

Lecture 23: Replication, Transformation, mRNA Processing, Carbohydrates.

Lagging strand synthesis – Replicated in sections, with replacement of multiple RNA primers by DNA pol I and joining of segments by DNA ligase.

Identify the differences between each image – what happened and who did it?

Black = template

Blue = RNA primers

Red = New DNA

5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-

↓ primase
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *direction of syn* (arrow pointing right), *helicase* (circled), *gyrase* (circled), *dSDNA opened*, *DNA helicase*

↓ Pol III
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *Pol III* (red), *Pol III* (black), *Pol III* (black)

↓ primase top strand
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *primase top strand* (blue), *AUCAG5'* (red)

↓ Pol III
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *Pol III stops* (red), *Pol III* (black), *RNA digest by pol I* (blue)

↓ pol I extends DNA
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *made by pol III* (red), *pol I extends DNA* (red), *broken phosphodiester (nick)* (red)

↓ repaired DNA ligase
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *repaired DNA ligase* (red)

↓ lagging strand (sections)
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *made in* (red), *RNA primer* (red), *Primer* (red)

↓ leading strand synthesis
5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-
Handwritten: *leading strand synthesis* (blue), *comp piece DNA* (blue)

5' CCCCTTAACCTCCAAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCGC-
3' GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGCG-

Online material to help with DNA Replication: <https://oli.cmu.edu/>

- Register using course key: DNAREP-D
- Complete pre-quiz, view material, complete post-quiz (2 pts bonus for course).
- Send comments/suggestions to me (rule@andrew.cmu.edu) for improvements.

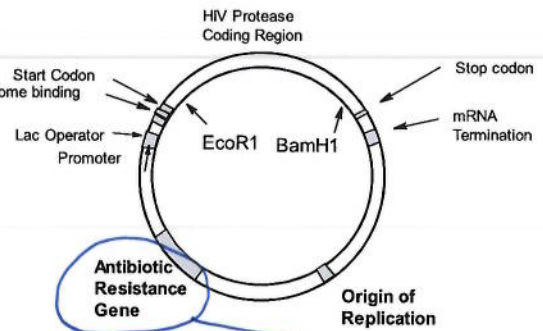
Bacterial Transformation & Antibiotic Resistance

Transformation – process of putting plasmid DNA into bacteria.

Selectable marker – gene contained on the plasmid that can produce a protein that makes the bacteria resistant to antibiotics.

- Growth of transformed cells in the presence of the antibiotic is referred to as selection, because it selects for those bacteria that contain the plasmid.
- It is necessary to have the antibiotic present at all times, otherwise the plasmid will be lost from the cells.

(image from http://2012.igem.org/Team:St_Andrews/Public-outreach)



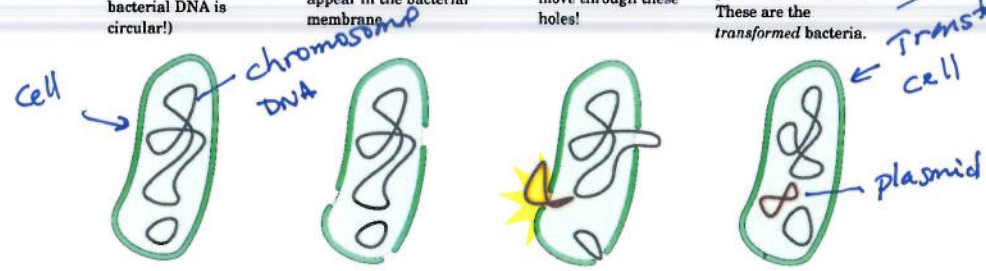
Here is an *E. coli* bacterium in natural state. (Notice how bacterial DNA is circular!)

Getting a plasmid into a bacterium

Extreme cold causes pores (small holes) to appear in the bacterial membrane.

Small DNA molecules like our plasmid can move through these holes!

When the bacteria are heated again, some of them end up with our plasmid inside them! These are the transformed bacteria.



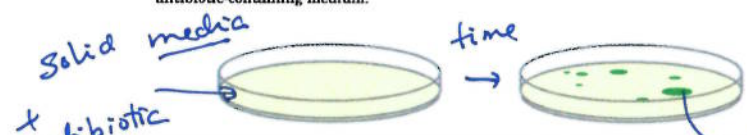
Transformed
Transformed cell
Produces protein that makes cells resistant



We can filter out the untransformed bacteria (the ones that got no plasmid) by growing all of the bacteria in an antibiotic-containing medium.

Untransformed bacteria are killed by the antibiotic in the medium. (They don't have the plasmid with the antibiotic resistance gene!)

The transformed bacteria grow though! Now we can pick them off the plate and grow more if we want.



1 bacteria → growth → many bacteria
colonies on surface (antibiotic resistant)

Summary of Plasmid elements:

– **Restriction sites (EcoR1 and BamH1):** Used to insert coding region into plasmid via sticky ends and DNA ligase.

Promoter – Binding site for RNA polymerase, generates mRNA from DNA sequence
Lac Operator – Lac repressor binds here, on/off switch for mRNA production.
mRNA termination – end of mRNA

Ribosome binding site – binds mRNA to ribosome, positions start codon in P-site.
Start codon – first codon, recognized by tRNA^{MET}, all prokaryotic proteins begin with a modified methionine residue.
Codons – coding for the amino acid sequence of our desired protein, anything can be made.
Stop codon – Signals protein release factor to release completed protein from ribosome, breaking bond between the last tRNA and the new protein.

Origin of replication – so that the plasmid will be replicated and passed on to daughter cells.
Antibiotic resistance gene – so that we can select for cells that have our plasmid.

mRNA production

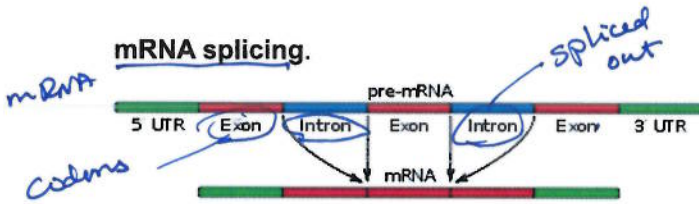
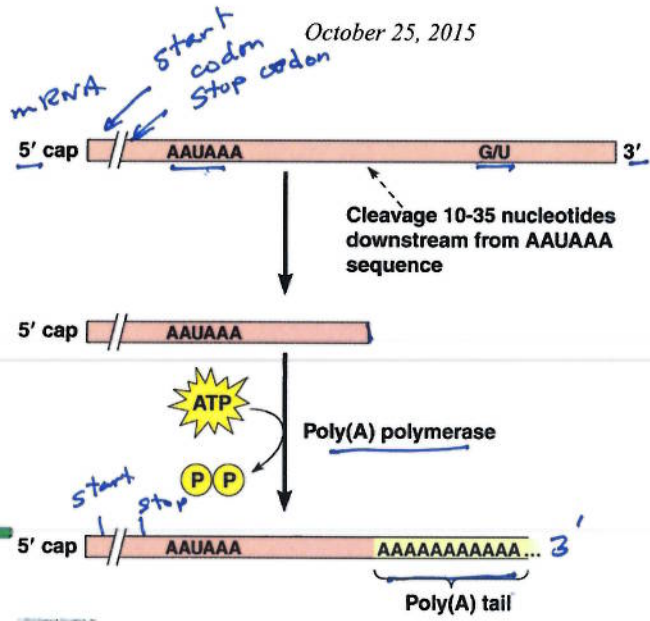
Protein Synthesis

Essential components of any plasmid.

mRNA processing in Eukaryotic Cells

Poly A addition. A series of A residues are added to the end of the mRNA by specialized enzymes. This is important for:

- Nuclear export
- Translation (protein synthesis)
- enhancing the stability of mRNA.



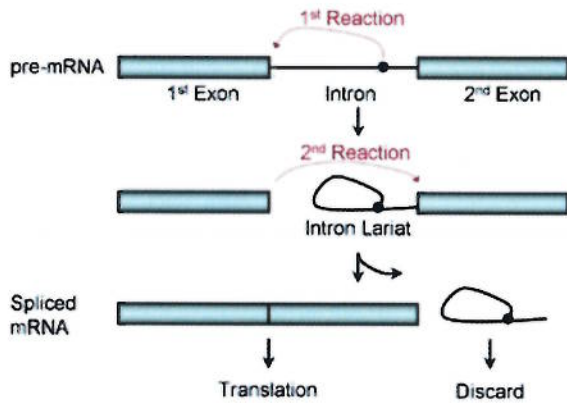
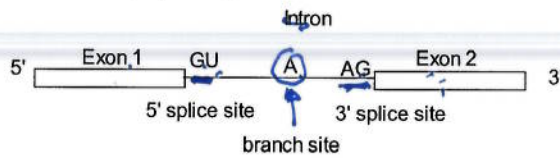
The initial transcript is composed of:

- Exons that code for amino acids
- Introns are intragenic regions that are removed during splicing.
- Splicing requires the following sequences in the intron to guide the splicing machinery:

- a 5' donor site
- a 3' acceptor site
- a branch sequence within the intron

• Steps:

- A in branch breaks phosphodiester at 5' splice site
- 3' OH at 5' splice site forms new phosphodiester with 3' splice site



Alternative splicing is common, with different exons retained in different tissues. This allows the same gene to produce many different proteins.

Genetic Diseases:

- Mutations in the splicing machinery can cause wide-spread problems in mRNA splicing.
- Mutations in the donor or acceptor site can cause incorrect splicing of individual mRNAs.

