'PLASMID'—WELCOME TO THE WORD POOL. BUT WHAT ARE YOU?

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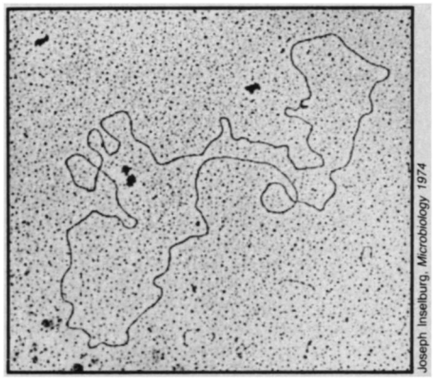

Plasmid: Continuous loop of DNA.

Some words enter the common language through the back door. They filter up from unseen sources and lie around on the grass until the public consciousness stumbles on them and picks them up. "Hassle" is this kind of word. So is "hunker." Rip-off. Hype. Chocolate City, streak, freak, funky, furry, deco, disco . . . ad creatum.

Other words and word connotations march down Main Street, accompanied by bugles and podiums and posters. They filter through proper channels into the evolving word pool. Vietnamization. Stonewalling. Hegemony, detente, sexism.

And then there are words that come quietly through the front door—in briefcases. Science words are like this. They are created methodically from Latin or Greek roots, and describe some observable event. They are documented and deliberate and well . . . a little dull. Ten years ago "enzyme" wasn't a new word, but it was just starting to inch its way into the common language. It hit its peak in a detergent commercial several years ago and is now firmly entrenched in the public consciousness.

During the last decade, there has been a quiet influx of science words, some old, some newly coined: nucleic acid, laser, pulsar, transistor, behavior mod, phage, plate tectonics, carcinogen, coronary. And now there is another one—plasmid. One can hear a collective sigh as cerebral entropy is dealt one more blow. "Funky" is fun, "plasmid" is no fun. But plasmid has popped up a lot recently, first in journals, then in specialty magazines, now in the popular press. One senses, in fact, more than a collective sigh. In the midst of a barrage of Back Door and Main Street words, there seems to be a bit of impatience. "What's so big about this little ring? Maybe I can get by without this word . . ." Probably not. Plasmid, like the other Front Door words, is here to stay. So, "plasmid," welcome to the word pool. But what are you, anyway?

The word itself comes from "plasm," as in germ plasm or cytoplasm, and means literally a "thing" in a cell's cytoplasm. It's come to mean more specifically a genetic "thing." It can have a fancy journal definition ("an autonomously replicating extrachromosomal genetic element") or a simple, newspaper definition ("a tiny ring of DNA" or, in one reporter's words, "genetic loose change"). But neither of these tells the whole story. Plasmids are rings of DNA, but they are also semi-life forms, separate mini-spheres within the universe of a bacterial cell. They teeter, like viruses, on the edge between life and nonlife and blur further that already hazy distinction. And *that* makes them fairly important.

The first plasmids were reported by Joshua Lederberg of Stanford in 1953. This was very early in the field of molecular biology—earlier, for instance, than the deciphering of the genetic code or the use of the electron microscope to probe biological tissues. Lederberg, through careful genetic experiments with bacteria, determined that there must be some genetic elements in addition to the main chromosome that directs the bacteria's life processes. He didn't know what exactly plasmids looked like or how big they were, but he deduced that the presence of some extrachromosomal genetic element was allowing certain bacteria to mate (rather than always propagate by dividing at the middle). He called these mating factors F-factors, as in fertility. For this show of deductive brilliance and other work on the sexuality of bacteria, he was awarded the Nobel Prize in 1958.

Since then, the field of molecular biology has matured technically, and researchers can now answer many of the basic questions about which earlier investigators could only speculate. Plasmids are found widely but have been most extensively studied in the enteric bacteria (those found in intestinal tracts) such as members of the genera *Escherichia, Shigella, Salmonella, Proteus, Aerobacter* and *Serratia*. Plasmids are not, however, limited to these genera. They are generally associated with the circular double strand of DNA that functions as a bacterium's chromosome. Plasmids, too, are circular and made of DNA, but they do not have the same sequences of nucleic acid base pairs. They have their own magic messages.

There can be from one to 20 or 30 plasmids in a cell, and each can carry from 2 to 250 genes, depending on the size of the ring. Large plasmids can be 50 microns across with a molecular weight of 100 x 10^6 and carry 250 genes. A small plasmid can be only 0.5 micron across, molecular weight 1 x 10^6 and carry two or three genes. Even a small plasmid is thousands of times larger than, say, a molecule of salt. But compared to the chromosome of a common *Escherichia coli* cell (1,250 microns, MW of 2,500 x 10^6 and 7,000 genes), a plasmid is like the ninth moon of a giant planet. Remember though, this is a miniature universe. One thousand *E. coli* cells could sit comfortably—and invisibly—on the head of a pin.

Researchers are fascinated by plasmids' small size, their self-replication and the messages they carry. They are on an equal footing with viruses—independent organisms that need a host cell for survival, but like to travel light—just some genes and a protein or two, no heavy metabolic machinery, cell wall or organelles. They are molecular parasites that hitch a ride on a bacterial cell, then move down the evolutionary road to somewhere.

Plasmids, although frequently attached to the host cell's circular chromosome, replicate independently. Separation of the double helix and duplication of the magic message can take place synchronously with bacterial DNA replication, but usually doesn't. Some researchers think that's why the plasmid often makes 20 or 30 copies of itself. It takes out a group policy to ensure that at least one plasmid will be randomly distributed to each daughter cell and the plasmid heritage will survive when the host cell divides.

A little head-scratching and armchair biology reveals a paradox in the plasmid story that must be addressed at this point. If a plasmid can go about its business, replicating whenever it wants, it can't be part of the bacteria's necessary genetic information and the direction of the cell's life support systems. But clearly, that plasmid heritage must be pretty important in order for the bacterial cell to carry it around and maintain it. Plasmids must be

paying some molecular room and board to justify the free ride, or the cell carrying it would be selected against rather quickly. But what's the price of that room and board?

The explosion of molecular biology during the last two decades has touched off an avalanche of answers. The plasmid genes must code for some characteristics that give the cell a competitive edge—and they do. They code for a wide range of characteristics that all seem to give the host cell a leg up on others of its species without them. One such characteristic is antibiotic resistance. Bacteria (sometimes harmless, sometimes pathogenic to animals) which contain resistance plasmids to specific antibiotics are not destroyed by those substances. Although this was not the case a few years ago, the common human pathogen *Staphylococcus aureus* can no longer be treated with penicillin. Over 90 percent of the "staph" germs found in hospitals now contain plasmids for resistance to penicillin.

Another plasmid-directed characteristic is the ability to colonize a specific animal tissue. *E. coli* cells, for example, sometimes cause severe diarrheal disease in humans and farm animals when they have a plasmid which allows them to colonize the animal's small intestine. Colonization is just the first step, though. They cause the enteric disease by releasing a toxin—and they can't do this unless they have a special plasmid for toxin formation. Plasmids for these characteristics occur in relatively few bacterial species in nature, and a fairly small percentage of the individuals of each species. But the edge they give to the cell makes it a survivor, and thus the plasmids are becoming more widespread.

Another important plasmid-coded characteristic is as advantageous for that semi-life form as it is for its host. A bacterium with Lederberg's F-factor will from time to time mate with another bacterium that lacks the fertility factor. When this happens, it deposits a single strand of plasmid DNA through a special little protein pipe called a sex pilus. The fertility plasmid directs production of this sex pilus. The mating, called conjugation, occurs only when the bacteria are crowded together—a disadvantageous environmental condition.

Plasmids and sex pili, says molecular biologist Stanley Falkow of the University of Washington at Seattle, come about as close to viruses as any other biosystems. Viral DNA is surrounded by a protein coat and the organism has a protein hypodermic mechanism for injecting its magic message into a host cell, where it gets translated into new virus particles. A plasmid, Falkow says, is really a special kind of virus that leaves, like shoes outside the door, its protein coat, its pilus, on the outside of the cell.

Falkow probably knows as much about plasmids as anyone, having just completed a book (to be released by Academic Press this month). He says plasmids always give their hosts nonessential genetic information (no information needed for the cell's immediate survival). Plasmids instead, give the bacteria "transient evolutionary advantage," allowing them to adapt quickly to immediate environmental pressures. Bacteria inhabit very specific niches, Falkow says, and normally propagate by binary fission, passing on the same genetic message over and over without the mixing of genes involved in sexual reproduction. This helps them retain the genes they need to cling to their narrow niches. Carrying around plasmids, on the other hand, while an energy drain, provides them with the mechanism (conjugation) to exchange useful genes, but also to meet short-term environmental pressures without the wholesale exchange of genetic information.

Besides their unique evolutionary advantage, plasmids are fascinating—and weird. They exhibit some truly novel biological behavior. When a sex pilus is being expressed, Falkow says, "a bacterium will mate with anything that moves." This includes bacteria of other species, and is clearly the plasmids' way of jumping ship and perpetuating itself. The plasmid is looking for the best ride and doesn't mind abandoning ship when it hits choppy waters. Resistance plasmids living in *E. coli* in the human gut, for example, have been found to transfer to other enteric species in the gut and to *Pseudomonas* living in wounds.

And plasmids can act even stranger than this. A new phenomenon called translocation has been reported recently by several major investigators. Frequently, plasmids that code for different characteristics will coexist in the same cell. For example, a big plasmid carrying resistance to the antibiotic ampicillin will coexist with a little plasmid carrying resistance to the antibiotics sulfonamid and streptomycin. In the past, it was thought that there could be no exchange of genetic information between two such plasmids unless they shared areas of homology—matching base pair sequences. And such different plasmids often wouldn't share these sequences. Now, researchers have found that bits of DNA can pop off one plasmid and onto another—translocate—even without these matching areas. The gene for ampicillin resistance, for instance, can jump off the big plasmid onto the little one, and create a new kind of plasmid that carries resistance to all three antibiotics. And this hopping around has been seen not only between plasmids, but also between plasmids and bacterial chromosomes. The traditional understanding of the transmission of genetic information has, in short, been set on its ear.

It's clear by now that plasmids are much more than little rings of DNA and that they are involved in some unusual and important cellular business. "Plasmid" is definitely in the word pool to stay, cerebral entropy notwithstanding. And coming with it may be some redefinitions of broader concepts—"life," for example. If a naked ring of DNA can pass from cell to cell to further its own evolution, what does this imply about traditional definitions of "life?" Perhaps DNA is no longer only what Nobel laureate Francois Jacob once called the "ultimate biological invariant." It's beginning to look like the ultimate biological definition, the smallest living unit. Or is it? In *The Ascent of Man*, Jacob Bronowski observed:

> "We are here face to face with the crucial paradox of knowledge. Year by year we devise more precise instruments with which to observe nature with more fineness. And when we look at the observations, we are discomfited to see that they are still fuzzy, and we feel that they are as uncertain as ever. We seem to be running after a goal that lurches away from us to infinity every time we come within sight of it."

Postscript: Now that we know what you are . . .

Now that we know "plasmid" isn't as dull as it looks—it's maybe even a little "funky"—where can we try it out? It seems appropriate to look briefly at some of its entrance points into the common language.

Plasmids are responsible for transferring resistance to antibiotics. This is a growing and well-publicized medical problem, since pathogenic bacteria are developing resistance to the commonly used antibiotics almost as fast as they can be marketed. This is no surprise, molecular biologist Richard Novick of the Public Health Research Institute in New York says, considering the cleverness of plasmids and their role as adaptive agents for short term environmental stress. The use of antibiotics has become so common in medicine and agriculture, Novick says, that we are, in effect, creating an evolutionary pressure which selects for the bacteria that carry resistance plasmids. Those without resistance plasmids are killed off; those with them survive and pass them on to their friends—related or not.

The second important context is genetic engineering experiments, such as those considered during the recent Asilomar conference (SN: 3/8/75, 3/22/75). Plasmids can be vehicles for carrying new genes into a cell and "markers" for monitoring the presence of certain characteristics. Many scientists, however, have expressed concern over using creatures so obviously wiley, unpredictable and devoted to their own self-preservation in genetic experiments. Research is now underway to build a "self-destruction capability" into plasmids so that they can't escape from the laboratory. □