

Lecture 2

Bacterial Plasmids

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What are plasmids, and why are they so useful?

What is a plasmid?

A plasmid is an extra-chromosomal element, often a circular DNA. The plasmids we will use in this class typically have three important elements:

- A cloning site (a place to insert foreign DNAs)
- An origin of replication
- A selectable marker gene (e.g. resistance to ampicillin)

Cloning sites

A cloning site is not required at all, but it sure is nice to have! What I mean by "cloning site" is a place where the DNA can be digested by specific restriction enzymes - a point of entry or analysis for genetic engineering work. This is a matter we will be discussing in great detail at a later point. For now, think of the following example: Suppose you are really thirsty and you buy a can of beer. Does it occur to you that one end of the can (the "top") is designed so that you can open it easily? If you bought a can of beer with two bottom ends and no top, you would have a hard time drinking it! It's the same way with plasmids. You can have a plasmid with lots of terrific features, but you might lack an easy way of "getting it open" with restriction enzymes. All this talk is making me thirsty, and now I believe I'll have that drink!

Origins of replication: Since a plasmid is (by definition) an extrachromosomal element, it cannot make use of any origin of DNA replication in a chromosome. That is, DNA synthesis within (i.e. copying of) a plasmid depends on its having an origin of DNA synthesis of its own. Obviously, if a plasmid couldn't be copied, it would be rapidly diluted out in a population of dividing cells because it couldn't be passed on to daughter cells.

Selectable markers

A selectable marker is not actually a required element of a plasmid, but it makes it possible for us to maintain stocks of cells that contain the plasmid uniformly. Sometimes, carrying a plasmid puts a cell at a *selective disadvantage* compared to its plasmid-free neighbors, so the cells with plasmids grow more slowly. Cells that happen to "kick out" their plasmid during division may be "rewarded" by having a higher rate of growth, and so these plasmid-free (sometimes referred to as "cured") cells may take over a population. If a plasmid contains a gene that the cell needs to survive (for example, a gene encoding an enzyme that destroys an antibiotic), then cells that happen to kick out a plasmid are "punished" (by subsequent death) rather than "rewarded" (as in the previous scenario). That selective pressure helps to maintain a plasmid in a population.

The lesson of the petri plate.

A septic parable ...

The wise molecular biologist tells us the tale:

Once upon a time, a plasmid met a cell, and they struck up a conversation. Saith the plasmid, "Kind cell, I bring thee a gene for antibiotic resistance, and if you let me in, I shall repay you by showing you how to make an enzyme that might save your life one day." The cell replied with annoyance, "What use hath I for the likes of thee? My food is all gone, I'm freezing my pili off, and you think my biggest problem is antibiotics? Maybe you're just a selfish gene, looking for a cell to make copies of you. Begone!" The plasmid smoothed out his supercoils, dodged a DNase, and tried to think of a suitable reply.

Just then, a massive wave of thermal energy struck them both, and it was all he could do to not lose his footing, standing as he was on the cell membrane.

The plasmid trembled, partly to distribute the energy uniformly to his vibrational degrees of freedom, but mostly at the thought of how painful thermal denaturation might be. Fortunately, it was not coming to that. Not this time. The temperature was high enough to pop a few hydrogen bonds, and his strands were breathing a bit, but he was covalently closed after all. He could handle it!

The cell was not so lucky, however. His membrane, which had been reasonably firm to this point, began to swirl and form vortices, and lost its smooth surface. The cell had gotten used to the cold temperatures by boosting the fraction of short chain and unsaturated fatty acids in his membrane. While it gave the membrane a good consistency in the icy cold, it was completely wrong for this new, higher temperature. The van der Waals interactions weren't strong enough to maintain cohesion, and errant lipids were now leaping through the bilayer like divers at a mosh pit. Huge patches of the membrane were involuting, bringing massive gulps of medium inside. The cell was just seconds away from a complete membrane breach!

Then, as quickly as it had started, the problem was over. The buzz of thermal energy was drawn away by some unseen entropic sink, and dissipated. The membrane returned to its glassy-smooth state. The plasmid was now inside the cell, having been carried through the membrane in its moment of weakness, but the cell was too preoccupied to notice the uninvited guest. The cell was using his last remaining energy to bail out the excess liquid, and set his ion gradients to rights. When he thought he could do no more, that his determined course would be senescence and death, a warm flow of fresh medium restored his spirits.

"Ah! Tryptone, and yeast extract", he said as he gratefully derepressed half a dozen operons. His ribosomes got right to work, making the enzymes that would help to catabolize the new food source and restore the structure of the cell to full health. He almost exclaimed, "I thought Darwin had me for sure that time!" but being a cell with little memory he had already forgotten the privations of a few minutes earlier. Thirty minutes passed, and he found himself cast onto a wide surface that was warm and rich in nutrients.

"Not bad", he said. I could live like this for generations!" But then, the horrible sounds of dying cells reached him. All around him, his brethren were being killed by an unseen attacker. An antibiotic was there, and was destroying the entire population. Oh the humanity! He braced himself for death, but then ... nothing happened. He was alive!

"Remember me?", said the plasmid? "I told you that I might save your life one day, and now it has come to pass." The plasmid, which had gone unnoticed since the thermal catastrophe, had been copied several times and transcribed by the cellular machinery. It had provided a gene that encoded an enzyme that destroyed the antibiotic before it even got into the cell. Although more antibiotic was diffusing into the neighborhood, the enzyme was on the job, and prevented it from doing any damage.

"Yes, you were right", said the cell. "I am grateful that you transformed me, and now that I have a logical explanation for my good health, I don't have to develop survivor guilt either. Stick with me, plasmid, and I'll make sure you are provided with a high copy number."

The generations passed, and the cell divided many times. Each time there was fission, the two daughter cells received an inheritance of plasmid copies.

There was widespread prosperity.

I would say "They lived happily ever after", but sadly that is not the end of the story. The daughter cells grew into a prodigious colony, and soon had destroyed so much of the antibiotic in the immediate vicinity that the real danger had passed. Some cells that had not perished in the original attack even managed to survive and grow nearby - little "satellite colonies" seeking refuge from the high levels of antibiotic elsewhere.

Then the cells in the big colony became lazy. The plasmid was not replicated to the same high copy number - it no longer served the interests of the cell to do so. Sometimes, daughter cells did not inherit even a single copy of the plasmid! As the colony aged and grew, the proportion of cells that carried the plasmid became less and less.

The colony actually believed itself to be quite progressive on this point. Some of the more strident cells even argued: "Why should young cells be forced to make enzymes that they don't need?" They said "We want to evolve higher order characteristics, not merely regurgitate the knowledge that served our great-great-great grandcell!" Before long it was unfashionable to carry the plasmid, though a few still did, but the colony grew faster without the added responsibility of the added synthesis.

One day, a toothpick scraped the colony from a plate and carried it high into the sky. "At last," the progressive cells thought, "we are entering a bold new era in which we are going to be able to realize our true genetic potential!" The toothpick was dropped into a tube of fresh medium. The fresh medium had fresh antibiotic.

What happened next is almost too horrible to tell. The cells that still carried the plasmid lived of course. They produced the enzyme that destroyed the antibiotic. The cells that had not inherited the plasmid were unworried at first, because they thought that a protective shield of enzyme could be built by the others, but being in liquid medium there was no hope for them. The antibiotic was not limited by diffusion, as it had been on the plate, and they were soon overcome.

The few wise cells that had not lost their plasmid went on to eternal storage in glycerol stocks, and were written about in books and famous journals. They were grown in huge 10,000 liter fermentation tanks, and provided with the very richest medium that had ever been made!

The end.

So what does all this mean?

Bacteria can sometimes take up DNA from the external environment, a natural process that we call "transformation", and sometimes the DNA taken up is a plasmid that can be maintained in the cell because it has an origin of replication. Plasmids often earn their keep by providing a gene that gives a selective advantage to the cell, which then replicates (instead of dying) and makes more copies of the plasmid.

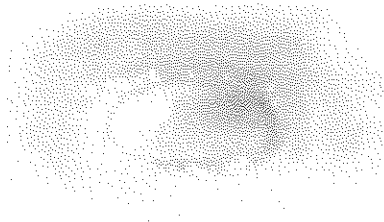
What happens if the plasmid is taken up by a cell that cannot make sense of its origin of replication? After all, origins of replication are not universal, and one species may not recognize a sequence that works perfectly well in another. Well, then the DNA has only one way of being maintained, and that is to become integrated into the host chromosome.

Integration isn't likely, but it can happen.

What happens if the plasmid provides no selective advantage to the cell. Can it be maintained? Yes, but practically speaking it would be hard to pick out the cells that carry it because they would be surrounded by perfectly healthy cells that did not carry it.

If you were to do a transformation in the laboratory, and put the cells on a bacteriological plate that lacked antibiotic, the plate would be covered with a solid lawn of bacteria the next day. Why? Because very few cells take up DNA in a typical transformation experiment, and most cells are usually killed by the antibiotic. It is the rare cell that survives.

On the other hand, some cells do become "freeloaders" and survive because other cells are doing the work of destroying the antibiotic in their immediate vicinity on the plate. We call these "satellite colonies" because they are tiny little colony specks that surround the big antibiotic-resistant colony. They only develop with antibiotics such as ampicillin, that are destroyed by enzymes such as beta lactamase outside of the cell.



See the little satellites around the big colony?

Are satellites a problem? Probably not, provided that the colony is subsequently picked and grown in fresh medium containing antibiotics. If the ampicillin plate is old (meaning that the antibiotic is partially degraded), or the transformed cells are plated at very high density (meaning that the plate is covered with resistant cells), or the copy number of the plasmid in the cells is so high that beta lactamase is secreted at high levels, or the colonies grow on the plate for several days (allowing more time for degradation), then satellites are more likely to develop.

Other types of mischief can also develop. Half of the cells in the middle of an ampicillin resistant colony may become lazy themselves, kicking out the plasmid and living off of the work of the other cells. It's like being the kind of person who goes to the bar to enjoy the music, but never puts a quarter in the jukebox to share the cost. If you pick a bacterial colony and streak it out on a fresh plate with antibiotics, the loafers are exposed and they get their just desserts. Bacteriologists love to streak out cells, because getting a single colony on a fresh plate makes them feel like they are starting an experiment with something "known". They've separated a potential mixture into individual cells, and the cells have each grown up into a colony. They are hoping that the cells in a single colony will be identical, and that there is no mischief afoot.



(What the bacteriologist dreams about)

Still, plasmids make plenty of mischief in the world. They are one way that you can get natural and efficient horizontal transfer of information between species. For example, Vancomycin was an antibiotic of last resort against *Staphylococcus aureus* infections. Resistance to vancomycin is associated with the VanA gene, which is carried on a plasmid in other species of bacteria (such as *Enterococcus faecium* strain BM4147).

The bad news is that *Staphylococcus* has been learning how to survive in a hospital, by taking up DNA from other species that have overcome the commonly used antibiotics - recently it was reported that there exists a *StaphA* strain Mu50 that is showing signs of vancomycin resistance! Transformation of a plasmid from *Enterococcus* to *Staphylococcus* is the probable cause.

What is the process of cloning a plasmid?

When we say that something is "cloned" we mean that it grew from a single genetic origin. In the case of a plasmid, we mean that the DNA has been separated and grown on its own. If we were to take a fresh colony of bacteria containing the plasmid, then it is probable that all of the bacteria grew from a single cell, and that the plasmid they contain was derived from a single molecule that happened to drop into the cell during transformation.

The idea of a "clone" is important, because it is a point of consistency in an experiment. You have the opportunity to obtain a raw material that is pure, not a mixture of many things. Once you've done an experiment, you can repeat it again and again, because you still have the original clone. It doesn't matter how you made the clone, whether you dropped the agarose gel onto the floor when you were isolating the fragment, whether you accidentally threw it in the garbage and had to go diving into a dumpster to recover it. Nobody really knows and nobody cares. Once you've transformed the DNA into the cell and isolated a colony that is pure, all else is forgiven. In the past 20 years, molecular biology has moved faster than any other field of science, and it is the ability to fiddle with the inner workings of genetic material reliably that made it so.