MMG 301, Lec. 25
Mutations and Bacteriophage

Questions for today:

1. What are mutations and how do they form?
2. How are mutant bacteria used in research?
3. What are the general properties of bacteriophage (viruses of bacteria)?
4. How do phage grow?
5. How do we assay for phage?

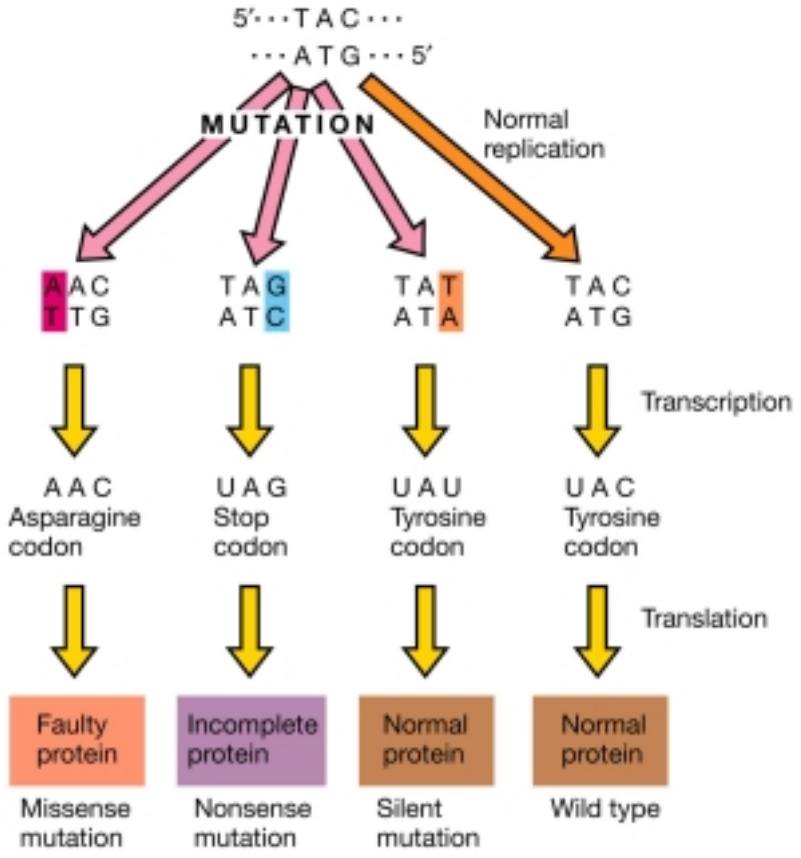
Overview of mutations

Definitions:

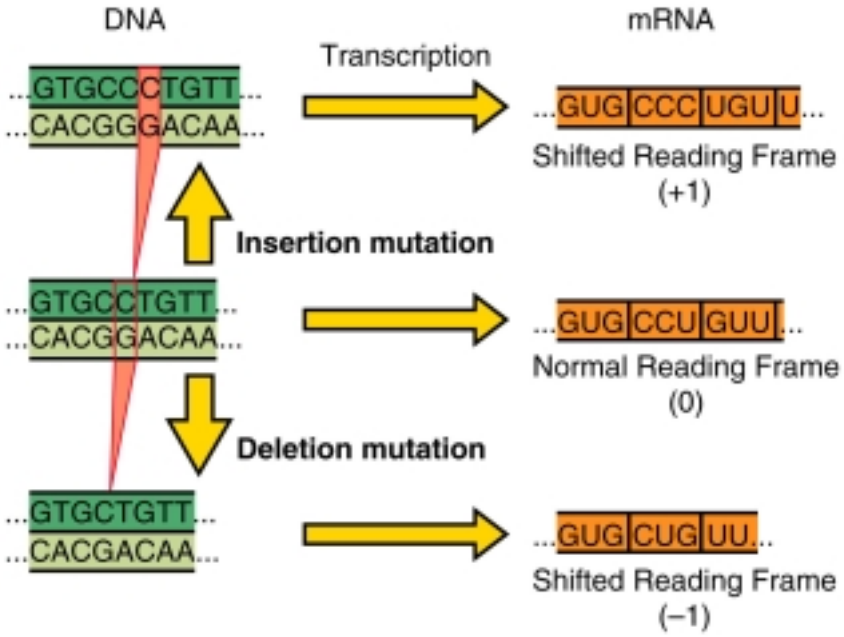
Mutation: an inheritable change in the base sequence of a genome
Mutant: organism carrying a mutation
Genotype:genetic makeup of an organism
Phenotype: observable characteristics of an organism (pigments, overall appearance such as flagella or capsule, nutritional growth requirements, etc.)

Types of Mutations:
Base pair changes and insertions/deletions

Possible base pair changes to a codon for Tyrosine



This figure shows the insertion or deletion of a single base, but larger segments can be inserted or deleted



Shifted reading frames are likely to result in the formation of stop codons

Effects on the cell (phenotype):

No effect: this is always the case for silent mutations, often seen with missense mutations, and may occur with insertions/deletions that retain the same reading frame.

Death:this can be hard to study! One approach to examine such mutants is to include 2 copies of the gene, where only one is mutated.

Conditional lethality: This is the best phenotype for genetics studies. Cells survive only under a subset of conditions

- New growth requirement: e.g., mutations in a *trp* gene will prevent cell growth unless tryptophan (Trp) is provided (the mutant is an *auxotroph* whereas the wild-type cell is referred to as a *prototroph*).

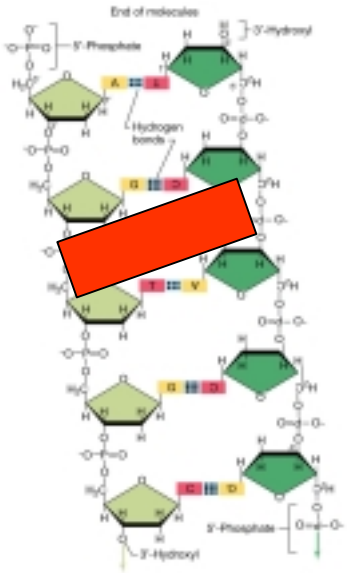
- Unique temperature dependence: e.g., the mutation alters an enzyme needed for bacterial survival. The variant enzyme works in cells grown at 30ºC, but is inactivated in cells grown at 37ºC.

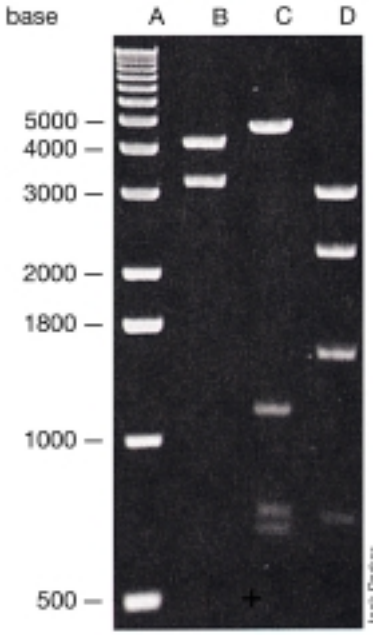
Causes of mutations:

Errors in replication: Spontaneous. Occurs at a rate of 10^{-7} to 10^{-11} per base pair. For a 1000-bp gene, this means 10^{-4} to 10^{-7} chance of a mutation per generation. If 10^{8} cells/ml will likely find mutant!

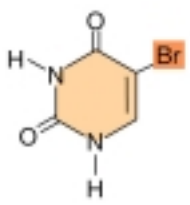
Chemical mutagens:

- Chemicals that modify DNA.
  - Nitrous oxide deaminates A and C
  - Hydroxylamine reacts with C
  - Ethylmethane sulfonate adds CH_3 group
    - (repair enzymes exist!)
  - Nitrosoguanidine crosslinks 2 DNA strands
- Intercalators insert into stacked bases to resemble an extra base. Example = ethidium bromide. This hazardous chemical is frequently used in labs to visualize DNA in gels due to UV fluorescence.

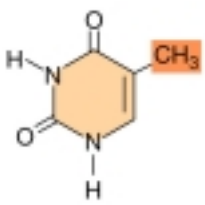




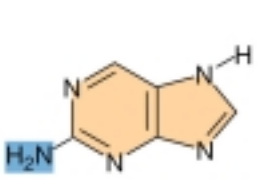
- Base analogs resemble true bases, are incorporated into DNA, but lead to incorrect base pairing.



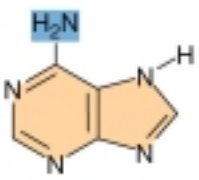
5-Bromouracil



Thymine



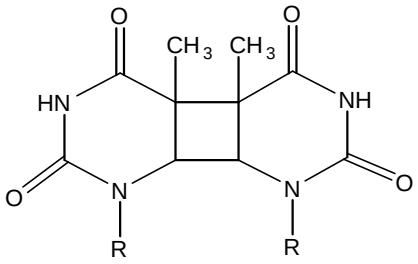
2-Aminopurine



Adenine

Radiation

- UV light causes thymine dimerization

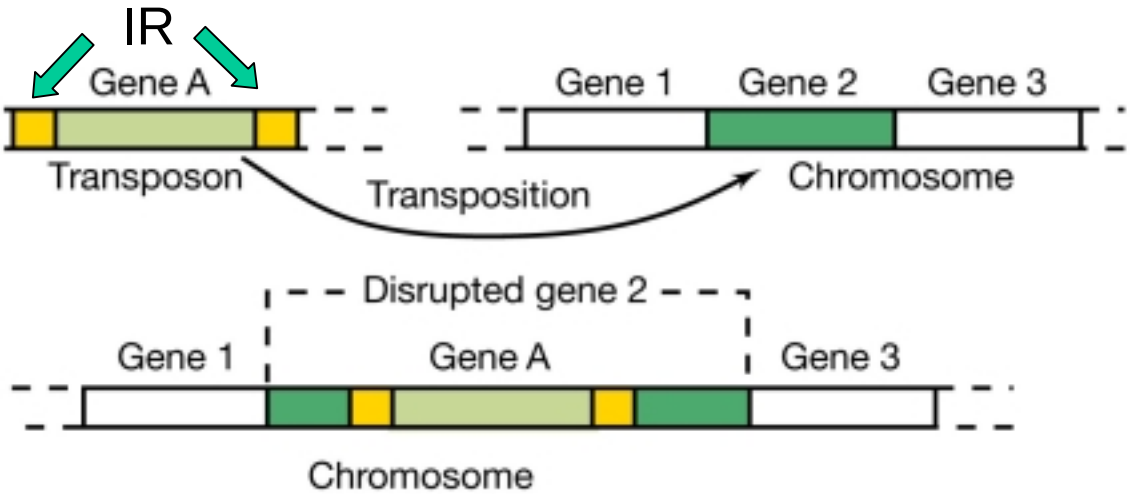


repair enzymes exist

- X-rays/cosmic rays/gamma rays lead to production of hydroxyl radicals (OH•) that can damage DNA in several ways including strand breaks. (Repair enzymes exist. *Deinococcus radiodurans* has special proteins that hold DNA together to allow repair)

Biological agents

- transposons are DNA fragments that "jump" from one position to another using inverted repeats (IR). They will cause a mutation if they land inside and disrupt a gene. They are likely derived from viruses.



- viruses can also disrupt genes if they become inserted into a genome (see prophage below)

Working with mutant bacteria

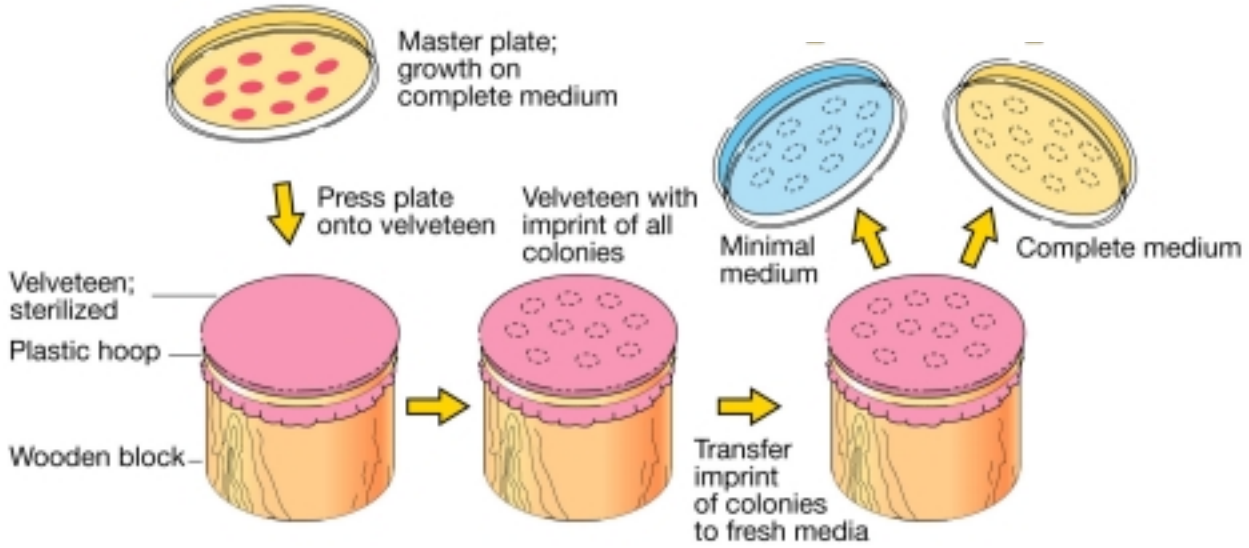
How do we isolate a desired mutant?

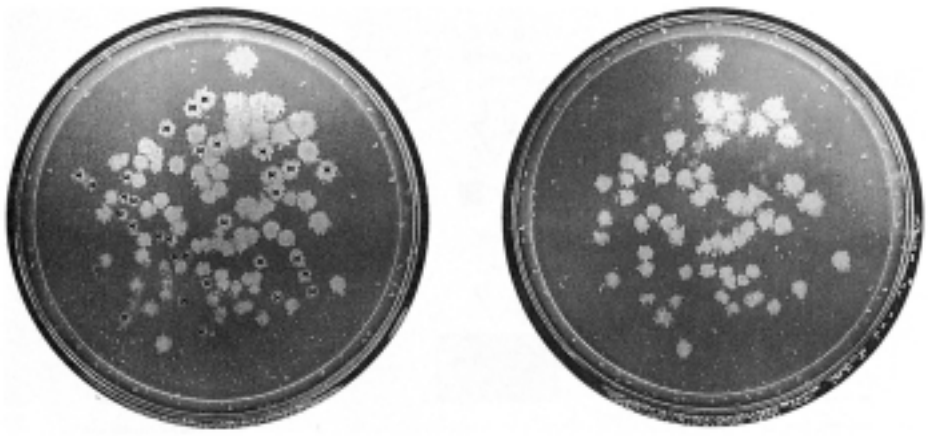
Example = Trp auxotroph

Spread a sample of mutated culture on complete medium (A, containing Trp) at a dilution to give individual colonies.

"Replicate plate" the colonies onto (A) and (B) containing medium lacking Trp.

Colonies growing on A, but not B, are likely Trp auxotrophs defective in a *trp* gene.





Penicillin selection

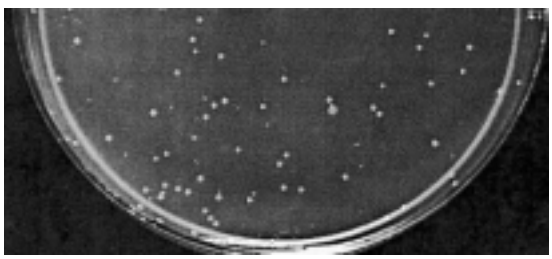
The above method works great if you have lot's of mutants, but this is usually *not* the case. A useful method to "enrich" for mutants is by penicillin selection.

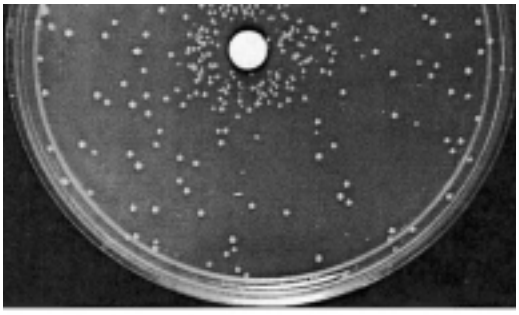
A culture is inoculated into liquid medium lacking the growth factor of interest (e.g., Trp) and containing penicillin. Cells able to grow without Trp are killed by penicillin, whereas the auxotrophs cannot grow so are not affected by the antibiotic. After transfer to a medium without penicillin, but with Trp, the mutants grow.

Cells → Medium A: - Trp, + penicillin → Medium B: +Trp, - penicillin → Enriched for Trp auxotrophs

Using mutants to test for mutagens:

Ames test: *his* auxotroph requires His for growth. The mutation spontaneously "reverts" so that ~10 of 10^8 cells can grow without His. Mutagens (added to a paper disk) increase the reversion frequency.





[Next lecture: using mutants to study gene transfer]

General Properties of Bacteriophage

Definitions

Bacteriophage: A virus or genetic element containing DNA or RNA that requires a host bacterial cell for replication, but has an extracellular state.

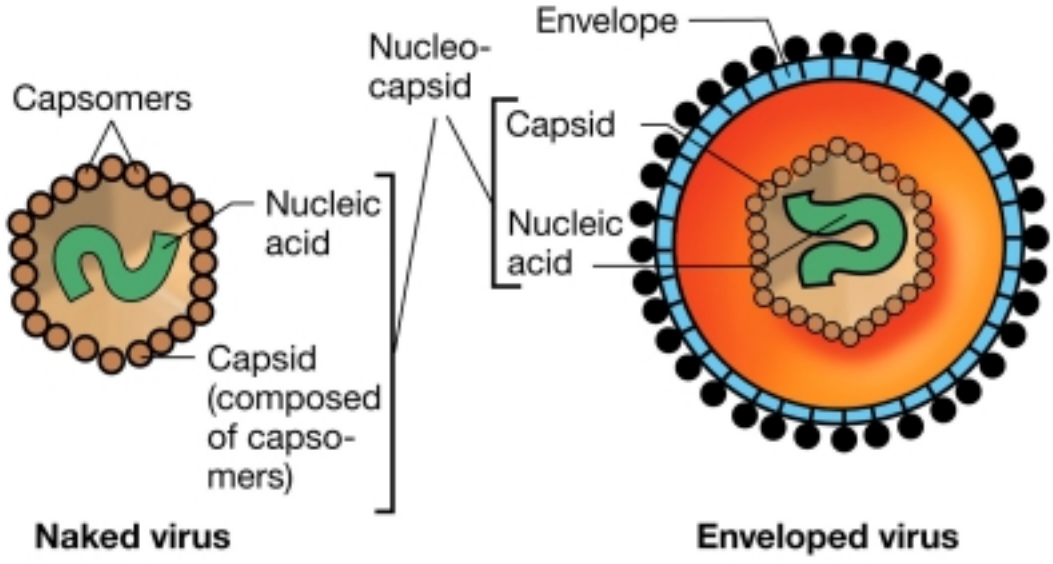
Virion: virus particle.

Structures

Too small to see by light microscopy, can only visualize by electron microscopy.

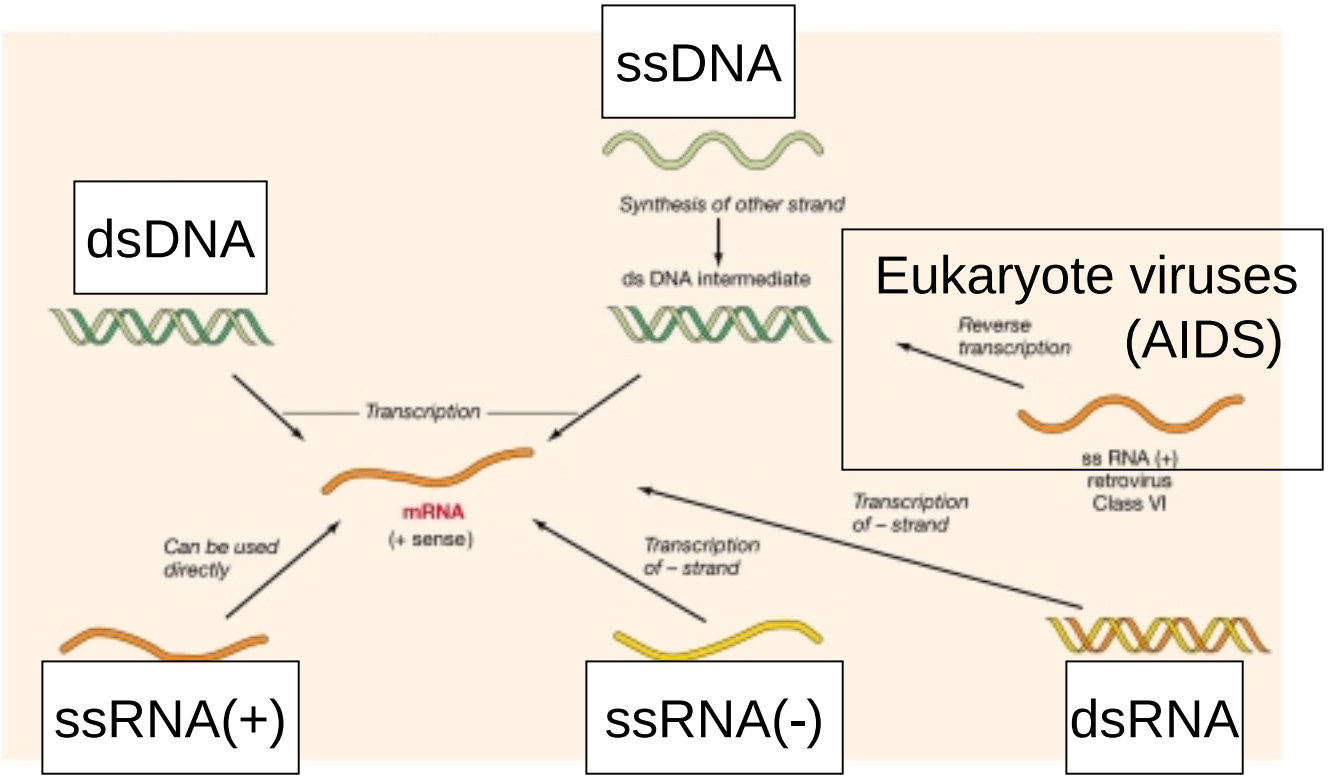
Naked phage (more common) contain the genetic material surrounded by a protein shell (capsid) composed of capsomer proteins. The capsid plus genetic material is the nucleocapsid

Enveloped phage additionally possess an envelope derived from the host.



Types of nucleic acid depend on the phage, with dsDNA (48,514-bp Lambda or 168,903-bp T4), ssDNA (ΦX174 of only 5,386-bp), dsRNA (ϕ6), or ssRNA (MS2) known.

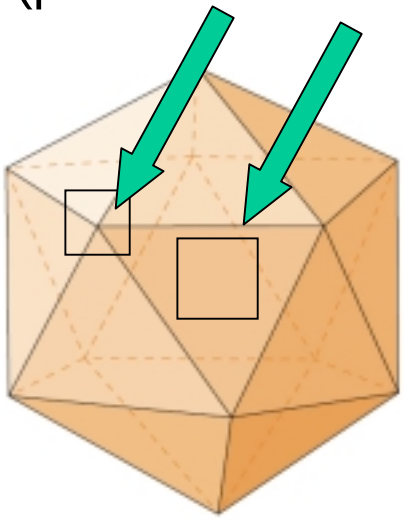
In the case of ssRNA, the strand may be the coding (+) sense (i.e., mRNA) or the opposite (-) sense. The method of replication/transcription will depend on the type of nucleic acid:

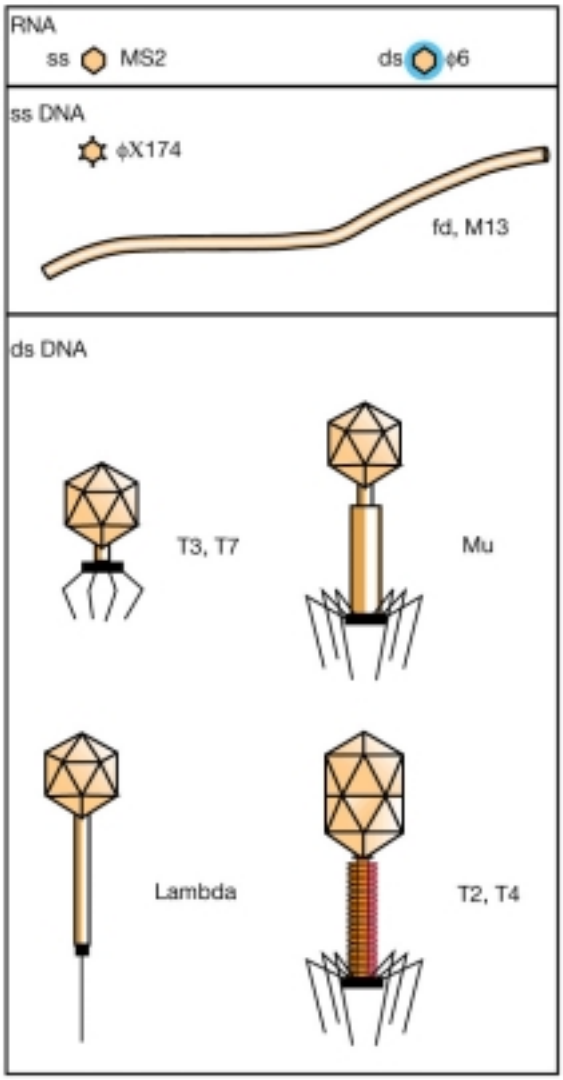


Properties of the capsid: Made of one or a few types of capsomer proteins that <u>spontaneously assemble</u> into various defined shapes.

- helical
- icosahedral
- complex

All the same protein, or distinct proteins in different parts of the structure (proteins A vs. B)



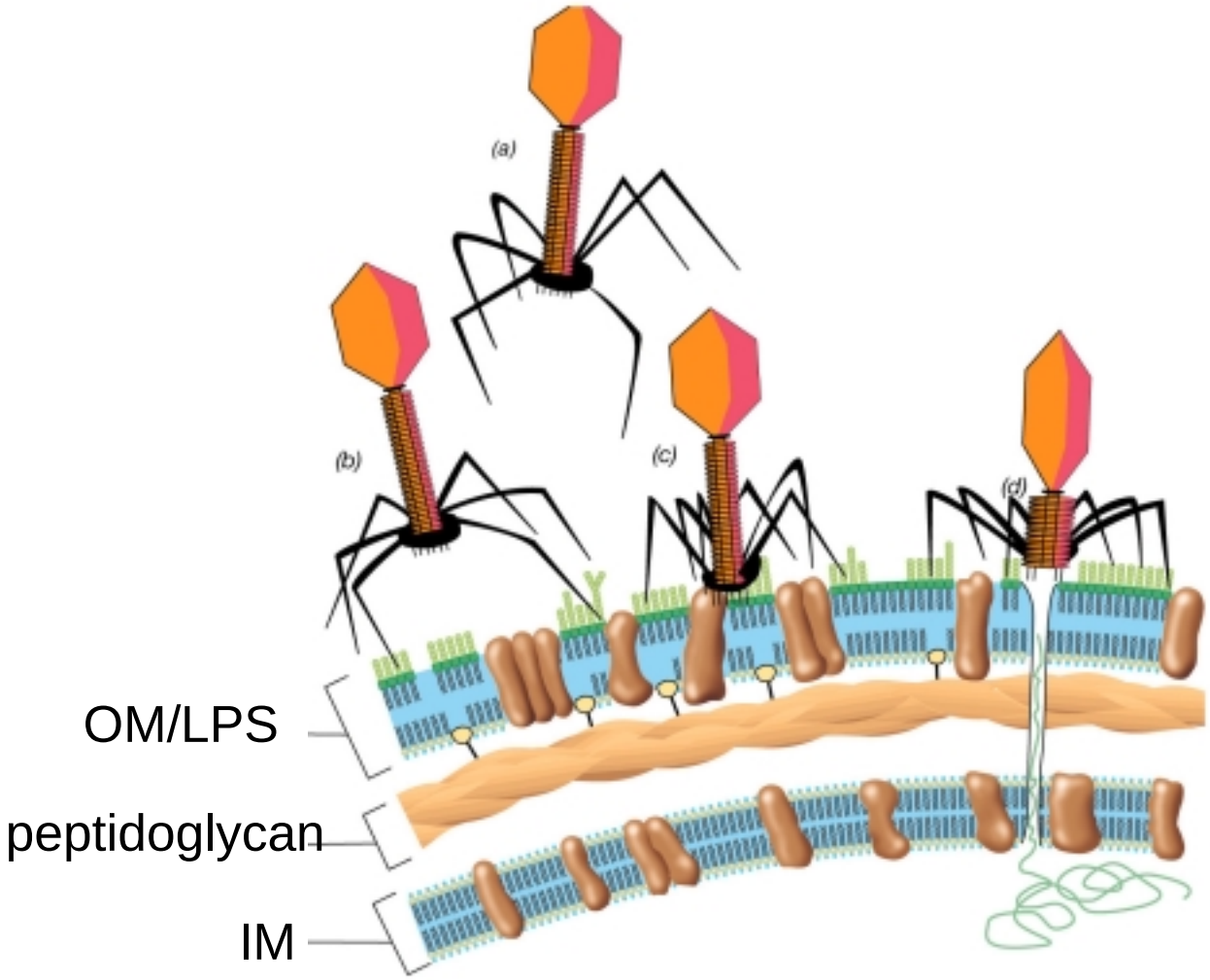


The capsomer also often carries phage-encoded enzymes made by the host cell. These enzymes may include phage-specific polymerases and nucleases to degrade the host nucleic acids

Growth cycle of phage

Attachment: highly specific due to phage proteins binding to a host cell receptor (protein, carbohydrate, lipid, etc.). The receptor is not designed to bind phage, rather it has a normal cellular role (e.g., Fe transport).

Penetration: Nucleic acid (and some proteins) enter cell, whereas capsomer often remains external. In the case of some phage (e.g., T4) nucleic acid is injected into the cell.



Early infection: early mRNA transcribed, early proteins made to begin replication process.

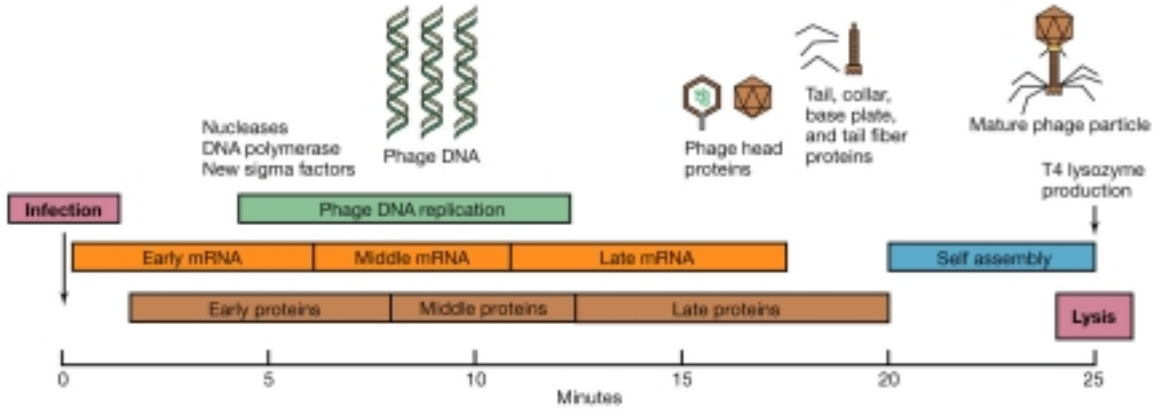
Replication: Phage nucleic acid is synthesized.

Viral protein synthesis: capsomer components formed.

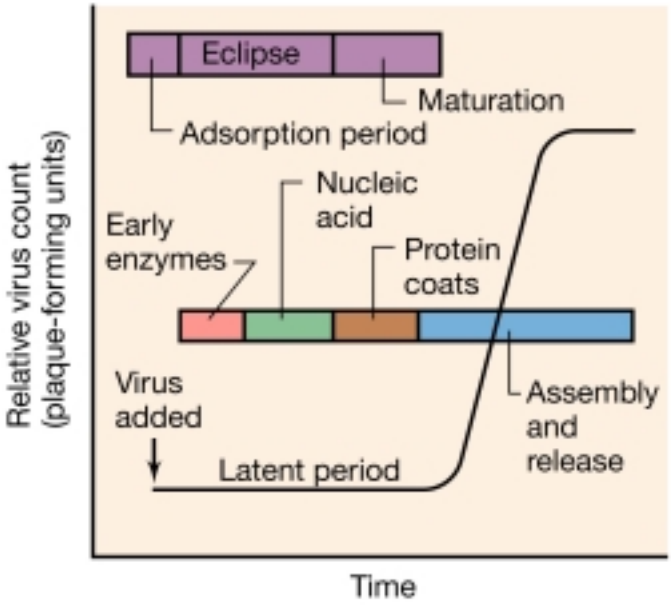
Assembly: spontaneous formation of new phage.

Release:

- in some cases, new phage “leak” out without killing the host (e.g., M13).
- in other cases, host is destroyed by lysis. This is completed in 25 min for T4.



One-step growth curve leads to “burst” in number of virions (e.g., each T4 phage yields 100 new virions)

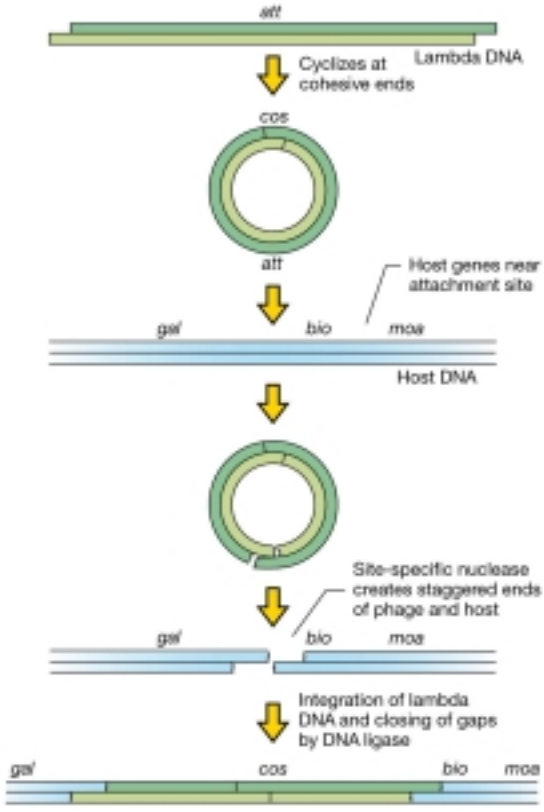


Temperate phage: an added complication.

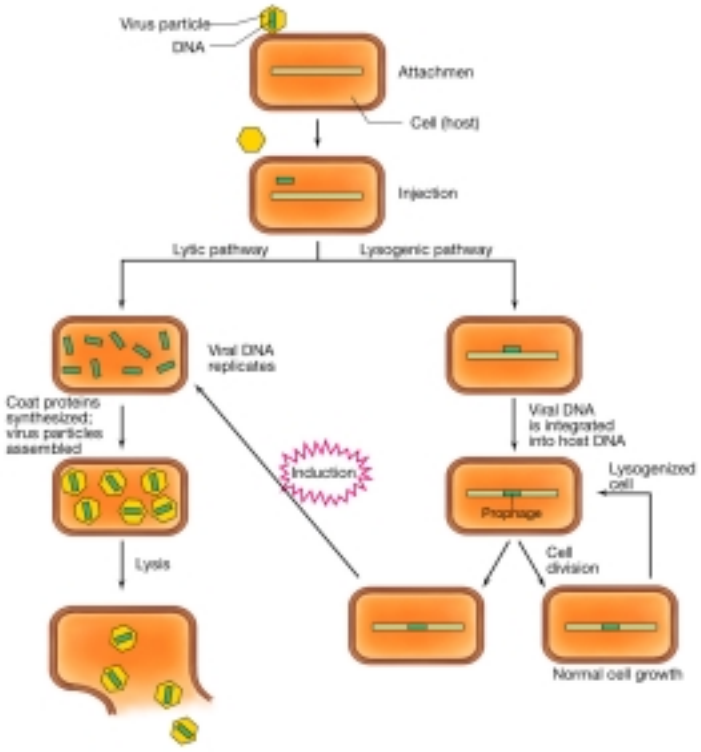
Rather than always being virulent (lysing the cell), the temperate phage (e.g., Lambda) participate in an alternative course of action after infecting a cell called *lysogeny*.

Lysogeny occurs when the phage DNA incorporates into the host DNA, creating a *prophage*, then simply replicates with the host.

The site of insertion may be very specific as in Lambda.



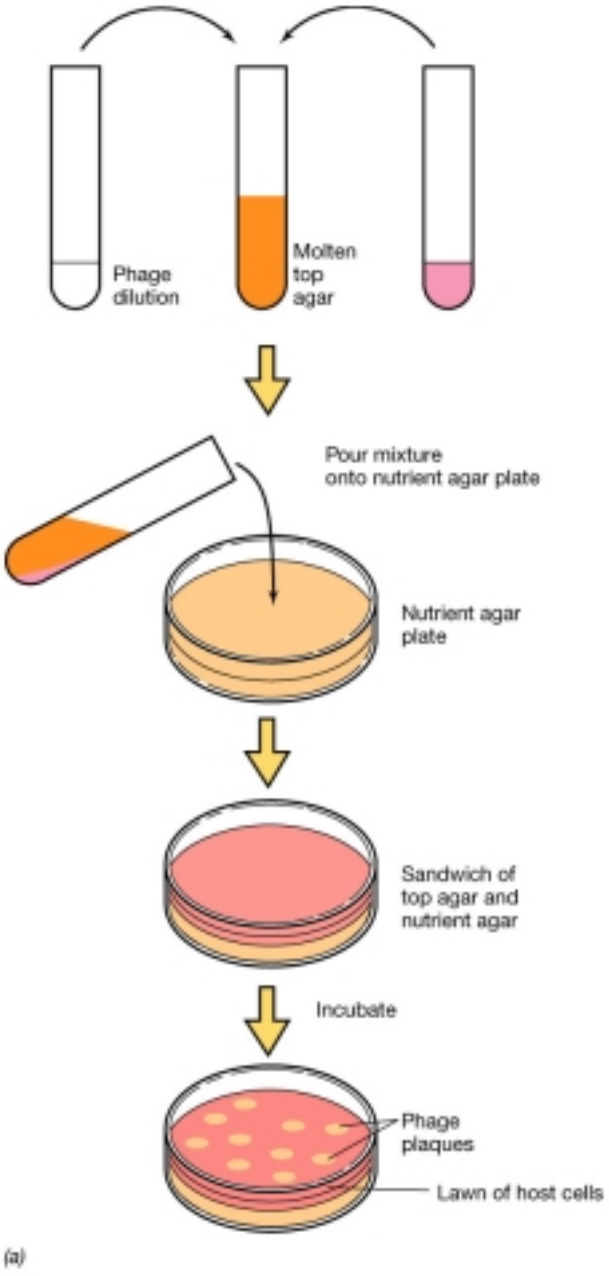
On rare occasions the prophage excises from (leaves) the host DNA, resulting in a lytic response.



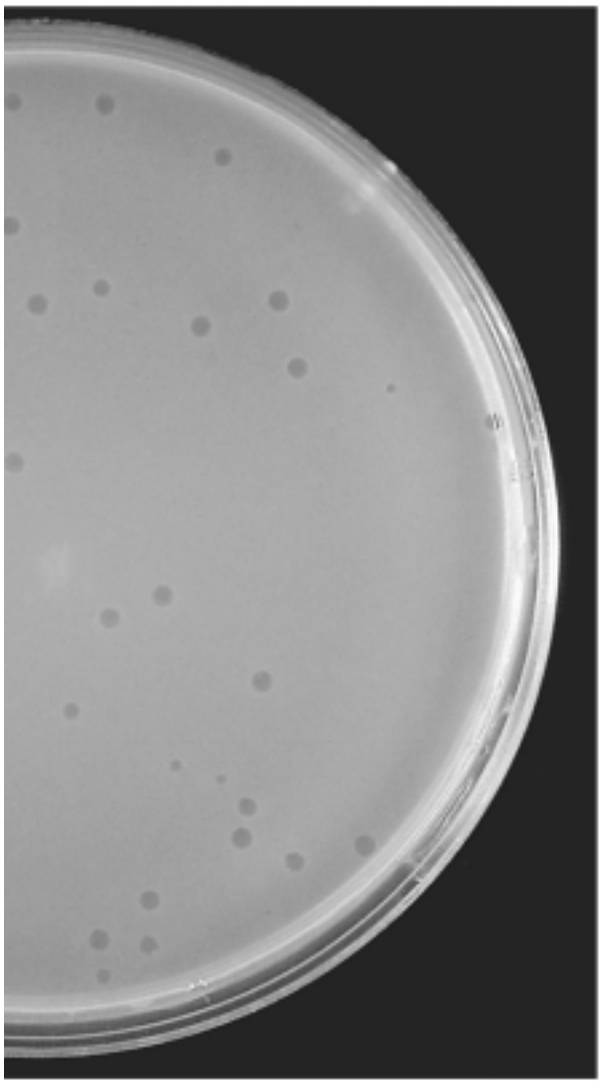
Prophage excision is stimulated by UV, X-rays, and DNA damaging chemicals

Assay (quantitation) of phage

For lytic phage, one uses a "*plaque assay*"



A lawn or confluent culture of bacteria is grown with phage. Lysis of the bacteria by phage results in clear spots or plaques



This approach also works with animal viruses, using tissue culture cells