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**“Antibiotics and the ribosome,
the cell's protein factory”**

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I want to start by saying it is a great honour for me to have been chosen to give this lecture, especially considering some of the past speakers. I am sorry that despite several years of studying Spanish, I don't have the fluency to deliver it in that language and must do so in English.

What you see here (Slide 1) are pictures of great writers and musicians who had one thing in common: they all died young of an infectious disease. Today we expect that if get a serious infection, we can simply go to a doctor, be prescribed an antibiotic and be cured. But is that really true?

A glimpse of the problem can be had by looking at the case of *Staphylococcus aureus* a bacterium that often likes to invade wounds and thus a problem after surgical operations in hospitals. On the left (Slide 2), you see a picture of the bacterium, which shows how it got its name due its gold color. On the right, you can see that when penicillin was discovered in the 1940s, nearly all *Staph. aureus* was sensitive to penicillin (ie no resistance). However, as time went by, resistance quickly became prevalent in hospitals (black squares), followed a couple of decades later by widespread resistance in the community (open squares).

Today bacterial resistance to antibiotics is acknowledged as a widespread problem. In Europe alone, over 25000 people die of infections just from resistant *Staph. aureus*. This has prompted leading financial journals such as the *Financial Times* (Slide 3) or more recently *The Economist* (Slide 4) to warn of a major impending crisis. Interestingly, *The Economist* points out that Fleming, the discoverer of penicillin, warned long ago in his Nobel Prize lecture that bacteria would evolve to beat antibiotics. So this leads to the question of how antibiotics were discovered, how some of them work and whether science has anything to contribute to solving this problem.

A modern view of antibiotics as chemicals selectively targeting certain types of cells was enunciated by Paul Ehrlich (Slide 5), who noticed that certain dyes would preferentially bind bacteria (rod-like particles on the right that are stained dark red) while not binding the much larger human cells. This gave rise to the idea of a “magic bullet” that would only kill bacteria. He himself tried a number of compounds, but real success came later.

One person who was influenced by Ehrlich was Gerhard Domagk (Slide 6). He worked for the Bayer chemical company and thus had access to hundreds of compounds, which he systematically tested for antibacterial activity. He discovered that a red dye, prontosil, was highly effective against many bacteria that caused potentially fatal infections. In those days, even a small cut could lead to an infection that travelled up the limb which then required amputation, and soon after the discovery of prontosil, he used to save his own daughter from having her finger amputated. However, he was unlucky in a number of ways. Just a year later, it was discovered that prontosil was broken down in the body to the colorless compound sulfanilamide, which was the active ingredient. This voided his patent on prontosil. He was also awarded the Nobel Prize for Physiology or Medicine in 1939, but because the Nazi regime had banned Germans from receiving the prize, he could not accept it, and was actually briefly arrested by the Gestapo. After the war, he was honored in Stockholm by the Nobel foundation, but they told him that because too much time had elapsed, the money was returned to the general funds and he could not get his prize money! But he must have had the satisfaction of being the father of modern sulfa drugs which saved countless lives.



A second approach to antibiotics based on natural compounds was the result of an accidental discovery. Fleming, a Scottish microbiologist working in London (Slide 7, left) accidentally had a plate contaminated by a mould spore (right - top). He noticed that the bacterial colonies were smaller when they were closer to the mould spore. He correctly surmised that the mould spore was secreting something that inhibited the growth of bacteria and called it penicillin after the *Penicillium* mould. But it took many years and a huge effort by Florey, Chain, Heatley and others to make and purify penicillin in sufficient quantities to test on patients and to be useful. Still, penicillin was only effective against certain types of bacteria (known as “gram positive” bacteria) and was not at all effective against diseases like tuberculosis.

The discovery of penicillin led the soil microbiologist Selman Waksman and his student Albert Schatz (Slide 8, left) to systematically screen many species of soil bacteria, mostly from the genus *Streptomyces*. They found one such species secreted a compound (Slide 8, right) that appeared to not only inhibit both gram positive and gram negative bacteria, but also inhibited the growth of *Mycobacterium tuberculosis* (tubes on bottom right) which causes tuberculosis.

The discovery of streptomycin led to a systematic search of soil bacteria and resulted in the discovery of a large number of antibiotics (Slide 9). Some of these were not clinically useful because they were toxic, but others, such as tetracycline and erythromycin (and their derivatives) remain widely used even currently. A large number of these antibiotics work by preventing bacteria from making proteins. Since proteins are essential to all life, inhibiting their production kills bacteria or at least stops them from growing so that they are subsequently killed by the body’s immune system.

This leads to the question of how proteins are made, so that we can try to understand how antibiotics inhibit the process. The famous “central dogma” (Slide 10) points out that information that resides as a gene in one of the strands of double-helical DNA. This strand is copied into mRNA, which is then translated into the protein that is coded for by the gene. There are a couple of major puzzles about this process that were solved in the 1950s and 60s. Firstly, proteins are made up of amino acids, of which there are mainly twenty. On the other hand, both DNA and mRNA consist of just four types of bases as building blocks. So clearly one needs at least 3 bases (a “triplet” or “codon”) to code for each amino acid, and indeed this was established by molecular biologists. A second problem is that an amino acid has little binding affinity for triplet codon that specifies it. Both these problems were solved by the discovery of tRNA, which has a triplet of bases that is complementary to a particular codon, and brings along an attached amino acid at the other end.

Initially, it was not clear how these amino acids would be joined up to make a protein. However, a major breakthrough was made when cell biologists showed by electron microscopy that newly synthesized proteins localized as particles on the endoplasmic reticulum (Slide 11, left). When these particles were isolated from the microsomal fraction, it was found that they were about 25 nm in diameter and in all species, they consisted of a large and small subunit. They were found to be about 2/3rd RNA and 1/3rd protein by mass, and were thus called “ribosomes.”



Ribosomes were biochemically analyzed and found to consist of over 50 proteins in bacteria (Slide 12), which combine with three large pieces of RNA to make up the two subunits, which then associate to form the whole ribosome. The overall mass of the ribosome is enormous by molecular standards: about 2.5 megadaltons in bacteria and over 4 megadaltons in higher organisms. An early cryoelectron microscopic structure is shown in the inset to provide an idea of the overall shape of the ribosome. Ribosomes from higher organisms are significantly larger and consist of as many as 80 proteins and even larger RNA.

About forty years of efforts by ribosome biochemists and geneticists established that the ribosome has three binding sites for tRNA (referred to as the A, P and E sites). The small subunit binds the genetic message in the form of mRNA, and the large subunit catalyzes the formation of a peptide bond between the growing protein chain (which begins as just the first amino acid) and the new amino acid brought by the tRNA that matches the codon on mRNA. The ribosome moves to the next codon, when the next tRNA joins, and the next bond is formed, and so on. This basic outline of ribosome function is shown in cartoon form in Slide 13.

A more realistic view of what the ribosome looks like began to emerge in the 1990s as a result of cryoelectron microscopy (Slide 14). This shows that the mRNA is wrapped in a cleft around the small (30S) subunit and the L-shaped tRNAs present their anticodon ends to the mRNA in the small subunit, while their aminoacyl ends are buried in the catalytic site in the large (50S) subunit. The growing protein chain – the so-called nascent chain – emerges through a tunnel in the large subunit.

These were tremendous advances, but if one wanted to know how the ribosome worked in detail and how various antibiotics disrupted its function, it was essential to obtain a detailed atomic structure for the ribosome. How could one obtain such a structure? The usual way to obtain a detailed image of a small object is by microscopy. In its simplest form, this involves looking at an object with a magnifying lens (Slide 15). Light rays that hit the object are scattered, and these scattered rays are recombined by the lens to form a magnified image.

The problem with ordinary light rays is that they have a wavelength of about 500 nm, whereas the distance between atoms is about 0.2 nm. This means that light has far too large a wavelength to resolve details at the atomic level, since a theorem in physics says that you cannot distinguish two objects that are closer than roughly the order of the wavelength of the light used to visualize them. There are indeed “light” rays of very short wavelength. When photons – or light – have a wavelength of around 0.1-0.2 nm, they are called x-rays. However x-rays have other problems. They are difficult to focus compared to ordinary light. Moreover, unlike ordinary light, they damage the object that is examined by x-rays, so even with focusing one would end up destroying a molecule before there was enough signal to visualize it properly.

This problem was surmounted almost exactly a hundred years ago, by using a technique called x-ray crystallography. In this technique (Slide 16), first one forms crystals of the molecule of interest, which is to say a regular three-dimensional array of the molecules. This is often a difficult and painstaking process, especially for very large molecules like the ribosome. The next step is to do a “diffraction experiment” in which the crystal is placed in a beam of x-rays. The crystal scatters the x-rays, but because of the interference or diffraction



from the regularly spaced molecules in the crystal, the scattered rays are reinforced along certain special directions, to generate “spots” or “reflections.” As the crystal is rotated, these directions change and new spots are generated. If one measures the intensity of all the possible spots that are diffracted in this way, then in principle it is possible to recombine them to produce a three-dimensional image of the object. This involves solving the “phase problem” in which it is necessary to know how far ahead or behind the crest of the wave corresponding to each spot is relative to the others. But once that is known, then a computer can do mathematically what a lens does each time it recombines scattered waves to produce an image, because effectively the lens is doing the analogue equivalent of a Fourier transform on the scattered light rays, which are waves.

Crystals from very large molecules like the ribosome diffract weakly, which is to say that the intensity of the diffraction spots is quite weak. So in order to measure them accurately, very powerful sources of x-rays are needed. These are synchrotrons, which are large electron accelerators that produce a fan of radiation as the electrons orbit around in a ring. The x-rays can be made even more intense by making the electrons oscillate during their path. Such large accelerators are very expensive and are run as shared facilities. Slide 17 shows some of the synchrotrons where we have collected data.

At a synchrotron, instruments are separated in small rooms distributed around the ring where the electrons orbit. Each room or “hutch” has specialized instrumentation. One such instrument is shown in Slide 18. The pipe on the right brings the x-ray beam to the center where the crystal is located, and on the left is a square face which is a large x-ray detector that measures the intensity of the spots.

The result of such an experiment is a detailed three-dimensional image of the molecule. However, such an image (Slide 19) does not directly tell you the atomic structure, because for large biological molecules, the resolution, or level of detail, is not usually sufficient to see individual atoms. So such an image must be interpreted in molecular terms. This is a bit like solving a large jigsaw puzzle, but with some important differences. The first is that because it is a real experiment, the image is imperfect. Parts of the image may be missing, and there may be “noise” that appears to be part of the image when it shouldn’t be there. This is a bit like having pieces of the jigsaw puzzle missing, or pieces from some unrelated puzzle being included. Another difference is that this is a three-dimensional rather than two-dimensional image. But the biggest difference is that the answer is not supplied on the cover of the box!

Just as with a jigsaw puzzle one starts with parts that are recognizable such as the edges or regular patterns, with an image, one looks for regions that have recognizable features. If you zoom in on the image, you see (Slide 20) that there is a region that shows two ridges of the image (or “electron density”) that appear to be interconnected, and each of them has regular bumps. To an expert, this is immediately recognizable as a piece of double-stranded RNA (Slide 21). So a portion of the image has now been interpreted in molecular terms (Slide 22). This portion is of course only a small part of the whole structure, and just as with a jigsaw puzzle, one interprets or “solves” the entire structure until there is nothing left in the image to interpret (Slides 23-28). At that point, you have solved as much of the molecular structure as is possible with the experiment.



Using x-ray crystallography, the atomic structures of both the large and small subunits of the ribosome were solved in 2000 (Slide 29). These structures shed light on how ribosomes may have originated. Since ribosomes make proteins, but they themselves consist of proteins, how could they have begun? One idea – the so-called RNA World hypothesis – is that an early form of life consisted mainly of RNA (perhaps with small peptides that were not made by translating a genetic code). In this view, ribosomes may have begun as enzymes that started to make small peptides and gradually evolved to make larger proteins under directions from a gene, and then evolved so some of these proteins became part of the ribosome itself. Before the discovery of RNA catalysis in the 1980s, nobody took this idea seriously, but once it was discovered that RNA could both carry information (like DNA) and do catalysis (like proteins) it became entirely plausible that the ribosome began as an RNA molecule and evolved into an RNA-protein complex. The structures showed that the binding sites for tRNA and the catalytic site where peptide bonds are formed consisted almost entirely of RNA that was highly conserved even among the various domains of life. This suggested that they formed part of an ancient core of the ribosome that consisted of RNA.

Some years after the atomic structures of the subunits were solved, and following on a lower-resolution structure of the whole ribosome, the atomic structure of the entire bacterial ribosome with mRNA and tRNAs bound to it was solved (Slide 30). This structure, with half a million atoms, was the largest molecular structure solved (except for viruses, which have repeats of the same molecule), until it was superseded by the even larger ribosome from higher organisms.

Solving the first structure of the ribosome is technically quite hard. However, once it is solved, it is quite straightforward to determine how antibiotics bind to it. Effectively, the same experiment is done on crystals of the ribosome with an antibiotic bound, and the extra density after the rest of the ribosome has been subtracted from the image shows the antibiotic. An example is spectinomycin (Slide 31), which can be seen in detail on the left. On the right, you can see that spectinomycin binds in a small crevice between the “head” and “body” of the small ribosomal subunit. In this manner, the structures of many different antibiotics bound to the small (30S) ribosomal subunit were solved (Slide 32). Each of them reveals how by binding to a critical pocket in the ribosome, they block a specific aspect of ribosome function.

One example are the tetracyclines, a group of antibiotics with a characteristic 4-ring structure (Slide 33). These compounds bind exactly where the tRNA that brings in the new amino acid to the ribosome would bind in the A site (movie in Slide 34). When tetracycline is bound, it blocks the binding of the new tRNA, which would clash with it. Without the binding of the new tRNA, protein synthesis simply stops, since new amino acids cannot be added to the growing protein chain.

Many antibiotics bind to the large subunit of the ribosome (Slide 35). Some, like chloramphenicol, bind right at the peptidyl transferase centre, which is the catalytic site where the peptide bond is formed. Others bind at the entrance to the “exit tunnel” through which the newly made protein must emerge. Zooming into this region of the ribosome (Movie in Slide 36) shows the first amino acid and tRNA (red) and the new amino acid brought in by the new tRNA (green). The task of the ribosome is to form a bond between these two amino acids to start the process of making a protein chain. Chloramphenicol



(magenta) binds exactly where the new amino acid would bind (Slide 37). In its presence, a new amino acid could not bind to the right place and therefore peptide bond formation could not occur, thus stopping protein synthesis. On the other hand, erythromycin and related “macrolide” antibiotics bind very near by at the entrance to the tunnel (Slide 38, movie). These antibiotics may prevent the passage of the growing protein chain through the tunnel, but they don’t stop the first few peptide bonds from being formed.

The atomic structures of the ribosome led to the formation of a company, Rib-X Pharmaceuticals, with the goal of developing new ribosome-based antibiotics (Slide 39). The proximity of the chloramphenicol and erythromycin binding sites (Slide 40) gave the scientists at Rib-X an interesting idea. Each of these antibiotics has problems, e.g. chloramphenicol is toxic and many strains are resistant to erythromycin and other macrolides such as azithromycin. Using the knowledge of the structures and binding sites, they designed a molecule (Slide 41) that binds simultaneously to both the chloramphenicol and erythromycin sites. This molecule binds as expected (Slide 42) and has antibacterial activity against many of the macrolide-resistant strains.

This shows that it is technically possible to come up with new molecules with promising properties. To go from these candidate molecules to a medicine that can be prescribed is a long and expensive process, because many of them will have undesirable side effects, may be too expensive to make in large amounts, or may be difficult to deliver to the sites of infection in the body. This means that the production of new antibiotics may cost well over a billion dollars, so any new such antibiotic will be quite expensive and thus restricted to the small cohort of patients who are infected with resistant strains against which current, cheap antibiotics are not effective. Moreover, a good antibiotic will cure the patient, who in a week or two will no longer need it. The small potential market and the short-course means that it may not be easy for a pharmaceutical company to recover its development costs compared to drugs for chronic illnesses or those with much larger markets, such as diabetes, hypertension, elevated cholesterol or cancer. It is true that a huge market for antibiotics exists in developing countries but they are precisely the ones who cannot afford very expensive new drugs. Thus the current model for antibiotic development is deeply flawed, and some other way of promoting it is badly needed.

An important point is that no matter what new drug is developed, resistance will always emerge, as Fleming had said a long time ago. Antibiotics work by a precise fit between the molecule and its target, such as a pocket in the ribosome. This tight binding in a critical pocket prevents the target from working. It is possible to prevent this tight binding in a number of ways (Slide 43). Cells can make enzymes that break down antibiotics, so that the pieces of the antibiotics no longer bind tightly in the pocket. Or they can modify either the antibiotic or the pocket by adding extra chemical groups to one or the other, so that the precise fit to the pocket is destroyed. Many cells also have “pumps” which are proteins that pump out foreign molecules from the cell including antibiotics, so that they are removed before they can cause harm. Regardless of the exact mechanism, natural selection ensures that at some point, resistance will emerge to a new compound.

Thus the problem of infections must be dealt with a multi-pronged approach (Slide 44). Some of these are matters of social awareness and public hygiene. It is important to have good surveillance so that epidemics are quickly identified and dealt with. It is also important not to



abuse the large number of existing antibiotics by promoting their rational use. This includes not using them to fatten up cattle in the animal farming business, and not making them available without prescription, and not using them for viral infections. I am told there are almost 20 million prescriptions annually in the USA for the common cold, for which antibiotics have no effect, and about 10 million kg used in feeding animals. A third aspect is infection control in the form of good public sanitation and even simple procedures such as washing hands before coming into contact with others, especially patients, has proved to be extremely beneficial.

Science can also help. Understanding precisely how a particular bacterium causes disease (microbial pathogenesis) can identify new targets for potential antibiotics. Better diagnostics can help to deliver antibiotics specifically targeted to the particular infection. Vaccine development can prevent infections from occurring in the first place. Finally, the development of new drugs and therapies can be done with the help of biochemical and structural work as discussed in this talk.

I would like to close by showing you a movie (Slide 45, also available at http://www.mrc-lmb.cam.ac.uk/ribo/homepage/movies/translation_bacterial.mov). This shows how the ribosome finds its starting point on mRNA with the help of protein initiation factors, how amino acids are delivered by tRNA and how the ribosome moves along the mRNA. Finally, when it reaches the end, special proteins cleave off the newly made protein (which has emerged from the tunnel) and split the ribosome apart, allowing it to start the process all over again. It is an amazingly complicated process that we are only beginning to understand in real detail, and is even more complicated in higher organisms.

Thank you very much for your attention.