**Escherichia coli (aka e. coli)**

(Assignment over February Break)

Your task over Winter Break is to get to know this little organism we call e. coli. When you return, we will be looking at how genes are regulated in prokaryotes and then performing a bacterial transformation lab using e. coli to see a mechanism of regulation in action. There have been some questions about plasmids that have arisen throughout the year, and I want you to be familiar with the e. coli organism before we start working with it. There are a few parts to this assignment. Don’t worry, it’s not massive, its’ meant to be informative as well as a little interesting (from a science teacher’s perspective, at least!).

**Assignment:**

1. Draw a full page (computer paper) picture of an e. coli and label it with the important parts *(cell wall, cytoplasm, nucleoid DNA, plasmid DNA, ribosomes, cell membrane, pilli, flagella)*.
2. Read the 2 passages about plasmids below.
3. Listen to the podcast on e. coli ([episode 37](http://thispodcastwillkillyou.com/2019/11/12/episode-37-e-coli-unless-its-beets/)) from the podcast This Podcast Will Kill You.

You can listen through any podcast app (apple podcasts or spotify or through the website).

(link available on our class website to the episode on the website for This Podcast Will Kill You)

1. Respond to the podcast and passages by writing a ½ page typed response. Turn in on the day after vacation.

Plasmids – an Overview

Plasmids, are naturally occurring and used by bacteria to transfer genetic information among themselves. Many plasmids isolated in nature encode antibiotic resistance genes. They also have sequences called an origin of replication that are recognized by the bacteria cell and signal the bacteria’s replicating enzymes to replicate the plasmid. Because the plasmids are small (a few thousand base pairs) they are mobile. Once they enter a bacteria cell, they are replicated several to many times. The plasmids can remain as free molecules that are not part of the bacteria’s larger chromosome. Bacteria that maintain plasmids with an antibiotic resistance gene and are then exposed to an antibiotic will live while bacteria that are susceptible to the antibiotic do not survive. Bacteria that are transformed or genetically altered by the introduction of a new plasmid have an incentive to maintain this new antibiotic resistance trait. Therefore they will make copies of the plasmid.

In the 1960's the properties of these plasmids described above were discovered. In the 1970s, Stanley Cohen and other researchers at the University of California discovered that these plasmids could be altered and maintain their transformation properties. They could combine new DNA sequences with the plasmids, making recombinant plasmids in a test tube. The recombination was directed to occur in a specific way by cutting the DNA with a restriction enzyme and then ligating or attaching the cut ends of different molecules together with DNA ligase. Bacteria cells could then be transformed with these recombinant plasmids and would replicate the entire plasmids regardless of the origin of the new DNA sequences that were recombined into the plasmids. If the recombination involved the insertion of new DNA into an antibiotic resistance gene, the resistance gene would be inactivated. The insertion mutated the antibiotic resistance gene coding sequence and the bacteria could no longer use that information to express the resistance trait.

“Gene and Cell Survival”

*by Guillermo de-la Cueva-Mendez and Belen Pimental. EMBO Reports. June 2007.*

R1 architecture: a winning design for DNA survival Plasmid R1 is a good example of how the need to be stably maintained in host cells shapes the architecture and building blocks of extrachromosomal elements. Four properties can be distinguished in R1, all of which contribute to plasmid survival: antibiotic-resistance genes, horizontal DNA transfer genes, stability systems and the basic replicon (Fig 1A; Womble & Rownd, 1988).

R1 carries ampicillin- (bla), chloramphenicol- (cat), kanamycin-(aph), streptomycin/spectinomycin- (aad) and sulfonamide- (sul ) resistance genes. These are clustered in the R-determinant, which contains three insertion sequences and a transposon, Tn4. Insertion sequences are minimal transposable elements that encode functions involved in insertion events, and they are found in plasmids and in the chromosomes of prokaryotes, eukaryotes and archaea (Mahillon & Chandler, 1998). They mediate various DNA rearrangements, including the integration of R1 into host chromosomes, which can also influence the transcription of neighbouring genes. Genes flanked by two identical insertion sequences become transposable elements; therefore, antibiotic-resistance genes in R1 can be mobilized as transposons in different combinations.

R1 can also move horizontally into plasmid-free cells by conjugation. To achieve this, R1 induces the synthesis of a pilus that attaches the donor to a recipient cell. One DNA strand of R1 is then cleaved at oriT (origin of transfer) and transferred through the pilus to the recipient cell, in which it is re-circularized and converted into double-stranded DNA. Most genes required for transfer are encoded in the tra operon. In R1, a regulatory network inhibits expression of this operon, limiting the frequency of conjugation to 1×10–3 per R1 copy. However, once transfer occurs, the absence of the limiting regulatory factors in recipient cells allows R1 to spread rapidly in dense bacterial populations (Pölzleitner et al, 1997). Resistance genes confer growth advantages to R1 hosts in niches in which antibiotics are present, and genetic mobility allows R1 to acquire and evolve these survival strategies (Frost et al, 2005).

However, few, if any, of these genes are under strong selection at all times and even if they were, their mobilization into the host chromosome would allow cell survival without the costs of maintaining the plasmid. Therefore, R1 has evolved additional functions to ensure that its survival does not depend on a single strategy, but rather on their additive effects. These functions lie outside the R-determinant. Therefore, loss of antibiotic resistance does not completely compromise plasmid survival. Low copy number also contributes to R1 survival, minimizing the metabolic burden that the plasmid imposes on host cells. In Escherichia coli, the ratio between R1 and the oriC (origin of chromosomal replication) is approximately 1:1. Chromosomal rep-

lication requires the initiator protein DnaA; however, R1 encodes its own initiator factor, RepA. This protein is limiting for the replication of R1, and the plasmid keeps its copy number low by tightly regulating RepA synthesis. Furthermore, RepA uncouples R1 replication from the host cell cycle, a process largely regulated by DnaA (Nordström, 2006).