MICROBIOLOGY TODAY

Physics under the microscope

Taxonomy for the new millennium

How molecules cross membranes

Let's have a debate!

Contents

Above: Part of the structure of the core of bluetongue virus.

Photo courtesy J. Diprose, J. Grimes, D. Stuart and R. Esnouf

This issue of *Microbiology Today* focuses on biophysics and the applications that are relevant to microbiology. It includes in-depth articles on Raman spectroscopy, Cryo-EM, imaging, NMR, X-ray crystallography and atomic force microscopy.

Molecular methods are revolutionizing microbial classification, but on p. 70 Howard Gest questions the validity of some of the resultant changes to taxonomy.

The SGM meeting at Edinburgh was a great success. The next meeting at Leeds in September promises to be of equal merit. The range of interesting symposia are outlined on p. 84 and a preview of the Main Symposium '*How Do Molecules Cross Microbial Membranes?* appears on p. 74. Also relating to Society meetings, Philip Mortimer raises the subject of debates as a means of scientific communication (p. 77).

Several important events to foster the public understanding of science, such as SET week, take place in the spring. SGM is an active participant and our recent activities are described on p. 90.

These articles appear in addition to all the regular features and reports of Society activities.

Articles

Regular Features

Other Items

Physics under the microscope
Dave Roberts

Richard Dawkins has pointed out that the essential difference between physics and biology is that physics is predictable: when you put a ball on a hill, you can predict in which direction it will move when you let it go. When you put a mouse on the same hill, you cannot predict what will happen. Physicists sometimes chide biologists for practising a soft science because we are unable to order our observations into testable theories which will allow prediction, with the noteworthy exception of Darwin's theory of evolution by natural selection.

In part our problems spring from an inability to gather the right kind of data. In 1962 Arthur Koch published a paper in the *Journal of General Microbiology* (now *Microbiology*) describing a model for the statistics of cell division. The problem is simply stated: if a cell grows until it doubles in size and then divides into two, how will the sizes of individual cells be distributed in steady-state? In fact this problem turned out to be incredibly complicated and Koch got the statistics wrong, but it stimulated some two decades of active study into the quantitative aspects of cell growth. It all petered out in the end because we were, and indeed still are, unable to measure the distribution of cell sizes with sufficient accuracy to be able to distinguish between radically different models of cell growth.

As a sweeping generalization (letters from readers are invited), biologists tend to gather what data they can and make of it what they may. The instruments with which we gather data are based on solid physical principles. New classes of instruments become available as new physics is discovered, but more commonly the decline in price or increase in availability of some technology makes new instruments possible, so that we can exploit a new level of resolution previously unavailable, to reveal the structure of ever bigger molecules for example. So there are two processes at work here: the discovery of new physical principles, such as digital computers, lasers, phase-contrast microscopy or superconductivity, and the advance of technology enabling us to push back the limits of machine performance, such as the improvements in magnetic field strengths or the combination of technologies in confocal microscopy.

In this issue of *Microbiology Today* we have sought to bring together a set of articles which discuss the physics of instruments, what limits their performance and what future developments there might be of relevance to our own discipline. In my experience it is not difficult to get physical

scientists interested in the problems of biology. In the USA there is a growing demand for super-computers to attack biological problems: these super-computer centres are usually run by physicists, of course, but the demand has largely been driven by the explosion of interest created by molecular biology. Earth scientists are also now asking what contribution microbes have made to processes previously considered to be inorganic and what kind of evidence might be left behind to help determine the answer. In these days of the large multi-disciplinary grant this must be an opportunity for microbiology to start to get the kind of data that we really want. If we fail to take the opportunity the physicists' criticism of biology will have been fairly made.

● *Dave Roberts, Editor*

It's all a question of image
Dave Roberts & Gianfranco Novarino

History

A picture is worth a thousand words, as the saying goes. The ability to visualize microbial structures and processes is a central part of the communication and, in many cases, the understanding of our discipline.

Your eyes are forming an image of the letters on this page because you are able to resolve the adjacent parts of each letter into its black and white components and so discern its shape. If you were to move the page away from you, the letters would form smaller and smaller images on your retina until you were no longer able to resolve the black and white parts and only see the text as a grey blur. The angular separation of any two points on the page which can be separated by your eye is a measure of resolving power and is called the acuity. Its absolute limit is when the two points fall on adjacent retinal cells.

To see smaller objects, we can use lenses to form images which appear to be larger and thus are resolvable. Magnifying glasses are an example of this principle, well known for centuries. If it has been known for so long it is reasonable to ask whether today's microscopes are really better than those from the turn of the century or are they just easier to use? Is there any new physics in the modern instrument?

Lens design and aberrations

The theory of microscope lenses and how to get the best from them was essentially worked out by Ernest Abbe working in the University of Jena, Germany. The formation of an image by a lens depends on the diffraction of light. School-level physics tells us how lenses work, but there are a number of imperfections in the image formed which are an inevitable consequence of the process of diffraction. For example, the diffraction of light depends on its wavelength, so that an image formed with white light has a slightly different place for each component colour, resulting in images having a coloured fringe around the edge, and generally reducing the sharpness of the perceived image. This is called chromatic aberration. There are a total of six basic kinds of aberration which are corrected by careful design of the component parts of the objective lens, all of which was well understood by the end of the 19th century.

Resolution

Traditional microscopy was, and in purist circles still is, judged by the simple measure of resolution. Resolution is a question of how close together two objects can be placed and be perceived as two objects. It is almost entirely governed by the angle formed by the light from the edges of the objects as it enters your eye and, therefore, the wider the cone of light, the better the resolution. The light-gathering potential of the microscope objective is measured by its numerical aperture and the greater the numerical aperture, the higher the resolution. The downside of this relationship is that the higher the numerical aperture, the shallower the depth of field will be. Real specimens are almost always thicker than the depth of field and parts of the specimen which are out of focus will clutter up and technically degrade the image. In real applications it is necessary to trade the greater depth of field of lower numerical apertures with the loss of resolution to get the best combination.

Contrast

The capacity to resolve an object is of no use unless you can see it; that is, it has to be different from its background. Many microscope test objects are high-contrast, typically being completely opaque, which helps to separate the different components of lens performance. A number of ways to enhance the level of contrast have been developed.

Stains. Perhaps the most obvious way to increase contrast is to apply a stain. Surely every microbiologist must have done a Gram stain, the first stage of which, staining with crystal violet, demonstrates a clear increase in the contrast of the objects. Bacteria that were once translucent become an opaque purple and stand out clearly against their background. The inverse of this process, negative staining with, for example, nigrosin has the same general effect.

Completion of the Gram stain illustrates a second principle of staining, which is the capacity to use chemical reactions to discriminate morphologically similar objects. This is one area in which considerable progress continues to be made.

Dark field. When light interacts with a specimen it may do so in several ways. It may be occluded by an opaque specimen, resulting in a silhouette, absorbed at one or more wavelengths, resulting in a coloured image, or the light may be re-directed or scattered. Light scattering by small particles is known as the Tyndall effect and is the mechanism by which we see dust motes in a sunbeam. The objects being seen by this method are often too small to be resolvable directly. Dark-field microscopy works by arranging for the objective lens to collect scattered light so that the specimen is seen as a bright object against a dark background. In technical terms this improves the signal-to-noise ratio and gives a particularly powerful technique for detecting low-abundance objects.

Phase contrast. Many microbes are essentially hyaline but that does not mean they have no effect on the light which passes through them, which may be changed in phase or polarity, although neither change is visible to the eye. In

ABOVE:
Two species of the peniculine ciliate *Frontonia* [*F. vernalis* (green) and *F. leucas*] from the sediment of Esthwaite Water. The black background indicates that the microscope was set up for dark-field illumination. Each cell is about 200 µm long.
COURTESY BLAND FINLAY, INSTITUTE OF FRESHWATER ECOLOGY

BELOW:
The sessile pertitrich *Vorticella similis* photographed under DIC. Note the illusion of oblique illumination caused by the brightening of the left side and the darkening of the right edge. The round object in the lower portion of the cell is the contractile vacuole. Bar, 100 µm.
COURTESY NICOLA MILLER, SGM

(a)

(b)

(a)

(b)
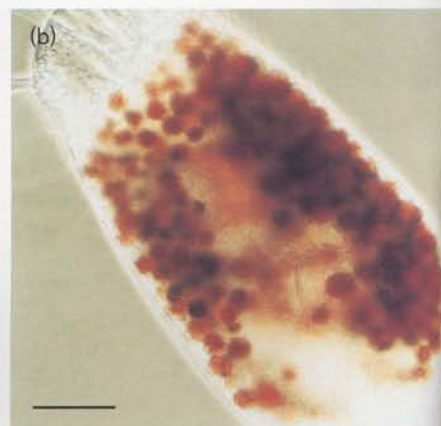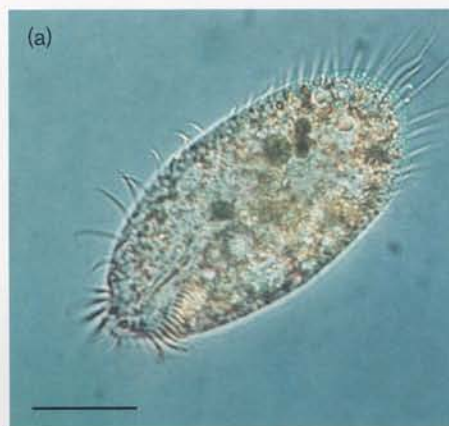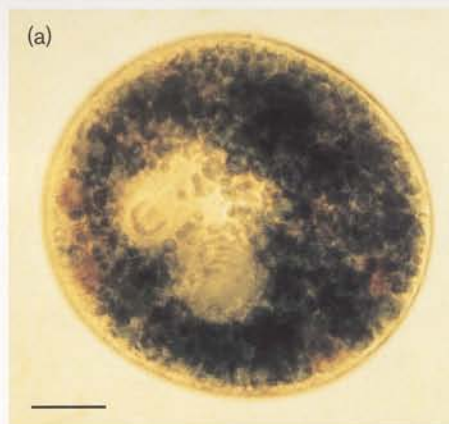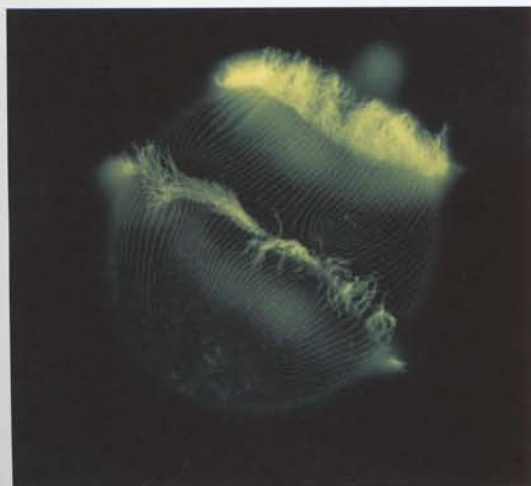
practice polarized light microscopes are most commonly found in a mineralogical context and are uncommon in biology, but Frits Zernike was awarded the 1953 Nobel Physics Prize for the phase-contrast microscope which allowed changes in phase to be made visible. Essentially this is done by making the light passing through an object interfere with light passing through the background. This interference may be additive, causing a brightening, or subtractive, causing a darkening in the image. The cell may be alive and moving and otherwise unstained. This technique is probably best used in microbiology to observe fine projections, such as eukaryotic flagella which are exceedingly difficult to observe in life by any other technique. The disadvantage of phase contrast is the halo which it creates around an object which can be so bright as to obscure detail.

Interference contrast. Strictly, all microscopes form their images through interference, but in the interference contrast microscope the optics are arranged so that the interference is most sensitive to the rate of change of the optical properties, hence the system is known as differential interference contrast or DIC. The optical principle of this method was first described in the late 19th century by Jamin but it was the work of Nomarski in the 1950s that made the technique easily available to the biological laboratory.

DIC images are difficult to interpret because the effect of the interference is as though the specimen was obliquely illuminated creating a pseudo 3-D appearance. The contrast effects are, however, demonstrating differences in the optical path properties, not the actual thickness. Real cells do not often look like fried eggs with a protruding nucleus.

Fluorescence. UV light was first used as a microscope illumination source in 1904 by Köhler, in an effort to improve spatial resolution. Instead, the far more useful property of specimen fluorescence was discovered. Like dark-field illumination, the fact that most materials do not fluoresce greatly improves the ability to study those that do.

However, like phase contrast, the halo generated by a strongly fluorescent object can obscure more weakly fluorescing objects nearby. Many fluorochromes also bleach when they are exposed to UV light and so fluoresce for only a very short time. The use of scanning high intensity light sources, typically lasers, can help to overcome this problem.

● Depth of field

The problem of depth of field is faced by those who want to observe complete systems rather than thin sections. The parts of the specimen which are out of focus exist as a diffuse blur which detracts from the clarity of the in-focus image.

Holography. In principle it is possible to take a holographic image of a drop of water which could then be re-created by a projector and studied using a conventional microscope. To our knowledge, there is no published record of this ever having been done. The formation of the holographic plate is a process of convolution which cannot readily be reversed by computer because of the complexity and, particularly, the size of the calculations necessary.

Confocal microscopy. The new-kid-on-the-block is still the confocal microscope, which once again has its roots in the 19th century. The true microscope, i.e. an instrument capable of forming a real-time image, was patented by Minsky in 1957 and mechanical instruments were produced a decade later. In the late 1970s the advent of comparatively low-cost digital computers and lasers made the instruments more widely available and by the early 1990s it was a practical proposition to have them in biological laboratories.

The principle of the confocal microscope is to use an optical stop, a small hole, at the objective's primary focus. The effect of this is to allow only in-focus light rays to pass through. To turn this pin-point into an image it has to be scanned over the specimen. This takes real time, of course, and renders the system of limited value to moving (live) specimens. The removal of out-of-focus information from the resulting image results in what is known as an optical section and repeated sections can be built up within a computer into an effective solid model.

The loss of total light by the very small confocal aperture has to be compensated for either by use of a photomultiplier or by an enormous increase in the intensity of illumination. With very intense illumination it is necessary to be able to discriminate the illumination (background) from the interference pattern that is the image. This is done most effectively by employing fluorescence to shift the wavelength of the light to be observed and to filter out the illumination source, exactly as in a standard fluorescence microscope. If the illumination source is suitably concentrated, normally a focused laser, then only the region of instantaneous interest is illuminated which considerably reduces the impact of photo-bleaching.

Deconvolution. The combination of in-focus and out-of-focus information is called convolution and is well defined mathematically. It is possible to achieve the confocal effect with a normal microscope by reversing the process, which can be done if you have a sufficiently powerful computer and you know a great deal about the optics of the instrument. The latter issue is the real stumbling block. In practice, however, microscope images can be greatly improved by deconvolution.

Time and the movies

There is more on the collective mind of modern biologists than mere description: the recognition of the dynamic nature of biological processes and interactions demands systems capable of producing images of changing specimens. The halo effect in phase contrast, for instance, rather limits its usefulness in the case of actively swimming flagellates, when DIC is a more appropriate choice. If the cells slow down then phase contrast once again becomes the method of choice.

Cameras

The original microscopes were intended to permit visual observations. Film cameras were added later to allow the images to be recorded, although anyone experienced with a microscope will know that the information captured on film is often only a fraction of that available down the eye-pieces.

The first constraint is the amount of light. Film emulsions trade the amount of light needed to expose them against the grain size, i.e. the resolution, of the photographic image. Advances in the chemistry of emulsion production and processing, especially of colour emulsions, have greatly relieved this limitation. Nonetheless, for motile cells the length of time needed for exposure inevitably leads to motion-blur. The use of a flash tube is still the best way to overcome this problem, to deliver a high-intensity burst of light of very short duration. Sadly few modern microscopes are, or can be, equipped with flash tubes.

Video

The amount of available light has been a limiting factor in the use of cine film in microscopy, but the arrival of affordable video cameras in the 1980s brought a new technique to microscopy. The first video cameras were based on tube technology and were prone to a number of problems in terms of accuracy and resolution. These days tubes are used only in specialized circumstances.

The charge-coupled device (CCD) is a semi-conductor array which accumulates charge when a photo lands on one unit. These charges are read off at a standard time and the system is re-set for the next image. The data are run together into a standard TV signal in most cameras which is passed to a video recorder or a computer digitizer. In the latter case, the digitizer board has to try and dismantle the signal back into the cell units of which it was first composed. One problem with CCD cameras is that the array accumulates random signal as a result of thermal noise. This can be considerably reduced, and the sensitivity of the camera consequentially increased, by cooling the chip.

The tumbling price of fast personal computers and large storage devices has now opened the field of digital video as a routine application in the laboratory to record and study dynamic processes under the microscope. There have, as yet, been comparatively few published studies in microbiology exploiting the potential of this technique.

Image analysis

Once you have a digital image, then many forms of image processing are now possible and these techniques are a powerful addition to the microscopist's arsenal. Apart from deconvolution and work on 3-D images, there has been very little progress in image analysis methods since the early 1980s. These techniques are, however, rather mathematical and tend to be driven from the command line of a computer. Few things seem to put biologists off more effectively, so progress in computer-assisted microscopy has largely come with improvements in software design making it easier to use.

Developments

It should, we hope, be clear from the above that very little new physics has entered the field of microscopy since the development of phase contrast. Nonetheless, today's instruments will, in most but not all circumstances, outperform their turn-of-the-century equivalents.

It is in the field of technology, of new combinations of known physics, chemistry and mathematics that microscopy is developing now. Since we started with a cliché, we should end with one too – *you ain't seen nuffin' yet!*

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Further reading

The Royal Microscopical Society (RMS) handbook series is recommended, particularly the titles listed below.

Bradbury, S. & Bracegirdle, B. (1998). *Introduction to Light Microscopy. Microscopy Handbook Series*, 94 pp. Oxford: BIOS Scientific Publishers, in association with the RMS.

Bradbury, S. & Evennett, P. (1996). *Contrast Techniques in Light Microscopy. Microscopy Handbook Series*, 118 pp. Oxford: BIOS Scientific Publishers, in association with the RMS.

Gall, J. G. (1996). *Views of the Cell. A Pictorial History*. American Society for Cell Biology.

Sheppard, C. & Shotton, D. (1997). *Confocal Laser Scanning Microscopy. Microscopy Handbook Series*, 106 pp. Oxford: BIOS Scientific Publishers, in association with the RMS.

Atomic force microscopy
Alastair Smith

● The principle of the atomic force microscope

Microscopes have historically been tools of great importance in biological science. The atomic force microscope (AFM) is one of a family of scanning probe microscopes which has grown steadily since the invention of the scanning tunnelling microscope by Binnig and Rohrer in the early eighties for which they received the Nobel Prize for Physics in 1986. The AFM uses a cantilever, usually made from silicon or silicon nitride, with a very low spring constant, on the end of which a sharp tip is fabricated using semi-conductor processing techniques (Fig. 1). When the tip is brought close to a sample surface the forces between the tip and sample cause the cantilever to bend and this motion can be detected optically by the deflection of a laser beam which is reflected off the back of the cantilever. If the tip is scanned over the sample surface then the deflection of the cantilever can be recorded as an image, which in its simplest form represents the three-dimensional shape of the sample surface. Many variants now exist which use special tips to probe the electric, magnetic (Figs 2 and 3) or thermal properties of surfaces, and even optical spectroscopy is now possible with about 50 nm lateral resolution using scanned probe techniques.

The resolution of AFM depends mainly on the sharpness of the tip which can currently be manufactured with an end radius of a few nanometres. Atomic resolution is easily obtained on relatively robust and periodic samples. Soft samples however, particularly biological samples, provide a more difficult surface to image because the forces exerted by the tip during imaging can cause deformation of the sample. The problems involved with imaging soft samples have been overcome to a large extent by the introduction of tapping mode AFM imaging. Instead of maintaining a constant tip-sample distance of a nanometre or so, the cantilever is oscillated in a direction normal to the sample resulting in only intermittent contact with the surface. This greatly reduces the lateral forces being applied in the plane of the sample which are responsible for most of the damage as the tip is scanned. The AFM is capable of better than 1 nm lateral resolution on ideal samples and of 0·01 nm resolution in height measurement.

There are some significant advantages of AFM as an imaging tool in biology when compared with complimentary techniques such as electron microscopy. Not only does AFM achieve molecular resolution but the technique requires almost no sample preparation and, most importantly, can be performed under fluids, permitting samples to be imaged in near native conditions. The fluid may be exchanged or modified during imaging and therefore there is the potential for observing biological processes in real time. Several instruments are commercially available for around £100K and in general the technology is straightforward to use and occupies only a small table top.

● High resolution imaging

There have been many studies of biological materials using AFM in the few years since its conception, including nucleic acids and their complexes with proteins, two-dimensional protein crystals and individual isolated proteins, membranes and membrane-bound proteins, and living cells.

One of the most extensively studied systems is nucleic acids which have now been imaged with sufficient resolution to measure the pitch of the double helix. Nucleic acids may be deposited on mica quite simply by using a divalent cation to bridge between the negative backbone of the nucleic acid and the negatively charged mica surface. Most recently there have been several reports of AFM imaging of the complexes formed between proteins and DNA which have, for example, provided detailed information about the changes in conformation of DNA in response to protein binding. The one-dimensional diffusion of RNA polymerase along DNA has been imaged in real time and the enzyme was seen to slide along DNA and even hop to neighbouring nucleic acids on the substrate surface. Fig. 4 shows one recent example of AFM imaging of a protein/DNA complex. Tapping mode AFM has been used to image an individual complex of human transcription factor 2 with DNA. It appears that a protein–protein interaction has facilitated looping of the DNA to allow two distal DNA sites to be brought together on the substrate. A goal of some workers is to develop AFM imaging techniques to a point where the DNA sequence can be read; however, this ambitious goal remains elusive.

The highest resolution images are obtained on tightly packed structures such as two-dimensional arrays of proteins because the packing affords a greater mechanical stability to withstand the imaging forces. In cases such as this the resolution of the images is better than 1 nm because the highly regular assembly of proteins also allows averaging to be performed which greatly improves the signal-to-noise ratio. The quality of information in such images has been demonstrated by Engel and co-workers who have reported real-time observation of the central pore of proteins in the HPI layer of *Deinococcus radiodurans* opening and closing. Another example, shown in Fig. 5, is the molecular resolution tapping mode AFM image of the periplasmic surface of OmpF porin from *Escherichia coli* taken in buffered solution. A rectangular unit cell arrangement ($a = 135$ Å, $b = 82$ Å) can be seen each containing two porin trimers with a lateral resolution of about 8 Å.

Imaging of living cells is not straightforward because the cell surface is extremely soft despite the structural support of the cytoskeleton. Obviously deformation of the sample during imaging can seriously limit the resolution which can be achieved on cells and although the overall shape can be
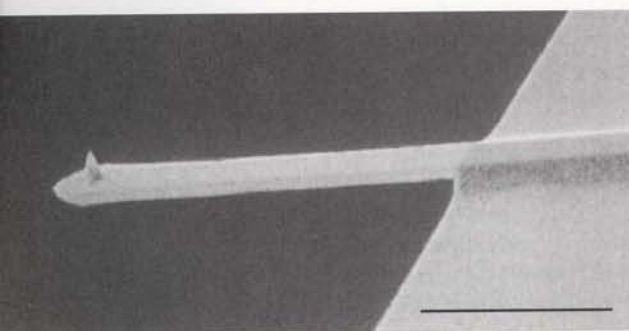
ABOVE:
Fig. 1. Electron micrograph of a typical tapping mode AFM cantilever shown 'upside-down' to reveal the sharp tip used for imaging. Bar, 100 µm.
COURTESY DIGITAL INSTRUMENTS, VEECO METROLOGY GROUP

TOP RIGHT:
Fig. 2. Magnetic AFM image of a garnet film showing flower-like magnetic domains.
COURTESY DIGITAL INSTRUMENTS, VEECO METROLOGY GROUP, AND V. FRATELLO.

BELOW:
Fig. 3. Magnetic AFM image of a praseodymium–iron–boron permanent magnet.
COURTESY DIGITAL INSTRUMENTS, VEECO METROLOGY GROUP, AND R. STREET
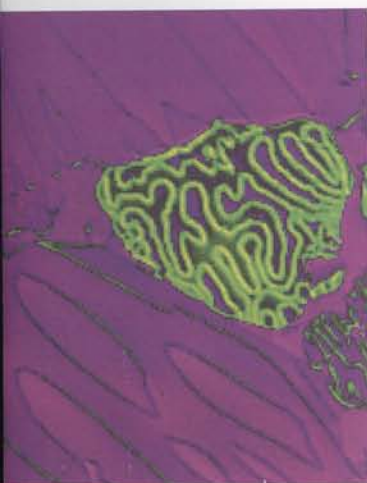
measured easily, surface detail is difficult to obtain. In some cases the nucleus can be clearly seen in the images, which suggests that it is less easily deformed than the cell membrane, and movement of the actin filament bundles beneath the periphery of the cell membrane has been observed.

● Measurement of biological interaction forces

In addition to the potential of the AFM to provide very high resolution images of biological samples and to monitor conformational changes and biomolecular processes in real time under native conditions, the instrument is also capable of manipulating molecules and measuring the strength of biomolecular interactions with piconewton sensitivity.

The forces exerted between the silicon nitride tip and the sample arise principally from van der Waal's interactions. These interactions are rather non-specific in the biological sense but lead to bending of the cantilever which provides the topographical information for the images. However, it is relatively straightforward to modify the tip surface chemically so that its interaction with the sample may be made highly specific. For example the tip may be modified to have a charged surface, one which readily forms hydrogen bonds or may be made very hydrophobic. In these simple cases it is the interaction with the sample which bends the cantilever and therefore the information that is contained in the images has chemical information about the tip–sample interaction. Tip modification is usually achieved using the well known process of thiol self-assembly on gold. The tip is first coated with a thin layer of gold by evaporation, then immersed in a solution of ω-functionalized alkyl thiol molecules. These molecules have a functional head group which will provide the tip with the desired physico-chemical property and an alkyl chain spacer of about 10 carbons terminated with a thiol group. The sulphur spontaneously forms a covalent bond with the gold coating on the tip and the molecules pack tightly to form a well ordered monolayer with the functional group uppermost, creating a new surface of tailor-made chemistry on the tip.

Tips may also be modified to have biological functionality. For example, antibodies can be tethered to the tip via flexible polyethylene glycol spacers, permitting the antibody to bind to an antigen on a sample surface. Using such a tip, the distribution of antigens over a cell or other surface may be mapped, but it is also possible to pull the antibody–antigen complex apart by moving the tip away from the substrate on which the antigen is absorbed and measure the strength of the interaction directly. The binding event between single pairs of streptavidin/biotin

molecules has been observed by several groups and measured to be of the order of a few hundred piconewtons. The resolution of the AFM as a force or binding strength measuring device is of the order of about 10 piconewtons, limited by the thermal noise of the cantilever at room temperature. An exquisite example of the measurement of such biomolecular interactions was reported by Lee and co-workers at the Naval Research Labs in Washington. Two complimentary DNA oligonucleotides, one tethered to the tip and one to a gold substrate, were allowed to interact and then pulled apart whilst the forces required to rupture the double helix conformation were measured.

Perhaps one of the most exciting demonstrations of the potential of the AFM to measure biomolecular interactions has been the recent demonstration by Gaub's group, and others, of the mechanical unfolding of single proteins. Gaub has shown that the forces required to unfold the subdomains of titin, the giant muscle protein, can be measured by AFM. In these experiments the AFM tip is used to pick up part of the vast titin molecule whilst the rest remains absorbed to a gold substrate. The tip is then withdrawn and a series of small tugs on the cantilever can be observed which have been attributed to the unfolding of the immunoglobulin and fibronectin domains. The forces measured are in two phases. First, the extension of unfolded chains which appear to fit well to a worm-like chain model and second, a dramatic step which is identified with the catastrophic unfolding of a domain. This type of measurement, which has initiated a flurry of activity in mechanically manipulating biomolecules, is only the first step in a developing new field of biophysics based on AFM technology.

● The future

The fact that AFM is relatively cheap technology and straightforward to use suggests that it will be accepted quickly as an imaging tool in biology. The ability to perform real-time observations of biological processes under native conditions will be of great interest and as the biochemistry is developed to modify tips in more complex ways to attach proteins, peptides and small organic molecules, then the detailed measurement of biomolecular interactions appears to be an area with very great potential. The simplicity of the instrument also lends itself to integration with other instruments. In the author's laboratory, as in several others, AFM is being combined with laser spectroscopy so that mechanically induced conformational changes can be monitored by fluorescence or infra-red spectroscopy. It seems that AFM is likely to become one of the most widely used high resolution microscopy techniques in the biological sciences.

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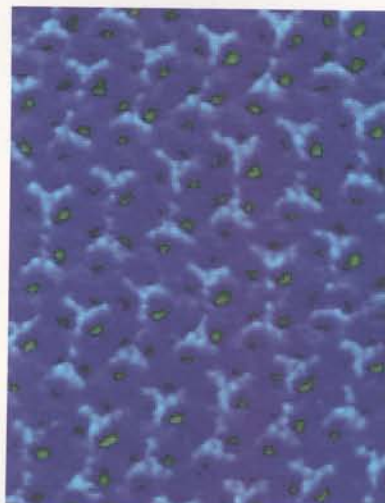
ABOVE:
Fig. 5. Image of the periplasmic surface of OmpF porin from *E. coli.*
COURTESY DIGITAL INSTRUMENTS, VEECO METROLOGY GROUP, AND A. ENGEL

LEFT:
Fig. 4. Tapping mode AFM image of an individual complex of human transcription factor 2 with DNA.
COURTESY DIGITAL INSTRUMENTS, VEECO METROLOGY GROUP, AND C. BUSTAMANTE

Further reading

Bookmark the following web sites:
http://www.di.com
http://www.thermomicro.com
http://www.molec.com

Cryo-electron microscopy: taking back the knight

Stephen Fuller

Why cryo-EM?

An instructor in an introduction to an X-ray crystallography course some years ago explained the difference between electron microscopy and X-ray diffraction as structural techniques. Imagine, he said, setting out to determine the structure of a man. One approach would be to cover him in a suit of armour, place him in a vacuum until all the water is gone and broil him under a high energy beam until the armour began to melt and flatten, and finally take a picture of the remnants and call it the structure. That, he said, is the electron microscopist's approach. The X-ray crystallographer, in contrast, maintains his sample in an aqueous environment and determines the structure of the entire sample in three dimensions rather than only that of a shell of a metal shadow or a heavy metal stain. From such a perspective, the choice between the two techniques was an obvious one to any serious student of structure.

Today the situation has changed dramatically and few structural biologists would omit electron microscopy as part of their characterization of a biological system. The advent of cryo-electron microscopy (cryo-EM) has allowed the observation of biological samples in a layer of vitrified water. This avoids the drying associated with classical approaches. The use of phase contrast to image the specimen allows one to perform unstained microscopy so that the entire density of the specimen contributes to the image. Image processing techniques have developed so that the contrast of these images can be accentuated and the three-dimensional structure determined by combining the projected densities in the individual micrographs. Finally, measures of the reliability of the resultant structures have been developed and validated by comparing them with structures derived from X-ray diffraction of the same complexes. This has led to the use of a divide and conquer approach to many systems. First, the atomic resolution structures of subcomponents of a macromolecular complex are determined by either X-ray crystallography or NMR. Then, cryo-EM is used to provide a context for these structures by showing their placement in the complex and indicating where the structure must be altered during assembly.

Vitrified samples

The preparation of a sample for cryo-EM takes advantage of the properties of water. When water is cooled slowly (a few degrees per second) it forms hexagonal crystals of ice, the form found in ice cubes and snow flakes. Very rapid cooling (thousands of degrees per second) traps the water in a vitrified state in which the structure has not been allowed to rearrange into a crystalline form. Practically, this is usually accomplished by a method developed 20 years ago by Jacques Dubochet and collaborators at the European Molecular Biology Laboratory (EMBL). A small aliquot of the sample in suspension is placed on a holey carbon film blotted to generate a layer of ~1000 Å thickness and plunged into a bath of ethane slush held in a container of liquid nitrogen. Ethane is a very efficient cryogen since, unlike nitrogen, it does not boil at the temperatures used for vitrification. Nitrogen is not used directly since the formation of gas around the specimen when it is introduced into liquid nitrogen slows the cooling and leads to the formation of ice.

The vitrified state is not an equilibrium one but rather a metastable one. It can be maintained at liquid nitrogen temperatures for long periods. Our group has examined specimens stored in liquid nitrogen for more than 10 years without loss of the vitrified state. Raising the temperature above about −140 °C causes devitrification and the formation of cubic ice. Paradoxically, vitrified water freezes upon warming. Cooling the sample again does not restore the vitrified state, it simply generates cooler ice. The vitrified sample is transferred to the microscope by passing it rapidly between baths of liquid nitrogen and then mounting it in a liquid-nitrogen-cooled specimen holder which is inserted into the microscope. Other schemes have been used for generating vitrified specimens. The requirements are sufficiently rapid cooling to avoid the formation of ice and maintenance of the vitrified state by keeping it at low temperature at all times, usually by storage in liquid nitrogen.

The requirement for very rapid cooling is a limitation of the technique. The method works best with specimens, such as suspensions of viruses or protein complexes, which can be made very thin so that cooling is rapid. Thicker objects such as cells must be handled differently and represent a challenge to the field. Suspension samples also present problems if they contain solutes which interfere with vitrification. Samples containing salt or sucrose show a phase partitioning after vitrification. Crystalline specimens are usually handled in a somewhat different way. These samples are often placed on a continuous carbon film in glucose or tannic acid and cooled directly in liquid nitrogen or in the holder rather than using ethane as a cryogen. This is possible because very high concentrations of these sugars act as a cryoprotectant and inhibit ice formation.

The vitrified sample is typically maintained at near −170 °C during microscopy. The specimen is extremely sensitive to radiation damage from the electron beam of

BELOW:
Fig. 1. Cryo-electron micrographs of SFV taken on a Philips CM200 equipped with a field emission gun at 2 μm (upper) and 4 μm (lower) underfocus. The 700 Å diameter virions sit in a layer of vitrified water suspended over the holes of a carbon film. The phase contrast nature of the image causes different resolution ranges to be accentuated at different defocuses.
COURTESY STEPHEN FULLER
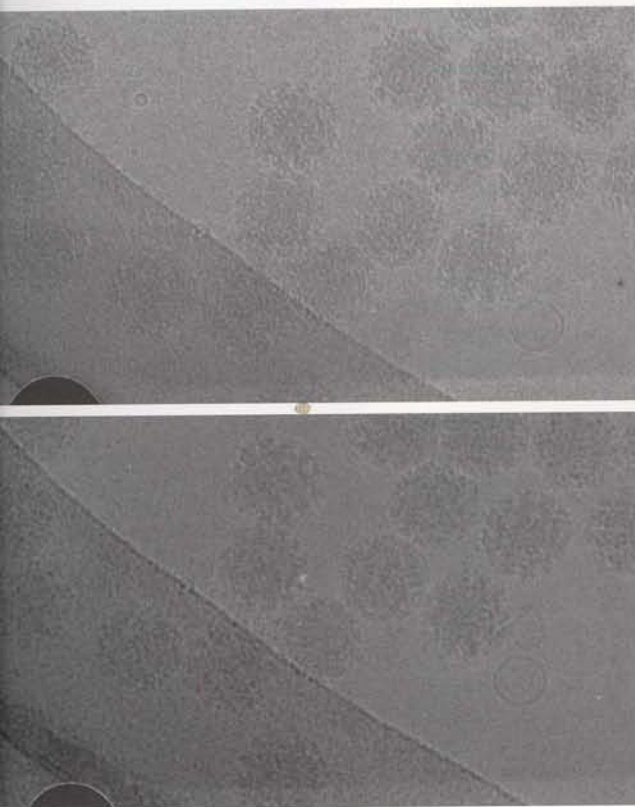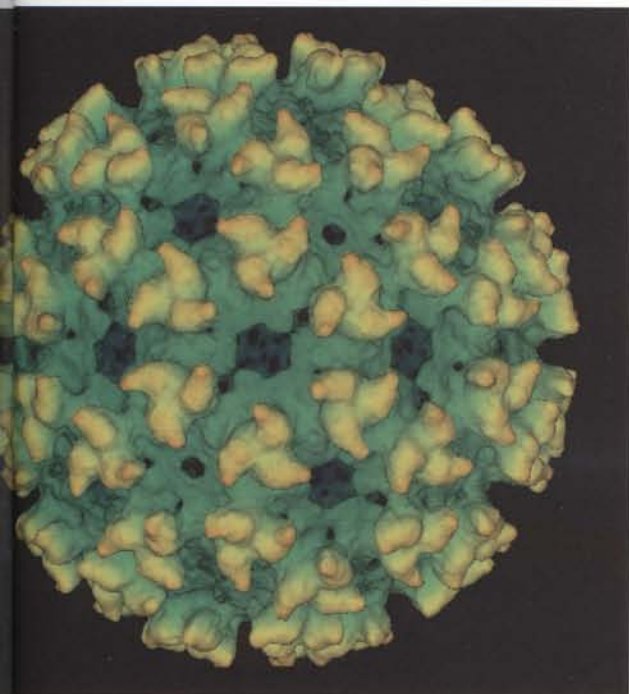
the microscope. An image of an undamaged specimen is obtained by using low dose techniques. The specimen is first imaged at low magnification so that areas of interest can be identified with minimal irradiation ($< 0\cdot1$ electrons Å^{-2}). The image is then captured at higher magnification with the first electrons to hit the specimen. Typically, the image is formed with a dose of 5–10 electrons Å^{-2}. Work with crystalline specimens at $-170\,°C$ indicates that this dose does not damage the structure at resolutions coarser than $10\,\text{Å}$.

The images obtained by cryo-EM show details of the entire specimen. In contrast to negative staining or metal shadowing which show the surface of the sample, as the armour models the features of the knight, the image is a superimposition of all of the density in the sample. Interpreting such a projected image requires either restricting oneself to the edges of the object or accounting for the projected nature of the image. Three-dimensional reconstruction techniques take advantage of the fact that the image is a projection. The most powerful approach is that of electron crystallography. However this can only be applied when the specimen is co-operative enough or the investigator inventive enough to produce the sample in an ordered two-dimensional crystal. The simplest of the alternative approaches takes advantage of the homogeneity of the specimen. A field of particles such as the one of Semliki Forest virus (SFV) shown in Fig. 1 can be viewed either as images of separate particles or as images of the same structure from a variety of orientations. The latter is key to determining the structure. The process of three-dimensional reconstruction involves identifying the orientations of each of the views represented by the particle images and then combining these images to determine the structure (Fig. 2). Obviously, the reliability of the assignment of the orientation and the resolution of the information contained in the image limit the final resolution of the structure. A process of refinement allows one to determine the orientations to higher reliability; however this process is only effective if the resolution of the data allows determination of the orientations with high precision. The homogeneity of the sample is critical to this process. The reconstruction method assumes that the

differences between separate particle images reflects the change in orientation rather than sample variation.

Three-dimensional reconstructions of particles with higher symmetry, such as icosahedral viruses, are particularly well developed. Building on algorithms formulated by R.A. Crowther (MRC Laboratory of Molecular Biology, Cambridge), a number of groups have developed methods for treating the case of icosahedral particles. More than 150 such structures have already been published and the number continues to grow. The use of a highly symmetric particle allows the symmetry to serve as a guide in determining the orientation and makes the process of computing the structure itself much more efficient. Tests for symmetry in the particle images allows identification of those particles in the population which have been distorted and hence would blur the reconstruction if they were included in the average. Reconstruction methods have also been developed for non-symmetric particles particularly by the groups of M. van Heel (London) and J. Frank (Albany, USA). The power of these methods has recently been demonstrated by high resolution reconstructions of the ribosome.

● Cryo-EM comes of age

Cryo-EM and three-dimensional reconstruction are undergoing a renaissance. A few years ago, the best icosahedral reconstructions achieved resolutions of slightly better than $30\,\text{Å}$. Higher resolution was the domain of electron crystallographers and others working with ordered specimens such as helices. Two developments have been key in bringing a new excitement to the field. The first is the establishment of methods for processing large numbers of images and combining them to produce a three-dimensional structure. Combining thousands of particles enhances the relatively low signal to noise ratio in the unstained, low dose image of a single particle. The second development is instrumental. The use of high voltage and field emission gun sources has increased the strength of the high resolution information transferred from the specimen to the film. This is a consequence of the fact that the image of a thin unstained specimen is dominated by phase contrast. A more coherent electron source produces a better phase image. The effect is particularly important for images of single particles. Accurate determination of orientations requires refinement against high resolution information which is, in turn, necessary for enhancing the signal to noise ratio at high resolution.

The determination of the fold of the hepatitis B capsid protein by the groups of R.A. Crowther (MRC, Cambridge) and A. Steven (NIH, Bethesda, USA) from the icosahedral reconstruction of hepatitis B cores signalled a coming of age in the field. The methods used by the two groups exemplified the two developments described above. The Cambridge group used thousands of images taken on a microscope equipped with a field emission gun source to produce a $7\cdot6\,\text{Å}$ resolution reconstruction which allowed visualization of the α-helices of the structure. Since this

marvellous demonstration of the potential of the methods, a number of groups have applied this approach successfully to larger systems. Work by W. Chiu's group (Baylor, Houston, USA) on herpesvirus and our group on SFV (Fig. 2) have produced structures at better than 10 Å resolution in which secondary structure can be identified. This progress is not limited to icosahedrally symmetric structures. The work on the ribosome mentioned above has now reached resolutions of 13 Å and is serving to map out the changes in conformation with the functional state of the system.

The increased resolution and demonstrable reliability of the information provided by the combination of cryo-EM and image reconstruction have altered the gentle rivalry which once existed between X-ray crystallographers and electron microscopists into a respectful working relationship. Three-dimensional structures derived from cryo-EM not only provide context for the higher resolution structures of components but are also increasingly used to investigate large macromolecular complexes by crystallography. The determination of the structure of the bluetongue virus core by D. Stuart's group (Oxford) (see p. 59) utilized a three-dimensional reconstruction from B. Prasad (Baylor, Houston, USA) to generate the initial model used for phasing. The progress on the crystallography of the ribosome has been aided by the use of reconstructions to serve as an initial phasing model and a control for heavy atom phasing at higher resolution.

● The future: promises and challenges

Cryo-EM also provides resolution in time. The vitrification method can be adapted to capture intermediates. The approach depends both on the timescale and the system but a large range of conditions is accessible. We employed a simple spray technique in which the blotted grid was exposed to a mist of low pH while it was plunging towards the ethane bath to examine the structures formed by the SFV spike during the first 50 ms after exposure to low pH. At this early stage, the virion remains icosahedrally symmetric and hence a three-dimensional reconstruction could be performed by standard methods. Simple mixing experiments have allowed us to examine the slower interaction of the pH-activated virion with the target vesicle. Naturally, the structure does not remain symmetrical upon contact and hence other approaches must be used to determine three-dimensional structure.

Despite the recent progress, challenges still remain. A typical example is shown in the image of immature HIV (Fig. 3). HIV is an ideal case for 'divide and conquer' since the structures of parts of all the structural proteins are now known. This image contains a wealth of information. It shows the layer of spikes on the surface, features within the membrane itself and the radial arrangement of the independently folded domains of Gag. It shows two other key features which represent challenges for the determination of the three-dimensional structure. First, the particles are not icosahedral. Rather they seem to be formed

of independent hemispherical sectors with closely packed, radially arranged Gag protein under the membrane with defects in the packing between the sectors. Second, the particles do not all have the same structure and hence averaging of images cannot make use of this basic property. Approaching such a system requires a continual interaction between biochemists and structural biologists to prepare homogeneous subassemblies such as the helical tubes formed by segments of the Gag protein. It also will require methodological developments such as the improvement of techniques for cryo-EM tomography to determine the unique structures of individual particles.

The future of the technique remains full of promise. Although methods are still under development, current techniques have reached such a level of sophistication that they can be applied in investigating a very broad range of biological problems. No longer are we restricted to working on the best behaved systems. Cryo-EM has passed from being a tool of the specialist to being an essential tool of the microbiologist.

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RIGHT:
Fig. 3. Cryo-electron micrographs of immature HIV produced by infected cells in the presence of protease inhibitors. The envelope proteins, features of the lipid bilayer, radial disposition of the Gag protein and heterogeneity in particle size and shape are easily seen.
COURTESY STEPHEN FULLER
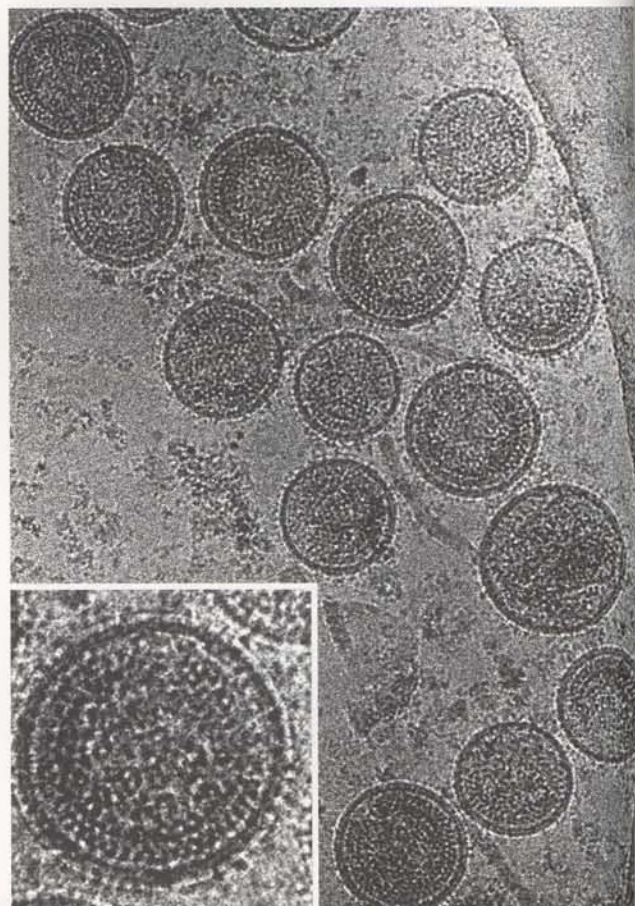
Crystallography and the atomic anatomy of viruses

David I. Stuart, Jonathan M. Grimes,
Nick Burroughs & Peter P. C. Mertens

David Baltimore in his 1976 Nobel Lecture observed *"The study of biology is partly an exercise in natural aesthetics. We derive much of our pleasure as biologists from the continuing realization of how economical, elegant and intelligent are the accidents of evolution that have been maintained by selection. A virologist is amongst the luckiest of biologists because he can see into his chosen pet down to the details of all of its molecules. The virologist sees how an extreme parasite functions using just the most fundamental aspects of biological behaviour…"*

These words are made flesh by X-ray crystallography. We use relatively soft X-rays (wavelength about 0·1 nm) that shine through our small crystals of virions, illuminating every detail and allowing atomic models to be constructed of the exquisitely ordered symmetrical shells of 'spherical' viruses. Knowledge of the three-dimensional positions of the atoms gives a complete static view of the virus and crystallography can, via the somewhat obscure B-factor, indicate molecular flexibility. The results are objective, however, the personal aesthetics of the crystallographer are betrayed in the often riotous choice of colours for their representation. The first virus structure was determined in 1977 by Stephen Harrison and co-workers at Harvard, and over the years a handful of groups around the world have built up a gallery of over two dozen virion structures that cover hosts ranging from bacteria, through plants, to animals. The structures almost exclusively relate to non-enveloped spherical viruses and a challenge for the future is to extend the technique to the less amenable lipid-containing viruses and the beautiful but complex machinery of some bacteriophages. Crystal structures provide snapshots of the virus life cycle which have profound implications for our thoughts on many aspects of their biology. Thus structure has begun to at least partially illuminate questions of virus assembly, evasion of the immune response and cell recognition and entry. Whilst crystallography has led to some unexpected simplifications in our thinking about viruses (for instance, a recurring structural motif suggests that most viruses studied to date may be related), it has also shown the tremendous richness of invention that has been achieved by selecting accidents of evolution.
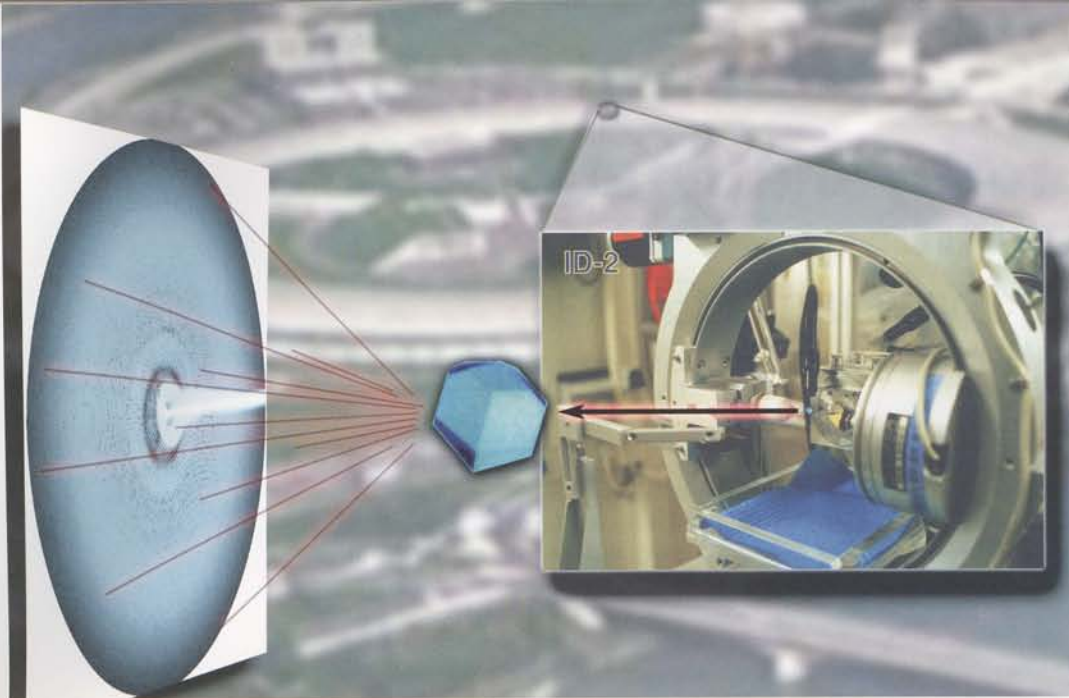
Small crystals / large viruses

A crystal of an organic compound or small protein contains many trillions of identical molecules whose molecular structure may be visualized by amplifying the feeble scattering of a single molecule. Thus, when illuminated by a nearly parallel beam of monochromatic X-rays, all the molecules in the crystal scatter together in certain directions to give detectable diffracted beams. The concentration of the signal into such diffraction spots, according to rules put forward by Bragg, allows relatively simple compounds to be analysed quite routinely in the lab. Virus crystallography is somewhat more complex but identical in its essentials. It turns out that the necessary first step, that of growing crystals, is often relatively easy for viruses. A solution of pure virus particles, at a concentration of a few milligrams per

millilitre, is simply treated as if it were a small protein and subjected to the battery of crystallization techniques now standardized in commercial kits. Whilst it is perhaps not surprising that robust spherical virions crystallize, it is astonishing that the crystals, held together by only a few flimsy non-covalent interactions, are so beautifully ordered. Naturally, since viruses are large, the diffraction from virus crystals is far weaker than for single proteins. For instance, in the case of the bluetongue virus crystals we have looked at, the number of repeating units across the width of the X-ray beam has been as small as 300, dramatically reducing the amplification of the signal upon which the method rests. For this reason, and because the number of diffracted beams to be measured increases in proportion to the particle size, virus crystallography still presents technical challenges and progress has been quite closely linked to the development of intense X-ray beamlines at synchrotrons. Several third generation synchrotrons are now coming on-line around the world (the first of which was the European Synchrotron Radiation Facility at Grenoble; Fig. 1) which present new opportunities to structural virologists. These machines produce X-ray beams thousands of times stronger than can be obtained with conventional X-ray generators and allow the accurate measurement of very weak data. Furthermore, undulator beamlines are available on these machines which produce very nearly parallel beams, allowing more reflections to be resolved on a detector of a certain size. With the technology now available it is hard to see the limit to what can be achieved. For instance, we have recently solved the structure of the bluetongue virus core that contains almost 1000 polypeptide chains (Fig. 2). Even larger structures could be analysed, provided that they have a well defined structure and provided there are dedicated microbiologists committed to making the many milligrams of pure virus required to analyse such complex systems.

Problem spots

X-rays cannot be focused sufficiently well to directly image atomic structures. Instead the information in the beams of scattered X-rays is reconstructed with a computer to provide the image. The diffraction pattern obtained is a series of spots which may be thought of as representing the scattering from the virus viewed through a grating. The periodicity of these spots simply reflects the periodicity of the crystal whereas the brightness of the spots reflects the distribution of electrons within the virus. To solve a crystal structure diffraction spots are recorded from a complete set of views of the crystal. Since X-rays quickly damage virus crystals we usually need to add together data obtained from many crystals. Next the spots must be transformed into a map of the electron distribution in the virus. Here we encounter a fundamental problem. The map is constructed from many component waves of different frequencies, amplitudes and directions (any object can be represented in this way, as pointed out by Fourier). Each diffraction spot defines one such component, whose frequency and direction

is described by the direction of scatter of the diffracted beam and whose amplitude corresponds to the spot brightness. To put the waves together to form the image we need to know their relative phases (this is crucial – in-phase waves augment each other whereas out-of-phase waves interfere!). Unfortunately, when a diffracted beam hits a detector, only its energy is recorded and all phase information is lost. The crystallographer has to find another source of information to reconstruct these missing phases. Fortunately, there are now routine techniques which, for viruses, usually start from rough phase estimates derived from a three-dimensional model. The model might be a crystal structure of a related virus, a lower resolution cryo-EM reconstruction or perhaps a combination of electron microscopy and high resolution X-ray structures of individual protein components.

Symmetry saves the day

The phases depend on the orientation and position of the model with respect to the axes of the crystal. The icosahedral symmetry of the virus provides us with a key not only to unlock these secrets but also to refine our rough initial estimates of the phases. Icosahedral symmetry consists of a set of 5-, 3- and 2-fold symmetry axes arranged in an absolutely fixed relationship to each other. If we look along a 5-fold symmetry axis, the virus structure will repeat every 360/5 = 72° around the axis. The same is true of the scattering from the virus. It is a simple matter, in a computer, to search for such repeats in the measured scattering and thereby lock on to the orientation of the particle. Once correctly orientated the model is moved systematically around the repeating volume of the crystal to find the position that best predicts the observed scattering. Phases may now be calculated, combined with the measured spot amplitudes, and an electron density map calculated. This map will be biased towards our rough model of the structure, adding noise to the image. Again icosahedral symmetry helps us, since the repeating crystal lattice cannot build in the icosahedral 5-fold symmetry. Inescapably, therefore, the virus particle has additional internal symmetry. Since we have located these symmetry axes it is a simple matter to impose icosahedral symmetry to clean up the picture. In practice we use a cyclic procedure, using the cleaned up image to get better phase estimates, which are combined with experimental amplitude measurements to give a new, improved image which is then subjected to further sanitization. Fortunately computers are not easily bored and carry this process through many cycles to produce maps of great clarity.

Early achievements

The seeming complexity of virus capsids can mask their underlying simplicity. In 1956 Watson and Crick realised that 60 identical subunits could self-assemble to form a closed particle with icosahedral symmetry, thereby satisfying the biological imperative of genetic efficiency. Some years later Casper and Klug proposed that even more complex assemblies might be made by relaxing the requirement for exact symmetry (giving rise to the term 'quasi-equivalence'). This can be achieved by breaking the icosahedral building block down into a number (T) of sub-triangles, such that the shell is made up of $60T$ chemically identical units. This explains the architecture of many viruses but does not provide a mechanism by which the exact size of such massive assemblies is determined. This is exemplified by tomato bushy stunt virus (TBSV, the first virus solved), where the $T = 3$ arrangement of the protein building blocks is exactly as predicted by Casper and Klug but where the architecture is defined by a pathway of controlled conformational switching of the chemically identical subunits during assembly. This combination of quasi-equivalence and conformational switching has now been found in many virus structures. The TBSV structure also revealed a new type of protein fold, an elongated wedge made from long strands of β-structure, often called a jelly roll since its strands are wrapped up as if the chain had been formed in the same way as the American confection of that name. This structure has now become a virtual trademark of viruses, being found in the capsid proteins of an enormous range of viruses (although it is relatively scarce in the proteins of bacteria, plants and animals). The tempting (but unproven) inference is that this reveals unsuspected ancient links between plant and animal, RNA and DNA, and enveloped and non-enveloped viruses. Even if this is true it remains a complete mystery why this structural signature is maintained over such enormous evolutionary distance in very different structural contexts. Very different questions were answered when the first animal viruses were solved in the mid-80s; poliovirus by Jim Hogle and human rhino-virus by Michael Rossmann. These structures immediately led to a clear rationalization of many of the antigenic properties of animal viruses and to an apparent resolution of the conundrum of how viruses maintain binding sites for a cellular receptor whilst evading the immune response. The canyon hypothesis proposes that receptor binding residues are concealed from antibody molecules within a crevice on the viral surface and is still the subject of much discussion.

Moving up a gear: bluetongue virus

Most viruses analysed crystallographically have been around 30 nm in diameter. The publication last year of the structure of the 70 nm bluetongue virus (BTV) core particle (Fig. 2)

demonstrates that much larger assemblies can now be tackled. BTV is a representative dsRNA virus and belongs to the most populous family of such viruses, the *Reoviridae*. The cores of these viruses act as transcriptional machines in infected cells, holding their genome and transcriptional enzymes within a protective shell so that dsRNA is never revealed to the infected cell, eliminating any chance of a cellular response (such as the interferon response) to this unusual nucleic acid. The structure suggests a compelling hypothesis for the assembly of nearly 1000 protein subunits. The core is made from two principal protein components. There is a thin skin covering the genome and enzymes, made from 120 copies of a large protein VP3(T2), which assembles into an icosahedral arrangement via conformational switching to yield a pattern of subunits not seen in other viruses and not predicted by the theory of Casper and Klug. This is clothed in 780 copies of the protein VP7(T13) in an arrangement that follows the theory of quasi-equivalence with greater precision than seen in other virus structures. There is no need for conformational switching in this layer since the VP3(T2) subcore acts as a scaffold upon which the VP7(T13) layer assembles. Intriguingly there is evidence from low resolution EM structures of other dsRNA viruses that the peculiar arrangement of VP3(T2) may be conserved even beyond the family *Reoviridae*. It is tempting to speculate that this reflects some fundamental involvement in the unique biology of these viruses. In most crystallographic analyses of viral capsids there has been little trace of visible structure for the genome. This does not mean that such structure does not exist, merely that the

process of crystallization has laid down viruses in random orientations, blurring the image beyond recognition. The BTV core is very unusual in this respect and we can get a fair idea of how much of the genome (20,000 bp), made up of ten unequal segments, is arranged. Unfortunately inappropriate blurring of the image limits the detail and means that interpretations are speculative. Nevertheless, by taking the structure together with information from previous electron microscopical and biochemical analyses we can propose a working model in which the enzymes form transcription complexes under each of the 5-fold apices of the protein shell. These contain three components, which (working outwards from the centre) unwind the genomic dsRNA (a NTP-driven helicase), transcribe a messenger RNA sense copy and cap it before it emerges from the core, ready to initiate protein synthesis. We suspect that the ten RNA segments are neatly coiled around these transcription complexes, so that each segment is set up to run as an independent machine without tangling the enormously long pieces of dsRNA.

Crystallography of viruses has come a long way in the last 20 years. Nevertheless, the structural bases of many of the fundamental biological functions are still unsolved. These functions are often carried out by large multi-component protein complexes. Improvements in the molecular biology and purification techniques for these complexes, along with developments in synchrotron radiation, now provide a way to tackle these difficult problems. We expect crystallography to be in the vanguard of structural approaches to the major functional problems in microbiology.

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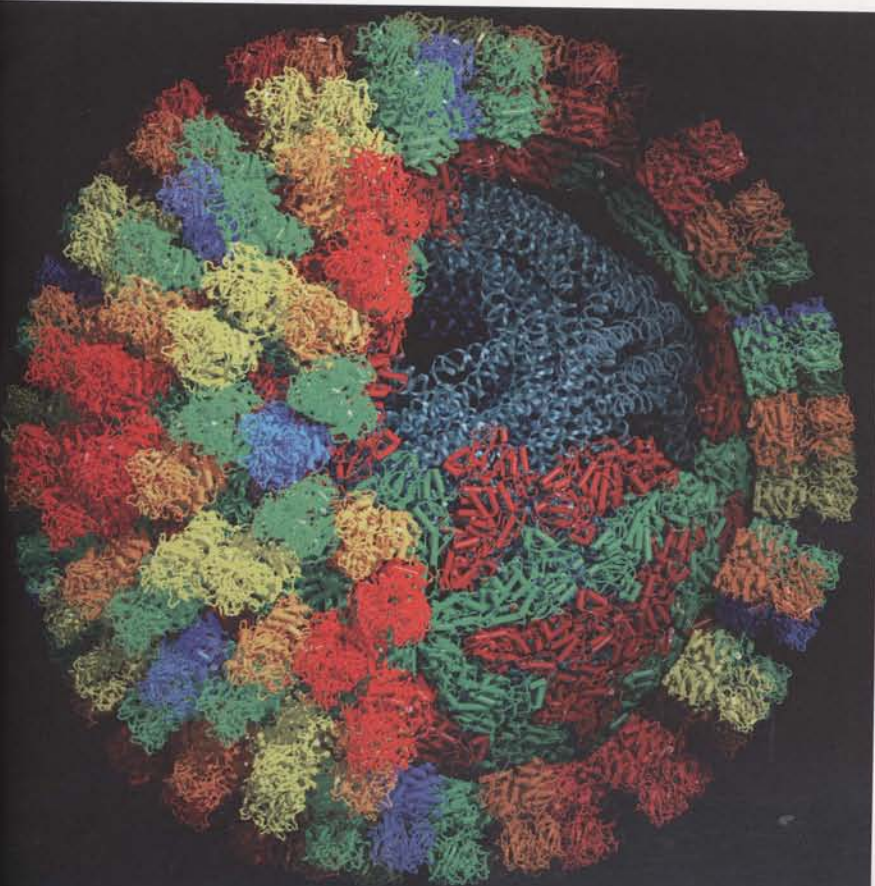
Nuclear magnetic resonance (NMR): keeping pace with microbiology

Stephen Matthews

A guide to NMR terminology

What is NMR?

Certain nuclei (e.g. ^1H, ^{13}C, ^{15}N) interact with high strength magnetic fields. These can be probed by irradiation with radio waves producing a spectrum containing a series of lines. The frequency of individual spectral lines is dependent on the chemical environment of the corresponding nuclei. Also, the relative three-dimensional location of nuclei can be measured using the nuclear Overhauser effect (NOE), enabling the structure to be deduced.

Nuclear Overhauser effect

An indirect way of measuring the interaction that occurs through space as the result of coupling between nuclear dipoles.

Liquid crystal NMR

A solution of disk-shaped micelles that induces molecular alignment of a macromolecule within the magnetic field and therefore creates directly measurable dipolar couplings.

Deuteration

The fractional or complete (perdeuteration) labelling of macromolecules with deuterons. Substitution of deuterons in place of protons facilitates simplification and improvement of NMR spectra.

TROSY

Transverse Relaxation Optimized Spectroscopy (TROSY) produces NMR spectra with significantly narrower line-widths than traditional experiments by the mutual cancellation of two relaxation mechanisms.

The primary biological application of nuclear magnetic resonance (NMR) is in the structural study of small macromolecules in solution. Although NMR can reveal high-resolution structures, it provides information complementary to both X-ray crystallography and electron microscopy (EM). NMR facilitates rapid generation of a three-dimensional map, delineating the protein fold in a progressive manner such that early data can be used to show clear functional implications. NMR can also be used to look at the dynamic processes involved in complex formation and folding. The main limitation of NMR is the size of the molecule under investigation; the larger it is the slower it tumbles in solution, which in turn increases the efficiency of the NMR relaxation process. This manifests itself as a severe broadening of the spectral lines that eventually degrades the resolution and efficiency of many NMR experiments. As a consequence an upper limit for molecular size has always been associated with NMR and has therefore precluded its use in studies of very large complexes.

Use with macromolecular complexes

Recently, the technique has entered a new period of accelerated growth, which should ensure that it remains a leading biophysical technique into the next millennium. The increased use of deuteration together with novel labelling strategies for proteins and nucleic acids, and the development of transverse relaxation optimized spectroscopy (TROSY) have all extended the applicability of NMR to much larger systems; molecular species in excess of 100 kDa are now tractable. Furthermore, liquid crystalline media together with the measurement of residual dipolar couplings enable structural information to be extracted accurately and easily without recourse to proton nuclear Overhauser effects (NOEs). NOEs allow the determination of interatomic distances that structures are traditionally modelled from but these are notoriously imprecise and often difficult to acquire comprehensively. NMR can now provide information on the structure of sizeable molecules and, perhaps more importantly, the intricate details of large macromolecular complexes are now attainable in solution. Although NMR does not compete with X-ray crystallography and EM for tackling huge structural problems, some major applications have become possible. In addition, NMR still retains its ability to generate structural data very quickly, which is often invaluable to the biochemist in the early design of new mutagenesis experiments or the search for target molecules.

Studies of enteropathogenic Escherichia coli

This new potential for NMR has recently been illustrated by the first structural study into the molecular basis of bacterium–host-cell interactions in enteropathogenic Escherichia coli (EPEC) infection. EPEC gives rise to persistent diarrhoea and is an important cause of mortality amongst infants in developing countries. These organisms,

together with the enterohaemorrhagic E. coli O157:H7 (EHEC), a cause of acute gastroenteritis, haemorrhagic colitis and haemolytic uraemic syndrome in humans, belong to a prevalent family of diarrhoeagenic enteric bacteria. EPEC and EHEC adhere tenaciously to enterocytes in the gut and induce the formation of classical sub-cellular structures known as attaching and effacing lesions. These E. coli strains produce a protein, known as TIR, which is transferred from bacterium to host cell, where it serves as a receptor for bacterial adhesion via the surface protein intimin. This unique mechanism was only recently reported and it implies a prominent role for intimin in disease progression. What is more, a 30·1 kDa fragment of intimin (Int280) plays a central role in attachment and lesion formation. Despite the clarity of the data presented on intimin–TIR interactions, several reproducible studies have emerged that suggest a more complex mechanism for EPEC/host-cell adhesion. These include the finding that intimin will adhere directly to mammalian cells in the absence of TIR.

Intimin structure

It seems remarkable that, in the light of the discovery of intimin about 10 years ago and the recent acceleration of research into EPEC pathogenesis, the wait for a detailed structural report has been so long. The very latest improvements have made proteins of up to 50 kDa routinely amenable to structural study by NMR and have accordingly opened the door for an NMR-based assault on the structure of Int280. The new strategy has proved particularly successful and has produced the first structural

$H_2O + {}^1H$
[^{13}C]Glucose
~0% D

$D_2O + {}^1H$
[^{13}C]Glucose
~86% D

$D_2O + D$
[^{13}C]Glucose
~97% D

^1H (p.p.m.)

D1

D2

D3

Putative Ig domains

EPEC membrane
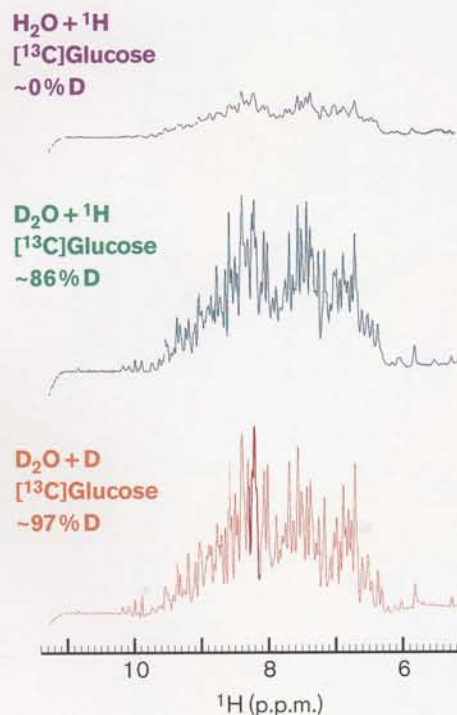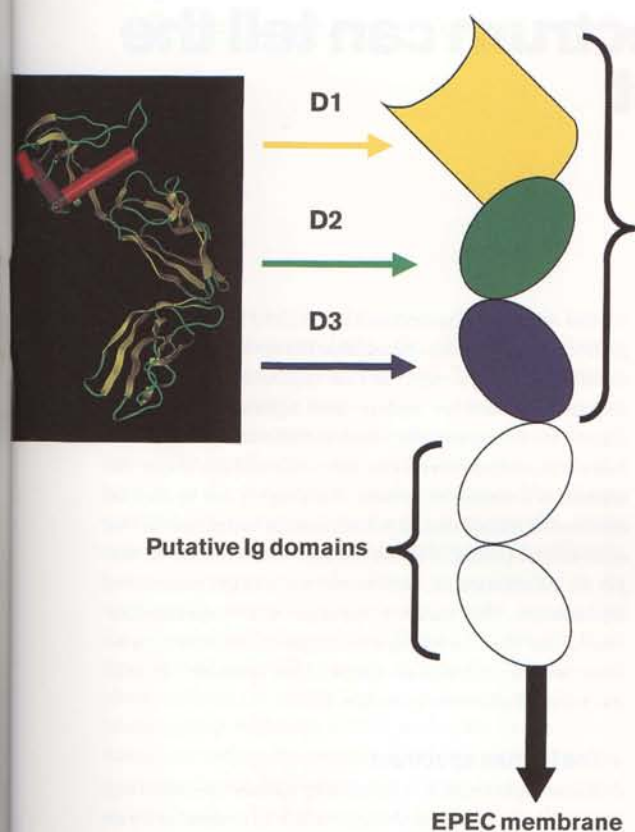
glimpses within 6 months of expressing the protein. This has allowed the microbiologists concerned to redirect their research efforts and make an impact from a biochemical perspective. Perdeuterated samples were produced, in which all the aliphatic protons were replaced by a deuteron and the amides were re-exchanged with protons. The standard NMR isotopes of nitrogen (^{15}N) and carbon (^{13}C) were also incorporated. The removal of the strong dipolar interaction involving protons drastically increases the short relaxation times normally associated with a molecule of this size. This improves both the spectral resolution and the sensitivity of many of the complex multidimensional, multiresonance NMR experiments that are necessary to complete a structural assignment. Uniformly deuterated ^{15}N/^{13}C-labelled material is readily prepared by growing bacteria containing the recombinant gene of choice in a defined medium that utilizes ^{15}NH$_4$Cl, [^{13}C]glucose and D$_2$O as isotope sources. If protonated glucose is used deuteration levels of up to 86% can be achieved, but to accomplish full deuteration, a deuterated carbon source is required. Fig. 1 shows the effect of deuteration on the one-dimensional ^{1}H NMR spectrum of Int280; the narrowing of the line widths and associated spectral improvement are clearly visible. Further perdeuterated samples, in which residues containing methyl groups are specifically protonated, afforded sufficient NOE distance restraints to facilitate the calculation of a global fold.

Fig. 2 shows a schematic representation of the tertiary and quaternary structure of intimin. Int280 is highly asymmetric, approximately 90 Å in length and built from three globular domains. The first two domains each comprise two β-sheet sandwiches that resemble the classic immunoglobulin superfamily (IgSF). Tandem repeats of IgSF domains are ubiquitous within cell-surface adhesion molecules and are responsible for a variety of molecular recognition processes. Sequence alignment indicates the presence of a third IgSF domain N-terminal to Int280

within full-length intimin and possibly a fourth. The IgSF domains in intimin form an extended, articulated linker that protrudes away from the bacterium surface and confers a highly accessible third domain for adhesion.

The topology of the third domain is reminiscent of the C-type lectins, a family of proteins responsible for cell-surface carbohydrate recognition that includes animal cell receptors and bacterial toxins. Although Int280 lacks calcium co-ordination, the similarity raises the possibility of carbohydrate recognition in the function of intimin and implies three models for intimin-mediated cell adhesion. Fig. 3 shows the three plausible models. In model A intimin binds a carbohydrate moiety that is attached to TIR. However, the rarity of bacterial glycosylation is inconsistent with this paradigm. Model B invokes a bipartite interaction, in which intimin interacts with TIR and a host-cell carbohydrate. Although studies showing that Int280 and fragments of Int280 will bind cultured epithelial cells in the absence of TIR add confusion to the intimin–TIR story, the data do provide circumstantial evidence to support this model. Despite this, we cannot rule out a final scenario, model C. Here the C-type lectin similarity is incidental and intimin interacts with TIR directly.

The structural similarities of Int280 with animal intercellular adhesion molecules are intriguing and provide remarkable insight into EPEC adhesion. This work is probably the first demonstration of the usefulness of combined perdeuteration/site-specific protonation and NMR spectroscopy strategy. A good molecular understanding of bacteria–cell interactions is crucial for targeted development of rational drugs and will have implications for the design of novel drug delivery systems.

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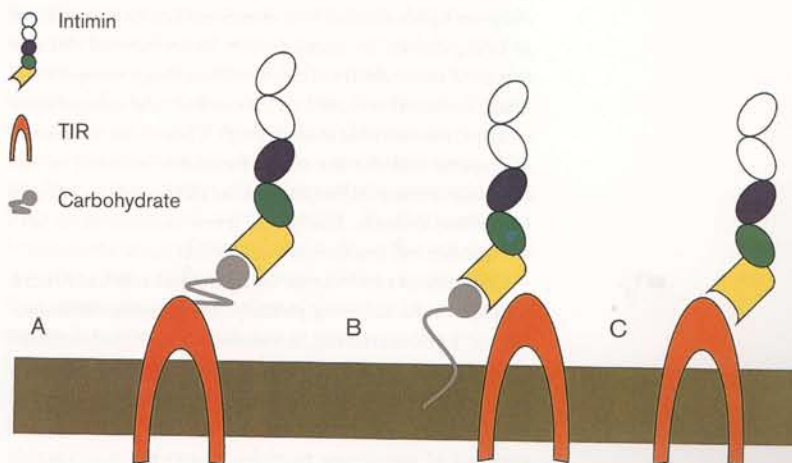
Intimin

TIR

Carbohydrate

A   B   C

What a Raman spectrum can tell the microbial ecologist

Nozomi Ytow

Microbial ecologists are mostly interested in the types of microbes present in communities and their contributions to ecosystem processes. It is, therefore, desirable to know which are the predominant species in a community, their abundance and their metabolic contributions. It is well known that most microbes in natural communities cannot be cultured in the laboratory, at least with current techniques, and this severely restricts identification of even predominant microbes (ordinary bacteria or archaea) because identification classically relies on responses to specific media and phenotypic expression under definite cultural conditions. Oligonucleotide molecular probes can help this situation, assuming that a microbial sample is available and we know what we should be looking for. They can also yield information on the abundance of microbes and, with some sophisticated treatment, can provide estimates of metabolic activity; hence their power as tools in microbial ecology.

Although molecular probing is a powerful and moderately straightforward technique, it still requires samples to be taken from the natural environment. Natural communities do not exist inside laboratory flasks but as components of ecosystems with spatio-temporal structures. The spatial and temporal structures of ecosystems play important roles in their dynamics because spatio-temporal heterogeneities can be driving forces for life processes, for example a redox gradient in soil is a driving force for the microbial soil ecosystem. Periodic material exchange by tidal action is an example of heterogeneity in time. These spatial gradients and temporal changes in environments can be generalized as spatio-temporal heterogeneities, especially when these two elements are conjugated in a single ecosystem. Upwelling in aquatic systems and hydrothermal vents are examples of such conjugated dynamic ecosystems. An understanding of the spatio-temporal effects on natural microbial dynamics requires continuous observation which cannot be accomplished by discretely sampling an ecosystem.

There are also cases in which sampling is difficult for technical reasons, such as in deep-sea hydrothermal vents. A deep-sea hydrothermal vent ecosystem can be summarized as being driven by nutrient-rich hydrothermal effluent supplied from small chimney orifices (high temperature black smokers) or from narrow cracks ('low' temperature effluent, referred to as shimmering). These sources are small and sparse and the use of submersibles (manned or unmanned) is essential for observation and for taking samples from these systems. This places severe constraints on both the number and amounts of samples that can be recovered.

Continuous observation using optical methods is one solution to the sampling problem. Measurement of turbidity, i.e. light-scattering, is commonly used to determine cell density in the laboratory, where it gives good results for pure cultures. It cannot, however, be applied to natural communities for two fundamental reasons. First, the presence of non-living particles creates an unacceptable level of noise in the system and second, the heterogeneity of microbial communities means that the optical contribution of each cell is not unique, so that calibration is effectively impossible. Another widely used optical technique is fluorescence measurement, often in combination with laser-based cytometry, which can give information about the amount of fluorophore present. Although it can be used for continuous measurement, it has a similar restriction to that of molecular probes; if we are not sure what is there, we may get no information or, even worse, we may get misleading information. We need a method with intermediate 'resolution' that can tell us which types of molecules, rather than which individual species, are present. Raman spectroscopy allows us to do this.

The Raman spectrum

A Raman spectrum is a set of very narrow spectral lines emitted from object molecules when illuminated by an incident light. The width of each spectral line is strongly affected by the spectral width of the incident light and hence tightly monochromatic light sources, such as lasers, are used. The wavelength of each Raman line is expressed as a wavenumber-shift from the incident light, which is the difference between the inverse wavelength of the Raman line and the incident light. The wavenumber-shift, not the absolute wavelength, of the Raman lines is specific to particular atomic groups in molecules.

Raman spectra measure the vibration states of molecules which are determined by their molecular structure, especially by atomic groups such as methylene, ethylene, amide, phosphate or sulphide. Most applications of Raman spectroscopy in biology are concerned with change in vibration states of macromolecules or related small molecules. Changes in either the wavenumber-shift of single Raman lines or the relative intensities of two or more Raman lines in an atomic group have been interpreted as indicating conformational changes in macromolecules. For these reasons Raman spectroscopy is mainly used for qualitative studies of molecules and molecular dynamics in biology. For easier and clearer interpretation of Raman spectra, use of the technique has been restricted mainly to purified materials and their systems, such as enzyme reactions. However, because Raman spectra are based on the specific vibrations of atomic groups we can also use them to characterize and quantify a mixture of molecules as compositions of atomic groups by a method akin to fingerprinting. Although unable to resolve the composition of a sample in terms of a list of chemical compounds, it does give a rough sketch of the molecular composition of the natural environment and how it changes with time.

Resonance Raman scattering

The Raman effect is an induced emission of light and its efficiency (intensity for the same incident intensity) depends on the energy interactions between photons and molecular groups. When object molecules have

Resonance Raman

Non-resonance Raman

Absorption

Fluorescence

chromophores with an absorption wavelength nearly matching the incident light, the efficiency of Raman scattering is considerably increased. This enhancement phenomenon, known as resonance Raman scattering, is different from non-resonance Raman scattering. Although they differ in efficiency and application, they share the same principle of non-elastic light scattering. Non-elastic scattering is a process involving energy transfer, hence the energy of incident and scattered photons is different. Since the energy of each photon is equivalent to its wavelength, non-elastic scattering can be detected as the emission of light with a different wavelength from the incident light. The energy difference of the emitted photon reflects the energy level in molecules, which is the atomic group vibration within molecules in the case of Raman scattering. By contrast, for elastic light scattering it is momentum, not energy, transferred between photon and molecule, which results in a change in the direction of the photon, i.e. reflection and refraction. This can be used to provide information such as the shape of whole objects as a result of the phase interference between scattered photons.

Resonance Raman scattering may sound like fluorescence, but it is a completely different process. Fluorescent light is generated after absorption and re-emission of a photon by the chromophore, but resonance Raman emission is induced without the absorption of a photon. They can be practically distinguished; when the wavelength of incident light is changed within a comparatively wide tolerance fluorescence spectra are not affected. The wavelength (i.e. absolute wavenumber) of a Raman line, on the other hand, varies with that of the incident light to keep the Raman shift constant. Also Raman scattering induced by a laser light gives much narrower spectral lines compared with the broad spectrum of fluorescence; the former is of the order of spectral band width of the incident light (commonly less than 1 nm with current lasers), but the latter is 10 nm or more and is not affected by the band width of the incident light. Using these differences we can distinguish, for example, between carotenoids and chlorophyll in phytoplankton cells *in vivo*, without any chemical treatment. Carotenoids can be detected as spiky Raman lines beside the broader fluorescence from chlorophylls. This is an unexpected result, considering the relative abundance of chlorophyll and carotenoids in phytoplankton cells. Either the energy transfer between chromophores or the intracellular structure in which these chromophores exist must suppress fluorescence from chlorophyll. Indeed the chlorophyll fluorescence of solvent extracts from phytoplankton cells makes detection of the carotenoid Raman band difficult. Thus, resonance Raman spectroscopy enables such 'optical' extraction of molecules. The choice of incident laser wavelength determines which molecules will be excited, and hence we can differentiate molecules of interest.
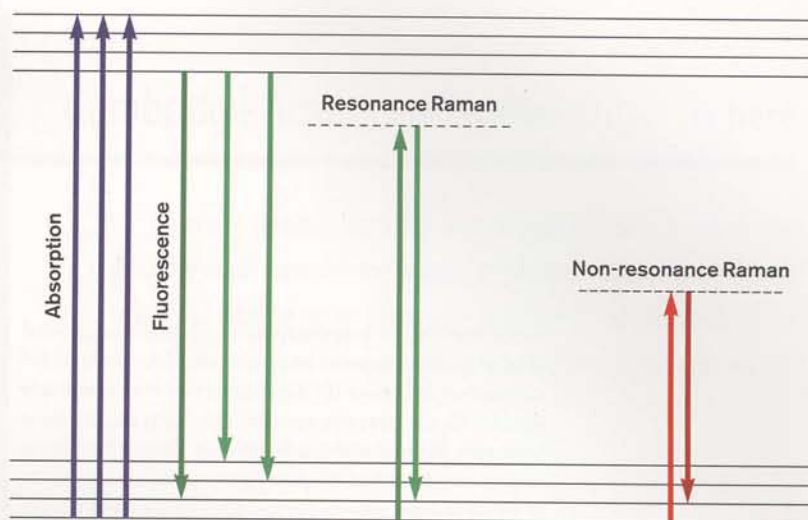
● Non-resonance Raman scattering

Similar Raman scattering also occurs even without resonance or absorption and is called, not surprisingly, 'non-resonance Raman scattering'. By contrast to resonance Raman scattering, which is only observed in molecules which resonate with the incident light, non-resonance Raman scattering occurs with all molecules, except in cases where Raman emission is prohibited by a physical law, the so-called 'selection rule', which relates to the shape of molecules determining their vibrational modes. This non-specificity means that non-resonance Raman spectroscopy can be used as a general tool to characterize and to quantify organic matter in the natural environment. All natural organic molecules contain either methyl or ethyl groups, so estimation of the concentration of these groups gives a measure of the organic carbon concentration. Simultaneous recording of Raman bands corresponding to methyl/ethyl, amide and phosphate groups provides a fingerprint of the organic molecules present. The generality of non-resonance Raman scattering means that it can provide a simultaneous record of a wide range of molecules. When we find a spectral pattern indicating the existence of a molecule of interest, we can then apply more specific methods to obtain greater detail. This is perhaps the greatest potential that the technique has to offer microbial ecology. It also provides the opportunity for a continuous monitoring method to give a spatio-temporally continuous view, which is highly desirable for understanding the nature of microbial ecosystems.

● Incident light

Resonance and non-resonance Raman scattering differ not only in specificity but also in sensitivity. Application of non-resonance Raman scattering is limited by the far weaker intensity of scattered light which arises from the nature of Raman scattering. Unlike absorption, Raman emission is the result of an interaction between an incident photon and the molecule, which is a much rarer event. This results in a much lower intensity with the same intensity (flux of photons) of incident light. Furthermore, the fingerprint region of organic molecules in Raman spectra is rather close to the wavelength of the incident light. Conventionally, tandemly aligned double or triple spectrometers have been used as filters to eliminate stray incident light. Sensitive detectors and long exposures are necessary, not only because of the weakness of Raman emission but also due to optical losses in tandem aligned multiple

spectrometers. For longer exposures, thermal background noise of the detector must be reduced and electrically cooled charge-coupled device (CCD) detectors are now commonly used for Raman spectroscopy. Incident light sources must have very narrow bandwidths because Raman lines carry over the bandwidth of incident light, and a broad spectrum of incident light reduces the spectral resolution of Raman spectra. The light source also must have high intensity and be well focused to optimize efficiency of the optical systems. For these reasons, lasers are commonly used as light sources in modern Raman spectroscopy.

Field applications

Raman spectroscopy has been largely used in laboratories with a specific interest in molecular vibration, so high-resolution spectrophotometers have normally been used. Spectrophotometers with longer optical paths have been preferred because these enable better spectral resolution. A longer optical path, however, results in greater optical loss in the system so demands longer exposure and a more intense light source, which again means a laser source. These problems have made the application of Raman spectroscopy in field research difficult. Smaller optics are preferable for field use not only for portability but also because they are brighter, enabling shorter exposure times (i.e. more data), and consume less power (the light source and the CCD cooler are the main power consumers in Raman spectroscopy). Field applications sometimes do not require the high spectral resolution demanded by the specialist laboratory and hence we can reduce the size of the optical system by restricting the spectral resolution within an acceptable range. Nowadays improved optical elements are commercially available, although some of them are restricted for military use, which permit the field application of Raman spectroscopy. Higher sensitivity resonance Raman spectroscopy would also expand the area of application by facilitating the detection at lower concentrations of the object molecules with lower power incident light and with shorter exposures to improve the time resolution of the process. Combination of resonance Raman spectroscopy and fluorometry would give another approach to quantifying biological pigments.

Conclusions

Raman spectroscopy has emerged from the physics world and has been applied in specialized biophysical laboratories as a technology to investigate molecular dynamics. Improvements in each component technology are creating an opportunity for wider application, including microbial ecology. Raman spectroscopy is now gaining the ability to be used for general field observations.

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Further reading

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Microbial Signalling and Communication
SGM Symposium Volume 57

Until quite recently, microbial cell–cell communication mediated by diffusible signal molecules was considered a curiosity and studied by few researchers. Certainly, signals such as A factor in *Streptomyces griseus*, cAMP in *Dictyostelium discoideum*, and the autoinducer N-(3-oxohexanoyl)homoserine lactone in *Vibrio fischeri* had attracted attention because of their spectacular effects on the producer organisms, but only in the last few years has it become clear that diffusible signals have a vital role in the development of many different micro-organisms. This Symposium volume is a tribute to a rapidly expanding area of research and brings together contributions describing cell–cell communication in major groups of prokaryotic and eukaryotic micro-organisms. The book also highlights the importance of signalling in certain microbe–plant and microbe–animal interactions – here, obviously, a choice of some well-documented examples had to be made. The chapters on bacterial signalling provide a useful and comprehensive overview on lactone and peptide signal molecules, and all groups of bacteria for which signalling mechanisms have been well characterized are adequately covered.

Book chapters offer their authors an opportunity to put research results into a broader perspective and to discuss unsolved problems. Several contributors to this volume have taken advantage of this opportunity. For instance, Kaprelyants *et al.* discuss the controversy about the 'viable but non-culturable' phenomenon in the context of bacterial cytokines and make a plea for clear operational definitions. By contrast, the apparent paradox that N-acylhomoserine lactone autoinducers cannot be indefinitely what their name suggests – because at some stage autoinduction must be terminated – does not seem to provoke much discussion. Fungal pheromone communication is represented by a chapter on *Schizosaccharomyces pombe*; perhaps a brief description of mating type signalling in *Saccharomyes cerevisiae* would have been a useful addition. In filamentous fungi, a multitude of morphologically defined phenomena contrasts with a scarcity of molecular data on signalling: lots to explore!

With its extensive bibliography and description of research in progress, this book is a highly useful and timely source of references.

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Bacterial classification and taxonomy: a 'primer' for the new millennium

Howard Gest

The notion that a single molecular marker, 16S rRNA sequence, can serve to decipher the evolutionary phylogeny of bacteria has apparently led to significant effects on the minds of many investigators. These effects include: (a) a general amnesia about the long history of thought and debate on the classification/taxonomy of bacteria, (b) some confusion about the distinction between actual bacteria and what can be called 'virtual computer bacteroids', (c) a mental void on the long, rich and important history of research into microbial nutrition, which led to the elucidation of many basic principles of biology and cell biochemistry, (d) belief in new myths such as the recently trumpeted 'discovery' of a great diversity of free-living, 'uncultivable' microbes as indicated by molecular biological probes, and (e) implantation of the misconception that the classification of bacteria should be largely, if not entirely, based on evolutionary phylogeny.

The immediate inspiration for this article was a sentence used by Sydney Brenner (1997) in an imaginary letter sent to a mythical nephew, namely *"I can't see the wood for the phylogenetic trees"*. To help see the 'wood', I have selected items from the extensive research literature that illustrate the problems encountered in the historical development of bacterial classification and taxonomy schemes. This 'primer' is offered as an elementary guide, as we enter the next century, to the somewhat chaotic status of this important aspect of microbiology.

The situation in 1946 as viewed by C.B. van Niel

In 1946, the great microbiologist C.B. van Niel published a thoughtful essay on *'The classification and natural relationships of bacteria'* in which he reviewed the history of earlier work aimed, in part, at developing a stable and generally accepted nomenclature that would eliminate duplication or multiplication of names for the same organism. He emphasized that even if we knew the phylogenetic relations among bacteria, a classification based on such relations would not necessarily be the best or most efficient for determinative purposes.

Changing names of bacteria

Although determinative keys are very important in practical matters (for example in medical microbiology, public health microbiology and plant pathology), this tends to be forgotten by those probing evolutionary relations using molecular markers. The latter press for revised taxonomic schemes and this inevitably leads to proposals for changing names of bacteria.

Particularly egregious instances of name changing have afflicted the anoxygenic photosynthetic bacteria group. *Example* – The purple photosynthetic bacterium *Rhodocystis gelatinosa* isolated in 1907 was renamed *Rhodopseudomonas gelatinosa* in 1944, redesignated *Rhodocyclus gelatinosus* in 1984 and in 1991 the name *Rubrivivax gelatinosus* was proposed. The bacterium does

indeed hydrolyse gelatin, but these reincarnations do not reflect appreciable advances in an understanding of its basic features or evolutionary relationships. This example indicates how confusion is introduced into the research literature, text books and computer information retrieval. It is already happening.

Genus and species

How are bacterial genera and species defined? An authority on this matter is *Bergey's Manual of Systematic Bacteriology*. The definitions are, in fact, a bit fuzzy, especially that of genus. From the *Manual*:

Genus: *"The bacterial genus is usually a well-defined group that is clearly separated from other genera, and the thorough descriptions of genera in the 1984 edition of Bergey's Manual exemplify the depth to which this taxonomic group is usually known. However, there is so far no general agreement on the definition of a genus in bacterial taxonomy, and considerable subjectivity is involved at the genus level. Indeed, what is perceived to be a genus by one person may be perceived as being merely a species by another systematist."*

Species: *"A bacterial species may be regarded as a collection of strains that share many features in common and differ considerably from other strains…One strain of a species is designated as the type strain; this strain serves as the name-bearer strain of the species and is the permanent example of the species, i.e. the reference specimen for the name. The type strain has great importance for classification at the species level, because a species consists of the type strain and all other strains that are considered to be sufficiently similar to it as to warrant inclusion with it in the species…"*

Numerical taxonomy

In 1963, Sokal and Sneath described an application of the Adamsonian approach to taxonomy in which as many diagnostic characteristics as possible are used and given equal weight. The degree of relationship between organisms was considered to be a function of the number of similar characteristics and is expressed as a 'similarity coefficient.' The utility of numerical taxonomy has been demonstrated in a number of studies. Sneath reviewed the 30-year history of numerical taxonomy in 1995 and concluded that *"Numerical taxonomy in the broad sense is the greatest advance in systematics since Darwin or perhaps Linnaeus. It has stimulated several new areas of growth, including numerical phylogenetics, molecular taxonomy, morphometrics and numerical identification."* Even though numerical taxonomy does not assume phylogenetic relationships, it is obvious that close correspondence of a large number of phenotypic characteristics has something to say about genetic connections.

Cowan's comments on taxonomy (1970)

"A hitherto undetected similarity exists between Lewis Carroll's Alice and taxonomists, and bacterial taxonomists in particular…taxonomy can – and does – drive taxonomists to a topsy-turvy Wonderland…" [Author's note: this paper was

The Pansy   The Chim-pansy

How To Tell The Birds From The Flowers And Other Woodcuts

Verses And Illustrations By Robert Williams Wood

based on a seminar *Alice in Taxonomyland* at the University of Maryland, 5 May 1969.}... *"It is surprising how many so-called microbiologists look upon the schemes published in Bergey's Manual (1923–1957) as if they were not only useful general classifications of bacteria, but ones that have received universal approval, both on earth and in heaven. I am assured by my colleagues that approval of Bergey's Manual is not universal, even on earth; I am not yet able to judge its reception in heaven..."*

Cowan pointed out that elaborate rules have been stipulated in codes of nomenclature in the attempt to regulate the formation and use of names, *"but these codes would delight the hearts of lawyers because they are too detailed and try to cater for all eventualities. In the event, they are confusing and self-contradicting..."*

"The Bacteriological Code {i.e., International Code of Nomenclature of Bacteria} should be simplified by deleting the Rules and Recommendations. It should consist of Principles, and discretion should be given to bacteriologists to apply them intelligently." The magnitude of the problem that Cowan discussed is indicated by List no. 22 published in the *International Journal of Systematic Bacteriology* (36, 1986), which contains 44 new names, new combinations, synonyms or revived names.

Example of a rule from the Code

"Rule 56b. A conserved name (nomen conservandum) is a name which must be used instead of all earlier synonyms and homonyms. Note 1. A conserved name (nomen conservandum) is conserved against all other names for the taxon, whether these are cited in the corresponding list of rejected names or not, so long as the taxon concerned is not united with another taxon bearing a legitimate name. In the event of union or reunion with another taxon, the earlier of the two competing names is adopted in accordance with Rules 23a,b."

The intent of this rule, to stabilize nomenclature, is excellent and is quoted here only to illustrate the quasi-legal (sometimes confusing) language of the Code.

Sneath's 1989 assessment of the utility of molecular sequence markers in classification and taxonomy

"A major difficulty with currently available molecular sequences is the fact that they are only samples of relatively small size. Sampling error will therefore continue to dominate the picture...

One area that is now being explored is the use of molecular 'signatures', – small sub-sequences that are characteristic of various bacterial groupings. Their value will depend considerably on how constant these signatures are within the newly defined bacterial taxa. At present there has been no detailed analysis of this, though some within-taxon variation is evident...

Another more pragmatic question is how far we can safely revise bacterial classifications on present sequence data. In many of the earlier papers on rRNA, strain numbers were seldom given, so we do not know which were type strains. Most workers examined only a single strain of a taxon such as a genus, yet the analyses were displayed as representative of the entire taxon – sometimes when the

validity of adjacent taxa was being questioned. Many genera are heterogeneous from the molecular viewpoint and this would imply that all their species must be studied before deciding on the new disposition of the genus. This still leaves unanswered the question of how homogeneous the species are."

More on molecular data

In 1994, Murray and Schleifer pointed out that the international nomenclature code *"is not able to provide sensible regulation of nomenclature for new taxa defined by very limited data, such as a nucleotide sequence for a small portion of the genome. The constructors of the original code (1957) and the Judicial Commission considering the 1976 and 1990 revisions did not foresee or act upon the possibilities for molecular description and typification of prokaryotes that were not yet cultivable. As a result, formal names are being proposed for uncultivated prokaryotes whose uniqueness is defined only by very limited characteristics, such as differences in a molecular sequence...*

A novel sequence isolated from nature merely indicates that there may be a unique organism in the environment. However, one has to take into consideration that sometimes sequencing and/or amplification errors simulate a novel sequence, suggesting some intraspecies diversity, or that formations of chimeric sequences may be possible. Moreover, the possibility that the sequence was retrieved from cell-free (naked) DNA cannot be excluded, and it may have originated from an organism not originally growing in the examined habitat..."

In fact, it was recently demonstrated in an 'environmental ecology' study, that RNA sequence data supposedly indicating the presence of certain bacteria in natural samples were due to contaminating 16S rDNA introduced in the exquisitely sensitive polymerase chain reaction.

Concluding remarks

The assumption that our current knowledge is sufficient to warrant new, presumably 'final', names for many genera and species is obviously questionable and arrogant, and evokes Cowan's admonition: *"It is often easier to create a new genus or species than to do the comparative work necessary to put an organism into its rightful place in an existing genus or species. The temptation to designate a new genus or species should be resisted."* Obviously, as knowledge advanced, numerous name changes were proposed and many were reasonable and useful, for example the change from *Streptococcus lactis* to *Lactococcus lactis*. During the past two decades, however, the notion that a single molecular marker can accurately reveal evolutionary phylogeny has driven a deluge of premature name changes. Since recent research suggests that microbial evolution was far more complex than commonly supposed, and probably involved extensive lateral gene transfer, conservatism in nomenclature changes seems well advised.

Carl Linnaeus originated the systematic classification of plants and animals. In 1737, before microbiology became a science, he said *"What difficulty has been caused to botanists from the revival of the sciences down to the present day by the invention of new names is known to everyone who has handled the subject."* Alas,

The Hen. The Lichen.

Lichens, regardless of conventions,
Exist in only two dimensions,
A life restricted to a plane,
On rocks and stones a greenish stain,
They live upon the simplest fare,
A drop of dew, a breath of air.
Contrast them with the greedy Hen,
And her most careless regimen,
She shuns the barren stones and rocks,
And thrives upon the garbage box.

In 1949, while still a graduate student, Professor Gest made the unexpected discovery that anoxygenic photosynthetic bacteria fix molecular nitrogen. He and his colleagues have isolated and characterized a number of new species of purple and green anoxyphototrophs, including the remarkable bacterium *Rhodospirillum centenum*. The latter was isolated in 1987 and given the species name in commemoration of the fact that it was discovered 100 years after the first isolation of a photosynthetic bacterium in pure culture (*Rhodospirillum rubrum*). When grown on agar, *R. centenum* cells produce numerous lateral flagella and the swarming colonies exhibit phototaxis (the first known example in such organisms). In conversation, Professor Gest refers to over-zealous bacterial taxonomists who would like to change the name of *R. centenum* as 'taxonomic pirates'.

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microbiology today is faced with this old problem. Santayana's 1905 observation is timely: "*Those who cannot remember the past are condemned to repeat it.*"

There is every reason to believe that as current genome sequencing projects mature and expand, we will be in a much better position to evaluate organismal relationships and deal with classification/taxonomy schemes more effectively. In the meantime, readers are referred to a very useful summary by R.G.E. Murray (1998) of current problems and procedures in dealing with taxonomy and nomenclature of bacteria.

Attention is also directed to a thoughtful review by Palleroni (1997) that discusses the limitations in using only molecular data for analysis of prokaryote systematics. On the brink of the new millenium, the significance of 16S rRNA as a major taxonomic criterion is receding. At the same time, recognition of lateral (horizontal) gene transfer as an important element in prokaryote evolution is gaining momentum (Lake *et al.*, 1999).

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2nd Workshop in Molecular Biology

28 December 1998–9 January 1999

University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam

■ **Robert E. Glass**

This two week course consisted of both practicals and lectures. It was delivered in English, with dual translations. Seven people from the UK and one person from The Netherlands participated. The UK co-ordinator was Dr Simon Cutting (School of Biological Sciences, Royal Holloway University of London) and Dr Pham Hung Van served as the Vietnam co-ordinator.

The course aimed to introduce molecular biology and its associated techniques to Vietnam, which is a developing country that contains a sound academic infrastructure established by the French (e.g. three Pasteur Institutes). Although Vietnam has a good background in the natural sciences and immunology, it has little understanding of molecular biology and how this can be applied to research and development.

The 43 enrolled students came from all over Vietnam (in reality many more people attended the lectures than just those who were enrolled). The enrolled students sat and passed an exam set by the organizers and were awarded a certificate indicating their successful attendance on the course. Each was given an extensive course manual containing details of all the practical classes and handouts for all the lectures.

During my week on the course I gave six 1·5 hour lectures on gene expression and its regulation. I also attended several of the practical classes and helped with these as well as dealing with questions arising from both the practicals and lectures.

Lecture topics

- Overview of gene expression and its regulation
- DNA transcription
- RNA translation
- Gene regulation

The students were a fantastic bunch of people. I found them incredibly keen to understand and learn molecular biology. Once the initial shyness of meeting a new person had worn off, they were more than ready to come up and ask questions after the lectures and during the practical classes. I really felt that the students got a lot from our presence, both in terms of the content of the course and the chance to speak scientific English.

Conclusions

I felt that the course was very worthwhile. It was clear that the students were learning a considerable body of molecular biology from us. This was indicated by the level of the questions as the course progressed and their performance in the written test. Since the students came from all over Vietnam, they will be able to take this knowledge back to a number of different departments.

● *Professor Robert E. Glass works in the Institute of Genetics, School of Clinical Laboratory Sciences, QMC, Clifton Boulevard, Nottingham NG7 2UH*
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International Development Fund reports

Through its International Development Fund, SGM is active in disseminating information about microbiology to scientists in developing countries. Here recent recipients of awards describe how Society sponsorship has been used to advance the knowledge of microbiologists in South East Asia and to sponsor scientists from other parts of the world to attend an international conference in the UK.

3rd International Conference on Anthrax

7–10 September 1998, University of Plymouth

■ Les Baillie

The conference attracted approximately 170 delegates representing all four corners of the world with the notable exception of Antarctica; apparently penguins do not catch anthrax. Through the generous support of the Society for General Microbiology we were able to sponsor the attendance of scientists from such diverse locations as Cuba, India, Russia, Turkey and Columbia.

Although it was a single subject conference, the presentations filled three full days and were divided into six main themes: Natural ecology and global incidence, Detection, Identification and classification, Structure and function, Molecular biology, Pathogenesis and vaccines. In all 36 oral papers and 54 posters were presented.

In support of the scientific programme there were a number of social events, notably a visit to the National Marine Aquarium, and the conference dinner at which delegates were encouraged to meet, discuss their work and make new contacts in spite of the best efforts of an excellent jazz band. These informal opportunities were of particular value to workers new to the field who had the chance to speak to many of the 'old hands'.

The stated aims of the conference were:

● to bring together the key workers in the field of anthrax research to review progress to date, and

● to create a critical mass which will lead to the formation of new networks, the exchange of ideas and stimulate new avenues of research.

In the light of the feedback that the organizers received during and since the conference, it was felt that these aims were achieved.

For those who were unable to attend it may be of interest that all the presentations were recorded on video, copies of which will be made available. In addition, the organizing committee intends to publish the proceedings.

For further details of the conference please contact me.

● *Dr Les Baillie was the Conference Chairman. He is based at DERA, Building 384, CBD Porton Down, Salisbury SP4 0JQ*
Tel. 01980 613881

Training Course on SRSV Virology

11–24 November 1998, Beijing

■ Binlei Liu

In November 1998 I conducted a two-week training course in Beijing, China. The course was jointly organized by the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing and the Department of Molecular Microbiology, University of Southampton. It included the introduction of recent advances in studies on small round structured viruses (SRSVs, or Norwalk-like viruses) and the application of the immunodetection (ELISA) techniques for SRSVs in China.

SRSVs cause acute, explosive diarrhoea and/or vomiting, and have a high associated infectivity, giving rise to rapid secondary spread. Food and water are major sources of SRSV transmission. SRSVs have a worldwide distribution and outbreaks of acute gastroenteritis have been reported in many countries. Although China has the largest population in the world, no SRSV outbreak has ever been reported and investigations of SRSV epidemiology and seroepidemiology have not been conducted there.

There were 14 participants, including staff, Masters and PhD students selected from the Institute of Nutrition and Food Hygiene. Professor Xiumei Liu, Director of the Institute, delivered an opening speech on the importance of study on SRSVs in China. I gave lectures on the molecular biology of SRSV and its detection using RT-PCR and ELISA. The participants performed ELISA experiments following my demonstration of the technique.

Traditionally, SRSV detection has been performed by electron microscopy. However, such expensive equipment demands the dedication of a skilled operator and unfortunately this is not available for routine diagnostic virology in developing countries such as China. ELISA offers a cheap alternative technology.

SRSVs are the most common group of viruses involved in food-borne contamination The development of ELISA technology for these viruses is a timely and important step forward that is particularly relevant to the interests of the Institute of Nutrition and Food Hygiene.

● *Dr Binlei Liu is currently a Career-Track Postdoctoral Fellow at the Department of Molecular Microbiology, Medical School, University of Southampton, SO16 6YD Tel. 01703 494941; Fax 01703 774316 e-mail bl@soton.ac.uk*

ABOVE:
Participants on the SRSV Virology Training Course held in Beijing, China.

BELOW:
Dr Binlei Liu giving a lecture.

Meeting preview
Delivering the goods
Bruce Ward

A preview of the topics to be discussed at the SGM Main Symposium *How Do Molecules Cross Microbial Membranes?* at the University of Leeds, 7–8 September 1999

Transport is an essential component of our lives. Yet cells display a dexterity in speed of conveyance, complexity of the variety of molecules carried and specificity of carriers that makes the traffic problems of the M25 look trivial. A major problem in both cases is getting to the right place at the right time.

In eukaryotes, some proteins are destined for particular organelles (mitochondria, chloroplasts, peroxisomes, lysosomes or the nucleus), some of which have multiple compartments. Others are retained within the general secretory pathway (endoplasmic reticulum and Golgi), exported to the plasma membrane or secreted from the cell. Even for the relatively simple prokaryotic cells, the problem of delivering molecules to the right place at the right time is not trivial. In a Gram-negative bacterium, macromolecules may be destined for the inner membrane, periplasm, outer membrane or the external medium.

No previous SGM symposium has focused entirely on microbial transport, although individual papers on transport of small molecules (late 1970s), protein secretion mechanisms (1989–1991) and, more recently, protein transport in relation to host infection (1993–1997) have been written for SGM symposia. The wealth of molecular information on transport systems has led to an improved understanding of the shared features of transport in prokaryotic and eukaryotic cells and the complexity of machinery required to ensure the safe delivery of molecules to the correct location in an active state. Some of the many current interests in microbial transport are: the nature of transmembrane channels and the regulation of their gating; the structure and mechanisms of protective chaperone proteins; and the mechanisms for ensuring correct targeting, modulating protein–protein interactions and controlling ligand specificity. Hence a symposium on transport of molecules across biological membranes seems timely.

Prokaryotic protein secretion

Bacterial membranes provide essential barriers between the inside of the cell and the external medium. Yet protein secretion is essential for secretion of virulence factors and extracellular enzymes, and also for the assembly of cell appendages like pili and flagella. Other proteins have to be integrated into the inner and outer membranes or exported to the periplasm. Gram-negative bacteria have evolved a number of systems for coping with the problem of traversing two rather different lipid membranes (the inner proton-impermeable membrane is composed primarily of phospholipid and proteins while the outer membrane contains lipopolysaccharide as a major component but relatively few proteins and has channels, called porins, that facilitate entry or exit of small molecules). The SecYEG translocase of *Escherichia coli* plays a vital role in transporting proteins into and across the inner membrane. Its eukaryotic counterpart is the Sec61 complex for protein translocation into and across the endoplasmic reticulum (ER) membrane to the ER lumen. The hydrophobic N-terminal signal peptide on the exoprotein is recognized by the Sec system. In many Gram-negative bacteria, Sec-dependent translocation serves to present a subset of proteins to the Type II secretion machinery, which is then responsible for the second (terminal) transport step across the outer bacterial membrane. Type II secretion requires some 12–16 proteins. Filloux will discuss the composition of the proteins in the Type II secretion system and speculates on how the 'patch' secretion signals of exoproteins are recognized by the machinery. Proteins can be delivered to the SecYEG translocase either via chaperones, most notably via the export-dedicated chaperone, SecB, or through their interaction with a signal recognition particle (SRP). In eukaryotes a cytosolic SRP binds the hydrophobic signal peptides of presecretory proteins as they emerge from the ribosome and delivers the nascent polypeptides to the translocase at the ER membrane. In their review High and colleagues will discuss the evidence for a bona fide SRP-dependent targeting pathway in *E. coli*.

By contrast Type I and Type III systems involve one-step mechanisms for protein translocation. Type III systems are found in *Shigella*, *Salmonella*, certain *E. coli* strains, *Yersinia* and plant-pathogenic *Pseudomonas*, *Xanthomonas* and *Erwinia* spp. These systems comprise a sizeable set of proteins capable of delivering a number of virulence factors into mammalian or plant host cells. Schneewind will review the Type III secretion machinery of the pathogenic yersiniae. Some of their proteins are directly injected into the cytoplasm of the eukaryotic host cell (so have to cross three membranes), whereas others are secreted into the surrounding medium, or remain bound to the surface of the bacterium. At least three of them require dedicated chaperones for successful targeting. Type I systems utilize a periplasmic ABC transporter (ATP-binding cassette) of which the α-haemolysin of *E. coli* is the best known example. Type I systems generally secrete only one or a few exoproteins, and these are recognized as substrates by the ABC protein exporter, largely by virtue of the secondary structure of their C-termini. The dynamics of the assembly of this relatively simple machinery and of the Type I secretion process are now beginning to be understood. Bacterial diversity is shown in Type II systems with alternative second steps and in the use of other transport systems, e.g. Type IV systems for export of T-DNA by *Agrobacterium tumefaciens*.

Tansy Peters

Eukaryotic protein transport

Proteins, which are destined for secretion, are firstly delivered to and transported across the ER membrane, then transported to the Golgi apparatus and thence to secretory vesicles. These vesicles move to and fuse with the plasma membrane from whence they are secreted. This process has to be tightly regulated all the way to control *inter alia* protein conformation and, in the case of hydrolytic enzymes, premature activation. Preproteins can either be co-translationally targeted to the ER membrane by SRP, or post-translationally by the Sec63-dependent route. In both cases the preprotein crosses the ER membrane via a transmembrane channel, which in both yeast and mammalian cells is composed primarily of three proteins (the Sec61 complex). The Sec61p of yeast shows homology to the SecY component of the *E. coli* SecYEG translocase. Recent work has revealed that the transmembrane channel is much wider than that required to accommodate a linearly extruded polypeptide, and thus to the realization that substantial folding of the protein may occur whilst it is still being translocated across the membrane. This and other recent advances, such as the revelation that gating of the translocase may be accomplished by transduction of conformational changes, that occur in the ribosome as it translates, will be discussed by Stirling.

Whilst a branch of this general secretory pathway is used for the delivery of proteins into lysosomes, most proteins of the mitochondria, peroxisomes, chloroplasts and the nucleus arrive at their destination as the result of post-translational import from the cytoplasm. Proteins transported from the plant cytosol to the thylakoid lumen of chloroplasts have to traverse three membranes: the outer and inner chloroplast envelope membranes and the thylakoid membrane. Chloroplast protein transport is more complicated than originally envisaged. Once proteins containing chloroplast transit sequences have been imported into the stroma they may undergo SRP-dependent targeting and Sec-dependent or ΔpH-dependent translocation across the thylakoid membrane. These pathways all reflect the prokaryotic origins of chloroplasts. Preproteins destined for the ΔpH-dependent pathway have N-terminal signal peptides that differ subtly from classical signal peptides by the inclusion of a twin-arginine motif bordering the hydrophobic region. The Sec- and ΔpH-dependent pathways seem to be mutually exclusive and, remarkably, the latter pathway acts on fully folded precursors. The discovery of the second ΔpH-dependent pathway has led to the recognition of the same type of pathway in bacteria for the export of folded periplasmic proteins requiring cofactors. A fourth pathway, that may also have a bacterial counterpart, appears to involve the spontaneous insertion of proteins into the thylakoid membrane.

Protein transport from the cytosol to peroxisomes is conceptually simpler than chloroplast transport as there is only a single membrane to cross. Whilst the vast majority of peroxisomal proteins are post-translationally imported from the cytosol (by virtue of short C-terminal targeting signals), it is becoming increasingly clear that peroxisomal lipids and some important peroxisomal membrane proteins derive from the ER. In her contribution Baker will focus on the biogenesis of peroxisomes.

Influx and efflux systems for small molecules

For uptake of nutrients the problems are slightly different. Often the major one is that the concentration of a metabolite outside the cell is much lower than that in the cell and uptake has to be against a concentration gradient. One solution is to convert the transported solute into a modified form as in the phosphorylation of sugars by the phospho-transferase system (PTS). Regulation of sugar transport at the protein level involves inducer exclusion for Gram-negative bacteria but inducer expulsion in Gram-positive bacteria. Primary and secondary transport systems for solutes respectively use energy from ATP hydrolysis and from ion gradients; they can catalyse both uptake and efflux. Poolman will review these systems with particular emphasis on transport systems used in adaptation to osmotic stresses.

Efflux systems are used to pump out antibiotics, heavy metals and toxic metabolites (including waste products from intermediary metabolism) from cells and thus help regulate the environment within the cell. Rosen will describe the ubiquitous arsenite efflux pumps and the ways in which accessory factors improve the efficacy of the resistance mechanism. Specific binding of a peripheral membrane ATPase to bacterial pmf-dependent arsenite carriers convert them into primary arsenite pumps, and possession of an arsenate reductase effectively extends the range of substrates that arsenite transporters can extrude.

Lewis will describe the multidrug resistance pumps (MDRs) that occur in bacterial, animal and plant cells. By contrast with solute influx transporters, MDRs have remarkably broad substrate specificity; amphipathic cations are generally the preferred substrates, even though MDRs

PROTEIN MANUFACTURING ROOM

ROUGH ER EXIT THIS WAY

VESICLE DOCKING BAY THIS WAY

ER LUMEN THIS WAY

GOLGI THIS WAY

TRANSPORT SYSTEM

LUMEN

CYTOSOL

"I'M NOT SURE WHAT I WAS MEANT TO BE IN LIFE, MY DESTINATION SEEMS SO UNCERTAIN."

Polly Peptide takes a wrong turn!

Speech bubble: "Sorry, this is the city protein-reprocessing yard for knackered proteins"

Tansy Peters

occupy four different membrane protein superfamilies. A variety of mechanisms and pathways for the ligand are used; these include a pathway whereby the ligand is flipped from the inner leaflet to the outer leaflet of the membrane and another where transport is from the outer leaflet to the external medium using a porin to traverse the outer membrane. The polarity of the ligand and its partitioning in the lipid bilayer may determine the route of efflux. Lewis will also discuss the possibility that naturally occurring plant alkaloids would be potent antimicrobials if not for the evolution of MDRs. As overexpression of MDRs causes clinically significant multidrug resistance, the possibility of combating this resistance by using the natural MDR inhibitors produced by plants (alongside the alkaloids) in combination with antimicrobials will also be discussed.

Sequence comparison studies have been applied to transport proteins and are particularly valuable when applied to membrane proteins, since so little high-resolution structural data exist. Such studies have proved useful in grouping proteins into evolutionarily related families, identifying conserved motifs are of functional significance, etc. Proteins with six transmembrane segments are found in many transport families and seem to have evolved independently in several families; the preference for this configuration is still unknown. Saier will discuss repeat elements within permeases, the recognition of fused domains and variations in permeases with multidomains.

Role of lipids

Lipids play crucial and often undervalued roles in transport of molecules across membranes. The phospholipid bilayer stabilizes the hydrophobic membrane-spanning α-helices from which most membrane proteins are constructed. Phospholipids may interact directly with the hydrophobic signal peptides of presecretory proteins. The internal and external layers of membrane bilayers may be asymmetric in their lipid composition and this may play a role in the recognition of sequences responsible for correct orientation of integral membrane proteins in membranes. For at least one MDR the membrane influences the substrate specificity of the MDR, by selection only of ligands that partition in the membrane. For example the mammalian P-glycoprotein works by a flippase mechanism (transporting molecules from the inner to the outer leaflet of the membrane by flipping them over). Lipids also play vital roles in vesicular transport of proteins between organelles.

Recurrent themes

Cells use different signal peptides as sorting codes for delivery via different systems. Bacterial, chloroplast and yeast proteins using the Sec-dependent route have the 'classical' signal peptide, whereas signal peptides for the ΔpH-dependent route in chloroplasts and bacteria contain twin-Arg regions next to the hydrophobic core. These two systems appear to have evolved to transport unfolded and folded proteins, respectively. In some cases bipartite signal sequences are used, each to cross one of two successive membranes. The old concept of proteins always being translocated in the unfolded state is obviously not true. Sequence comparisons have proven useful in identifying motifs characteristic of particular proteins, e.g. MDR efflux translocases can be identified by sequence analysis. Chaperones are important in controlling the folding and unfolding of proteins. Proteins like SecB and Bip bind unfolded proteins to prevent unwanted hydrophobic interactions. Some chaperones, as in the Yop system, are specific to particular proteins to ensure delivery in good shape. *You'll never walk alone* could well be the theme tune of the transport world.

Space regrettably precludes further discussion of recurrent themes or of the many applications like antibiotic resistance, inherited disease, inhibitor design, etc., that stem from the fundamental science.

However, the organizers of the Symposium on *How Do Molecules Cross Microbial Membranes?* hope that this preview of the programme is sufficient to whet your appetite to attend the session at Leeds in September.

See you there and may you arrive safely!

● *Dr Bruce Ward helped to organize this symposium and can be contacted at Institute for Cell and Molecular Biology, University of Edinburgh Tel. 0131 650 5370; e-mail bward@srvo.bio.ed.ac.uk*

Other symposium organizers
● *Professor S. Baumberg*
Department of Biology, University of Leeds
● *Professor C.J. Stirling*
School of Biological Sciences, University of Manchester
● *Dr J.K. Broome-Smith*
School of Biological Sciences, University of Sussex
● *Dr P.M. Goodwin*
The Wellcome Trust, London

Let's have a debate!

Philip Mortimer

To be successful, a scientific meeting must provide a mix of data and interpretation that instructs and stimulates the audience, and leads them to modify their opinions and possibly adapt their research. Sadly, meetings often fail by these criteria. Instead, keynote speakers jet into the venue, bearing slides. They deliver a well-honed speech to a passive audience and they leave the same day. Other speakers (who may at least have paid their way) give talks of variable quality. A cargo of offered papers and posters is added to the freight of scientific data, and the audience begins to reel under the weight of information. Coping strategies are adopted, such as lingering in the coffee bar, getting out of bed late or leaving the meeting early. The next time, faced with the prospect of another conference of the same sort, people consult their bulging diaries and decide that they would prefer instead to have the information filtered through the refereeing process of the journals. In particular, veterans who might have the most to contribute to a meeting and are best placed to discuss or challenge from the floor are the most likely not to find the time to attend.

Is it then still possible to organize a meeting that, rather than being a failed data transplant, is a successful interaction between old and young, experienced and naïve, knowledgeable and ignorant? One ploy, which the SGM Clinical Virology Group has been trying out with some success, is to include a debate in the programme. A topical issue is chosen and four or more main speakers are invited to argue a point. This gets people, both seniors and young turks, on to their feet to set out and defend their opinions about significant issues. At the recent meeting of the Society at Warwick, we debated universal screening of antenatal patients for HIV infection. The proceedings lasted over two hours and virtually no-one left the lecture theatre.

Where the form of disputation known as 'the debate' originates is unclear, but it is a structured contest in which opposing views about important issues are set out and tested in front of a non-partisan audience which then votes for one side or the other. They do so on the merits of the case as presented by the main speakers. The speakers may well see both sides of an argument, and indeed might have been able to argue the opposite one, but their role is to advocate one cause and refute the other. The analogy with English and American courts of law is obvious.

The exercise is academic – no-one is bound by the outcome of the motion or by the arguments advanced. A debate, like a cricket or football match, is simply a game played to rules but it has a serious purpose. It airs and tests arguments, it encourages speakers and listeners to evaluate their opinions and, because there is a time constraint, it forces people to structure, prioritize and refine their arguments. It often reveals the weaknesses of a generally held opinion or the merits of a minority one. Above all, it is rarely dull.

Debating, an important sedentary sport in British schools and universities until more visceral recreations took over, has long been in decline, and may be unfamiliar to younger scientists. If so, it is time to revive it. It has often been argued that science is about facts and not about opinions, but this is unconvincing. Ideas, not facts, are what count. If it is agreed that scientific meetings need less undigested fact and unchallenged assertion, and more discussion and argument, then a debate is the way to break the mould. Just as no scientific data should be taken on trust until it has been replicated elsewhere, no scientific proposition should be accepted until it has been aired in public. If a proposition cannot be defended in debate there is probably something wrong with it. Moreover (and this is an important consideration), practice in debating will help scientists when, in committees or elsewhere, they are called upon to present and explain technical issues, and argue for funds.

The subject and the wording of the motion are essential to the success of a debate. It is said that the defeat of the motion that *'This House would fight for King and Country'*, at the debating society of an ancient English university in 1933 fed Adolf Hitler's ambitions and altered the course of history. It will take more than the outcome of a debate to change the course of science, yet a debate can bring a somnolent post-prandial session to life. It can turn the final hours of a meeting from a dispiriting succession of exits by an audience that has been furtively consulting timetables to identify the earlier train, to an arresting clash of arguments that holds the attention of all.

Nor should the impact on informed opinion of a well fought debate on a topical issue be discounted. The most famous British scientific debate that ever took place was that between Bishop Wilberforce (known on account of his oleaginous manner as Soapy Sam) and Thomas Huxley. The issue was the descent of man and the outcome was that man's relationship to the apes came to be accepted. That occasion has not been forgotten after over 100 years, so it should not be thought that a debate in a current scientific meeting will count for nothing.

Attendance at scientific societies, and the SGM is no exception, is suffering from the pressures that administrative responsibilities, greater accountability and growing teaching commitments put on people's time. Yet societies need members' attendance and their participation at meetings just as much as they need their subscriptions. Without such fellowship a society, however rich it may be, is moribund. The debate is an occasion where serious issues can be thrashed out openly, and in a form where no-one is irrevocably committed, or immune by virtue of their position from challenge. It is a place where tenured scientists can be called upon to defend their work and opinions, and where the young and less secure can promote theirs.

A final warning to the organizers of scientific gatherings: *a debate could seriously enliven your meeting!*

● Dr Philip Mortimer is Convener of the SGM Clinical Virology Group. He can be contacted at PHLS Hepatitis and Retrovirus Laboratory, CPHL, 61 Colindale Avenue, London NW9 5HT
Tel. 0181 200 4400; Fax 0181 200 1569

The Rules of Debating

A motion is chosen – *'This House believes/ agrees/prefers/ supports/deplores…'*, and two proposers and two seconders speak alternately for and against the motion. When the seconder against the motion has finished (time limits of 10 minutes are usually imposed on each speaker) the chairperson calls any number of speakers 'from the floor'. When the chairperson decides that proceedings should draw to a close, the proposers are each invited to summarize the arguments for their side and dispose of those of the other (each may have 5 minutes). A vote is then taken and the chair declares, by a majority of *x* to *y*, 'that this House does or does not believe, agree, etc'.

SocietyNews

February Council Meeting

Microbiology Today

● Council placed on record its congratulations to all concerned, especially Janet Hurst, Ian Atherton and Janice Meekings, the production team at Marlborough House, on the excellent appearance, content and impact of the first issue of *Microbiology Today*.

Terms of office

● The President outlined some concerns he had regarding the lengthy terms of office of senior Officers of the Society which were possibly a serious deterrent to able candidates who might take on these important posts. Council may decide to bring a resolution to the next AGM covering this issue, and comments from ordinary members of the Society would be welcome.

Contacts

● Difficulties sometimes arise in filling not only Officer vacancies, but also on occasion those of ordinary membership of Council, and in attracting high quality candidates for the Society's prize lectureships. Members agreed that a valuable tool for trawling departments would be a comprehensive, up-to-date list of senior contact persons in relevant departments in universities, institutes and industry. Anyone who could fulfil such a role in their department is asked to contact Janet Hurst at SGM Headquarters.

Investment policy

● The efficient handling of its capital investments is vital to the future financial security of the Society. Due to changes in the organization of Dresdner RCM Global Investors, our investment managers, Council instructed the Treasurer, Finance Manager and Executive Secretary to make a thorough review of investment policy and the options for fund management. With guidance from our auditors and after extensive discussion by the Treasurer's committee, Council agreed to transfer the portfolio to Mercury Asset Management for investment in large, common managed funds for charities. Ironically, in their last year managing the Society's independent fund, the Dresdner team have performed very well on our behalf and the Society's assets have increased significantly in notional value.

Public understanding of science

● With the appointment of our Education Officer, a number of new initiatives are coming forward and Council was pleased to approve a proposal to extend the use of the Society's Developments in Teaching Fund to include aspects of public understanding of science. For details of procedures for application for funds see p. 81.

Small prizes in university departments

● Council also supported enthusiastically a proposal to fund small prizes in university departments for undergraduates with high levels of attainment in microbiology. Details will be announced in a future issue of *Microbiology Today*.

● *Charles Penn, General Secretary*

Notices

Annual General Meeting 1999

The Annual General Meeting of the Society will be held on **Tuesday, 7 September 1999**, at the Society Meeting at the University of Leeds. Agenda papers, including reports from Officers and Group Conveners, and the Accounts of the Society for 1998 will be circulated with the August issue of *Microbiology Today*.

SGM Symposium Volumes

The contributions to the April 1999 symposium on *Microbial Signalling and Communication* are available as Volume 57 in the series. A review of the book appears on p. 66. As usual, there is a 60 % discount to members buying their personal copies. The prices are as follows:

Members
£26.00/$46.00

Non-members
£65.00/$115.00

Student Members
£16.00

The book can be ordered by post using the form in this issue of *Microbiology Today*. This form can also be used to order any past volumes in print that you missed at the time of publication.

Student Members wishing to purchase Symposium Volumes at the discounted rate should write to the Grants Office at Marlborough House for a special order form.

Wanted

Gil Domingue, University of Exeter, is trying to find a **Fixed Head 10 × 100 ml** centrifuge head suitable for an **MSE Automatic Superspeed 50**. If you can help, please contact Gil or Frieda Jorgensen at The PHLS FMRU, Church Lane, Exeter EX2 5AD (Tel. 01392 402955; Fax 01392 412835; e-mail g.domingue@exeter.ac.uk)

News of Members

● The Society notes with regret the untimely death of **Professor Gordon S.A.B. Stewart**, University of Nottingham. Professor Stewart had a distinguished research career and was the Society's 1997 Colworth Prize Lecturer. The recent symposium volume (no. 57, *Microbial Signalling and Communication*), to which he contributed, has been dedicated to Professor Stewart's memory.

● The Society also notes with regret the death of **Professor P. Novotny** (member since 1974). An obituary can be found on the web at http://www.bc.ic.ac.uk/research/fairweather/obit_novotny.html

Staff News

In February we were pleased to welcome two new recruits to the External Relations and Grants Office. **Mrs Josiane Dunn** has taken over running the Society's meetings, but her job title – Events Administrator – reflects a change both in that role and the ever-broadening out-reach activities of the SGM. Josiane has come to us after 11 years working at the Institute of Food Research Reading Laboratory which is shortly to close. Latterly she ran the Business Development Office where one of her many functions was to organize scientific meetings. Her considerable administrative skills have been brought into use immediately, organizing the Society's spring meeting at Edinburgh, a complex event which was very well attended. Delegates will now be able to put a face to the name.

The new post of Education Assistant has been filled by **Mrs Dariel Burdass**, a microbiology graduate who has not only carried out scientific research but in recent years has taught both in secondary and primary schools. The SGM's role in promoting microbiology to schools, through the creation of teaching resources, organization of training courses in practical techniques for teachers and technicians and the provision of an information service, has grown considerably over the past two years. Dariel will be handling most of these activities, in conjunction with Janet Hurst and Jane Westwell and the new Education Officer on Council, Liz Sockett. The benefit of an extra pair of hands has already been made apparent through Dariel's enthusiastic participation in our SETweek activities (see p. 91).

In the Journal Sales office we were sorry to say goodbye to **Janine Wooldridge** who is moving on to a job elsewhere. The work will be covered by **Debbie Smith** who is already a familiar face in the building where she often lends a hand with journals distribution and other casual work.

Finally, we wish a happy retirement to **Anne Gurr** who worked for many years as a part-time proofreader for *Journal of General Microbiology*. Since going freelance in the early 1990s she has continued her proofreading activities for *Microbiology* and has frequently helped out in busy periods with copy editing, particularly for IJSB.

Grants

Education Development Fund 2000

Awards for PUS projects now available

Only a small number of applications to the Developments in Teaching Fund have been received in recent years. This is probably due to the changing nature of microbiology teaching at all levels and constraints such as the diminishing time that academic staff have to devise novel applications. In recognition of this situation and in furtherance of Society policy to promote the public understanding of microbiological issues, Council has decided that the remit of the fund should be changed. From now on members will be able to apply for small grants for relevant science promotion initiatives as well as for funding to support developments likely to lead to an improvement in the teaching of any aspect of microbiology relevant to secondary or tertiary (including postgraduate) education in the UK. Funding for tours to overseas higher education institutions to study methods of teaching large classes will no longer be available.

Applications from members are now invited for either category of award. The full rules of the scheme are given below.

Rules

1. Applicants must be members of the Society, currently residing in the UK or Republic of Ireland.

2. *Practical teaching aids*

(a) Applicants may seek support, normally within the range £200–£3,500, for:

(i) purchase of consumable materials, but not capital equipment;

(ii) short-term assistance, e.g. vacation employment of an undergraduate, or exceptionally a post-graduate after expiry of a studentship.

(b) Examples of projects which might be funded include the provision of teaching materials (e.g. videos, slides, posters), the development of reliable, novel practical exercises, new approaches to teaching/learning familiar concepts (e.g. computer simulations or tutorials) or any other appropriate aspect. It is not intended that the Fund should subsidize normal departmental teaching practices; the Society wishes to encourage innovation.

(c) Successful applicants will be notified in February to facilitate forward planning for their project. They will normally be required to make the results of their work available to Society members, within 18 months of the award being made. This will include a presentation at a Society meeting and publication of an article in *Microbiology Today*. Physical materials, whether offprints, videos, slides, computer programs, microbial strains or in other forms, should be readily available to Society members on free or low-cost loan or purchase for a period of at least five years after termination of the project.

(d) The Society would encourage commercial or other dissemination of the results of the project to a wider public. All Intellectual Property Rights, including copyright and design rights, in any materials produced as a result of the grant will be vested in the Society.

3. *PUS awards*

(a) Applicants may seek funding of no more than £1,000 for small projects to promote the public understanding of microbiology. These might include talks, workshops, demonstrations, posters, leaflets, broadcasts, activities at science festivals and audio-visual or computer-based packages. These activities can take place as part of a SET event at the applicant's place of work but PUS activities that are part of the programme of an open day to promote the institution are ineligible for funding.

(b) Applicants must provide a detailed description of the proposed initiative, which it is anticipated will take place in 2000, full costings and evidence of any collaborations or other sponsorship. Each application should also include a safety risk assessment and evidence of, or costing for, appropriate public liability insurance cover if the activity is to be held at a public venue. Payments to helpers such as undergraduates who are giving up their free time to deliver the activity may be included in the costings.

Applicants should also indicate how they will assess the success of their event.

(c) Successful applicants will be notified in January to facilitate their arrangements. A report of the activity must be submitted to the Society within 3 months of the completion of the project. This should take the form of an article for publication in the 'Going Public' section of *Microbiology Today*. A copy of the results of the assessment exercise (a simple questionnaire or summary of public comments on the event will suffice) should also be provided.

Application forms

Application forms are available from the Grants Office at SGM HQ or may be downloaded from the web site.

Please state clearly whether a form is required for a teaching aid or a PUS award.

The closing date for applications is **29 October 1999**.

Fund for Developments in Teaching

1999 Awards

Only four applications were received and the Award Panel agreed that the following two projects should be funded:

Dr M. Tait, CAL Unit, School of Health, University of Wales Swansea: to develop a case-based exploration of clinical microbiology (£2,921.70).

Dr P. Dyson, School of Biological Sciences, University of Wales Swansea: to run practical workshops on microbiology in primary schools (£765).

Past awards

The reports have now been received for several completed projects. These include Dr Sarah Watkinson's account of her trip to the USA where she learnt about the uses of the web for teaching large classes and some novel practical exercises in microbial genetics; Dr Mark Roberts' insights into the uses to which computer-assisted learning is put in Australia for teaching veterinary bacteriology and mycology; and details of the Biodiversity Consortium Unit's 'Microbial Field Trip' CD-ROM developed by Professor Johanna Laybourn-Parry and her colleagues. Shortened versions of these reports will appear in *Microbiology Today* in due course.

The Watanabe Book Fund

Members who are permanently resident in a developing country are reminded that they may apply for funding to acquire for their libraries books, or possibly journals, relating to microbiology. These annual awards are available as a result of a generous donation from Professor T. Watanabe of Japan. Full details of the scheme were published on p. 24 of the February issue of *Microbiology Today*. The closing date for the receipt of applications, which should be made to the Grants Office at SGM Headquarters, is **4 October 1999**.

Non-SGM award

The Daphne Jackson Memorial Fellowships Trust

These fellowships help women return to their scientific careers after taking a break for family reasons. The fellowships are normally for two years, worth over £17,000, flexible as they are part-time, tenable at a university or an industrial laboratory designed for retraining and new research and restricted to science, engineering and technology.

Applicants must have a first degree, have taken a break for three years and be resident in the UK. Contact: Jennifer Woolley, The Daphne Jackson Trust, Department of Physics, University of Surrey, Guildford GU2 5XH (e-mail djmft@surrey.ac.uk).

International Development Fund

Council aims to assist microbiologists in developing countries and Eastern Europe through the International Development Fund. Awards are made by competition.

Purpose

1. Support visits (travel and accommodation) by members of the SGM to laboratories in countries where microbiology is inadequately developed but where its further development may assist education or the economy of these countries. The purpose of the visits must be to give short lecture courses and laboratory training in subjects designed to meet the needs of these countries. The countries may vary from time-to-time but at present these include many places in the Far East, Africa, South and Central America, the Indian sub-continent and Eastern and Central Europe. Host laboratories are usually expected to provide some evidence of local support for the courses.

2. Allow purchase of basic equipment essential for the needs of such training courses.

3. Provide Society journals, symposia and special publications to established libraries for a limited period of time at reduced or zero cost, especially when it can be shown that these publications are not currently reasonably available in the country concerned.

4. Support national microbiological facilities, e.g. culture collections (which underpin microbiology), where these run into temporary difficulties.

5. Support any other small project to assist in technology transfer from Western Europe to the areas mentioned above for which other sources of funding do not exist. This might include provision of equipment to a nominated centre at which a member is working permanently.

Guidelines

1. Applications for sums between £1,000 and £5,000 will be considered first. No applications above £7,000 will be accepted.

2. Applicants must be members of the Society.

3. In making applications for support for giving short lecture courses or laboratory training, detailed information must be provided about the relevance and quality of the training course and the degree of local support for course.

4. Each application must be accompanied by full supporting documents.

5. A condition of funding (except for provision of publications) is that a brief report, suitable for *Microbiology Today*, be provided.

Applications to the Fund are now invited. Four copies, including full supporting documents, should be sent to the International Secretary, (Professor J.W. Almond, Pasteur Mérieux Connaught, 1541 Avenue Marcel Mérieux, 69280 Marcy L'Etoile, France).

The closing date for applications is 1 October 1999.

COURTESY *READING EVENING POST*

Seminar Speakers Fund 1999/2000

The purpose of the Seminar Speakers Fund is to promote talks on microbiological topics in departmental seminar programmes. Applications are invited from higher education institutions where microbiology is taught for grants of up to £200 towards the travel, and if necessary, accommodation, expenses of an invited speaker. Applications will be dealt with on a first come, first served basis during the academic year. Written submissions should be sent to the Grants Office at SGM Headquarters for consideration. The rules of the scheme are detailed below.

1. The scheme is open to higher education institutions in the UK and Republic of Ireland where microbiology is taught. Normally only one department within an institution will be eligible for an award within each academic year, which is defined as running from September 1999 to June 2000. It is expected that departments will collaborate in selecting a seminar speaker.

2. Applications will only be accepted from departments, not from Student Microbiology Societies.

3. Up to two speakers may be funded each year, provided the total award to the institution does not normally exceed £200.

4. Seminars must be advertised regionally as sponsored by the Society.

5. Awards will be paid retrospectively on receipt of evidence of the actual expenses incurred.

6. Applications should contain the following information.

(a) The names and addresses of the speaker(s) to be invited and the topic of the talk(s).

(b) Evidence, in the form of a programme, that an active seminar programme is already established in the department(s). Where no previous programme exists, good reason should be given for the request, such as the establishment of a new department.

(c) Details of any sponsorship for seminars that the department already has (or is anticipating).

(d) An indication of the target audience for the seminar, which may include undergraduates and postgraduates.

John Redwood MP visits Marlborough House

It is Council policy to promote understanding and knowledge of microbiological issues on the widest stage. This includes submitting evidence to Parliamentary inquiries and Government Department consultations, and maintaining contacts with politicians on a non-party partisan basis.

Marlborough House, the Society's Headquarters, lies in the Wokingham parliamentary constituency of the Rt. Hon. John Redwood MP, who is Shadow Minister for Trade and Industry. As such, he has overall responsibility for science and technology issues within the Shadow Cabinet, and speaks on these issues for the Conservative Opposition in Parliament. It was therefore appropriate that he was able to visit Marlborough House on 12 March, to learn about the range of SGM's activities, meet the staff and discuss matters of science and education policy with representatives of Council and senior staff. Matters covered included the importance of support for basic research, the effects of excessive monitoring and centralization, employment conditions for academic and research microbiologists, including their ability to exploit findings commercially, and the role of the scientific community in giving advice and promoting public understanding of contentious issues such as GM food and BSE.

The microbiological highlight of a working lunch was provided by three excellent cheeses produced by local cheesemakers.

The photograph above shows (from left to right) Howard Dalton (SGM President), Don Ritchie (SGM Professional Affairs Officer), John Redwood MP, Ron Fraser (SGM Executive Secretary) and Janet Hurst (SGM Deputy Executive Secretary).

Society for General Microbiology
Prize Lectures and Awards

New procedure for nominations

In recent years Council has been disappointed by the lack of nominations for the range of prestigious awards made by the Society in recognition of distinguished contributions to microbiology. It has therefore been decided to publish annually in the May issue of *Microbiology Today* the rules for each prize lecture due to be awarded in the following year. It is hoped that this new system will assist members in making nominations. The award panel will consider the nominations in the autumn and their recommendations will be taken to November Council for approval. The outcome will be announced in the February issue of *Microbiology Today*.

Nominations are now sought for the prize lectures listed on this page. A nomination form may be downloaded from the SGM web site or obtained from the Executive Secretary at Society HQ. Please complete the form and send it to Dr Charles Penn, School of Biological Science, University of Birmingham, Birmingham B15 2TT. Dr Penn will be pleased to discuss the criteria for nominations, should any queries arise.

Closing date for all nominations:
31 August 1999.

Fleming Award 2000

The Fleming Lecture is awarded annually for outstanding research in any branch of microbiology by a young microbiologist in the early stages of his/her career. The award is £1,000.

1. Nominees should normally have been engaged in research for not more than 10 years after doctoral qualification or equivalent. Years may be added to this total in respect of career breaks, for parenthood or other substantive reasons.

2. There should normally have been a connection with the scientific activity of the Society, either by means of past and continuing membership of the Society (a minimum of 3 years' membership of the Society would normally be expected), or past presentation(s) at a Society meeting or publication(s) in a Society journal, or an organizational or administrative contribution to the scientific work of the Society.

3. Candidates, who need not be members of the Society, should submit an outline CV including details of qualifications, scholarships, research grants obtained, etc., a list of publications, an outline of their career progression (posts held in postdoctoral research) and the names of two members who are familiar with their work, who will be asked to provide a statement detailing the candidate's contribution to microbiology and merit for the award. Alternatively, members who wish to make a nomination should provide such a statement and should arrange for a second member willing to support the nomination to provide a statement, and should ask the candidate to provide the CV and publications list. The General Secretary will be pleased to advise members preparing nominations about the information to be supplied.

4. The recipient will be expected to give a lecture based on his or her work to a meeting of the Society, which will usually not be that which takes place in the spring. He or she may be asked by the Council of the Society to repeat the lecture at another centre in this country or in Europe. Expenses of the lecturer will be paid by the Society. Requests for such a second lecture should be made to the General Secretary and will be considered by Council. The text of the lecture will be published in either *Microbiology* or in the *Journal of General Virology*, whichever is the more suitable. The choice will be at the discretion of the Editors of the two journals.

5. In the event of there being no successful nominee in any particular year, the Award money will be returned to the funds of the Society. Any given nominee may be chosen once only.

Kathleen Barton-Wright Memorial Lecture

The lecture is awarded every other year by SGM on behalf of the Institute of Biology for an outstanding contribution to research in a more applied area of microbiology, or in an area where microbiology impinges on other areas of biology, and where the topic would be attractive to a wider biological audience. The prize is £500. Contact Charles Penn for further details.

Marjory Stephenson Prize Lecture 2000

The first call for nominations was published in the February issue of *Microbiology Today* (Vol. 26, p. 23). Please note that the closing date for nominations has been changed to fit in with the new procedure.

Rules

1. The Marjory Stephenson Prize Lecture shall be awarded biennially for an outstanding contribution of current importance in microbiology, without restriction on the area of microbiology in which the award is made.

2. Nominations for the Marjory Stephenson Prize Lecture shall be made by any two members of the Society; the nominee need not be a member of the Society. Nominations should be accompanied by a statement of the contribution to microbiology made by the nominee, supported by reprints or other appropriate documentation. A brief *curriculum vitae* of the nominee and a full bibliography of his or her work should also be included.

3. There shall be no restriction by means of age or nationality of those eligible for the Marjory Stephenson Prize Lecture. Recipients of the Lectureship may not be nominated on a subsequent occasion.

4. The recipient of the Marjory Stephenson Prize Lectureship will be expected to give a lecture based on the work for which the Prize Lectureship has been awarded to a meeting of the Society, normally the Spring meeting following the announcement of the award. The recipient will be strongly encouraged to publish the lecture in either *Microbiology* or the *Journal of General Virology*, whichever is the more suitable. The choice will be at the discretion of the Editors of the journals.

Fleming Lecturer 1999

The 1999 Fleming Lecture has been awarded to Dr David Richardson, School of Biological Sciences, University of East Anglia, in recognition of his contribution to the understanding of fundamental physiological and molecular aspects of bacterial respiration. The title of his lecture, which will be delivered at the Society meeting at the University of Leeds in September 1999, is *Bacterial respiration: a flexible process for a changing environment*. A profile of Dr Richardson will appear in the August issue of *Microbiology Today*.

Meetings

Meetings on the web

Up-to-date information on future Society meetings is available on the web site http://www.socgenmicrobiol.org.uk

Meetings organization

The programmes of the Society's meetings are organized by the committees of the special interest groups, co-ordinated by the Scientific Meetings Officer, Dr Pat Goodwin. Suggestions for topics for future symposia are always welcome. See p. 95 for contact details of Group Conveners. Administration of meetings is carried out by Mrs Josiane Dunn of the Meetings Office at SGM Headquarters, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AE Tel. 0118 988 1805 Fax 0118 988 5656 e-mail meetings@socgenmicrobiol.org.uk

Abstracts book

Edinburgh Meeting April 1999

The full text of the abstracts book is now available as a PDF file on the SGM web site.

144th Ordinary Meeting
University of Leeds
7–10 September

Details of many of the symposia were published in the February issue of *Microbiology Today* (pp. 28–29). See web site for full information about the programme, changes as they occur and to download a booking form if you do not wish to use the one on p. 99 of this magazine.

● **Main Symposium (7–8 September)**
How Do Molecules Cross Microbial Membranes?

This symposium will be published in book form. See p. 74 for a preview of the topics to be covered.

● **OTHER SYMPOSIA**

● **7 September**
Teaching Microbiology to Non-microbiologists: the Challenge of Modular Structures

Education Group
Organizer: Helen O'Sullivan (osullih@livhope.ac.uk)

● **7–8 September**
Cell Lysis in Fermentation and Bioprocessing

Fermentation & Bioprocessing Group
Sponsored by New Brunswick Scientific (UK) Ltd
Organizer: Rob Cumming
Titles and abstracts for offered papers or posters should be sent to Reg England (r.england@uclan.ac.uk) **by 1 June 1999**.

● **8–10 September**
Food-borne Infections and Intoxications

Microbial Infection/Systematics & Evolution/Physiology, Biochemistry & Molecular Genetics Groups jointly with The Pathological Society
Organizers: Ian Poxton, Simon Foster, Adrian Eley and Niall Logan
Titles and abstracts for offered papers and posters should be sent to Ian Poxton (i.r.poxton@ed.ac.uk) **by 1 June 1999**.

● **9 September**
Adhesive Structures

Cells & Cell Surfaces Group
Organizers: Anthony Smith (prsaws@bath.ac.uk) and Mike Wilson (mwilson@eastmand.ucl.ac.uk)
Titles and abstracts for offered papers and posters should be sent to the organizers **by 1 June 1999**. This is an ideal opportunity for researchers in the structural and functional aspects of microbial adhesion to present their work. Posters are welcome; there are currently two slots free for oral presentations.

● **9 September**
Molecular Machines: Mobile Protein Complexes in Micro-organisms

Physiology, Biochemistry & Molecular Genetics Group
Organizer: Liz Sockett (liz.sockett@nottingham.ac.uk)
A speaker in this session is the Nobel prizewinner J. Walker. Titles and abstracts for offered papers and posters should be sent to the organizer **by 1 June 1999**.

● **9–10 September**
Deep Subsurface Biosphere

Environmental Microbiology Group jointly with the Geological Society Marine Studies Group
Organizer: J. Parkes (j.parkes@bristol.ac.uk)
Titles and abstracts for offered papers and posters (young scientists particularly welcome) should be sent to the organizer **by 1 June 1999**.

● **SPECIAL EVENTS FOR YOUNGER MEMBERS**

● **7 September**
Promega Prize Final

Promega sponsors this competition to encourage excellence in scientific communication by young scientists. Group Committees have now judged the oral or poster presentations by members under 28 related to recent Group symposia. The finalists go forward to compete for Promega Prizes in a special session of short oral presentations on their research at Leeds. There are two prizes of £200 each to be won and the winners will go on to compete for the title of *Young Life Scientist of the Year* in 2000 against finalists from other learned societies.

Younger Members' Reception

In the evening following the competition there will be a special event for younger members – postgrads and postdocs. A short presentation on CVs and Interviews will be followed by a wine reception and finger buffet. For a successful career in microbiology it is necessary to be as skilled in promoting yourself as communicating your research findings – this session will provide invaluable advice. The event is free, but entry will be by ticket only which must be booked when registering for the meeting. Contact Jane Westwell at Marlborough House for details.

● **OFFERED POSTER PAPERS**

Offered poster papers are invited on any aspect of microbiology. Titles and authors (including full addresses) should be sent to the Meetings Office at Marlborough House, to arrive **no later than 1 June 1999**. This date is earlier than that previously advertised. If possible abstracts should also be submitted at this stage; these should arrive **no later than 30 June 1999** and be sent by e-mail for publication in the abstracts book. A maximum of 200 words is permitted.

Future Meetings

WINTER 1999/2000
145th Ordinary Meeting
5–7 January 2000
University of Surrey, Guildford

● Virus Infection – Life or Death of a Cell

Virus and Clinical Virology Groups

Organizer: Geoff Smith (glsmith@molbiol.ox.ac.uk)

Day 1: A. Wyllie (Cambridge) Overview of apoptosis / D. McCance (Rochester, USA) HPV / T. Wileman (Pirbright) ASFV / H. Thomas (St Mary's London) HBV / A. Greenberg (USA) CTL and apoptosis / Offered oral papers

Day 2: P. Gallimore (Birmingham) Adenovirus transformation / W. Wold (St Louis, USA) Adenovirus apoptosis / L. Young (Birmingham) EBV / C. Boshoff (Chester Beatty London) HHV-8 / B. Fleckenstein (Erlangen, Germany) HVS / Offered oral papers

Day 3 (am): P. Freisen (Madison, USA) Baculovirus / C. Bangham (St Mary's London) HTLV-1 / J. Fazackerley (Edinburgh) Alphaviruses / H.-J. Thiel (Giessen) BVDV

Titles and abstracts for offered papers or posters should be sent to the organizer **by 21 September 1999.** All submissions should contain the names of all authors, their affiliations, the title of the paper, the name of the author who will make the presentation and whether they are eligible for consideration for a Promega Prize (see p. 89) together with an abstract (maximum 150 words).

Evening Workshops

Influenza Virus

Organizer: John McCauley (john.mccauley@bbsrc.ac.uk)

Exotic Viruses

Organizer: Susan Jacobs (jacobs_susan@hotmail.com) Anyone wishing to participate in the workshops should contact the appropriate organizer **no later than 30 November 1999.**

SPRING 2000
Millennium Meeting
10–14 April 2000
University of Warwick (jointly with Society for Applied Microbiology)

● Main Symposium Fighting Infection in the 21st Century

Organizers: P. Goodwin, P. Andrew, G. Smith, D. Stewart-Tull, M. Easter and P. Oyston

Prestigious speakers from around the world, including a UK government representative and spokesmen from WHO, will cover the following topics: Lessons from the successes and failures of global vaccine programmes / The global threat of emerging infectious diseases / The worldwide epidemic of antibiotic-resistant bacteria / Protecting farm animals from infectious disease / Can molecular techniques have a role in the prevention of contamination of processed food by pathogens? / New approaches to public education in the prevention of communicable disease / Is global clean water attainable? / The use of microassays in understanding infections and in diagnosis / Live attenuated vectors / The promise of DNA vaccination / Vaccine development / Immunotherapy of infectious cancers / Vaccine production in plants / Therapeutic antibodies against endotoxaemia and sepsis / New strategies for identifying and developing new antimicrobial drugs / Are molecular methods the optimum route to antimicrobial drugs? / Prospects for a new and a rediscovered form of therapy: probiotics and phage

To be published

Other symposia: Microbial ecology of food-poisoning micro-organisms (EM Group & SfAM. Organizer: Linda Lawton l.lawton@rgu.ac.uk) / Molecular epidemiology: sub-specific classification and identification (SE & CV Groups) / Potable water treatment (FBP Group & SfAM) / Proteases, proteolysis and control (PBMG & CCS Groups. Organizers: C. Stirling colin.stirling@man.ac.uk & D. Hodgson dm@dna.bio.warwick. ac.uk) / Public education in safe food and water (Ed. Group & SfAM. Organizer: R. Bishop rh.bishop@ulst. ac.uk) / Transcriptional control circuits in fungi (PBMG Group. Organizer: A. Brown al.brown@abdn. ac.uk) / Vaccine delivery (MI Group. Organizer: P. Oyston poyston@hotmail.com) / Virus entry and exit (V Group. Organizer: G. Smith glsmith@ molbiol.ox.ac.uk)

Also: Evening workshops, social events and trade exhibition Further details of all symposia are available on the SGM web site and will be published in the next issue of *Microbiology Today.* Where no names of symposia organizers are given above, please contact the appropriate Group Convener for information (see p. 95 for addresses).

AUTUMN 2000
147th Ordinary Meeting
12–15 September 2000
University of Exeter

● Main Symposium Community Structure and Co-operation in Biofilms

To be published

Organizer: Hilary Lappin-Scott (h.m.lappin-scott@exeter.ac.uk)

Other symposia: Applications of recombinant technology to industrial fermentations / Biofilms in infection and disease / Control of biofilms / Mathematical skills and microbiologists

SPRING 2001
148th Ordinary Meeting
24–30 March 2001
Heriot-Watt University

● Main Symposium New Challenges to Health: the Threat of Virus Infection

To be published

Organizer: Geoff Smith (glsmith@molbiol.ox.ac.uk)

Irish Branch

Commercialization of Microbial Biotechnology
16–17 September 1999
University of Ulster at Coleraine

For further information and to offer papers and posters contact Nigel Ternan (ng.ternan@ulst.ac.uk). See also the SGM web site.

Recent Advances in Molecular Microbial Ecology
7–8 April 2000
University College, Galway

Title t.b.a.
Autumn 2000
National University of Ireland, Maynooth

For details of Irish Branch activities contact the Convener, Martin Collins (m.collins@qub.ac.uk)

Group News

CLINICAL VIROLOGY

An excellent symposium and keenly contested debate at the SGM meeting at Warwick in January were marred only by the disappointing attendance. Members are respectfully reminded that the short term demands of their work in the Health Service and elsewhere should not invariably take precedence over their own scientific and professional development. In the long term the needs and interests of patients will surely be better served by coming to SGM meetings and sharing problems in formal and informal interactions with colleagues, rather than spending every day in your laboratory. Please delegate and come to SGM whenever you can.

I shall soon be handing over as Convener to Tim Wreghitt. I congratulate him and wish him a happy and successful reign. I would like to thank warmly the committee for their support and help and Lisa Snowden for most efficiently seeing to the clerical side of the Clinical Virology Group's business. The SGM is the main avenue through which clinical virology in the UK accesses the rapid and continuing advances in microbiology. I congratulate members of the Group on their wisdom in belonging to (and so benefiting from) the Society and I urge them to invite colleagues to join and attend meetings. There is no better deal among the learned societies!

■ *Philip Mortimer*

HotofthePress

Science journalist Meriel Jones takes a look at some papers in current issues of the Society's journals which highlight new and exciting developments in microbiological research.

Archaeology meets microbiology

G.M. Taylor, M. Goyal, A.J. Legge, R.J. Shaw & D. Young

Tuberculosis is one of the ancient scourges of humanity. It is still a feared disease, although it is now rare in the UK and usually treatable with antibiotics. Although the most familiar symptoms of TB are damage to the lungs, in a small number of cases it attacks bones. This produces very characteristic scars that last as long as the bones. This enduring damage has been used to study the historical spread of TB both among human communities and between people and their domestic animals. Indeed, one hypothesis for the origin of the human disease is that about 15,000 years ago one strain spread from newly domesticated cattle and goats to people.

Scientists are now hoping to get clearer information about the origin and spread of TB through applying molecular biological methods. These have been developed for hospitals to identify sub-groups of the causal bacterium *Mycobacterium tuberculosis* and to check antibiotic sensitivity. DNA is a remarkably persistent molecule and a collaboration of medical scientists and archaeologists have now reported their analyses of DNA from bones in the graveyard of the Abbey of St Mary Graces. This central London cemetery was founded in 1350 and used until the dissolution of the monasteries by King Henry VIII in 1538. The researchers found *M. tuberculosis* DNA in bones bearing TB scars and not in bones lacking these signs, indicating that the DNA had not become scattered during the centuries. A quick check for evidence of resistance to modern hospital antibiotics showed that, as anticipated, these old bacteria would have been sensitive. After working through a series of tests, the authors could see that *M. tuberculosis* DNA from bones of one young man had subtle differences from bacterial DNA in another man's bones. This holds out the prospect of tracking individual strains of the disease through long-dead communities and taking archaeological epidemiology to an exciting new level.

■ Genotypic analysis of *Mycobacterium tuberculosis* from medieval human remains. *Microbiology* 145, 899–904.

A fatal break

N. Lea, J.M. Lord & L.M. Roberts

The infamous *Escherichia coli* O157 has made a place for itself as the cause of a particularly lethal form of food poisoning. Researchers at the University of Warwick have been trying to understand how one of its toxins works. This toxin, called SLT-1, is a protein released by the bacteria that clings to the surface of gut cells and then enters them. Once inside, one of the normal constituents of the cell makes the fatal mistake of cutting the toxin. This lethal fragment then interferes with normal protein production within the cell and the result is death.

There has been a scientific argument about the importance of this fragmentation. Some scientists have detected toxic activity before cleavage, although the level certainly increased afterwards. Others, including members of the Warwick group, have made changes to the toxin that should have prevented it being cut, and then recorded no decrease in toxicity. This has led to speculation that SLT-1 can be snipped in more than one place.

Michael Lord and his colleagues have now reported what happened when they worked through the toxin systematically removing every possible cleavage site. They then tested these novel versions of toxin for both cleavage by cell enzymes and toxicity to mammalian cells. The outcome was quite clear. The toxin indeed had two possible cleavage sites. Once these were both removed, the cells could no longer cut the toxin. Although this intact version of SLT-1 could cause some damage, the cleavable versions were very much more toxic.

■ Proteolytic cleavage of the A subunit is essential for maximum cytotoxicity of *Escherichia coli* O157 : H7 Shiga-like toxin-1. *Microbiology* 145, 999–1004.

5 cm

Microbes from the deep blue sea

Microbiologists are gradually meeting the bacteria that live deep under the sea. In the April 1999 issue of the IJSB, three new species of bacteria and archaea from the sea bed and a new method for identifying one specific group were described. There is increasing interest from scientists worldwide in the biology, chemistry and geology of deep-sea environments, where we can learn more about the formation of the continents and the incredible diversity of life.

An international group (Marteinsson et al.) has found one species that likes its seawater at 400 times normal atmospheric pressure at temperatures of up to 98 °C! It came from samples taken from a hydrothermal vent at a depth of 3550 m in the Mid-Atlantic Ridge by the submersible Alvin in 1993. Boiling water and rock fragments erupt here from the seabed. Once at the surface, the samples were kept oxygen-free in pressurized syringes in a hot oven. Repeated dilution left the scientists with one spherical, swimming bacterium that grew fastest in the very hot, high-pressure conditions. The group has now reported much more about its nature. It turns out also to be able to grow at much lower pressures (only 3 times atmospheric pressure) and temperatures (75 °C), although not so well. Thermococcus barophilus, as they have named it, is a member of the domain Archaea. It possesses the fatty acids unique to this group, along with a characteristic apparatus for protein synthesis. The archaea have some features in common with animal cells, along with their bacterial characteristics. These organisms probably resemble the earliest life on this planet and are considered by some to be a separate kingdom.

Although this species comes from the ocean's depths, a Japanese group (Takai et al.) has managed to isolate another one, which was called Thermaerobacter marianensis, from mud on the world's deepest sea floor. This is 10897 m below sea level (yes 11 km deep!) in the Mariana Trench Challenger Deep, off Guam Island in the Pacific Ocean. One odd aspect of its growth requirements was that despite coming from such a high-pressure environment, Thermaerobacter marianensis would not grow when the researchers put it into pressurized containers. This long, thin, rod-shaped bacterium required seawater, oxygen and a temperature around 75 °C.

Both these organisms have fairly conventional nutritional requirements, relying on a mixture of proteins, sugars and vitamins. However, marine bacteria can have very different needs. There is one distinct group that can use only carbon dioxide and hydrogen as sources of energy, generating methane gas as a waste product. In the first of two papers in the issue, Jeanthon et al. identified two new species of these so-called methanogens, Methanococcus vulcanius and Methanococcus fervens, from hydrothermal chimneys in the East Pacific Rise and Guayamas Basin, more than 2000 m beneath the surface. Again, these organisms prefer extremes of temperature and pressures for growth, and this is reflected in their names: vulcanius refers to Vulcanus, the Roman fire god, and fervens means boiling hot in Latin.

Perhaps inspired by the difficulties of identifying organisms from the extreme environments mentioned above, in their second paper in the issue, Jeanthon et al. described a rapid method for identifying hyperthermophilic methanococci. It exploits the Polymerase Chain Reaction (PCR) to amplify a region of genes known to contain features unique to methanogens; then, using enzymes that cut the amplified DNA into several chunks, the organisms can be identified by the pattern produced – this is also known by the rather long-winded tongue-twister Restriction Fragment Length Polymorphism (RFLP). The accuracy of the new method was determined by comparing predicted and actual patterns from known methanococci. When Jeanthon et al. tried this out on methanogens obtained from as far apart as the Mid-Atlantic Ridge and the East Pacific Rise, they could identify some species that had a worldwide distribution.

Considering the large number of microbes that have been found on the Earth's surface, culturing and identification may continue well into the next millennium before these inhospitable deep-sea habitats yield the true diversity of their inhabitants.

■ V.T. Marteinsson, J.-L.Birrien, A.-L. Reysenbach, M. Vernet, D. Marie, A. Gambacorta, P. Messner, U.B. Sleytr & D. Prieur. Thermococcus barophilus sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int J Syst Bacteriol 49, 351–359.

■ C. Jeanthon, S. L'Haridon, A.-L. Reysenbach, E. Corre, M. Vernet, P. Messner, U.B. Sleytr & D. Prieur. Methanococcus vulcanius sp. nov., a novel hyperthermophilic methanogen isolated from East Pacific Rise, and identification of Methanococcus sp. DSM 4213T as Methanococcus fervens sp. nov. Int J Syst Bacteriol 49, 583–589.

■ C. Jeanthon, S. L'Haridon, S. Pradel & D. Prieur. Rapid identification of hyperthermophilic methanococci isolated from deep-sea hydrothermal vents. Int J Syst Bacteriol 49, 591–594.

■ K. Takai, A. Inoue & K. Horikoshi. Thermaerobacter marianensis gen. nov., sp. nov., an aerobic extremely thermophilic marine bacterium from the 11000 m deep Mariana Trench. Int J Syst Bacteriol 49, 619–628.

THIS PAGE:
The unmanned submersible Kaiko used for collection of samples from the deep trenches of the Pacific Ocean.
COURTESY KEN TAKAI, JAMSTEC

OPPOSITE PAGE TOP:
Freeze-fracture electron micrograph of Methanococcus vulcanius strain M7, a novel hyperthermophilic methanogen isolated from the East Pacific Rise.
COURTESY C. JEANTHON, CNRS, ROSCOFF

OPPOSITE PAGE BOTTOM:
Spinal tuberculosis. Large cavitating lytic lesions in two adjacent lumbar vertebrae from a male aged between 15 and 25 years at the time of his death. The samples were excavated from the large graveyard overlying the site of the old Royal Mint, London. The cemetery was associated with the Abbey of St. Mary Graces, the last Cistercian foundation in England, founded in 1350 AD. Genotyping suggests disease was due to a strain resembling present day Mycobacterium tuberculosis rather than Mycobacterium bovis.
COURTESY MICHAEL TAYLOR

viral RNA

recombinant RNA

viral replicase

transgene RNA
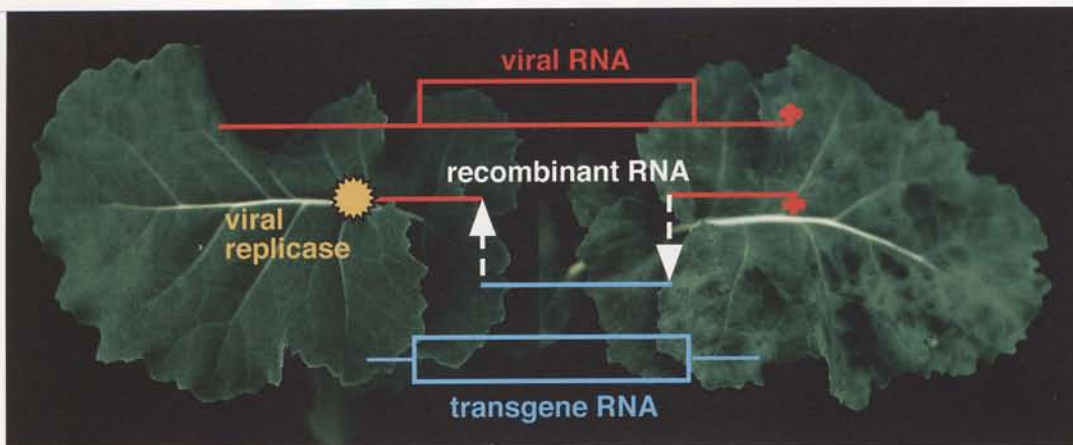
Microbiology Streptomycete special issue

To honour the career of Professor Sir David Hopwood, *Microbiology* is publishing a special issue of the journal in autumn 1999 in which many of the papers will be devoted to the genetics and molecular biology of streptomycetes and related actinomycetes. This issue will include a review article by David Hopwood (*Forty years of genetics with Streptomyces: from in vivo through in vitro to in silico*), as well as peer-reviewed research papers from leading groups.

For further information and an order form see our web site at http://www.socgen microbiol.org.uk

Viruses and transgenic crops

R. Aaziz & M. Tepfer

Viruses are a strange form of life. They are stripped down to the bare essentials of a container which can recognize a host cell and then release a set of genetic instructions to subvert it. This cell, whether in an animal, plant, fungus or bacterium, immediately ignores its normal duties and commences replicating the virus. The new viruses can then travel to new unsuspecting host cells leaving their exhausted or dead benefactor behind. This sort of activity will usually result in a disease, and one that can be very difficult to treat because most of the virus' activities are actually carried out by the normal constituents of the healthy host.

For viruses that infect plants, one good way to prevent disease is to use pesticides to eliminate the insects, fungi or nematodes that spread the virus from plant to plant. An alternative is to breed resistance into commercial crops, although this has often proved impossible. The problem arises when no resistance gene exists, or when it proves impossible to introduce it into the crop. In addition, the viruses can keep ahead of the plant breeder through mutating and recombining into new versions.

The large group called RNA viruses are known to have many mutant versions. Whenever an organism copies its genes, it needs to check the accuracy of the new set. The RNA viruses have no way of doing this, and indeed gain from the errors that inevitably creep in. What is more, if two different RNA viruses happen to be close together when they are copying themselves, the copy process can get confused, resulting in a new recombinant virus which is a mixture of both. The first record of recombination between RNA viruses was reported in 1962 from a culture of human cells infected with two strains of poliovirus. A third strain with mixed features of its two parents was isolated from the cells. There have been many more reports since. Although recombination can occur between regions of genes that are very similar, it also happens between areas with no obvious similarity. Rachid Aaziz and Mark Tepfer from INRA-Versailles, France, have been reviewing current

knowledge about this topic and have concluded that we still know surprisingly little about the details. Experiments are generally designed to select one particular type of advantageous recombinant, and so underestimate the quantity of minor and deleterious variants.

These changing populations of RNA viruses are only restricted by the need to maintain viable organisms in the face of environmental selection pressures. The authors point out that this continual natural shuffling may have ecological implications for a new type of virus-resistant crop. Plant breeders have achieved this type of crop by constructing transgenic plants containing a viral gene. The first virus-resistant transgenic plants were described in 1986 but it is particularly timely to think about their impact now because three varieties have already been licensed for commercial use in the USA.

The authors point out that although we cannot know the frequency of recombination, the very large amount of a commercial virus-resistant crop suggests that even rare recombination events are likely to happen. The plant-produced viral RNA will inevitably be encountered sometime by an invading virus during its own copying cycle. The authors therefore go on to explore possible scenarios with the emphasis on hazards that could arise. Since recombination occurs naturally when plants are infected simultaneously with different viruses, the emphasis must be on discovering whether any completely novel types of virus can be produced during infection of a virus-resistant transgenic plant. The most worrying situation might be the appearance of new viral strains with new properties persisting in the environment.

The reviewers have only found a few experimental reports exploring these possibilities. For example, tobacco plants expressing a gene essential for viral movement throughout the plant have been infected with modified viruses in which this gene is inactivated. Although recombinant viruses appeared, none of them produced more severe symptoms than the normal virus. In another series of experiments, new recombinant viruses were produced in the test-tube that gave novel symptoms when plants were infected. Unfortunately, there was no comparison with the original viral strains, since in real life any new recombinant virus only persists if it has some advantage over other strains.

After evaluating the limited published information, the authors outline the experiments they think would permit proper safety evaluation of virus-resistant transgenic plants. The key ones are comparisons between events in non-transgenic plants infected with two viruses and virus-resistant transgenic plants infected with each virus separately. A series of these experiments should show whether results are the same as over previous millions of years or whether something quite new happens.

■ Recombination in RNA viruses and in virus-resistant transgenic plants. *J Gen Virol* **80**, 1339–1346.

E. coli aids the fight against sunburn

Vacation studentship leads to published paper

The SGM Vacation Studentship scheme has proved very successful in giving undergraduates some experience of working in a research laboratory during the summer holidays before the final year of their BSc. For Sarah Smith, an awardee in 1997, it has led to co-authorship of a scientific paper – a valuable addition to her CV. Sarah's supervisor, Dr Robert Jones of the University of Portsmouth, reports that the paper, entitled 'A microbiological assay for the sun protection factor of sunscreen products', was published in *J Pharm Pharmacol* (1998) **50** (supplement), p. 138. The paper describes the development of an assay using *Escherichia coli* to measure the protection factor of commercially available sunscreen products as an alternative to the current tests which use human volunteers to determine the effectiveness of the creams against ultraviolet radiation from the sun. Indications from the study are encouraging and a microbiological system may well provide the basis for an animal-free testing system.

● Contributions for gradline from younger SGM members are welcome.

Young Life Scientist of the Year

Promega sponsors an annual competition to encourage young life scientists and to promote scientific communication skills. Contestants are drawn from five learned societies, including the SGM. The finalists are selected by their respective societies on the basis of poster or oral presentations and each receives a £200 prize. They then go forward to compete for the title of *Young Life Scientist of the Year*. Profiles of the two SGM contestants, Liz Mathew and Susan McGrath, appear below.

The 1998 finals of the competition took place at the Genetical Society meeting at the University of Warwick on 24 March 1999. The panel of judges included representatives from all of the learned societies participating. The 10 competitors each made an oral presentation about their research and Adele Marston from the University of Oxford, a finalist from the Genetical Society, was the winner of a hard-fought contest. She received £2,000 and a glass trophy.

■ Liz Mathew

For me, further education began at Imperial College, London. My interest in molecular microbiology really developed after completing a vacation project in Deborah Smith's group on a *myoD* homologue in *Trichinella spiralis*. I went on to do a DPhil in Geoffrey Smith's group at Oxford, which involved the characterization of a vaccinia virus envelope protein, B5R. As with most other postgraduate degrees, mine was also 'character building' and my time in Oxford as a student was fun.

As part of the training I received during both under- and postgraduate studies, presenting work and participating in Journal Clubs was strongly encouraged and proved to be suitable training for finally presenting data from my own thesis. The opportunity to talk at SGM conferences has provided a relaxed environment within which to communicate my data and exchange ideas with other scientists. Also, winning the Promega prize for best open paper was a nice bonus!

I am very interested in learning about host–pathogen interactions and I am eager to complement my experience in molecular virology with a better understanding of the immune system. I am currently working with S.K. Alex Law (MRC Immunochemistry Unit, Department of Biochemistry, Oxford) on leukocyte integrins. This position is temporary and has provided me with an ideal stepping stone from which to apply for postdoctoral positions abroad in molecular immunology.

e-mail emathew@molbiol.ox.ac.uk

■ Susan McGrath

I graduated with a BSc Hons in Biomedical Sciences from the University of Ulster at Coleraine (UUC) in 1995 and it was during my sandwich placement year that my interest in research began. On completion of my primary degree, I decided to pursue a career in research and began my PhD studies under the supervision of Dr R. Haylock and Dr J Dooley in the Biotechnology Research Group at UUC. My PhD studies involved the development of an RNA assay for *Clostridium botulinum* toxin gene expression and during my 3 years I gained invaluable laboratory experience in a wide range of molecular biology techniques. I attended several conferences during my PhD where I presented my work in the form of poster and oral communications. My first contact with the SGM was at the Irish Branch meeting at University College Dublin in September 1997, at which I was awarded the prize for best oral communication. As a result of this I was nominated to compete for a Promega Prize at the SGM meeting at the University of East Anglia in September 1998 where I was one of the two winners. I feel that it is extremely important to publicize research and one of the most important mediums for this is through attending conferences and presenting both in oral and poster format. It is also an important means of learning about new and upcoming areas of research and vital for forming collaborations.

At present, I am studying the possible applications of competitive RT/PCR for the detection of food-borne pathogens and toxins at UUC. I hope to extend my knowledge of molecular detection systems which may be beneficial to the food industry through future postdoctoral learning and industrial experience.

e-mail mcgrathsusan@hotmail.com

Promega Prize Competition

● Are you a member of the SGM?

● under 28 years of age?

● a postgraduate or first postdoc?

● thinking of presenting an offered paper or poster at an SGM meeting?

Why not enter for the Promega Prize Competition? You could win £200 in the SGM section of the competition and go on to compete for a further £2000 in the *Young Life Scientist of the Year* event.

Please contact the SGM Meetings Office (meetings@socgenmicrobiol.org.uk) for details of how to enter.

Finals 1999

The SGM finals to decide the winners of the 1999 competition will take place in the afternoon of Tuesday, 7 September at the Society's meeting at the University of Leeds. £200 prizes are at stake. The two winners of this session will go forward to the next *Young Life Scientist of the Year* competition.

Further details are available from the SGM Meetings Office or on the Society web site.

GoingPublic

In this issue, *Going Public* focuses on some of SGM's own activities to promote the public understanding of microbiology. Contributions from readers on any aspect of science communication are always welcome.

New posters

Microbes & the Environment

A set of two posters on the theme of environmental micro-organisms has been added to the range of SGM educational resources.

● *Natural Wonders* covers the amazing diversity of microbial habitats, microbial communities and their essential role in keeping the life cycles of the planet turning.

● *Wonderful Workers* gives examples of the applications developed by biotechnologists for microbes in agriculture, food and pharmaceutical production, pollution control and bioremediation.

Most of the illustrations came from two SGM-funded projects to produce a portfolio of micrographs of micro-organisms at different magnifications. These were carried out by summer students who have produced an invaluable resource for use in future educational posters and booklets.

Single sets of posters are free from the External Relations Office at SGM Headquarters. Contact Jane Westwell for details (j.westwell@socgenmicrobiol.org).

Pasteur competition for schools

A competition put on by the Institute for Animal Health, Compton, attracted a good range of entries from children aged 8–14. They were asked to design a poster illustrating the importance of Louis Pasteur's discoveries about micro-organisms and disease.

The entries were judged in two age groups (8–11 and 12–14) by staff from the Institute, representatives of BBSRC (one of IAH's major sponsors) and Liz Sockett, the SGM Education Officer. The results were announced in SETweek and the winning schools will receive their prizes of £350 for science books and equipment at the IAH's Pavilion at the Royal Show, Stoneleigh, where the top entries will also be on display.

ABOVE:
Liz Sockett, Tracey Reader (BBSRC), Marion Baker (primary school teacher), Dave Cavanagh (IAH & Schools' Liaison Officer), Elaine Collier (IAH) and Barry Freeman (IAH), judging entries in the Pasteur poster competition.

UPPER RIGHT:
The *Bioscience at Work* exhibition stand at *Careers 2000*, Glasgow.

LOWER RIGHT:
Children displaying their 'microbial' art, painted on handkerchiefs, during the *Family Science Fun Days* event at the University of Reading. See p. 91.

Microbial art workshops

The workshops described opposite were very popular with young children and we have since taken some of the activities to the Edinburgh Science Festival. If there is enough interest from readers who are involved in promoting microbiology to schools, then we will prepare worksheets for these activities. Please contact Dariel Burdass for details (d.burdass@socgenmicrobiol.org.uk).

'Careers Я us' rolls on

Jane Westwell

The last few days of February saw External Relations Office staff spending time in windswept and rainy Glasgow at *Careers 2000*, a national careers fair for school pupils, mature students and people seeking a career change. The SGM organized the *Biosciences at Work* exhibition stand in collaboration with the Biochemical Society, the British Society for Immunology and the British Pharmacological Society.

John Schollar from the National Centre for Biotechnology Education put on a 'live' demonstration which drew groups of fascinated onlookers. Once the crowds were hooked, volunteers from the societies moved in to give advice and information about the different biosciences and choices in higher education.

Over 29,000 people passed through the doors of the exhibition hall over three days and a good proportion were interested in biological sciences. Whether this is a reflection of the education system in Scotland we do not know, but it was very satisfying to see so many young people who were interested in science. A significant number of graduates also came to the stand seeking advice on finding work in their chosen field. This was less encouraging, but the general theme that came through was that their difficulties were probably due to poor CV presentation.

Biosciences at Work attends several careers fairs each year and is the only source of impartial information at these events about higher education and careers in the different disciplines. Future outings will be to Manchester GMEX, 3–5 October, and London Olympia, 13–14 October.

● *Jane Westwell, SGM External Relations Office*

SET99 – The Secret World of Microbes

Jane Westwell, Dariel Burdass & Janet Hurst

The 1999 National Week of Science, Technology and Engineering was marked by the SGM on 20–21 March at the *Family Science Fun Days*, University of Reading. An exhibition called *The Secret World of Microbes* illustrated the wide diversity of micro-organisms on earth and the impact that they have on our lives.

Thousands of visitors came to the Fun Days, ranging from pre-school children to grandparents. *The Secret World of Microbes* catered for all age groups with extensive poster displays and several types of hands-on activity ably supervised by SGM staff and volunteers.

Pre- and primary school aged children enjoyed making salt-dough models or painting pictures of environmental microbes on cloth handkerchiefs, guided by SGM's new Education Assistant, Dariel Burdass. After a short introduction to several micro-organisms which affect their every day lives, the children were let loose on the salt-dough and pens. Creative juices flowed freely and some beautiful drawings and models resulted. Handkerchief artists took their pictures away and the 'best' salt dough model from each workshop earned the winner some time as 'cameraman' on a video microscope. Several children left the exhibition proudly clutching a video containing 30–45 minutes footage of freshwater protozoa and algae.

Other visitors looked at various blue cheeses under lenses before spending a messy half hour inoculating toilet rolls with oyster cap mushroom culture. They left with clear instructions on mushroom care ringing in their ears!

More ambitious, and usually older, visitors learned the art of doing a Breed smear to calculate the number of yeast cells in a 5 litre fermentation vessel. The prize of a bottle of wine or microbial mouse-mat offered incentive to these intrepid individuals.

Microscopes brought previously unseen pond life into view and families were fascinated by the diversity of shape of various algae and the sight of protozoa zooming around slides in front of their eyes.

Passers-by were also invited to have a go at the *Lucky Dip*. Winners received a coaster depicting a range of micro-organisms. Thanks are due to *SuperSnaps* for their contribution towards the cost of the coasters.

The SGM activities and posters were complemented by display material on composting from the Henry Doubleday Research Association; posters about sewage treatment from Thames Water; posters illustrating the water and nitrogen cycles from the BBSRC and NERC and a small display on Quorn provided by Marlow Foods. Staff from the Environment Agency brought an exhibition about their work and a large dish of pond water containing grubs and bugs for children to examine. SGM member Nina Jenkins from CABI Bioscience, Silwood Park, set up a display of posters, fungi and locusts (dead and alive) to illustrate the LUBILOSA project which has developed a mycoinsecticide for use against locusts. John Grainger and Bob Rastall, SGM members who are staff at Reading University, gave up their weekend to support *The Secret World of Microbes* and staff from the National Centre for Biotechnology Education also contributed enormously.

Our grateful thanks are owed to John Schollar, Kate Porter, Bene Watmore and Laura Pountney (NCBE); John Grainger and Bob Rastall (University of Reading); Kathy Hurst and Gary Holmes; Nina Jenkins and Jane Gunn (CABI Bioscience) and staff from the Environment Agency.

● *Jane Westwell, Dariel Burdass & Janet Hurst work in the SGM External Relations Office*

THIS PAGE:
Scenes at the *Family Science Fun Days* event at the University of Reading.

Reviews

A classified compendium of book reviews from 1996 to the present is available on the SGM web site.

Practical Plant Virology: Protocols and Exercises

By J. Dijkstra & C.P. de Jager
Published by Springer-Verlag GmbH & Co. KG (1998)
DM 148.00/öS1081.00/sFr135.00/ £57.00/US$99.00, pp. 459
ISBN: 3-540-63759-1

This laboratory manual is aimed at students and teachers of plant virology, and also at research workers and technicians. It provides a comprehensive guide to classical and modern methods of handling and characterizing plant viruses: the authors' avowed intention has been to stick with tried and tested procedures and avoid the most recent developments. The great majority of the material in the book will be familiar to the experienced plant virologist: its merit is in bringing such a wide range of methods together in one place. In sum, 89 protocols and sub-protocols, ranging from infectivity assay to PCR, are described; there are 17 practical exercises. The descriptions of the methods are very detailed and thorough, with background information on how things work. Most sections are illustrated with helpful diagrams. Recommended as a teaching aid and for laboratories embarking on plant virus studies, but at the price, perhaps beyond the means of the individual student.
■ Ron Fraser
SGM Marlborough House

Molecular Biomethods Handbook

Edited by R. Rapley & J.M. Walker
Published by Humana Press (1998)
US$89.50, pp.704
ISBN: 0-89603-501-8

I liked this book and, probably more importantly, so did my PhD student. Each chapter covers one particular technique e.g. RFLP, HPLC, Southern blots. The chapters vary in their approach but all give a reasonable overview of the basis of the techniques and some go into a little more detail and include some recipes. I would not recommend it if you wanted to know exactly how to construct, for example, a YAC, but if you want to know the rationale behind the technique, it's a good resource. The chapters are on the whole well referenced and so

details can be easily found. A good book for those who have been 'brought up' on kits and for those who like to understand a little bit more about how a technique works rather than simply use it.
■ Bob Dalziel
University of Edinburgh

Molecular Variability of Fungal Pathogens

Edited by P.D. Bridge, Y. Couteaudier & J. Clarkson
Published by CAB International (1998)
£55.00/US$100.00, pp. 336
ISBN: 0-85199-266-8

The fungi are mutable beasts, continually exhibiting variability. This can be annoying when using them as experimental tools, but is clearly part of their nature. This book is an assemblage of 19 separate accounts of various aspects of fungal variability. It is the outcome of an EC workshop in September 1997, and does not attempt to give a comprehensive review of the subject. It covers a wide range of fungi, mostly plant, invertebrate and human pathogens. The insect pathogens get most coverage, with six chapters devoted chiefly to Metarhizium and Beauvaria, one, for example, describing variability due to the Restless transposon in Beauvaria-related fungal stains. There is also a brief account of fungal viruses, which are unusual by being transmitted only by intracellular routes. In conclusion, this is a pot-pourri of well written accounts, among which most mycologists should find something of interest.
■ Graham Gooday
University of Aberdeen

Immunochemical Protocols, Second Edition. Methods in Molecular Biology, Vol. 80

Edited by J.D. Pound
Published by Humana Press (1998)
US$69.50
ISBN: 0-89603-388-0

This book provides protocols of a range of immunochemical methods which are likely to be of interest to molecular biologists. It is a treasury of detailed information, both within the protocols and in the Notes sections, which in some chapters are extensive. However, for some of the techniques covered, such as

hybridoma production, reference to a more specialist text would probably be necessary if the reader was initiating the technique in the laboratory. As the chapters are organized under specific applications, rather than general techniques, there is repetition of some methodology, for example 'Western' blotting. For the reader who has experience of these techniques this gives an interesting insight into the variety of ways in which different laboratories successfully reach essentially the same end-point. I have one minor quibble in that Freund's complete adjuvant (FCA) is described in one chapter as containing 'heat-killed pertussis bacteria' rather than Mycobacterium tuberculosis. Also the dangers of FCA for the user if a needle-stick injury occurred should have been highlighted. This book will be useful to both those who already have some experience in this area and, probably in conjunction with other methods texts, to those who wish to embark on new techniques.
■ Sheila Patrick
The Queen's University of Belfast

Animal Cell Culture Techniques

Edited by M. Clynes
Published by Springer-Verlag GmbH & Co. KG (1998)
DM188.00/öS1,373.00/sFr170.00/ £72.50/US$119.00, pp. 618
ISBN: 3-540-63008-2

This book provides insight into a wide range of animal cell culture techniques, particularly those associated with biochemistry. Sections on General methods, Cell proliferation and death, Models of cell differentiation and Models of toxicology and pharmacology are included. Each chapter lays out procedures giving precise, step-by-step instructions which are easy to read and carry out. The background information for each topic is detailed, up-to-date and accurate. The book is particularly suitable for researchers who are relatively new to the practice of cell and tissue culture, providing a sound introduction to many of the modern applications in research and development. However, as such a wide range of topics is covered, the depth of information provided is, in some cases, limited. In such cases

access to more comprehensive texts may be required. This volume is highly recommended as a basic reference text for all tissue culture laboratories.
■ Andy Spencer
Veterinary Laboratory Agency, Addlestone

Bartonella and Afipia Species Emphasizing Bartonella henselae. Contributions to Microbiology, Vol. 1

Edited by A. Schmidt
Published by S. Karger AG, Basel (1998)
CHF146.00/DM175.00/US$127.00, pp. 218
ISBN: 3-8055-6649-2

This book is the first in the Contributions to Microbiology series. It consists of 14 chapters written by an international panel, giving an overview of infection caused by Bartonella and Afipia spp. The layout is clear, with tables and diagrams that are easy to understand, and the text is well written. The sections concerning laboratory methods, including molecular techniques, are particularly useful. The chapters can be read independently, as each covers a discrete subject. However, a minor irritation is that there is considerable repetition between them. Several comprehensive reviews of Bartonella infections have appeared recently in major journals, including two by some of the chapter authors. Given the cost of this book and the fact that this field is likely to progress rapidly, I wonder who will need to buy it. It would be valuable for institution libraries, and perhaps larger departments, but not for the individual, who can get the same information from review journals.
■ Nick Brown
Addenbrooke's Hospital, Cambridge

Genetic Engineering: Principles and Methods, Vol. 20

Edited by J.K. Setlow
Published by Plenum Publishing Corporation (1998)
US$95.00, pp. 292
ISBN: 0-306-45911-6

The first volume in this series was published in 1979, new editions appearing each year since then. In

the early years the series had an easily perceived identity, since there were relatively few principles and methods to describe. Genetic engineering is now used in such a wide variety of situations, however, that it is inevitable that a series which attempts to cover the whole subject will lose focus. Consequently, this edition includes chapters specific to mammalian, plant and amoebic systems, together with more generic *in vitro* and computer methods. This diversity is mirrored by the range of approaches taken by the contributors. Some chapters give superficial overviews, whilst others go into great detail and they range in length from 8 to 48 pages. The complete series may provide a useful resource in a library, but it is unlikely that this single edition would be missed from most personal bookshelves.

■ **Glenn Matthews**
Queen Elizabeth Hospital, Birmingham

● Gene Regulation. A Eukaryotic Perspective. Third Edition

By D.S. Latchman
Published by Stanley Thornes (Publishers) Ltd (1998)
£24.99, pp. 329
ISBN: 0-7487-3977-7

This is an improved edition of an already useful basic text. It has been brought up-to-date and extended, thus offering more to the reader. Part of the revision has been the inclusion of more basic material on the gene expression process. However, it is particularly pleasing to see the additional sections on post-transcriptional control. These may be somewhat limited in coverage in comparison to the considerably more extensive treatment of transcription, but do make this book more balanced. Overall, this book is a well written and lucid work that provides a sound introduction to this important field for advanced undergraduates, postgraduates and research scientists who are not specialists in the field. This reasonably priced paperback will be a worthwhile purchase for many such individuals, but given the rapid pace of advances in research on gene expression, the author may be invited to prepare a fourth edition very soon.

■ **John McCarthy**
UMIST, Manchester

● DNA Damage and Repair, Vol. 2: DNA Repair in Higher Eukaryotes. Contemporary Cancer Research

Edited by J.A. Nickoloff & M.F. Hoekstra
Published by Humana Press (1998)
US$125.00, pp. 672
ISBN: 0-89603-500-X

The second volume in this ambitious pair covers higher eukaryotes, which turn out to be mammals. Plants, the nematode *C. elegans*, and frogs were in Vol. 1. The articles are summaries either of different repair pathways, sets of enzymes, or analytical methods. Authors are acknowledged experts in the field and the result is articles of generally very high quality.
Overviews of transcriptional and post-translational responses to genotoxic stress pull together a huge amount of data. There is overlap between a few chapters, but in many cases this presentation of different viewpoints works to the reader's advantage. There are exhaustive reference lists, though not every chapter has clear figures and tables. In general there is an excellent and up-to-date (as of early 1998) scope of coverage that will be useful to active researchers in DNA repair, students exploring a particular area, or outsiders wanting an overview of a subject.

■ **Rick Wood**
Imperial Cancer Research Fund, Clare Hall Laboratories

● Therapeutic Applications of Ribozymes. Methods in Molecular Medicine, Vol. 11

Edited by K.J. Scanlon
Published by Humana Press (1998)
US$ 99.50, pp. 480
ISBN: 0-89603-477-1

Ribozymes are a class of ribonucleic acid that possess enzymatic properties which can be used to inhibit gene expression in a highly sequence-specific manner by catalysing the *trans*-cleavage of target RNA. As such these molecules (a.k.a. RNA enzymes) have been increasingly used as biological tools to understand gene expression, as drug-target validation agents and, the focus of this volume, as potential therapeutic agents. This timely

volume is the most comprehensive review to date of the methodology associated with synthetic and endogenously expressed ribozymes in cell culture and animal models. Details pertaining to ribozyme synthesis, purification and biological activity testing are included for the most commonly studied ribozymes (Hammerhead, Hairpin, Hepatitis Delta Virus and RNaseP) for a diverse range of applications, including the targeting of viruses such as HIV, hepatitis B and influenza, and cancers of the breast, pancreas and lung. I thoroughly recommend this volume to all newcomers and established groups working with ribozymes. A good companion to have by your side on the lab bench!

■ **Saghir Akhtar**
Aston University

● Virus Life in Diagrams

By H.-W. Ackermann, L. Berthiaume & M. Tremblay
Published by CRC Press/Springer-Verlag GmbH & Co. KG (1998)
DM145.00/öS1059.00/sFr132.00/£56.00/US$69.95, pp. 221
ISBN: 0-8493-3126-9

This is a collection of previously published diagrams. As a result each diagram is drawn in a different style and for each virus a varying amount of information is given. There has been no attempt to standardize the format of each entry such that all aspects of the life cycle are covered. There has also been no apparent attempt to edit the material and many diagrams cover the same processes as those on adjacent pages, for example there are seven different diagrams outlining the pox virus life cycle from entry to maturation. This is repeated for many viruses, yet there is, for example no diagram outlining the replication cycle of the autonomous parvoviruses. I am unsure at whom this book is aimed: I would perhaps use it as a teaching resource; however, if you are only interested in a single virus, Fields is probably a better bet.

■ **Bob Dalziel**
University of Edinburgh

● Specificity, Function, and Development of NK Cells. NK Cells: The Effector Arm of Innate Immunity. Current Topics in Microbiology and Immunology, Vol. 230

Edited by K. Kärre & M. Colonna
Published by Springer-Verlag GmbH & Co. KG (1998)
DM224.00/öS1,636.00/sFr202.00/£86.00/US$149.00, pp. 248
ISBN: 3-540-63941-1

This book is a timely collection of excellent articles on natural killer (NK) cell activation, receptor interaction and development. Lysis of target cells and secretion of cytokines by NK cells is controlled by distinct combinations of inhibitory and activation receptors. Since the latter remain mostly elusive, many of the chapters deal with the receptors and co-receptors involved in inhibitory function. The role of NK cells in the lysis of tumour and virally infected cells is also dealt with under a number of headings. However, NK cells also function by secreting cytokines, especially IFN-γ, which has an important role in innate defence early in infection and can selectively enhance the type 1 arm of the adaptive immune response. From the microbiologist's point of view, perhaps one weakness of the book was the scarcity of information on the function of NK cells in immunity to infectious diseases; less than a page of text is devoted to the role of NK cells in anti-bacterial immunity.

■ **Kingston Mills**
National University of Ireland, Maynooth

● Viruses and Human Cancer

Edited by J.R. Arrand & D.R. Harper
Published by BIOS Scientific Publishers Ltd (1998)
£35.00. pp. 200
ISBN: 1-872748-44-9

This is an excellent introduction to human tumour virology, suitable for advanced undergraduates, postgraduates and research workers entering the field. As well as treating each major group of human tumour viruses in reasonable detail (hepatitis viruses, papillomaviruses, Epstein–Barr viruses, Kaposi's sarcoma, human herpesvirus 8 and oncoretroviruses), the book is enhanced by an

excellent and stimulating introduction by Robin Weiss, which conveys both an accurate sense of history and real excitement of things to come in the human tumour virus field. In the final chapter, most of the authors of the other chapters combine forces to provide a good overview of the prospects for vaccination and therapy of virally mediated human cancer. A small reservation is that by focusing on human tumour viruses by group, and not giving much detail on non-human tumour viruses (e.g. SV40, polyoma) and human viruses that cause tumours in animals (e.g. adenoviruses), some very interesting common evolved mechanisms in tumorigenesis are lost from consideration here. However, this is a small point balanced against what is a highly recommended book.

■ **Eric Blair**
University of Leeds

● Adenovirus Methods and Protocols. Methods in Molecular Medicine, Vol. 21

Edited by W.S.M. Wold
Published by Humana Press (1998)
US$99.50. pp. 704
ISBN: 0-89603-551-4

The use of adenoviruses as gene delivery systems guarantees a strong interest by many research laboratories in good protocols for construction of adenovirus mutants and recombinants and their use. This book caters quite well for those who simply want to grow adenoviruses, assay them and use them. The protocols are written in a standard, detailed way in each chapter and seem easy to work from. More advanced topics are included, such as growth of fastidious adenoviruses, the study of the immune response to adenoviruses and molecular and cell biological methods for study of infection and cell transformation. Overall, this is a very comprehensive methods book that will be a laboratory standard for some years to come.

■ **Eric Blair**
University of Leeds

Feedback

Readers' reactions to the articles on GM crops and food in the February issue of *Microbiology Today*

What about the positional effects?

I greatly appreciated the four articles on genetic engineering of plants, for their content and the courage of the views expressed. However, I was surprised that no mention was made of position effects, described abundantly in genetics textbooks, at least those of a generation ago. The random insertion of a genetic element into a genome by non-homologous recombination is likely to affect the information present and expressed from the region of insertion, giving rise to unpredictable side-effects separate from the functionality of the inserted sequence. Insertions might affect not only growth pattern and morphology, but also metabolic equilibrium which would be less obvious and more difficult to detect.

The textbooks mention examples of positional effects and transposable elements from animals ranging from *Drosophila* to humans, and Barbara McClintock's pioneering studies with maize are justly famous. Later studies with haploid micro-organisms, such as those of Werner Arber, established the importance of insertions and inversions in causing spontaneous mutations, especially insertions into plasmids and bacteriophages.

With plants, the increasing availability of sequence information may make it possible to target the transgene to specific regions of the genome, by adding host sequences to each end in an attempt to achieve insertion by homologous recombination. But this, and the continued need for screening for positional side-effects, will be time-consuming and counter to industrial pressures for commercially usable products that give profits within a short timescale.

● *Professor Emeritus Eduard Kellenberger*
IGBM de l'Universite de Lausanne, Switzerland

Dear Editor

In the first issue, Peter Cotgreave rightly berates sloppy writing about microbes. We must be just as careful to avoid it when venturing into non-microbiological contexts.

Among the interesting articles on GM foods and crops, your editorial observes: "*What is undoubtedly motivating the biotechnology companies at present is profit and the creation and expansion of market share*". "*At present*", you write: are you expecting change? Like all businesses, biotechnology companies are always looking for profit. A company is not a charity; if it never expects to be profitable, there will be no investment and no company, biotechnological or otherwise.

One of the complaints sometimes levelled at the scientific community is its unfamiliarity with the realities of business and economics. Surely that cannot include the Editor of *Microbiology Today*.

With fraternal greetings.

● *Professor Vivian Moses*
Division of Life Sciences, King's College London

Conference

Genetically Modified Foods & Ingredients: the way forward
22–23 June 1999
Royal Marsden Hospital
NHS Trust, London
Contact IBC Global
Conferences Ltd
Tel. 0171 453 5496

Further information

The feature articles on GM plants and food in *Microbiology Today* coincided with a huge amount of media interest in the topic. Public concern has been such that many supermarkets and food companies have now banned GM ingredients from their products. Inquiries into aspects of the subject have also been set in motion by the UK government and bodies such as the Royal Society. As a result of all this interest several useful leaflets have been produced by the research councils.

● *GMOs and the Environment: scientific certainties and uncertainties*
A 6 page A4 briefing note from NERC which examines the facts associated with genetic modification and assesses the risks for the natural environment. Available from: NERC Planning & Communications Directorate, Polaris House, North Star Avenue, Swindon SN2 1EU (e-mail nerccomm@nerc.ac.uk).

● *InGENEious: the science and issues of genetic modification*
A DL size, 8 page leaflet from the BBSRC covers the background to the topic, the debate, issues and concerns, GM and animals, GM and crops, applications and a useful reading list. Available from Public Affairs Branch, BBSRC, North Star Avenue, Swindon, SN2 1UH (e-mail public.affairs@bbsrc.ac.uk).
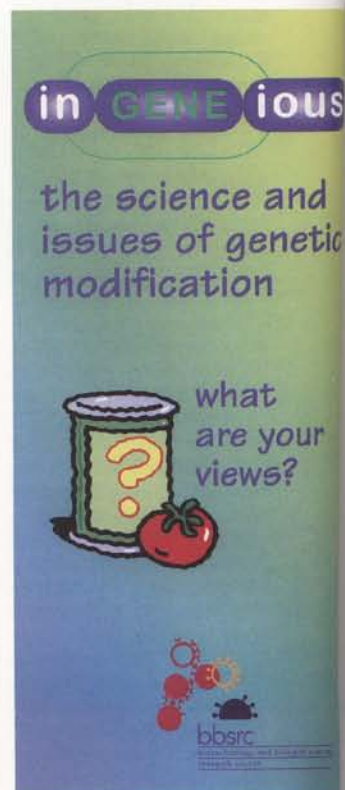
Diary

june 99

MOLECULAR BIOENERGETICS OF CYANOBACTERIA: MECHANISMS OF ENERGY CONVERSION AND ELECTRON TRANSFER

Gmunden, Austria
5–10 June 1999

CONTACT: J. Hendekovic, European Science Foundation, Office of European Research Conferences (EURESCO), 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France (Fax +33 3 88 36 69 87; e-mail euresco@esf.org; http://www.esf.org/euresco)

july 99

XIX INTERNATIONAL WORKSHOP/ SYMPOSIUM. RAPID METHODS AND AUTOMATION IN MICROBIOLOGY. SHAPING THE NEW MILLENNIUM

Kansas State University, USA
9–16 July 1999

CONTACT: Janice Nikkel, Conference Co-ordinator (Tel. +1 785 532 2548; e-mail jnikkel@dce.ksu.edu; http://www.dce.ksu.edu/dce/conf/ microbiology/)

NMR IN MOLECULAR BIOLOGY: STRUCTURE, BINDING AND MOLECULAR RECOGNITION

Granada, Spain
10–15 July 1999

CONTACT: J. Hendekovic, European Science Foundation, Office of European Research Conferences (EURESCO), 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France (Fax +33 3 88 36 69 87; e-mail euresco@esf.org; http://www.esf.org/euresco)

ENZYMES IN THE ENVIRONMENT: ACTIVITY, ECOLOGY AND APPLICATIONS

Granada, Spain
12–16 July 1999

CONTACT: Secretariat, Dr Jose-Miguel Barea, Consejo Superior de Investigaciones Cientificas, Estacion Experimental del Zaidin, Profesor Albareda 1, 18008 Granada, Spain (Tel. +34 58 12 10 11; Fax +34 58 12 96 00; e-mail jmbarea@eez.csic.es; http://www.css.orst.edu/grenada/)

E. COLI: FRIEND OR FOE. SOCIETY FOR APPLIED MICROBIOLOGY SUMMER CONFERENCE

University of York
14–16 July 1999

CONTACT: Dr Ann Baillie, Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ (Tel. 01234 326661; Fax 01234 326678; e-mail sfam@btinternet.com)

THE BIOCHEMICAL SOCIETY MEETING

University of Keele
20–22 July 1999

CONTACT: The Meetings Office, Biochemical Society, 59 Portland Place, London W1N 3AJ (Tel. 0171 580 3481; Fax 0171 637 7626; e-mail meetings@biochemsoc.org.uk; http://www.biochemsoc.org.uk)

august 99

49TH HARDEN CONFERENCE. FUNCTIONAL ASPECTS OF ENERGY METABOLISM IN BRAIN: RELATIONSHIP TO BRAIN DEVELOPMENT AND NEURODEGENERATIVE DISEASE

Eynsham Hall, North Leigh, nr Oxford
14–18 August 1999

CONTACT: The Meetings Office, Biochemical Society, 59 Portland Place, London W1N 3AJ (Tel. 0171 580 3481; Fax 0171 637 7626; e-mail meetings@biochemsoc.org.uk; http://www.biochemsoc.org.uk)

september 99

MICROBIAL AQUATIC SYMBIOSIS. PRESENTED BY THE JOINT AQUATIC ASSOCIATIONS OF THE UK

Scottish Association for Marine Science, Oban
1–3 September 1999

CONTACT: Dr Douglas McKenzie, Scottish Association for Marine Science, Oban PA34 4AD (Tel. 01631 567832; Fax 01631 571150; e-mail dmck@dml.ac.uk; http://www.nerc-oban.ac.uk/dml/ meetings/meetfram.htm)

50TH HARDEN CONFERENCE. ANNEXINS

Wye College, Kent
1–5 September 1999

CONTACT: The Meetings Office, Biochemical Society, 59 Portland Place, London W1N 3AJ (Tel. 0171 580 3481; Fax 0171 637 7626; e-mail meetings@biochemsoc.org.uk; http://www.biochemsoc.org.uk)

FUNDAMENTAL ASPECTS OF SURFACE SCIENCE: STRUCTURE AND DYNAMICS OF ORGANIC AND BIOLOGICAL MOLECULES AT INTERFACES

Castelvecchio Pascoli, Italy
3–8 September 1999

CONTACT: J. Hendekovic, European Science Foundation, Office of European Research Conferences (EURESCO), 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France (Fax +33 3 88 36 69 87; e-mail euresco@esf.org; http://www.esf.org/euresco)

HUMAN FUNGAL PATHOGENS: FUNGAL DIMORPHISM AND DISEASE

Granada, Spain
4–8 September 1999

CONTACT: J. Hendekovic, European Science Foundation, Office of European Research Conferences (EURESCO), 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France (Fax +33 3 88 36 69 87; e-mail euresco@esf.org; http://www.esf.org/euresco)

THE BIOCHEMICAL SOCIETY MEETING

University College Cork
7–9 September 1999

CONTACT: The Meetings Office, Biochemical Society, 59 Portland Place, London W1N 3AJ (Tel. 0171 580 3481; Fax 0171 637 7626; e-mail meetings@biochemsoc.org.uk; http://www.biochemsoc.org.uk)

MOLECULAR BIOLOGY OF RNA: PROCESSING OF EUKARYOTIC PRE-mRNA AND NUCLEO- CYTOPLASMIC TRANSPORT

Castelvecchio Pascoli, Italy
11–16 September 1999

CONTACT: J. Hendekovic, European Science Foundation, Office of European Research Conferences (EURESCO), 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France (Fax +33 3 88 36 69 87; e-mail euresco@esf.org; http://www.esf.org/euresco)

FOOD MICRO '99. ECOLOGY AND PHYSIOLOGY OF FOOD RELATED MICRO-ORGANISMS. THE 17TH SYMPOSIUM OF THE INTERNATIONAL COMMITTEE ON FOOD MICROBIOLOGY AND HYGIENE (ICFMH)

Veldhoven, The Netherlands
13–17 September 1999

CONTACT: ICFMH, Congress Service Brabant, The Netherlands (Fax +31 40 2546566; e-mail KSB@koningshof.nl; http://www.cbs.knaw.nl/foodmicro)

DIET, GUT FUNCTION AND HEALTH

The Royal Society of Medicine London
27–28 September 1999

CONTACT: Emma Bryce, The Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE (Tel. 0171 290 3919; Fax 0171 290 2977; e-mail: events@roysocmed.ac.uk)

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october 99

PROTEIN TARGETING: MECHANISMS AND COMPONENTS OF PROTEIN SORTING TO SUBCELLULAR COMPARTMENTS

Obernai, nr Strasbourg, France
1–6 October 1999

CONTACT: J. Hendekovic, European Science Foundation, Office of European Research Conferences (EURESCO), 1 quai Lezay-Marnésia, 67080 Strasbourg Cedex, France (Fax +33 3 88 36 69 87; e-mail euresco@esf.org; http://www.esf.org/euresco)

november 99

SECOND FOCUS ON FLUORESCENCE SYMPOSIUM: EXCITING DYES AND THEIR APPLICATIONS

Leiden, The Netherlands
26–27 November 1999

CONTACT: Ms Irme van der Heijden, Molecular Probes Europe. Poortgebouw, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands (Tel +31 71 523 3378; Fax +31 71 523 3419; e-mail eurotech@probes.nl)

december 99

ADVANCED COURSE ON MICROBIAL PHYSIOLOGY AND FERMENTATION TECHNOLOGY

Delft University of Technology, The Netherlands
6–17 December 1999

CONTACT: Dr Ir.L.A. van der Meer-Lerk, Institute for Biotechnology Studies Delft Leiden (BODL), Kluyver Laboratory, Julianalaan 67, 2628 BC Delft, The Netherlands (Tel. +31 15 2781922; Fax +31 15 2782355; e-mail bodl@stm. tudelft.nl; http://www.kluyver.stm.tudelft. nl/BODL/ACS.htm)

july 2000

BEYOND THE GENOME. 18TH INTERNATIONAL CONGRESS OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

International Conference Centre, Birmingham
16–20 July 2000

CONTACT: Andrea Buxton, Centre Exhibitions, The NEC, Birmingham B40 1NT (Tel. 0121 767 3755; Fax 0121 767 3535; e-mail genome@necgroup.co.uk)

Comment

Your views on the new *Microbiology Today*

I took *Microbiology Today* home over the weekend to read. I thought it was really stupendous. You and your team have certainly made a really excellent job of it. The '*Quarterly*' has been transformed into a really professional, high-class magazine for the Society. The articles are really very good and comment on really important issues. The whole layout is also very professional looking, the red bullets are remarkably effective. Please feel free to pass these hearty congratulations on to others in your team. Well done.
Professor John C. Fry, Cardiff School of Biosciences

The contents of the February issue are interesting, useful and enjoyable. However, the type gives me problems both for size and legibility. In particular the compressed, thin sans serif type used in legends, news items and the meetings section is a real problem – and others agree with me. Furthermore, you have reduced the size of type for the articles to a point that is barely tolerable and a bit tiring for people like me. I am appreciative of the evident intention to make *Microbiology Today* a necessary and useful read for the members, but please do not make it too difficult.
Professor Robert G.E. Murray, Microbiology and Immunology, University of Western Ontario, Canada

The pages are much more visually striking than previously and the whole magazine has a fresh and trendier look about it. You must be very pleased with it. The changing font styles make the text look busy and the squashed upright format is difficult to read...
Howard Jenkinson, Convener, Cells & Cell Surfaces Group

Congratulations on a smart and highly professional new publication. The first issue of *Microbiology Today* suggests that it has transformed from a good publication of the members (The *Quarterly*) to a magazine that can be passed to non-members for information and opinion on matters of importance. The timing of the articles on GM crops was fortuitous and you should run on some spare copies of those articles for distribution to our opinion-formers. My view of the new format and title is, of course, entirely unbiased by the fact that it contains an article by one of our 1998 finalists and the announcement of a Prize Lectureship to one of our staff!
Professor Nigel L. Brown, School of Biological Sciences, University of Birmingham

A brief note to congratulate you and your colleagues on the first issue of *Microbiology Today* – I started at page 1 and read solidly through to the end – it was absorbing, interesting and educational.
Dr M.O. Moss, Surrey

Microbiology Today is an extremely disappointing replacement for the *SGM Quarterly*. The font throughout is too small and in certain sections has made the text unreadable. Did anyone open Reviews and compare it with the Book Reviews in the last *Quarterly*? I cannot believe they would still have mailed it if they had. The bullet points are an irritating mess. Please do not compress the text so much in future issues then we might see the content.
Mrs D. Wyatt, Regional Virus Laboratory, The Queen's University of Belfast

Micro Today looks excellent – well done on a great job.
Reg England, Convener, Fermentation & Bioprocessing Group

Microbiology Today looks extremely smart in its new design but I am afraid that for my ageing eyes improved style has been achieved at the sacrifice of readability – main stories I can just about cope with but MicroShorts, Meetings and Reviews are all something of a challenge.
Professor Roy R. Russell, The Dental School, University of Newcastle-upon-Tyne

GREAT!!!... well done.
Professor Nick Russell, Microbiology Laboratories, Wye College University of London

MT launch

A lunch was held to mark the launch of the new magazine. The production and distribution team at Marlborough House was joined by the Editor Dave Roberts, the designer, Ruth Gregory, from Graphics International who was responsible for the new look, Ina Cocks from NWH Sales, and Paul Yates and John Young from Warwick Printers who made such an excellent job of turning the design into print. We were sorry that the editorial board members were unable to join the celebrations.

The editorial team has taken note of comments on the style and size of typeface. These will be changed in due course to improve the readability of *Microbiology Today*. Please let us know your views.