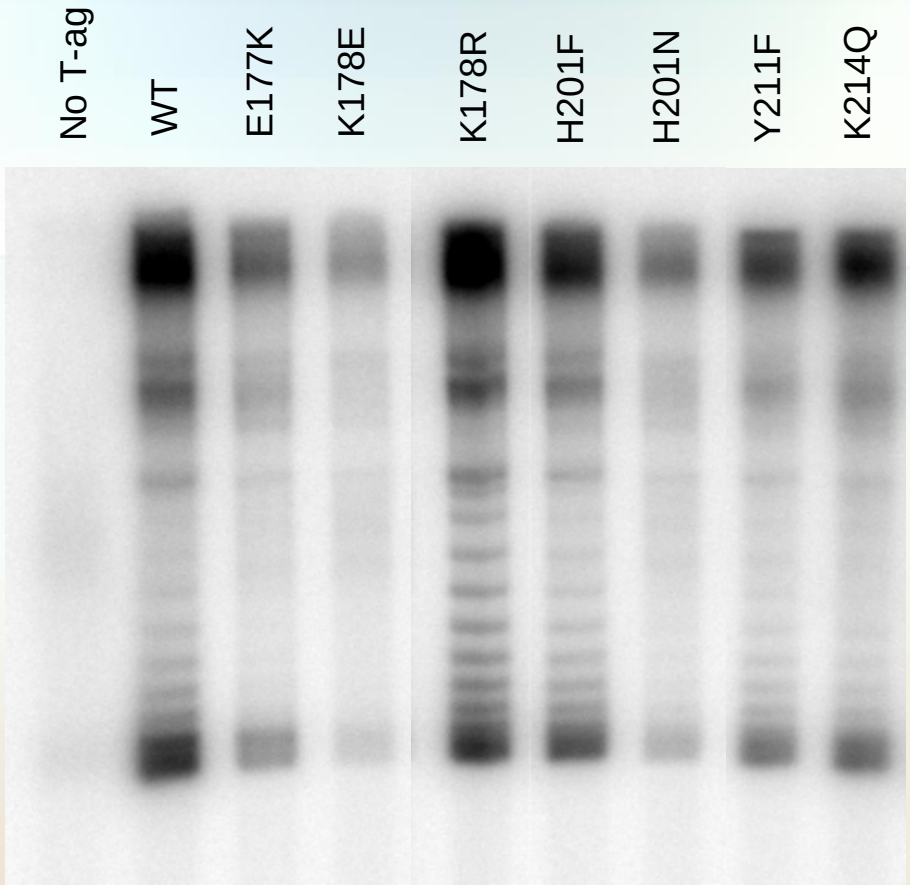


What about my research?



- I am making mutations to investigate the ability of T-ag to interact with DNA
- By mutating these amino acids, I hope to disrupt DNA binding thus proving the function of these amino acids.

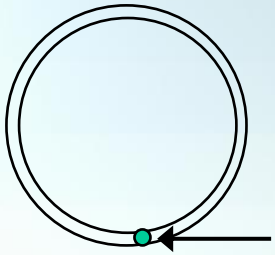
In vitro DNA Replication



Reactions include origin containing plasmid DNA, 1.5 µg FL T-ag, 293 cell extract supplemented with 100 ng Topo I and ^{32}P labelled dCTP.
After 1 hr incubation at 37C reactions were stopped and DNA was purified and run on a 1.5% agarose gel. Replicated DNA was visualized by autoradiography.

Thank you!

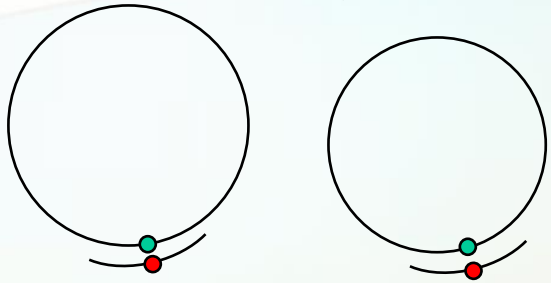
How I make mutant T-ag



Plasmid DNA containing gene for SV40 T-ag

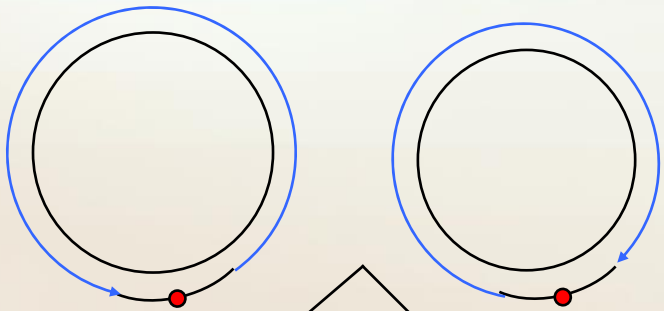
Target for mutation

Primer with mutated base



Denature/separate DNA

Allow primers DNA to attach



Copy the rest of the plasmid

Enzymes